

INVESTIGATION OF CELL CYCLE NETWORK OF YEAST USING LAB-ON-A-
CHIP PLATFORM

by

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to my beloved family...

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ABSTRACT

INVESTIGATION OF CELL CYCLE NETWORK OF YEAST USING LAB-ON-A CHIP PLATFORM

Genome instability has long been implicated as a salient causal factor in aging and age related diseases such as cancer and neurodegeneration. However, the molecular mechanisms associated with genome instability remain unclear. Recent studies suggest that growth signaling in the organism *Saccharomyces cerevisiae* and in higher eukaryotes might affect oxidative stress and aging/age-related diseases by activating DNA replication stress that causes DNA damage. Within the scope of this thesis, to investigate the role of Sld7p in yeast cells, a network biology approach, combining the interactome and transcriptome data of budding yeast, was applied. The protein-protein interaction networks of cell cycle and chronological aging processes in budding yeast were reconstructed. The ultimate aim was to determine the putative molecular function of the Sld7p by using microfluidic technology, where the behavior of the wild-type and *sld7Δ* mutant cells were followed on a Lab-on-a-Chip (LoC) platform under different conditions and consequently to support the computational studies by experimental findings. The wild type and mutant yeast cells were grown in glucose rich YNB or YPD media with or without an alkylating agent. The brightfield microscopy images of the trapped cells were captured by Nikon Eclipse-T inverted microscope at regular time intervals and then processed via ImageJ software. The results of cell count, area and perimeter values were analyzed and used to calculate the cell sizes (mother and bud). The durations of G₁ and S/G₂/M phases of wild type and *sld7Δ* mutant cells were determined under control and environmental stress conditions. Our multi-omics approach indicated a dual role for Sld7: it participates in “macromolecular complex binding” in cell cycle and has “oxidoreductase activity” in chronological aging in budding yeast. These potential functions of Sld7 in yeast can offer new insights on the role of its putative homolog MTBP in humans.

ÖZET

ÇİP-ÜZERİNDE-PLATFORM KULLANARAK MAYA HÜCRESİNİN YAŞAM DÖNGÜSÜNÜN İNCELENMESİ

Genom istikrarsızlığı, kanser ve nörodejenerasyon gibi yaşlanma ve yaş ile ilgili hastalıklarda rol oynayan belirgin bir faktör olarak gösterilmiştir. Bununla birlikte, genetik istikrarsızlık ile ilişkili moleküler mekanizmalar belirsizliğini korumaktadır. Son çalışmalar, maya ve daha kompleks ökaryotlarda bulunan büyüme sinyalinin DNA hasarına sebep olan DNA eşlenme stresini aktive etmesi ile oksidatif stres ve yaşlanmaya bağlı hastalıkları etkileyebileceğini göstermiştir. Bu tez kapsamında, maya hücrelerinde Sld7 proteininin rolünü araştırmak için, mayanın interaktom ve transkriptom verilerini birleştiren bir ağ biyolojisi yaklaşımı uygulanmıştır. Gelişmekte olan mayadaki hücre döngüsü ve kronolojik yaşlanma süreçlerinin protein-protein etkileşim ağları yeniden oluşturulmuştur. Nihai amaç, farklı koşullar altında Çip-üzerinde-Laboratuvar platformu kullanılarak yabancı tip doğal fenotipli ve sld7Δ mutant hücrelerinin davranışlarının izlendiği mikroakışkan teknolojisi kullanarak Sld7 proteininin varsayılan moleküler fonksiyonunu belirlemek ve hesaplama çalışmalarını deneysel bulgularla desteklemektir. Yabancı tip hücreler ve mutant maya hücreleri, hem alkilleyici bir faktör ile hemde normal koşullarda glikoz açısından zengin YNB veya YPD ortamında yetiştirildi. Tutulan hücrelerin ışık mikroskobu görüntüleri Nikon Eclipse-T ters mikroskop ile düzenli aralıklar ile çekilmiş ve daha sonra ImageJ yazılımı ile işlenmiştir. Hücre sayımı, alan ve çevre değerlerinin sonuçları analiz edilmiş ve hücre boyutlarını (ana ve tomurcuk) hesaplamak için kullanılmıştır. Yabancı tip ve sld7Δ mutant hücrelerinin G1 ve S / G2 / M fazlarının süreleri, kontrol ve çeşitli çevresel stres koşulları altında belirlenmiştir. Çoklu omik verileriyle sistem biyolojisi yaklaşımı sonucunda, Sld7 proteininin iki farklı görevi olduğu görülmüştür, ve bunlar: hücre döngüsünde "makromoleküler kompleks bağlanmaya" katılması ve tomurcuklanan mayada kronolojik yaşlanmada ise "oksidoredüktaz aktivitesi" şeklindedir. Mayada bulunan Sld7 proteininin bu muhtemel fonksiyonları, insandaki homolog proteini olarak varsayılan MTBP'nin insan metabolizmasındaki rolü hakkında yeni bilgiler sunabilir.

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LIST OF ACRONYMS/ABBREVIATIONS

ACT	Annotation collection table
BSA	Bovine serume albumin
CAN	Chronological aging network
CCN	Cell cycle network
CLS	Chronological life span
COC	Cyclic olefin copolymer
COP	Cyclo olefin polymer
GO	Gene ontology
MMS	Methyl methanesulfonate
MTBP	Mdm two binding protein
N1	YPD medium
N1R	Fresh YPD medium
N2	YPD medium (0.01 % MMS)
OD	Optical density
PC	Polycarbonate
PDMS	Poly (dimethylsiloxane)
PEEK	Polyether (ether ketone)
PMMA	Poly (methylene methacrylate)
PS	Polystyrene
RLS	Replicative life span
ROS	Reactive oxygen species
SC	Synthetic complete
SPA	Selective permissibility algorithm
WT	Wild type
YNB	Yeast nitrogen-base
YPD	Yeast extract-peptone-dextrose

1. INTRODUCTION

The study of protein-protein interactions is essential to the understanding of how proteins function within the cell. Characterization of the proteins in a given cellular interactome (whole set of molecular interactions in a particular cell) is important to understand the biochemistry of the cell. Structure of cell and expression of the genes encoding the proteins change at different conditions to orchestrate the myriad of several processes. These processes include differentiation, signalling, cell-cycle control, protein folding, transcription, translation, post-translational modification and transport (Alberts *et al.*, 2002).

The improvement in healthcare has been mainly based on the research on organisms, which have high common genetic information with *Homo sapiens*. Yeast is a unicellular (single-cell) eukaryotic microorganism, which duplicates itself by budding, asexually. The yeast cells have more than a thousand genes that match with human genome. But, this genetic match was not the only reason to use the yeast cells instead of human cells in research. The time of duplication is also short compared with a human cell (90 min vs. 24 hours). In addition to these, the manipulation of the DNA of yeast cells is also easy (Alistair and Brown, 1998). The cellular response in yeast cells, chronological lifespan and aging processes can easily be associated with those in human cells as there are certain similarities in cellular organization. These facts provide an opportunity to detect diseases at their early stages and/or improve treatments.

In recent years, microfluidic devices have become popular because of its numerous advantages as research tools. In these systems, at least one dimension of the channel should be at the order of micrometer. The microfluidic devices were first developed in 1980s by producing silicon based microfluidic devices, and gave rise to industrial microfluidic applications. These devices are used in chemical and biological applications such as DNA analysis, cell culturing or organ on a chip. Some of the advantages of these systems are high-throughput analysis with low cost, effective visualization of cells and reproducibility of devices. For the applications of yeast cells, cell trapping has an important role on visualizing the response of the single cells or a colony under different environmental

conditions (Whitesides, 2006). To visualize the reactions of the yeast cells against stressful conditions is more convenient in a microfluidic device than conventional bioreactors (Halldorsson *et al.*, 2015).

The fabrication of microfluidic devices are cheaper than those of other conventional systems. To produce the microfluidic devices from polymers such as PDMS, PMMA, COP or PS, the soft lithography and photolithography techniques are used. (Yeo *et al.*, 2011). These polymers are convenient to create microfluidic devices due to their optical transparency, mechanical strength, biocompatibility and ease of fabrication. The scheme of the three different microfluidic devices that were designed for different purposes is given in Figure 1.1.

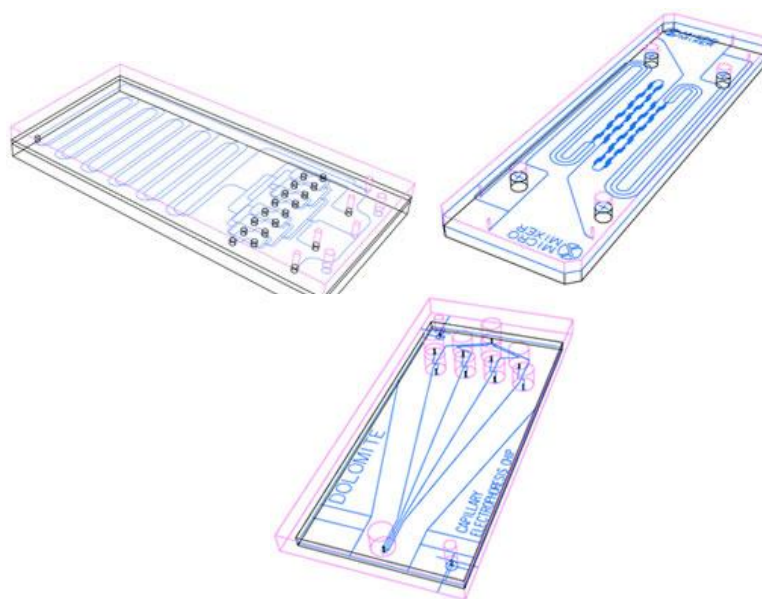


Figure 1.1. The microfluidic devices with different designs

In this thesis, the putative molecular functions of Sld7 in chronological life span (CLS) and replicative life span (RLS) were first studied by computational methods using an integrated systems biology approach. The ultimate aim is to determine the putative molecular function of the Sld7p by using microfluidic technology, where the behavior of the wild-type and *sld7Δ* mutant cells were followed in a Lab-on-a-Chip (LoC) platform under different circumstances and consequently to support the computational studies by experimental

findings. The fabrication of the microfluidic device was performed using polystyrene by hot-embossing and thermal bonding techniques. Yeast cells of different genetic background were trapped in the 0.5 nL c-shaped trapping regions and culturing of these cells under different environmental conditions were performed successfully in the PS made microfluidic device. The bright-field microscopic images were processed by ImageJ software to analyze the cell growth.

To provide an insight about the fabrication of microfluidic devices and their applications on yeast cells, the second chapter gives literature information and is designated as “Theoretical Background”. The third chapter, entitled as “Materials and Methods”, explains the system biology approaches, fabrication steps of the microbioreactor and image processing steps in detail. In the fourth and fifth chapters, the computational findings on the putative function of Sld7, and the experiments done by the microfluidic device and the remarks from the experiments are given, respectively. The durations of the cell cycle phases of the wild type and *sld7Δ* mutant cells under different environmental conditions are discussed in chapter 6. The last chapter consists of the conclusions achieved by this study and the recommendations for future works.

2. THEORETICAL BACKGROUND

2.1. Role of Sld7

Dpb11 is a protein expressed in *Saccharomyces cerevisiae*, and it is responsible for DNA replication initiation. Proteins that have synthetic lethality to Dbp11 are called as Sld- such as Sld2, -3, -5 and -7, which also have a role in DNA replication. Altmann and Westermann (2005) did a study that resulted in a putative molecular function of Sld7. In this study, the essential cellular proteins with mitochondrial morphogenesis as their molecular function were identified by deleting non-essential proteins. The mutant cells were grown on YPD medium in 96-well plate. Each mutant's cell cycle was observed under the fluorescence microscope. Four mutants with unknown protein function were found, which were *erg29*, *fcf1*, *pga2* and *sld7*. These proteins might have a role in cell physiology or lead to pleiotropic defects (Altmann and Westermann, 2005). Sld7 is a limited abundance protein that regulates DNA replication initiation by decreasing the association of Sld3 with Cdc45, both of which are essential proteins in the G1 phase of cell cycle (Araki, 2010; Tanaka *et al.*, 2011b). The depletion of Sld7 slows down DNA replication and cell growth in budding yeast (Tanaka *et al.*, 2011b). The crystal structures of Sld3 and Sld7 were determined by X-Ray crystallography, and binding of Sld7 to N-terminal of two Sld3 molecules was noted earlier (Itou *et al.*, 2015). The quaternary structure of Sld7-Sld3 complex (Figure 2.1) is suitable for binding Cdc45 and GINS onto Mcm2-7 on a replication origin (Itou *et al.*, 2015). Sld7 is localized in nucleus and co-localized with spindle pole (Huh *et al.*, 2003). Despite the comprehensive research, the molecular function of Sld7 is still unknown.

A functional relation between yeast Sld7 and human MTBP has been proposed (Boos *et al.*, 2013). MTBP is one of the binding proteins of Treslin/Ticrr, which is the homolog protein of Sld3 in yeast. The reduction of MTBP affects human cells in the same way as the depletion of Sld7 affects growth in yeast cells. The expression of MTBP was reportedly elevated in breast and ovarian cancers (Boos *et al.*, 2013; Grieb *et al.*, 2014). The data from The Cancer Genome Atlas (TCGA) confirmed that 19 % of breast cancer patients showed amplification and overexpression of MTBP. The clinical research indicated that the increase in the expression of MTBP led to the reduction in the survival of breast cancer patients (Grieb

et al., 2014; Bassi *et al.*, 2016; Chen and Butte, 2016; Han *et al.*, 2016). Thus, targeting MTBP might be a novel promising therapeutic approach in the treatment of cancer. Indeed, the research in mouse haplo-insufficient in MTBP revealed a decrease in the growth and development of lymphoma (Odvody *et al.*, 2010). The potential of MTBP in human or Sld7 in yeast as a drug target needs to be explored for therapeutic purposes.

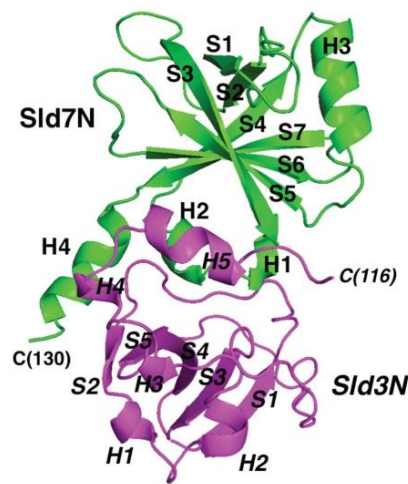


Figure 2.1. The quaternary structure of Sld7-Sld3 complex

Gene expression data is used to estimate the molecular function of an interested protein at different conditions. Spellman *et al.* (1998) formed a genomic dataset for cell cycle regulated genes of *Saccharomyces cerevisiae*. Eight hundred genes regulate cell cycle process in yeast. More than 400,000 individual measurements were done under different circumstances by using DNA microarray that was used to analyze the fold change in the cell cycle genes' expression levels. The co-regulated genes in cell cycle network were found by using cluster algorithm (Spellman *et al.*, 1998).

2.2. Microfluidic devices for yeast cell applications

In 1980's microfluidic devices for biological and chemical applications became popular (Whitesides, 2006). These devices were fabricated by using photolithography or soft lithography techniques from polymers as polydimethylsiloxane (PDMS), polymethyl methacrylate (PMMA), cyclic olefin copolymer (COP) and polystyrene (PS), which have

high mechanical strength, elasticity, biocompatibility and transparency. These devices are designed for applications such as DNA analysis, cell culturing and eventually for organ-on-a-chip. The created designs provide high-throughput analysis at low cost, accurate data collection with small amount of reagent consumption and effective visualization of cells (Pierce, YEAR). For the applications of yeast cells, cell trapping becomes an important point to visualize a single cell or a colony during the replicative lifespan for aging (Alistair and Brown, 1998; Yucel and Ulgen, 2011; Bell *et al.*, 2014; Crane *et al.*, 2014), genetic interaction, cell cycle dynamics (Lee *et al.*, 2012) and environmental response (Crane *et al.*, 2014; Werner *et al.*, 2011). The studies with pioneering designs reported in literature in the last 10 years are summarized below and listed in Table 2.1.

A micro-fabricated elastomer chip was designed by Groisman *et al.* (2005), which was suitable for both bacteria and yeast in shallow microscopic microchamber arrays. The microchip was fabricated by using PDMS and it consisted 16 flow through symmetric channels and 340 chambers. The channels were used for nutrient flow and chambers were used for trapping the microorganisms. These chambers allowed chemical diffusion, but impassable to cells. To grow the colonies in each chamber, they used the advantage of height differences between channels and capillaries. The design and operation of microfluidic device was illustrated in Figure 2.2. Due to the high flexibility of PDMS, depths of capillaries were increased by gauge pressure. The nutrient flow was continuous that helped to refresh the chamber environment. The response of the trapped cells was observed with respect to exogenously added auto-inducer and temperature change. It was seen that the addition of auto-inducer decreased the growth rate of cells, in contrast, its removal increased growth rate. Cell growth was affected in a positive way by increasing the temperature. Chamber size was increased at different temperatures to observe its effect on cell density. The cell density was not changed at 20 °C whereas it decreased at 35 °C. Fluorescence microscope was used to observe the GFP tagged cells (Groisman *et al.*, 2005).

A chemostat is a bioreactor in which the interested microorganism can be growth at steady state, keeping the media composition constant. Chemostats provide opportunities for collecting data in metabolic, biochemical and physiological areas because of significant number of experiments at high accuracy. Nevertheless, these experiments had expensive

instrumental system with low throughput and they consumed large volume of culture medium and reagents.

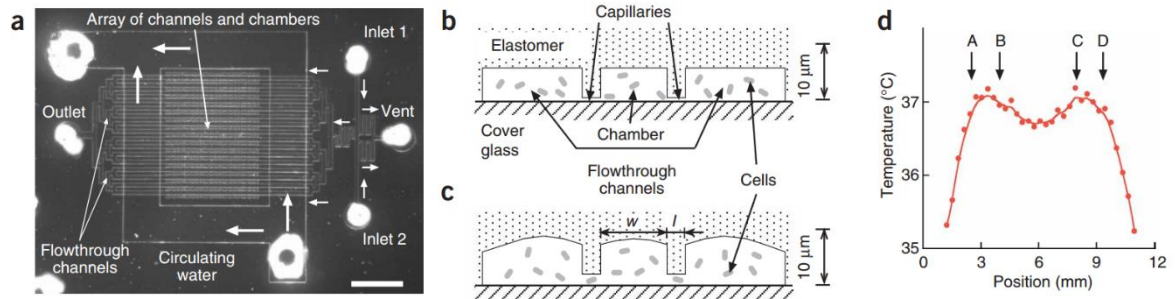


Figure 2.2. (a) Micrograph of the PDMS device (b) Schematic drawing of a cross-section of the array showing two channels at low gauge pressure (c) Capillaries depth at high gauge pressure (d) Temperature profile along a line

Park *et al.* (2013) developed fully automatic microchemostat to control cell growth at steady state by using periodic replenishment with high throughput and low cost. The microchemostat system was designed as two-depth chamber to prevent fluctuations at steady state and allowed monitoring the single cells directly. Therefore, system was divided into two regions according to their depths. These were a circulation region with 100 μm depth (deep region), where cell movement occurred by using peristaltic pumps, and a replenishment region with 20 μm depth (shallow region), which was segmented as 1 % of the bioreactor volume. Two pneumatic valves provided isolation to replenishment region from the bioreactor. The isolated part was replenished with fresh culture medium and the fresh part was back to the bioreactor with an arrangement of valves as on or off (Figure 2.3). The microchemostat system was fabricated by 15 μm - thick PDMS and *Saccharomyces cerevisiae* was used as the microorganism. The cell density was calculated for different dilution rates at steady state. It was observed that increasing the dilution rate decreased the cell density at steady state as expected. The budding of cells were observed clearly in the shallow region of chamber. The images were taken with Nikon optical microscope. The developed system could not only be used for culturing cells but also for studying and monitoring metabolic screening and drug response of microorganisms (Park *et al.*, 2013).

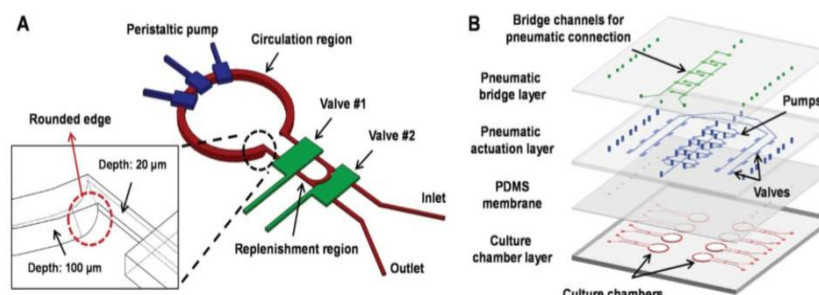


Figure 2.3. (a) A schematic illustration of a single small-volume fraction replenishment microchemostat unit composed of a two-depth culture chamber structure, a set of pneumatically actuated peristaltic pumps, and two valves. (b) An illustration of the 8-unit microchemostat array composed of four PDMS layers. (Park *et al.*, 2013).

The microfluidic device was designed as an array of 1152 microchemostats, which were divided into three independent sections (Figure 2.3). Medium was loaded into the system by two different sources, whereas the nutrients were loaded by diffusion to each chamber. The device was composed of two layers, which were a flow and control layer. These layers were fabricated on separate molds by using standard photolithography processes. In order to prevent clogging in the microchemostat, the divided cells were carried away by the medium stream and eluted from the device. The cells were forced to grow in a monolayer due to the pressure difference across the chambers and sieve channels were used to trap the cells, and hence cells could not go out from the bottom of the chemostat. To control the automated fluorescence microscope a Visual Basic program was written and images were taken at 20 minute time intervals. The time consumed for imaging the cell growth and changing the media was 6 and 7 hours, respectively. By the help of custom written image analysis software, chamber and cell segmentation, background correction, signal deconvolution and protein localization analyses were completed at the single cell level.

Another microfluidic device was developed by Crane and co-workers, called ALCATRAS (A Long-Term Culturing And TRApping System), to observe the behaviour of 1000 cells of *Saccharomyces cerevisiae* in a controlled environment. The device was designed in AutoCAD software and fabricated from PDMS by using conventional soft-lithography techniques. The produced device had three inlet ports. The cells were loaded

into central inlet and media were refreshed by using the side inlets that were controlled by a programmable syringe.

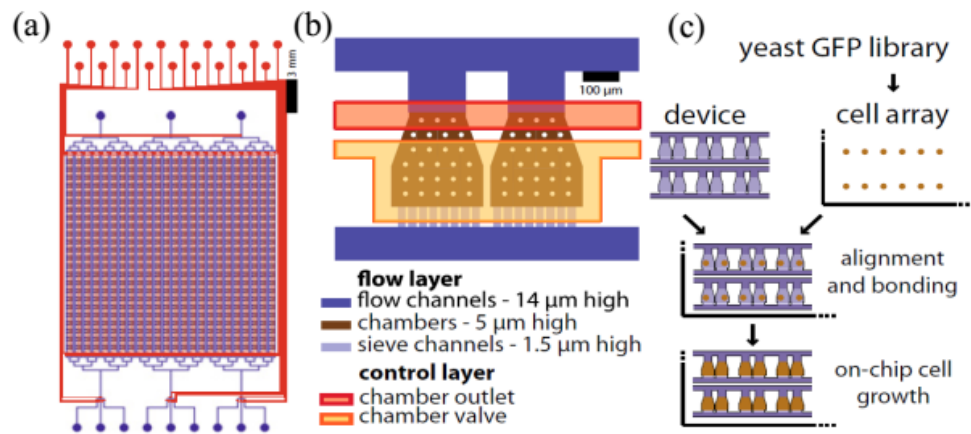


Figure 2.4. (a) Design of the parallel microchemostat array for culturing 1,152 yeast strains with flow and control layers (b) Schematic of a pair of microchemostats. (c) The chip programming process (Denervaud, 2013).

The chamber included more than 1500 individual cell traps with two pillars for each (Figure 2.5). The design of the chamber provided positive feedback, which prevented accumulation on the same pillars, and helped the removal of daughter cells. The daughter cells were removed according to their polarity. The daughter cells were distracted between pillars if the bud formed downstream of the mother cell. The diverted flow removed the daughter cells that were budded from the top of the trap (Figure 2.6). The hydrodynamic resistance of the trap prevented the escape of trapped cells by generating pressure difference from top to bottom of the trap. The cell cycle dynamics, cell viability and cell stress response were studied. The interested proteins were tagged with GFP to observe the cell behaviour under given circumstances. Heat shock protein Hsp104p –GFP was used to observe cell viability. More than 1000 cells were monitorized at 10-minute intervals in 50 hours. Whi5p – GFP fusion, which was localized in nucleus during late M and early G1 phase, was imaged to characterize cell division dynamics. Whi5p – GFP fusion provided periodic signal and helped to extract statics on the division of each cell. Msn2p - GFP was used to observe the cell stress response to glucose rich and glucose limited environments. It was observed that the tagged protein (Msn2p) was localized in cytoplasm in glucose rich medium and in

nucleus in glucose limited medium. Nikon Eclipse Ti inverted microscope was used to monitorize cells (Crane *et al.*, 2014).

High-throughput yeast aging analysis chip (HYAA-chip) was produced by Jo *et al.* (2015) to investigate life span and aging processes of *Saccharomyces cerevisiae*. The developed device was capable of investigating 8000 budding yeast cells individually. The designed HYAA-chip had 16 discrete parallel channels that were grouped into four modules. It enabled to run experiments with 16 different strains in a single type of medium or four different strains up to four different media. PDMS was used to fabricate the designed chip with distinctive column and row spacing parameters such as 6 μm , 12 μm and 18 μm . It was observed that retention time and number of trapped mother cells were high in 6 μm x 6 μm chambers, but washed daughter cells caused clogging. Therefore, the chip size was determined as 12 μm x 12 μm .

The traps were designed as cup shaped to capture mother cells; continuous flow was applied to remove daughter cells (Figure 2.7). It was observed that the single cell trapping reached to 96 %. The analysing time was reduced from 3-4 week to 3-4 days. The BY4742 strain was used as background for all mutant strains. Olympus IX-81 inverted microscope was used to observe cells. Positions of the cells were recorded at 10-minute intervals for 96 h by using 40X objective lens or 20 minute intervals for 72 h with 60X high N.A. oil immersion objective lens. The amount of glucose in the medium and terminal morphologies were followed to determine their effect on life span. The observation of fluorescence signal of single cells during the entire experiment provides high-throughput examination of the aging phenotype, including organelle morphology, gene expression, and protein localization. (Jo *et al.*, 2015).

Hansen *et al.* (2015) designed a microfluidic device with 5 channels by using PDMS and SU8 photo resist to localize Msn2 protein that was tagged with CFP and YFP. Photolithography was used in production of silicon wafer mold and soft lithography was used in molding of PDMS and fabrication of the final device. This device involved control valves. Time-lapse microscopy was used to visualize and analyse the localization of these proteins (Hansen *et al.*, 2015).

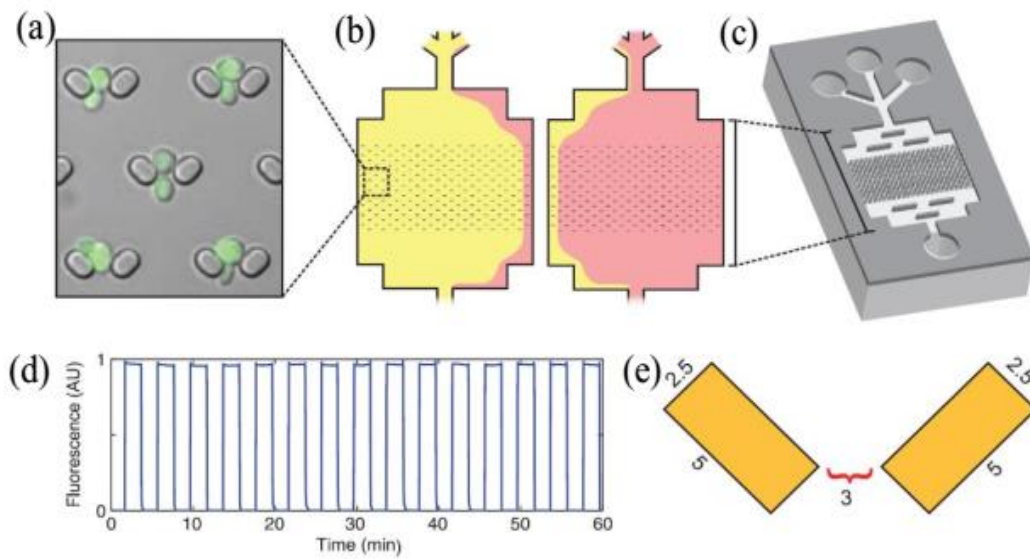


Figure 2.5. (a) A DIC image of the cell traps. (b) A schematic depicting fluid flow in the flow cell during media switching. (c) Overview of the microfluidic device. Each device contains more than 1500 individual traps. (d) The switching rate and reliability of media switching in traps. (e) Schematic showing trap dimensions in microns (Crane *et al.*, 2014).

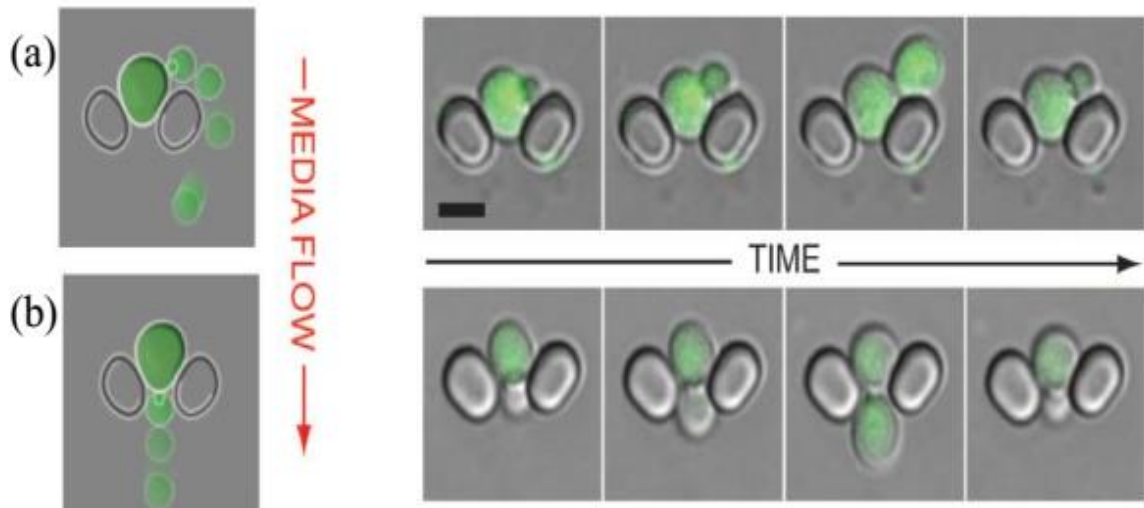


Figure 2.6. Chip design principle (a) the removal operation of a daughter cell by the media flow when the mother buds at the top of the trap or (b) at the bottom (Crane *et al.*, 2014)

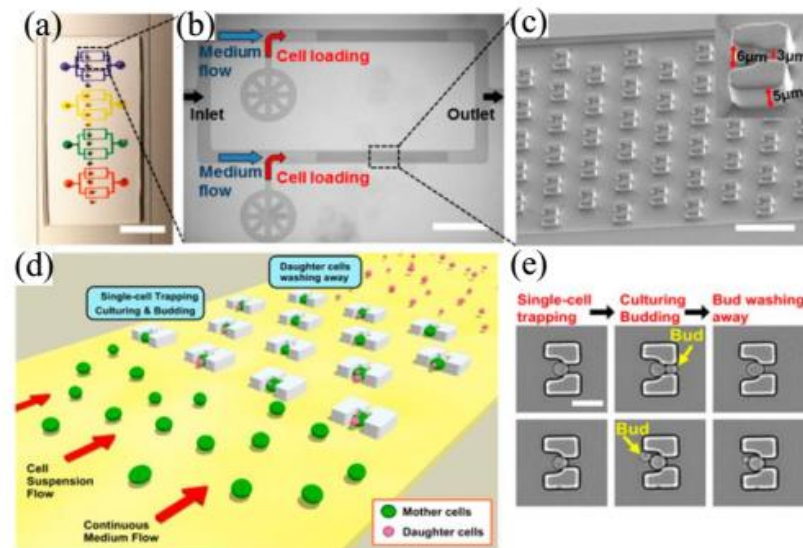


Figure 2.7. Chip design (a) optical image of the chip (b) microscopic image of branched trapping channels (c) SEM of trap arrays in the chip (d) chip working mechanism (e) a single yeast cell showing the working mechanism procedure (Jo *et al.*, 2015).

Table 2.1. Examples of Microfluidic Devices used for Cell Culturing

Reference	Device Material	Active/Passive	Microscopy	Yeast & Medium	Image Processing System	Single/Multiple
Park <i>et al.</i> , 2013	Microchemostat -15 µm thick PDMS –SU8	- Active - Peristaltic Pump (hydraulic) passive	- Nikon Optical Microscope -Scale bar 100 µm -Bright field	<i>S.cerevisiae</i> 100 mL Glucose limited YNB	-8 h (to observe different initial cell density affect) -	-Single cell
Crane <i>et al.</i> , 2014	- Glass -PDMS pillars	- Two programmable syringe pump (hydraulic resistance) passive	- Nikon Eclipse Ti inverted microscope - 60X 1.2 NA water immersion objective (Nikon) or -100X 1.4NA oil objective (Nikon)	<i>S.cerevisiae</i> cultured in glucose rich SC medium -1000 cells	- 10 min Hsp104-GFP expression -15 min → Whi5p-GFP expression	Single 1000 cells

Table 2.2. Examples of Microfluidic Devices used for Cell Culturing (cont.).

Reference	Device Material	Active/Passive	Microscopy	Yeast & Medium	Image Processing System	Single/Multiple
Jo <i>et al.</i> , 2015	PDMS SU-8 3005	-syringe pumps	CCD Camera(DP72; Olympus) inverted microscope (IX-81; Olympus)	- <i>S.cerevisiae</i> - BY4741 and BY4742 background	-10 min intervals → 96 h (40X)	- 8320 cells - Investigated individually

2.3. Microfluidic devices fabricated from PS and used in cell-based applications

Although PDMS is a preferred polymer for cell-based research, it has adverse effects such as high gas permeability and surface hydrophobicity. Polystyrene (PS), which is the material of petri dish and available commercially, is most common material for mammalian cell culture. Therefore, the performance of a PS made microfluidic device was tested by using PS of 1.2 mm thickness and compared to PDMS (Young *et al.*, 2011). Hot embossing technique was used for stamping the pattern to the PS surface. In this process, COP (at the bottom) and cellulose acetate (CA-between COP and PS) were used to flatten the surface and prevent stiction between PS and COP, respectively. Moreover, foam and teflon were put on the PS and mold to distribute pressure equally (Figure 2.7). The devices were fabricated via thermal bonding instead of solvent bonding, which is not preferable for biological applications because of the toxic residuals. The thermal bonding is applied below T_g to reduce the deformation of the channel, i.e its dimension, which may affect aspect ratio.

Delamination distance determines the bonding strength (shim stock) of the microchip, the less delamination distance, and the stronger bonded the microfluidic device. Increasing bonding time increases the collapse in channels by decreasing the height of the channels. To change the surface hydrophobicity and increase cell adhesion to the surface, oxygen plasma was locationally applied through the channels after bonding process, not to change bonding parameters. Human umbilical vein endothelial cells (HUVEC cells) were cultured in PS made chips for 48 hours (Figure 2.8). It was observed that culturing cells for long term was compatible in PS just as in PDMS.

PDMS is a very hydrophobic polymer with high level of adsorption and absorption. The stability of a converted hydrophilic PDMS was less than an hour, which is undesirable situation during experiments. Then, the surface of the biocompatible polymers, PS, PC, PMMA and COC, were tested by converting the hydrophobic surface to hydrophilic surface via UV-ozone or oxygen plasma to prevent adsorption of hydrophobic compounds. For all polymers, the hydrophilicity of the surface was more stable after UV-ozone treatment compared to oxygen plasma treatment. The microfluidic devices were fabricated via hot embossing and thermal bonding with Carver machine. The design of the microfluidic device was illustrated in Figure 2.10. The viability and proliferation of cultured HepG2 cells on microfluidic devices that were fabricated from each polymer were observed. The channels were coated with collagen to increase the adhesion of cells to the surface. The gas permeability, which is an important parameter to keep the level of oxygen and carbon dioxide constant in the medium, of the polymers were tested. All in all, PC and COC were the polymers that were better than PS and PMMA with respect to low gas permeability, high chemical resistance to organic solvents and cell viability (van Midwoud *et al.*, 2012).

Dong and Zhao (2015) designed a biochip to determine the specific pathogen among 13 types of uropathogenic microbes that cause urinary tract infections (UTIs) by employing immunosorbent ATP-bioluminescence assay (IATP-BLA) and to find the relevant antibiotic for patience according to detected microbial organism.

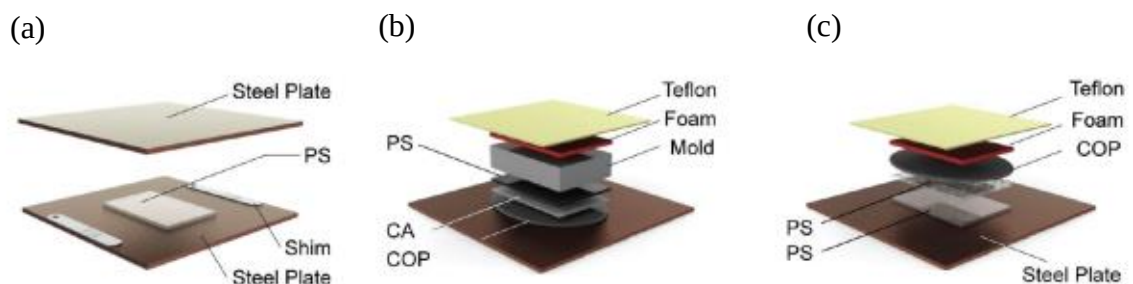


Figure 2.8. (a) Flattening of the PS sample to adesired thickness by use of metal shims. (b) Hot embossing of the PS sheet for through-hole fabrication (remove CA and COP for non-through hole approach). (c) Thermal bonding of two PS sample pieces (Young *et al.*, 2011)

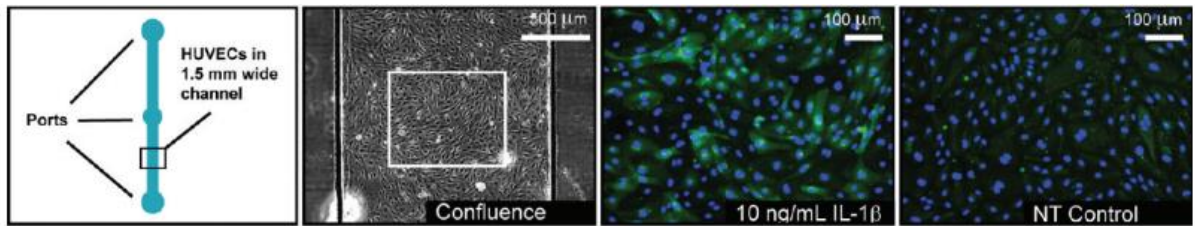


Figure 2.9. HUVEC monolayer culture and activation with IL-1 β in a straight PS microchannel. E-selectin expression (green fluorescence) was upregulated after 10 ng/mL IL-1 β treatment for 4 h. Nuclei were stained with Hoechst dye (blue) (Young *et al.*, 2011)

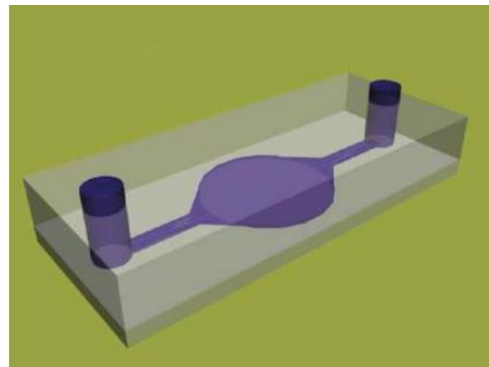


Figure 2.10. Schematic view of the chip used for adsorption and cell culture studies. The chamber dimensions are diameter 4 mm $\times$ 200 μ m (total volume $\sim$ 3.5 μ L), and the flow rate of medium used was 5 μ L/min (van Midwoud *et al.*, 2012).

The biochip consisted of 5 layers, two glasses as cover, two PS sheets of 2-mm thickness with 384 vertical reaction chambers as sample layer (urine and oxygen/air) or culture layer (microbes and other reagents) and fibreglass membrane, in which 8 different types of antibodies were immobilized, fixed between two PS sheets to target and capture microbes (Figure 2.12). The immobilized antibodies captured the specific microbes in loaded urine sample. Calcium alginate gel was used to encapsulate the captured cells and these encapsulated cells were evaluated by ATP-BLA in a microplate reader by using different methods for different channels. As a result, the biochip decreases the detection time of the pathogens from days to minutes and can be a model for the diagnosis of other diseases.

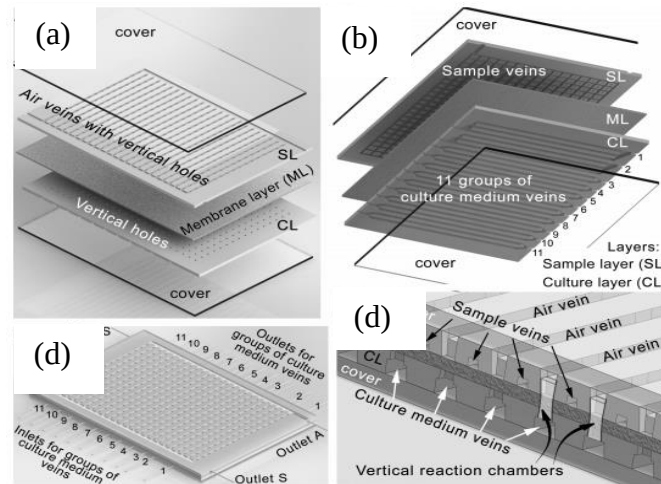


Figure 2.11. Architecture of the microfluidic simulator for the diagnosis and treatment of UTI. (a) Downward view of the chip layout. (b) Upward view of the chip layout. (c) The inlets and outlets of the device. (d) The meticulous structure of vertical reaction chambers is shown in the cross-sectional view of the device. (Dong and Zhao, 2015).

3. MATERIALS AND METHODS

3.1. Reconstruction and Analysis of Cell Cycle Network

The cell cycle network was reconstructed to determine the function of Sld7.

3.1.1. Network Reconstruction by Selective Permissibility Algorithm (SPA)

The aim is the determination of the “candidate proteins” that may be responsible in cell cycle of *Saccharomyces cerevisiae* by using Selective Permissibility Algorithm (SPA) (Arga *et al.*, 2007). The file entitled as ‘gene association’ (Carbon *et al.*, 2009) of *S. cerevisiae* was downloaded from Gene Ontology database (Wilson and Hunt). The first step of this process is to find the proteins that participated in cell cycle in terms of their Gene Ontology (GO) terms. To find these proteins the steps given below were followed. The ‘cell cycle’ term was searched in AmiGO Gene Ontology database (Carbon *et al.*, 2009). The cell cycle related proteins of *Saccharomyces cerevisiae* S288c (+) strain was downloaded, released on 04.09.2016. 860 known proteins in yeast were responsible for cell cycle. Annotation Collection Table (ACT) was formed by separating GO terms according to molecular function (MF), biological process (BP) and cellular component (CC) terms. This table was used to determine the “candidate proteins”. The GO terms of all the proteins in yeast were compared with those in the ACT to determine the proteins, which were related to cell cycle. The downloaded file ‘gene association’ included the GO terms of all proteins. These terms were separated into MF, CC and BP to match with ACT. The proteins, whose GO terms have an exact match with ACT, were taken into the list of candidate proteins. The interactors of these proteins were taken from the data given in Karagoz and Arga (2013). These interactors form the cell cycle protein-protein interaction (PPI) network (Figure 3.1). The chronological aging network was reconstructed by following same procedure as the reconstruction of cell cycle.

3.1.2. Cluster Identification and Functional Enrichment

Cytoscape Software was used to visualize and analyse the formed network. The cytoHubba plug-in was used to find important hubs (nodes) in a network. The proteins with the highest interactions can be found by using degree tool. The tool related to betweenness centrality gives a result for the proteins existing mostly in the determined pathway. The MCODE plug-in was used to find highly interacted proteins as a cluster. The MCODE results were given in terms of clusters, which include highly interacted proteins in the network (Figure 3.1). There were 23 clusters, yet none of them involved SLD7, the protein of interest. On the other hand, YDR277C, the interactor of SLD7, was in one of the clusters.

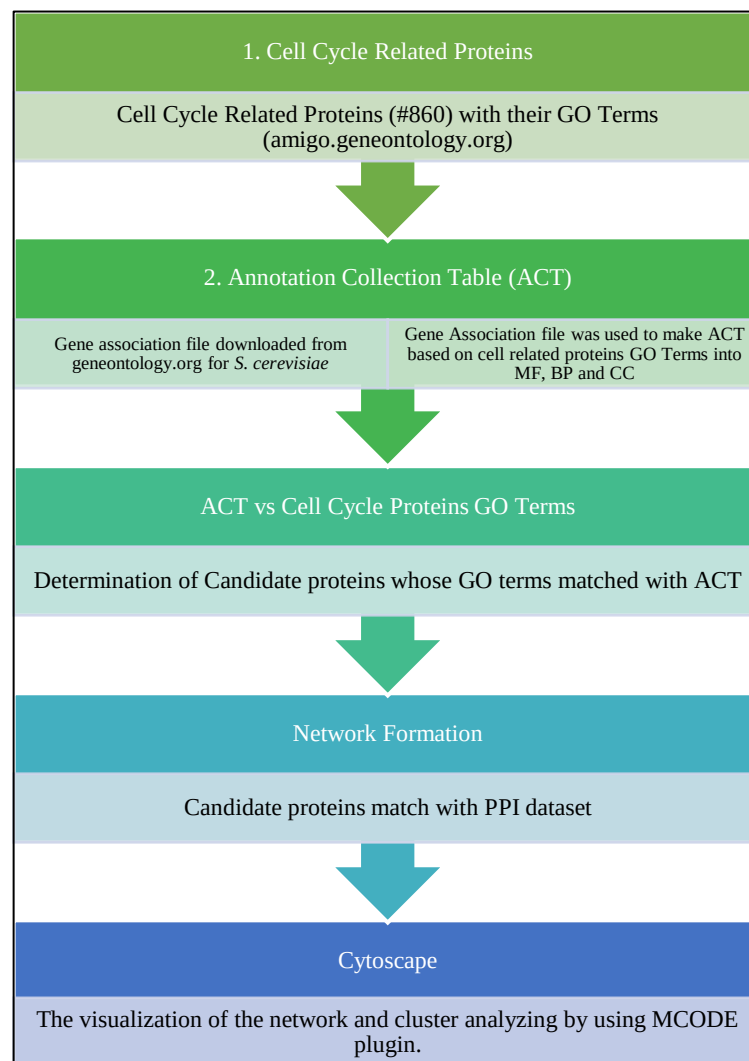


Figure 3.1. The flowchart of the cell cycle network formation

3.2. Linear Pathway Analysis

There are four different types of pathways in a cell, that are linear, branched, cyclic and equilibrium pool. Linear pathways convert one compound through a series of intermediates to another compound, such as glycolysis. In linear pathways, the proteins are directly connected with the previous and next protein. NetSearch algorithm (Steffen *et al.*, 2002) was used to determine the linear pathways of the cell cycle proteins and then to relate them with the interested protein, which is Sld7.

3.3. Chip Fabrication

A microfluidic bioreactor was successfully designed by Gençtürk E. (2015) and scaled down by Üçpınar S. (2016), to grow yeast cells, as a part of their MSc Thesis. In this study, the master mold that was produced via photolithography and electrochemical wet etching techniques as a part of Üçpınar S. (2016) MSc. Thesis was used to fabricate the 1nL microbioreactor.

3.3.1. Hot Embossing

Hot embossing is a technique which is used to fabricate microchannel from mask to thermoplastics (can be reshaped with temperature without losing physical and chemical properties) by controlling pressure and temperature (high pressure and elevated temperature was required). It has 3 main steps and these were embossing, cooling and demolding (He *et al.*, 2007). In the embossing step substrate (polymer) and mask were heated above the glass transition temperature (T_g) of polymer; then the mask was pressed into substrate, that process was called embossing. The transition temperature was unique for polymers and differs for each type. Below this temperature, the polymer was hard and brittle, whereas above this temperature the material was softened. Therefore, the temperature was kept above this glass transition temperature in embossing processes that makes it easier to imprint the pattern by decreasing the force. In the cooling step, system was cooled. Yet, the cooled temperature was important to reach perfect imprinted pattern. The temperature should be smaller than T_g , where the material becomes brittle. The last step was demolding where the separation of mask and polymer was called demolding or deembossing. The mold was released and ready

to using in the planned process. The steps of hot embossing process were illustrated in detail in Figure 3.2.

The thermoplastic materials that can be used in hot embossing processes were polymethylmethacrylate (PMMA), polystyrene (PS), polycarbonate (PC), polyvinylchloride (PVC), polyethylene naphthalate (PEN), cyclic olefin copolymer (COC) and cyclic olefin polymer (COP) (Wereley, 2006). This embossing technique was used in BioMEMS, BioSensors, micro total analysis systems and micro optics (Fiorini and Chiu, 2005).

For this study, polystyrene was used as the polymer substrate. The designed mask was transferred into PS pieces (3x3x1.5 mm) by using hot embossing technique. In this technique, hydraulic press machine was used (Carver 3851CE-0, The USA). The temperature of the hot plates was set to 98 °C. The height of the polymer piece was 1.5 mm, the width and length were 3 cm. The polymer piece was rinsed with distilled water and was dried with nitrogen gun. The prepared PS piece was set over the mask; the glass piece (0.2x5x5 cm) was put on PS (to emboss the design onto PS piece properly) and covered with aluminium foil. The aluminium-covered piece was pressed between hot plates under 35 bar pressure for 10 minutes. After 10 minutes, the hot embossed polymer piece was demolded from the mask (Figures 3.3 and 3.4).

3.3.2. Bonding

The inlet and outlet holes were drilled on the hot embossed piece for fluidic connections. The hot embossed and cover PS pieces were rinsed by isopropyl alcohol, distilled water and isopropyl alcohol. Then, both pieces were dried with nitrogen gun. Cover and hot embossed pieces were aligned and put in between the glass pieces (5x5 cm) which were used to prevent defects on the polymer pieces caused by aluminum foil (Figure 3.5a). The aligned pieces were bonded thermally at 93 °C under 10 or 8 bar for an hour. After 1 hour, the hydraulic press machine was turned off and the fabricated device was left to be cooled for 30 min under the same pressure at room temperature (Cao *et al.*, 2015). The fabricated microbioreactor was illustrated in Figure 3.5b.

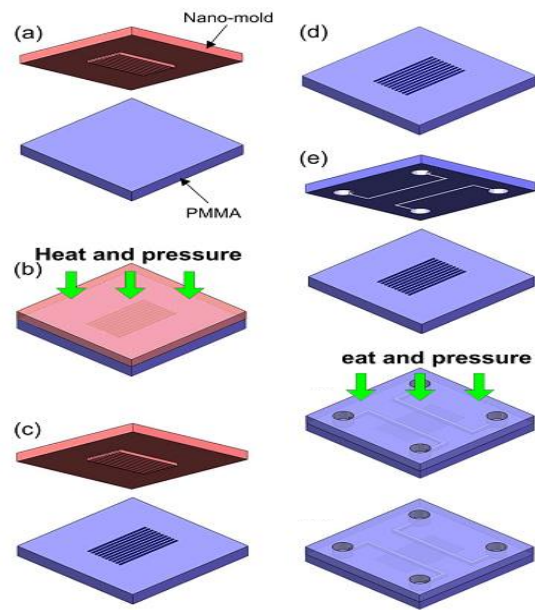


Figure 3.2. (a) the alignment of PMMA substrate and nano-mold, (b) hot embossing, (c) the separation of PMMA substrate and nano-mold, (d) fabricated 2D PMMA nanochannels, (e) the alignment of PMMA substrate and cover plate (Yin *et al.*, 2015).

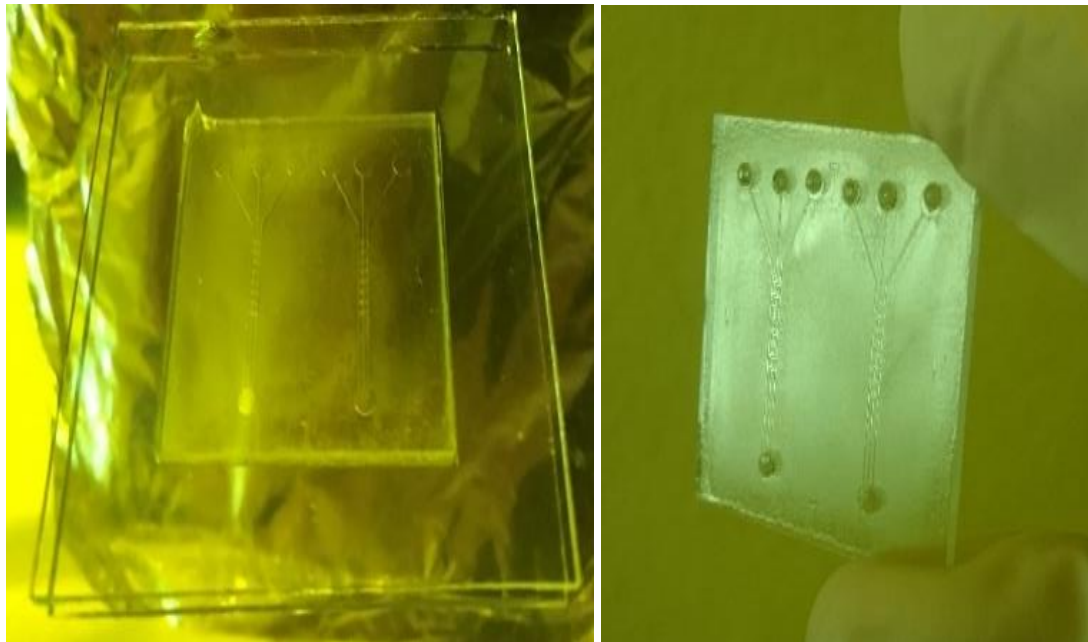


Figure 3.3. Hot embossing process (a) PS alignment on steel mask (b) hot embossed PS piece



Figure 3.4. The image of the chambers on hot embossed PS piece at 10X

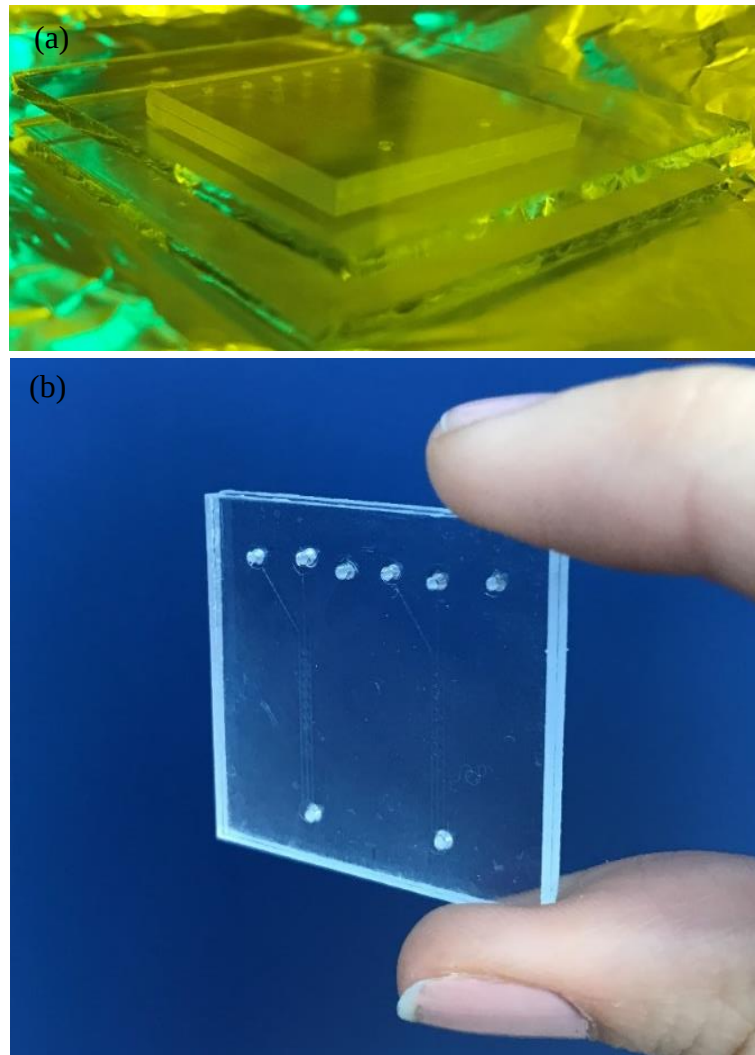


Figure 3.5. (a) PS pieces between two glass slides before bonding (b) the fabricated PS microfluidic device

3.4. Yeast Culturing in Liquid Medium

3.4.1. Sterilization

The laboratory equipment, which were graduated cylinders, erlenmeyer flasks of different sizes (100 mL, 250 mL and 1000 mL), 250 mL beaker, schott glasses and micropipette tips, were sterilized in autoclave at 121 °C for 15 min. The sterilized equipment were put under hood to keep them clean.

3.4.2. Yeast Culturing in Liquid Medium

The required medium for wild type BY4741 strain (Sld7-GFP) should be without histidine. The yeast cells are grown in low auto-fluorescence synthetic defined medium, which involved YNB (1.7 g), ammonium sulphate (5gr), glucose (2 % w/v), leucine (0.1 gr), methionine (0.02 gr) and uracil (0.02 gr). The glucose (20 gr) was dissolved in 300 mL distilled water and other ingredients were dissolved in 700 mL distilled water. On the other hand, Sld7Δ mutant cells of W303 background should be grown in a medium involving G418, which is an antibiotic used for selection of eukaryotic cells, and it is important that the media does not contain ammonium sulfate as the primary nitrogen source. In this case, monosodium glutamate (MSG) can be used as nitrogen source instead of ammonium sulfate in YNB media with G418. Unfortunately, MSG was not readily available in our laboratory. Therefore, 500 mL rich YPD medium, involving yeast extract (10 g), peptone (5 g) and glucose (10 g) was used. The glucose was dissolved in 150 mL distilled water and other ingredients were dissolved in 350 mL water. The YNB or YPD medium was prepared in sterilized erlenmeyer flasks and sterilized in autoclave at 121 °C for 15 min. To prevent the caramelization of glucose molecules, glucose solution was sterilized by using autoclave at 121 °C for 3 min. The solutions were mixed after sterilization. 1 mL (100mg) G418 (200 mg/L) was added to 500 mL YPD media just before the preparation of pre-culture. The precultures were prepared in 20 mL liquid YNB or YPD medium in 100 mL erlenmeyer flask. At least two precultures were prepared as a precaution. The cell and medium types that were used for experiments were given in Table 3.1.

Table 3.1. The cells and mediums that were used in the experiments

Strain Type	Haploid/diploid	Cell Type	Medium Type	Glucose Ratio (w/v)	Inhibitor (MMS)
BY4741	Haploid	Wild type Sld7 – GFP	YNB	2 %	-
BY4741	Haploid	Wild type Sld7 – GFP	YNB	2 %	+
W303	Diploid	sld7 Δ mutant	YPD	2 %	-
W303	Diploid	sld7 Δ mutant	YPD	2 %	+
W303	Diploid	sld7 Δ mutant	YPD	0.2 %	+
BY4743	Diploid	Wild type	YPD	2 %	+

3.4.3. Preparation of Frozen Stock

3.4.3.1. Agar Plate Culture preparation from frozen stock. Yeast culture can be reproduced on agar plates from frozen stock cultures. At first, the appropriate liquid culture, which is YPD or YNB without histidine was prepared for the yeast cells with respect to their genetic background. To prepare 500 mL YNB medium, methionine (0.01 g), uracil (0.01 g), leucine (0.05 g), ammonium sulphate (2.5 g) and yeast nitrogen base (0.85 g) were dissolved in 350 mL distilled water in a 500 mL flask. The glucose (10 g) was dissolved in 150 mL distilled water in 250 mL flask. The YNB medium and glucose solution were sterilized at 121 °C in autoclave for 15 min and 3 min, respectively. The flasks were cooled down, then 70 mL YNB medium and 30 mL glucose were mixed in 200 mL schott glass/bottle. The agar (8 g) was added into remaining medium (280 mL) and sterilized in autoclave at 121 °C for 15 min. The remaining glucose solution (120 mL) was stored in an oven at 70 °C. The sterilized medium with agar was mixed with glucose solution in the oven. The prepared agar medium culture was poured into petri dishes immediately. The petri dishes were wrapped with parafilm, after the medium was solidified.

The frozen cells were kept in 1 mL screw cap eppendorf tubes at -80 °C. The GFP tagged yeast cells from frozen stock were used to prepare preculture. These cells were let to

grow in liquid medium in an incubator at 28 °C. The inoculation loop was heated for sterilization and then cooled down on agar solid to not to kill yeast cells. The inoculation loop was immersed into flask, which contained liquid preculture, and then cells were spread on agar plate. At last, the agar plates were wrapped with parafilm and put upside down into oven at 28 °C for overnight. The prepared agar culture was given in Figure 3.6.

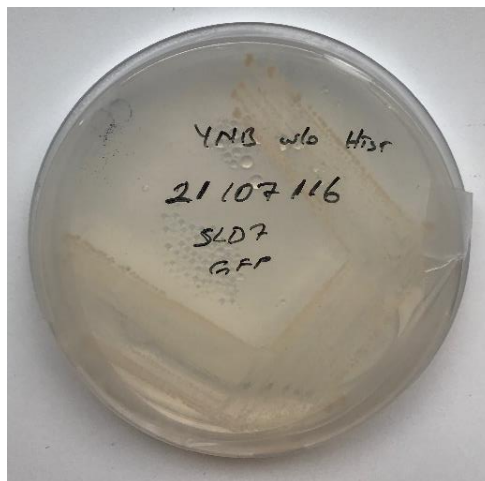


Figure 3.6. The prepared agar plate from frozen stock culture

3.4.3.2. Frozen Stock Preparation from Preculture. The prepared liquid preculture was used to prepare frozen stock culture. 30% glycerol containing solution was sterilized in autoclave at 121 °C for 15 minutes. By using micropipette, 0.5 mL glycerol and 0.5 mL preculture were mixed in screw cap eppendorfs and these eppendorfs were kept in refrigerator at -80 °C (Figure 3.7).

3.4.4. Experimental Setup

The connections to the fabricated microfluidic device were arranged with 200 µL yellow pipet tips (TipOne, USA SCIENTIFIC), silicon tubings (SCI, Micro Medical Tubing, 0.86 mm ID x 1.52 mm O.D.), epoxy (BISON 5 minute epoxy), PMMA (3x1x1) block and PDMS (3x1x0.1) piece. First, the PMMA blocks for inlet were drilled such that they matched to the inlet holes on the microbioreactor. Then, the pipette tips were cut until they tightly fitted the drilled hole on PMMA. The intersection of the pipette tip and the polymer were glued with the help of epoxy and let to dry at room temperature. The PDMS (3x1x0.1

cm) piece was put between PMMA block for inlet and PS microbioreactor to prevent leakage (Figure 3.8a). The metal piece was used to compress PMMA block inlet apparatus (Figure 3.8b). The 5 mL syringes were used to feed medium and liquid cultures into the microbioreactor. The syringes were inserted into syringe pumps. The diameter of the syringes and the pumping velocity were set as 15 mm and 5 $\mu\text{L}/\text{min}$, respectively. Then, one tip of the tubing was connected to the syringe and the other tip was connected to the inlet of the microbioreactor (Figure 3.9).



Figure 3.7. The prepared frozen stock that were kept in refrigerator

3.5. Image Processing

3.5.1. Brightfield Image Processing

The processing of the images of the cells were done via Fiji - ImageJ. The purpose of analyzing this image was to determine the dimensions and number of the yeast cells inside the chamber. The bright field image of a chamber in a microbioreactor was illustrated in Figure 3.10. The followed steps were shown in detail in Figures 3.11-3.13.

The interested region was drawn with *Polygon selection* in toolbox and *Edit* $\rightarrow$ *Clear Outside* was used to eliminate the images out of the interested area. The region of interest was shown in Figure 3.11.

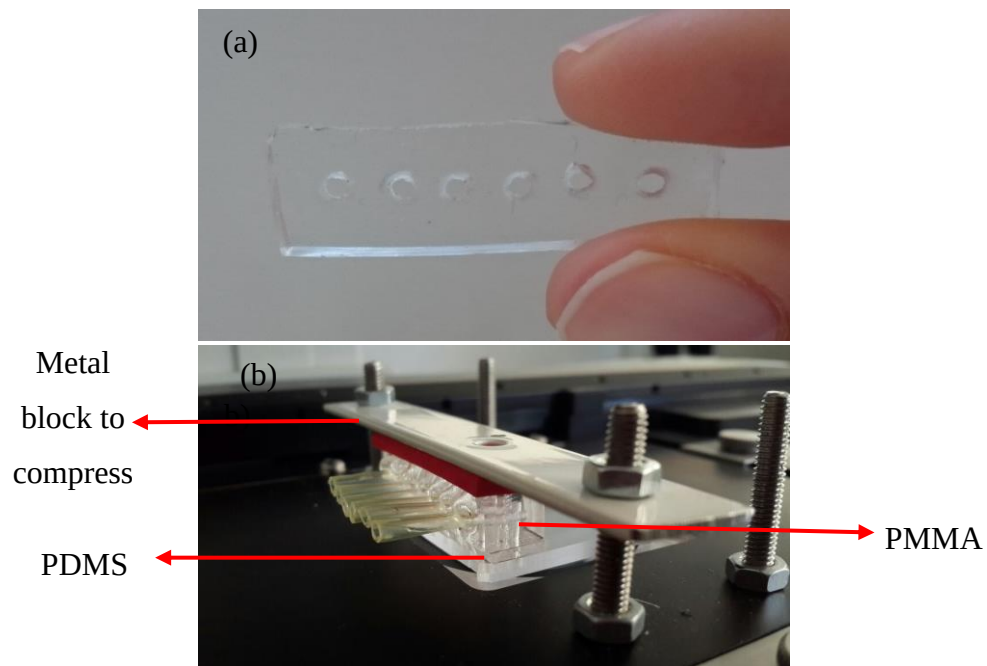


Figure 3.8. (a) The PDMS piece with inlet holes (b) The inlet set up with PDMS-PMMA-PS



Figure 3.9. Experimental set-up system with syringe pumps and Ti inverted microscope

The selected area was processed by using plugins under *Process* option menu lists all commands related to image processing, including point operations, filters, and arithmetic operations between multiple images. *Process* → *Sharpen* increases contrast and accentuates detail in the image or selection, but may also accentuate the noise (Figure 3.12a). *Process* → *Filters* → *Variance* was applied with 2.0 pixel radius to highlight edges in the image by replacing each pixel with the neighborhood variance (Figure 3.12b). *Process* → *Math* → *Subtract* commands were used and the subtract value was set to 50 (Figure 3.12c). *Process*

→ *Enhance Contrast* was used to make images clear by contrasting with the background (Figure 3.12d).

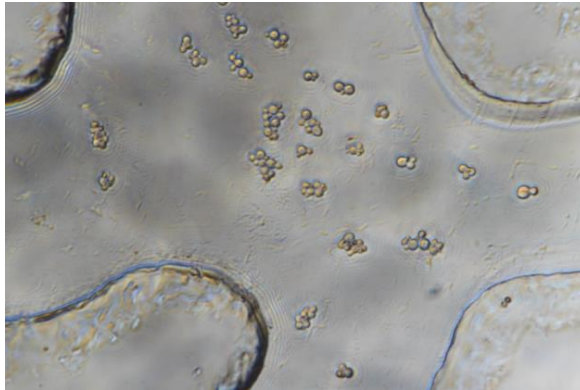


Figure 3.10. Bright field images of a channel

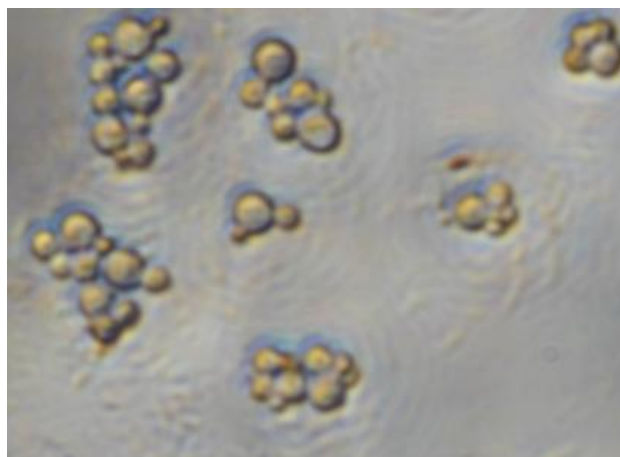


Figure 3.11. The region of interest in microbioreactor

The format of the image was RGB which was not suitable for thresholding process. In this case the image was transformed into 8-bit image by *Image* → *Type* → *8-bit*. Threshold tool was used to set lower and upper threshold values, segmenting grayscale images into features of interest. *Image* → *Adjust* → *Threshold*, *Otsu* threshold with *Red* was selected and *Dark Background* was chosen (Figure 3.13). There were some noises on the images. Then, *Process* → *Filters* → *Median* commands were used at 3.0 pixel radius to avoid these noises. The outline of the images was cleared by using the shaped tools in tool box and then the *Edit* → *Clear* was chosen.

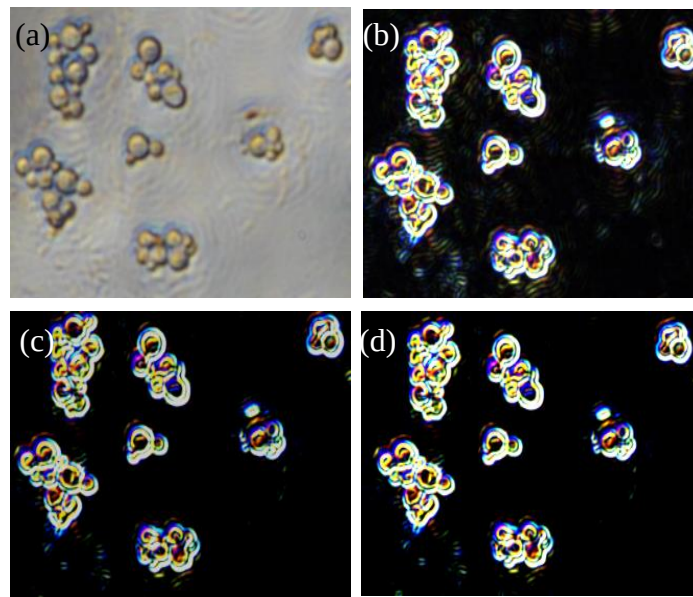


Figure 3.12. Image processing steps (a) sharpen selected area (b) 2 pixel radius variance (c) subtract with 50 (d) enhanced contrast

The processed images were analyzed by using *Analyze* → *Set Measurements* tool. The area, minimum and maximum grey value, mean grey value and perimeter were chosen. Then to analyze the number of cells in the ROI, *Analyze* → *Analyze Particles* tool was used and the size was arranged between 20- infinity and it was adjusted to show outline. Display results, clear results summarize and add to manager choices were chosen. The clusters might be counted as a cell, which was not reliable. In the present case, these clusters should be divided into cells; *Process* → *Binary* → *Watershed* tool was used to divide clusters into cells. After the division of clusters into cells, the same process was followed for particle analyzing.

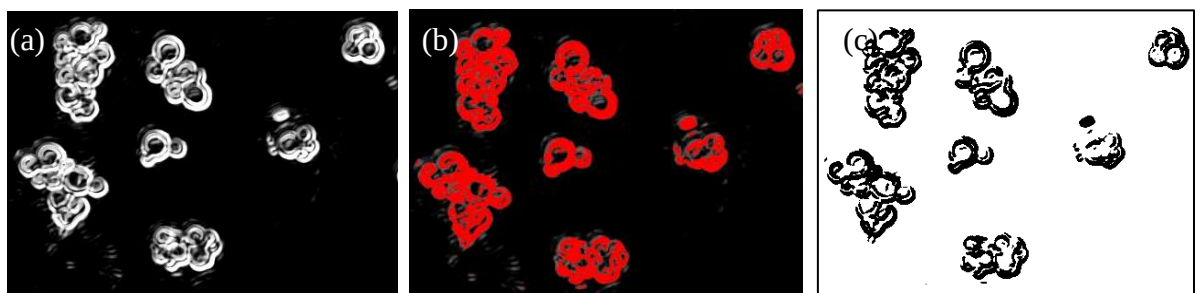


Figure 3.13. Threshold adjusting steps (a) 8-bit image (b) otsu-threshold with red (c) after applying threshold

3.5.2. Macro for brightfield image

The processing steps mentioned in previous section were applied for each images. Therefore, the macro was saved up to threshold value, which was different for each image. The steps after threshold were applied manually. The macro code for brightfield images was given in Figure 3.14.

```
run("Smooth");  
run("Sharpen");  
run("Variance...", "radius=2");  
run("Subtract...", "value=50");  
run("Enhance Contrast...", "saturated=0.4");  
run("8-bit");
```

Figure 3.14. Macro for brightfield images

4. COMPUTATIONAL RESULTS

4.1. Reconstruction and Analysis of Cell Cycle Network

Five cell cycle networks were constructed:

- CCN1 – cell cycle network including proteins with unknown GO terms (P, F, C),
- CCN2 – cell cycle network excluding proteins with unknown molecular function (F)
- CCN3 – G1 phase proteins with unknown GO terms (P, F, C).
- CCN4 – S phase proteins with unknown GO terms (P, F, C).

The list of proteins interacting with Sld7 (YOR060C) in the final network were given in Table 4.1. Sld3 (YGL113W) interacts with Sld7 (YOR060C) in each network.

The tool MCODE was run to find any cluster, which involves Sld7 (YOR060C). This process is called “top to bottom” approach.

Table 4.1. Comparison of PPI networks numerically

	CCN1	CCN2	CCN3 – G1	CCN4 - S	CAN
Number of Core Proteins	860	518	64	71	30
Number of Candidate Proteins	5284	2362	4091	4160	4038
Number of Interacted Proteins	4989	2288	3722	3822	3601
Number of Interactions	73579	28410	44311	45723	33772

4.1.1. Reconstruction of CCN1 – cell cycle network including proteins with unknown GO terms (P, F, C)

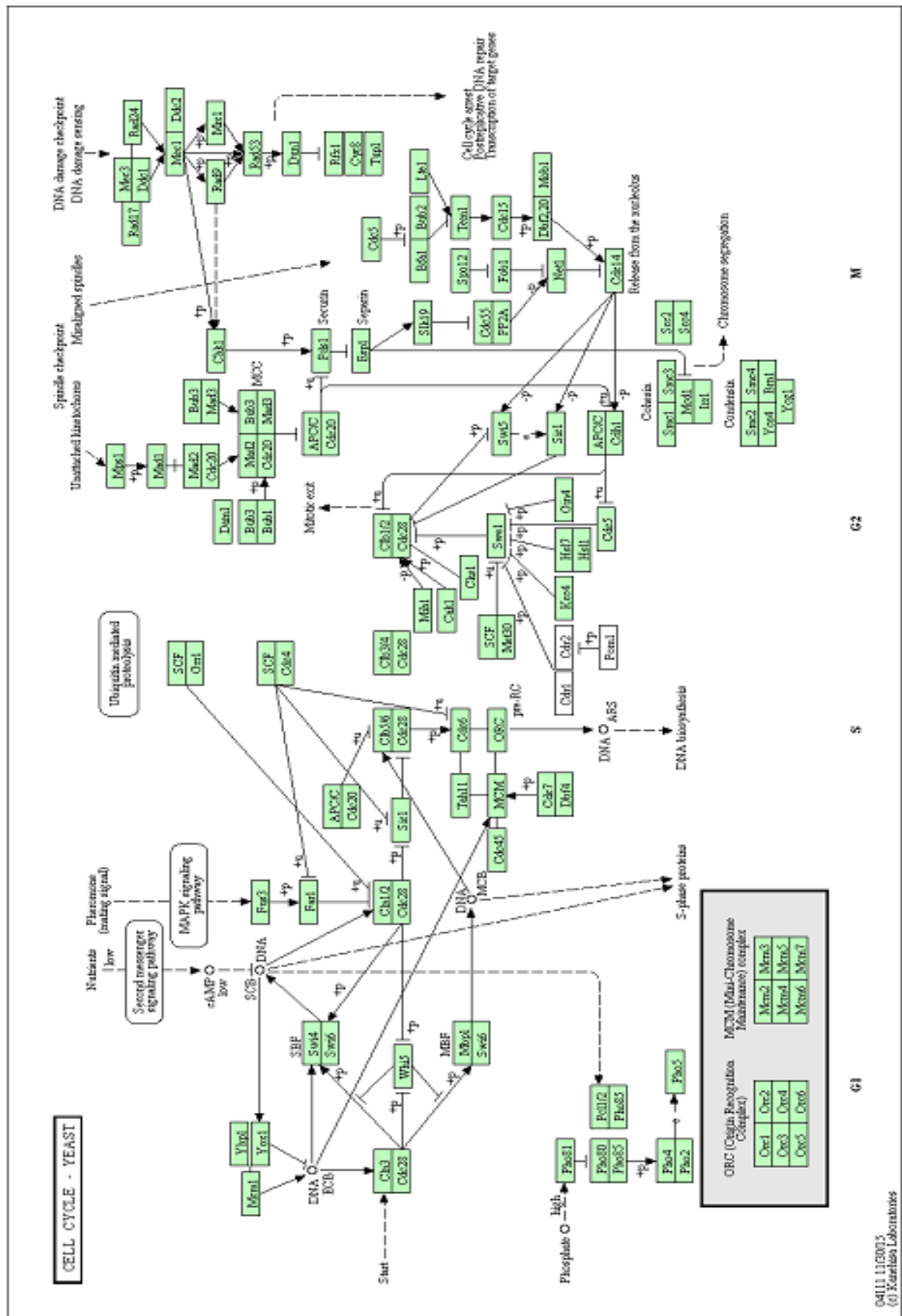
Starting with 860 proteins, 5284 proteins were found to be related to cell cycle network- CCN1. These 5284 candidate proteins were matched with the protein-protein interactor dataset (Karagoz and Arga, 2013). The interactions with a confidence score higher

than 0.5 were taken to get reliable results. If these candidate proteins match with both interactors (A and B), then they were accepted into the network. Among the 5284 candidate proteins, there were only 4989 interactors. The number of interactions was 72579.

In *Saccharomyces* Genome Database, three known proteins are reported to physically interact with YOR060C. These are HEK2 (YBL032W), (NAB2) YGL122C and SLD3 (YGL113W) (Hasegawa *et al.*, 2008; Batisse *et al.*, 2009; Tanaka *et al.*, 2011b). Moreover, other databases such as MINT, MIPS, IntAct, DIP, BioGrid include more than these three interactors, and the proteins SSA2 (YLL024C), NUP116 (YMR047C), (NAB2) YGL122C, HEK2 (YBL032W), SRP40 (YKR092C), MTH1 (YDR277C) are also reported as physically interacting with YOR060C (Karagoz and Arga, 2013). However, there was no data about the interaction between SLD3 (YGL113W) and YOR060C in Karagoz and Arga (2013), from which the list of interactors were extracted. Therefore, the missing protein and its interactions were added into the core list and cell cycle network was reconstructed. After these additions, the interactors of YOR060C in cell cycle network are SSA2 (YLL024C), NUP116 (YMR047C), (NAB2) YGL122C, HEK2 (YBL032W), SRP40 (YKR092C), SLD3 (YGL113W) and MTH1 (YDR277C). The constructed cell cycle network (CCN1) cannot be analysed any further since the number of interactions is extremely high (73579).

Table 4.2. Existence of the interactor proteins of YOR060C in the final networks

Interactors of YOR060C	CCN1	CCN2	CCN3	CCN4	CAN
YDR277C	+	-	+	+	+
YKR092C	+	-	+	+	-
YGL113W	+	+	-	-	+
YGL122C	+	-	-	-	-
YBL032W	+	-	+	+	+
YLL024C	+	+	-	-	-
YMR047C	+	+	-	-	-

Figure 4.1. Yeast cell cycle pathways (Alberts *et al.*, 2002)

4.1.2. Reconstruction of CCN2 – cell cycle network excluding proteins with unknown molecular function (F)

2362 proteins are related to cell cycle network- CCN2 (37.9 % of total proteins). These 2362 candidate proteins were matched with the protein-protein interactor dataset (Karagoz and Arga, 2013). The interactions with a confidence score higher than 0.5 were taken to get reliable results. If these candidate proteins match with both interactors (A and B), then they were accepted into the network. Among the 2362 candidate proteins, there were only 2288 interactors. The number of interactions was 28410. In this network-CCN2, only the proteins with unknown molecular function were eliminated. The cell cycle network was visualized by using Cytoscape software. The proteins that were out of the network were YEL020W-A (TIM9) and YPL257W-B (transposable element gene) on the right top of the scheme.

4.1.2.1. Cluster Identification and Functional Enrichment. The tool MCODE was run to find any cluster, which involves YOR060C. There are 34 clusters, yet none of them involves YOR060C, that means there is no clear information about it. On the other hand, SSA2 (YLL024C), NUP116 (YMR047C) and SLD3 (YGL113W), the interactors of YOR060C, were in clusters 7, 10 and 16, respectively (Tables 4.3, 4.5 and 4.7). The molecular functions of the clusters that involved these proteins were examined with GO enrichment analysis. The results of GO enrichment analysis were given in Tables 4.4, 4.6 and 4.8.

CCN2 - Cluster 7 – YLL024C: Cluster 7 had 62 proteins (Table 4.3) including SSA2 (YLL024C) that is one of the ATP binding proteins, which has 1103 physical interactions in yeast. It is also an interactor of SLD7 (YOR060C) and involved in cell cycle network. The results of GO enrichment analysis of cluster 7 were given in Table 4.4. The ratio of cluster frequency over background frequency was calculated for each function term to find the statistically meaningful result for the function of cluster properly. By comparing with p-values, the percentage results of GO enrichment analysis indicated that, this cluster was mainly responsible for microtubule plus-end binding, microtubule binding, oligosaccharyl transferase activity, dolichyl-diphosphooligosaccharide-protein glycotransferase activity and RNA-directed RNA polymerase activity.

Table 4.3. List of proteins in cluster 7 (YLL024C) at CCN2

Protein Names			
YBR010W	YGR113W	YKR037C	YGR186W
YFR013W	YPL203W	YLL024C	YDR201W
YLR378C	YAL032C	YDL209C	YNL031C
YNL182C	YER006W	YKL052C	YGL150C
YDR130C	YDL115C	YOR085W	YJL002C
YOR103C	YDR404C	YEL002C	YER125W
YOR116C	YJL140W	YBR089C-A	YLR074C
YPR101W	YDR496C	YOR150W	YDR359C
YOL005C	YGL071W	YDR320C-A	YKR001C
YDR405W	YDR225W	YKR083C	YDR190C

CCN2 - Cluster 10 – YMR047C: Cluster 10 had 15 proteins (Table 4.5) with NUP116 (YMR047C) that is one of the transporter proteins between nucleus and cytoplasm, and it has 191 physical interactions in yeast. It is also an interactor of SLD7 (YOR060C) and involved in cell cycle network. The results of GO enrichment analysis of cluster 10 were given in Table 4.6. Based on both p-value and percentage results, this cluster was found to be mainly responsible for structural constituent of nuclear pore, nucleocytoplasmic transporter activity, structural molecule activity, transporter activity, mRNA binding, poly(A) RNA binding. These results overlap with the GO enrichment results of cluster 8 (YMR047C) at CCN2.

Table 4.4. List of proteins in cluster 10 (YMR047C) at CCN2

Protein Names		
YJL124C	YMR153W	YMR047C
YLR018C	YJL039C	YPL169C
YKR082W	YKL057C	YJL130C
YOL149W	YDL160C	YLR347C

Table 4.5. The results of GO enrichment analysis belonging to cluster 7 (YLL024C) at CCN2

GOID	GO_term	Cluster frequency	Background frequency	P-value	%
51010	microtubule plus-end binding	9 out of 62 genes. 14.5%	13 out of 7165 background genes. 0.2%	8.17e-15	69.23
8017	microtubule binding	10 out of 62 genes. 16.1%	26 out of 7165 background genes. 0.4%	4.15e-13	38.46
15631	tubulin binding	10 out of 62 genes. 16.1%	35 out of 7165 background genes. 0.5%	1.35e-11	28.57
32403	protein complex binding	13 out of 62 genes. 21.0%	91 out of 7165 background genes. 1.3%	3.66e-11	14.29
44877	macromolecular complex binding	16 out of 62 genes. 25.8%	202 out of 7165 background genes. 2.8%	6.06e-10	7.92
8092	cytoskeletal protein binding	10 out of 62 genes. 16.1%	67 out of 7165 background genes. 0.9%	1.47e-08	14.93
384	first spliceosomal transesterification activity	6 out of 62 genes. 9.7%	13 out of 7165 background genes. 0.2%	4.23e-08	46.15

Table 4.6. The results of GO enrichment analysis belonging to cluster 10 (YMR047C) at CCN2

GOID	GO_term	Cluster frequency	Background frequency	P-value	%
17056	structural constituent of nuclear pore	7 out of 15 genes. 46.7%	24 out of 7165 background genes. 0.3%	2.39e-13	29.17
5487	nucleocytoplasmic transporter activity	4 out of 15 genes. 26.7%	13 out of 7165 background genes. 0.2%	1.84e-07	30.77
5215	transporter activity	6 out of 15 genes. 40.0%	409 out of 7165 background genes. 5.7%	0.00225	1.47
3729	mRNA binding	4 out of 15 genes. 26.7%	172 out of 7165 background genes. 2.4%	0.00747	2.32
44822	poly(A) RNA binding	4 out of 15 genes. 26.7%	173 out of 7165 background genes. 2.4%	0.00764	2.31

CCN2 - Cluster 16 – YGL113W: Cluster 16 had 33 proteins (Table 4.7) with SLD3 (YGL113W) that is one of the chromatin binding proteins located in nucleus and has 25 physical interactions in yeast. It is also an interactor of SLD7 (YOR060C) and involved in cell cycle network. The results of GO enrichment analysis of cluster 16 were given in Table 4.8. The ratio of cluster frequency over background frequency was calculated for each function term to determine the function of cluster properly by comparing with p-values. Based on both p-value and percentage results, this cluster was mainly responsible for ribonuclease MRP activity, RNA polymerase II C-terminal domain phosphoserine binding, protein phosphorylated amino acid binding, transcription factor activity and RNA

Polymerase II transcription factor binding. These results overlap with the GO enrichment results of cluster 6 (YGL113W) at CCN2.

Table 4.7. The results of GO enrichment analysis belonging to cluster 16 (YGL113W) at CCN2

GOID	GO_term	Cluster frequency	Background frequency	P-value	%
171	ribonuclease MRP activity	4 out of 33 genes. 12.1%	9 out of 7165 background genes. 0.1%	2.91e-06	44.44
4526	ribonuclease P activity	4 out of 33 genes. 12.1%	12 out of 7165 background genes. 0.2%	1.13e-05	33.33
4549	tRNA-specific ribonuclease activity	4 out of 33 genes. 12.1%	16 out of 7165 background genes. 0.2%	4.11e-05	25.00
1990269	RNA polymerase II C-terminal domain phosphoserine binding	3 out of 33 genes. 9.1%	5 out of 7165 background genes. 0.1%	5.57e-05	60.00
45309	protein phosphorylated amino acid binding	3 out of 33 genes. 9.1%	8 out of 7165 background genes. 0.1%	0.00031	37.50
50815	phosphoserine binding	3 out of 33 genes. 9.1%	8 out of 7165 background genes. 0.1%	0.00031	37.50

Table 4.8. List of proteins in cluster 16 (YGL113W) at CCN2

Protein Names		
YML103C	YOR123C	YJR045C
YER111C	YDR159W	YNL251C
YPR133C	YKR010C	YGR129W
YBR279W	YKL032C	YDR227W
YNL221C	YDL074C	YEL034W
YKL139W	YGL113W	YPL128C
YGL244W	YNR011C	YDL229W
YOR257W	YCL011C	YHR062C
YDL019C	YBR257W	YBR202W
YBR167C	YDL140C	YOR181W
YGL092W	YOL006C	YMR284W

4.1.2.2. CCN2 Features: Degree and Betweenness Centrality. Cytoscape Software was used to visualize and analyse the constructed network. The cytoHubba plug-in was used to find important hubs (nodes) in a network (Figures 4.2 and 4.3). The proteins with the highest interactions can be found by using degree tool. The proteins with high degrees are more likely to be essential (Tables 4.9 and 4.10). The betweenness tool gives a result for the most existed proteins in the shortest pathway.

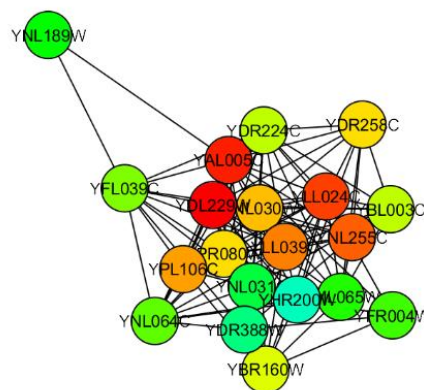


Figure 4.2. The interactions of top 20 proteins with the highest degree values at CCN2

Table 4.9. The list of top 20 proteins with highest degree in CCN2

Standard Name	Systematic Name	Degree
SSB1	YDL229W	1033
SSA1	YAL005C	744
SSA2	YLL024C	652
GIS1	YNL255C	332
UBI4	YLL039C	293
SSE1	YPL106C	270
HHF2	YNL030W	259
TEF1	YPR080W	254
HSP78	YDR258C	254
CDC28	YBR160W	236
HTA2	YBL003C	234
HTB1	YDR224C	234
ACT1	YFL039C	218
YDJ1	YNL064C	212
RPN11	YFR004W	207
ORC1	YML065W	191
SRP1	YNL189W	183
HHT2	YNL031C	178
RVS167	YDR388W	174
RPN10	YHR200W	163

The top 20 proteins with highest betweenness centrality and degree values were taken as clusters (Cluster-b and Cluster-d) and these clusters were examined by GO enrichment analysis for molecular function. The result of this analysis for both clusters was found to be same, that was protein binding. The proteins in the intersection of these two clusters of betweenness centrality and degree were examined. The proteins that were not found in the intersection set are YNL030W (HHF2), YKL049C (CSE4), YNL031C (HHT2) and YPL031C (PHO85).

Table 4.10. The list of top 20 proteins with the highest betweenness centrality results in CCN2

Standard Name	Systematic Name	Betweenness Centrality
SSB1	YDL229W	625847.97
SSA1	YAL005C	394175.24
SSA2	YLL024C	283640.21
GIS1	YNL255C	114887.97
UBI4	YLL039C	86456.72
SSE1	YPL106C	58760.57
TEF1	YPR080W	54197.12
CDC28	YBR160W	48882.38
HSP78	YDR258C	41779.90
YDJ1	YNL064C	37071.56
CSE4	YKL049C	33410.33
RVS167	YDR388W	32625.39
ACT1	YFL039C	32056.61
RPN11	YFR004W	31819.81
ORC1	YML065W	27587.94
RPN10	YHR200W	24633.29
HTB1	YDR224C	24309.65
PHO85	YPL031C	22878.79
HTA2	YBL003C	22817.59
SRP1	YNL189W	22464.17

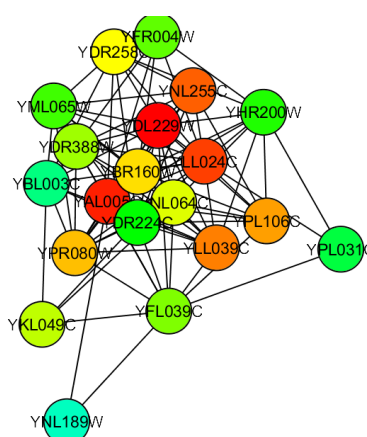


Figure 4.3. The interactions of top 20 proteins with the highest betweenness centrality values in CCN2

4.1.3. Reconstruction of CCN3 – G1 phase proteins with unknown GO terms (P, F, C)

The cell cycle of the yeast cell represented in Figure 4.4. There are 64 proteins responsible for G1 phase in yeast. The same procedure was followed to reconstruct G1 phase PPI network in yeast, CCN3. The number of interactions and proteins of this network were compared with the other reconstructed cell cycle networks in Table 4.11 (CCN1, CCN2 and CCN2).

Table 4.11. Comparison of the total number proteins and interactions in the final networks

	CCN1	CCN2	CCN2	CCN3 – G1	CCN4 - S
Number of Core Proteins	860	403	518	64	71
Number of Candidate Proteins	5284	949	2362	4091	4160
Number of Interacted Proteins	4989	915	2288	3722	3822
Number of Interactions	73579	8988	28410	44311	45723

4.1.3.1. Cluster Identification and GO Enrichment Analysis. The tool MCODE was run to find any cluster, which involves YOR060C. This process is called “top to bottom” approach. There are 29 clusters, yet none of them involves YOR060C, that means there is no clear

information about it. On the other hand, SRP40 (YKR092C), HEK2 (YBL032W) and MTH1 (YDR277C) the interactors of YOR060C, were in clusters 6, 7 and 22, respectively. The molecular functions of the clusters that involved these proteins were examined by GO enrichment analysis.

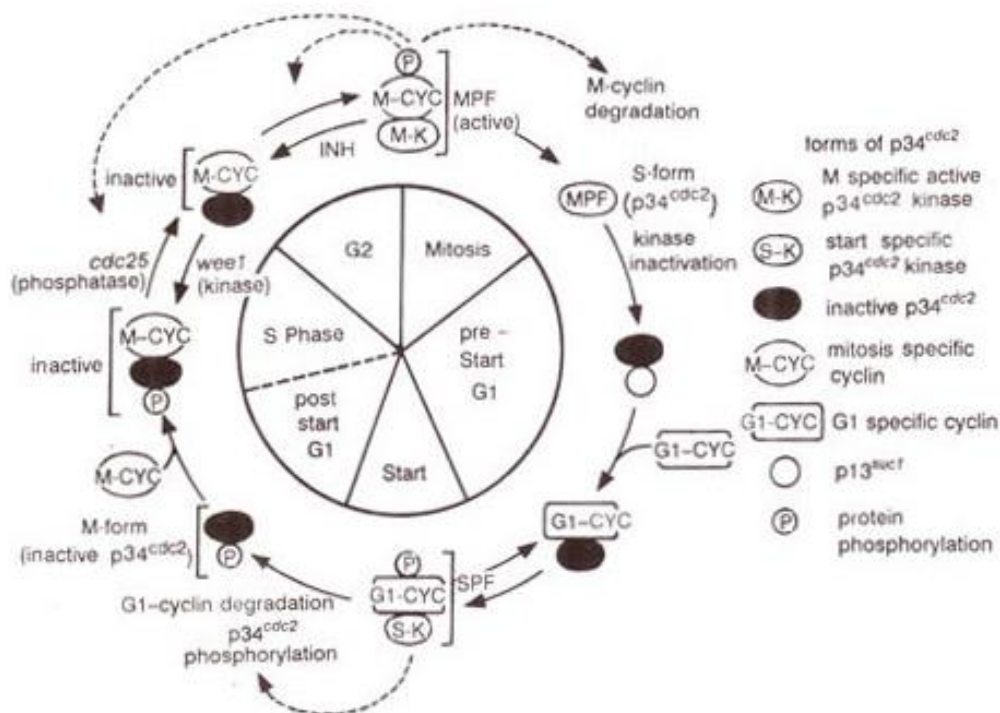


Figure 4.4. Schematic representation of cell division cycle in *Saccharomyces cerevisiae* (Castedo *et al.*, 2002).

CCN3 - Cluster 6 – YKR092C: Cluster 6 had 98 proteins (Table 4.12) including SRP40 (YKR092C) that is one of the serine rich proteins that has a role in pre-ribosome assembly of transport. It has 7 physical interactions in yeast, one of them is SLD7 (YOR060C). The results of GO enrichment analysis of cluster 6 were given in Table 4.13. The ratio of cluster frequency over background frequency was calculated for each function term to find the statistically meaningful result for the function of cluster properly. By comparing with p-values, the percentage results of GO enrichment analysis indicated that, this cluster was mainly responsible for small molecule binding, binding, heterocyclic compound binding, organic cyclic compound binding, protein binding, RNA polymerase II C-terminal domain phosphoserine binding and purine nucleotide binding.

CCN3 - Cluster 7 – YBL032W: Cluster 7 had 54 proteins (Table 4.14) including HEK2 (YBL032W) that is one of the RNA binding proteins that localizes to cytoplasmic mRNA processing body and the telomeric region of nuclear chromosome. It has 25 physical interactions, one of them is SLD7 (YOR060C). The results of GO enrichment analysis of cluster 7 were given in Table 4.15. The ratio of cluster frequency over background frequency was calculated for each function term to find the statistically meaningful result for the function of cluster properly. By comparing with p-values, the percentage results of GO enrichment analysis indicated that, this cluster was mainly responsible for ribonuclease MRP activity, ribonuclease P activity, tRNA-specific ribonuclease activity, endoribonuclease activity, producing 5'-phosphomonoesters, endonuclease activity, active with either ribo- or deoxyribonucleic acids and producing 5'-phosphomonoesters, endoribonuclease activity and endonuclease activity.

Table 4.12. List of proteins in Cluster 6 (YKR092C) at CCN3

Protein Names						
YNL207W	YPR175W	YKL032C	YDL029W	YDR255C	YNL287W	YNL216W
YNR052C	YLR196W	YNL139C	YML067C	YBR088C	YNL041C	YLR430W
YNL006W	YNL253W	YCR021C	YDR025W	YGL227W	YOR144C	YJL130C
YML074C	YOL068C	YNL016W	YDL213C	YDL225W	YMR135C	YGL244W
YDR168W	YGL241W	YGR134W	YMR237W	YPL204W	YKL054C	YIL017C
YBR049C	YPR105C	YJR068W	YKR029C	YKR024C	YFL039C	YCL039W
YGL195W	YGL030W	YDL175C	YPL246C	YCR033W	YCL037C	YHL001W
YLR103C	YPL010W	YDR258C	YML073C	YMR078C	YOR123C	YJR032W
YKR092C	YNL051W	YNL288W	YGR120C	YOR113W	YBR130C	YGR173W
YDR138W	YGL171W	YJL098W	YIL097W	YER095W	YBL003C	YPR161C
YDL208W	YDL055C	YFL008W	YBL049W	YLR330W	YOR038C	YGR274C

Table 4.13. The results of GO enrichment analysis belonging to Cluster 6 (YKR092C) at CCN3

GOID	GO_term	Cluster frequency	Background frequency	P-value	%
36094	small molecule binding	9 out of 98 genes, 9.2%	89 out of 7165 background genes, 1.2%	0.00037	10,11
5488	binding	48 out of 98 genes, 49.0%	1960 out of 7165 background genes, 27.4%	0.00046	2,84
1901363	heterocyclic compound binding	36 out of 98 genes, 36.7%	1267 out of 7165 background genes, 17.7%	0.00061	2,84
97159	organic cyclic compound binding	36 out of 98 genes, 36.7%	1278 out of 7165 background genes, 17.8%	0.00075	2,82
1990269	RNA polymerase II C-terminal domain phosphoserine binding	3 out of 98 genes, 3.1%	5 out of 7165 background genes, 0.1%	0.00302	60,00
17076	purine nucleotide binding	6 out of 98 genes, 6.1%	52 out of 7165 background genes, 0.7%	0.00853	11,54

Table 4.14. List of proteins in cluster 7 (YBL032W) at CCN3

Protein Names					
YDR310C	YLR083C	YAL034W-A	YGR052W	YFR027W	YNL221C
YHR084W	YBL018C	YNL183C	YJL099W	YJR138W	YHR152W
YDR451C	YNL233W	YML117W	YNL311C	YOR299W	YBR257W
YGR218W	YDR110W	YBL032W	YKL171W	YMR010W	YGR030C
YKL029C	YGR040W	YDL003W	YDR141C	YHR062C	YOL090W
YLR005W	YDR311W	YKL080W	YJL074C	YDL108W	YDR383C
YDR202C	YML124C	YNL197C	YCR093W	YER151C	YBR060C
YER068W	YDL229W	YJR033C	YPR104C	YPL049C	YJL092W

Table 4.15. The results of GO enrichment analysis belonging to cluster 7 (YBL032W) at CCN3

GOID	GO_term	Cluster frequency	Background frequency	P-value	%
171	ribonuclease MRP activity	7 out of 54 genes, 13.0%	9 out of 7165 background genes, 0.1%	1.93e-12	77,78
4526	ribonuclease P activity	7 out of 54 genes, 13.0%	12 out of 7165 background genes, 0.2%	4.19e-11	58,33
4549	tRNA-specific ribonuclease activity	7 out of 54 genes, 13.0%	16 out of 7165 background genes, 0.2%	5.91e-10	43,75
16891	endoribonuclease activity, producing 5'-phosphomonoesters	7 out of 54 genes, 13.0%	21 out of 7165 background genes, 0.3%	5.84e-09	33,33
4521	endoribonuclease activity	7 out of 54 genes, 13.0%	33 out of 7165 background genes, 0.5%	2.00e-07	21,21

CCN3 - Cluster 22 – YDR277C: Cluster 22 had 52 proteins (Table 4.16) including MTH1 (YDR277C) that is one of the ATP binding proteins, which has 15 physical interactions in yeast. It is also an interactor of SLD7 (YOR060C) and involved in cell cycle network. The results of GO enrichment analysis of cluster 22 were given in Table 4.16. By comparing with p-values, the percentage results of GO enrichment analysis indicated that, this cluster was mainly responsible for identical protein binding, protein homodimerization activity and protein binding.

Table 4.16. List of proteins in cluster 22 (YDR277C) at CCN3

Protein Names			
YBR109C	YIL026C	YOR204W	YDR277C
YKR020W	YJL047C	YFL003C	YLR150W
YBR102C	YDR211W	YLR086W	YBL037W
YLR319C	YDR027C	YPR007C	YFR009W
YJR109C	YGL115W	YHR079C	YDL155W
YJR090C	YOR326W	YGR234W	YDR293C
YDR381W	YDR484W	YJR017C	YER035W
YER177W	YLR362W	YBL076C	YKL104C
YDR325W	YDL131W	YBL097W	YJR005W
YJL029C	YGL252C	YHR033W	YDR103W

4.1.3.2. CCN3 Features: Degree and Betweenness Centrality. The top 20 proteins with highest degree score and betweenness centrality values in CCN3 were given in Tables 4.24 and 4.25. The results of GO enrichment analysis belonging to the clusters formed by these proteins were examined. The proteins in cluster-d were mainly responsible for transcription factor activity, RNA polymerase II transcription factor binding, RNA polymerase II transcription cofactor activity, transcriptional repressor activity, RNA polymerase II activating transcription factor binding, mRNA binding and poly(A) RNA binding. The proteins in cluster-b were mainly responsible for cyclin-dependent protein serine/threonine kinase activity, protein binding, ATPase activity and unfolded protein binding (results not shown). The difference between the GO enrichment results of these two clusters was caused by the 10 proteins, which were not in the intersecting group. The proteins

that were not in the intersecting set were YFR028C (CDC14), YBL003C (HTA2), YDR224C (HTB1), YHR027C (RPN1), YBR127C (VMA2) and YPL031C (PHO85).

Table 4.17. The list of top 15 proteins with highest degree in CCN3

Standard Name	Systematic Name	Degree Score
SSB1	YDL229W	1545
SSA1	YAL005C	1147
HSP82	YPL240C	595
HEK2	YBL032W	570
SSB2	YNL209W	553
HSP78	YDR258C	381
SSZ1	YHR064C	337
TEF1	YPR080W	321
HSC82	YMR186W	318
CDC28	YBR160W	312
JSN1	YJR091C	303
HHF2	YNL030W	292
RPN11	YFR004W	292
YDJ1	YNL064C	277

Table 4.18. The results of GO enrichment analysis belonging to cluster 22 (YDR277C) at CCN3

GOID	GO_term	Cluster frequency	Background frequency	P-value	%
42802	identical protein binding	3 out of 52 genes, 5.8%	12 out of 7165 background genes, 0.2%	0.00318	25.00
42803	protein homodimerization activity	3 out of 52 genes, 5.8%	12 out of 7165 background genes, 0.2%	0.00318	25.00

Table 4.19. The list of top 15 proteins with the highest betweenness centrality results in CCN3

Standard Name	Systematic Name	Betweenness Centrality Score
SSB1	YDL229W	1784479,12
SSA1	YAL005C	1222309,14
HEK2	YBL032W	585610,99
HSP82	YPL240C	399069,37
SSB2	YNL209W	294763,70
JSN1	YJR091C	159584,73
SSZ1	YHR064C	134331,56
HSP78	YDR258C	131567,43
TEF1	YPR080W	116079,37
CDC28	YBR160W	108491,85
RPN11	YFR004W	107889,41
YDJ1	YNL064C	91488,96
HSC82	YMR186W	88176,58
BZZ1	YHR114W	83753,20
PHO85	YPL031C	75136,67

Information flow around Sld7 within the G1 phase cell cycle network - CCN3: As Sld7 might have a role in the initiation of DNA replication in G1 phase of budding yeast (Tanaka *et al.*, 2011b), the information flow around Sld7 within the reconstructed G1 phase cell cycle network (CCN2) was analyzed through the linear path analysis, which is a valuable method to determine possible signaling pathways between the proteins in an interested organism (Steffen *et al.*, 2002; Arga *et al.*, 2007). The six subunits of the origin recognition complex, ORC (namely, Orc1, Orc2, Orc3, Orc4, Orc5 and Orc6), which are the initiator proteins that bind to replication origins during cell cycle (Labib, 2010), were selected as the input proteins, and the proteins that have a role in the initiation of DNA replication in G1 phase were set as the target proteins (Cdc7, Cdc45, Dpb11, Sld2, and Pol2) (Araki, 2011). The simulations were performed via Netsearch algorithm (Steffen *et al.*, 2002) with different

path lengths up to 7, which was the network diameter of CCN3, to identify the paths involving Sld7.

Previously, it was reported that Sld7 forms a complex with Sld3 and decreases the affinity of Sld3 towards Cdc45 (Tanaka *et al.*, 2011b). To analyze the interaction of these proteins in the initiation of DNA replication (early firing origin), the information flow from ORC to Cdc45 was analyzed in CCN3. Sld7 was encountered in the paths from all ORCs to Cdc45. Sld7 was found to be either the fourth or fifth protein in the paths involving 6 or 7 proteins, respectively. The upstream proteins of Sld7 were either Srp40 or Hek2; the downstream protein was always Sld3. Mcm2, Mcm5, Mcm7, Dot6 or Rad53 were also encountered in these paths. The analyzed information flows support the interaction of Cdc45 with Mcm2-7 recruited by Sld3, and also confirm the validity of our computational approach.

Sld is abbreviation of “synthetic lethality with Dpb11” (Tanaka *et al.*, 2011b), which is one of the DNA replication initiation proteins, and is responsible for the formation of CMG complex via the interaction between pre-LC and Sld3 (Muramatsu *et al.*, 2010; Tanaka and Araki, 2013). Dpb11 is phosphorylated by CDK. This phosphorylation removes Sld3 and Sld2 from Dpb11, then GINS-Pol ϵ bind to pre-RC and forms pre-IC. To estimate possible role of Sld7 associated with Dpb11, the linear paths from ORC to Dpb11 were analyzed. We identified 287 paths starting with Orc1, Orc2, Orc3, Orc6 or Mcm2-7 and involving Sld7, which was encountered as the third or fourth protein in all the paths with seven proteins. The upstream proteins that interacted with Sld7 were either Mth1, Srp40, Sld3 or Hek2; the downstream protein was Sld3. The proteins that were encountered in these paths were Mis1, Hta2, Ssb1, Hmo1, Mth1, Ssa1, Pex13, Psf1 or Ssa2. The analyzed paths were supported by the reported information flow (Masumoto *et al.*, 2002).

Sld2 was one of the checkpoint proteins, which binds to Dpb11 and forms pre-LC complex, which is required for the initiation of DNA replication (Masumoto *et al.*, 2002). It also has an essential role for CDK dependent activation, which is related to chromosomal DNA replication (Muramatsu *et al.*, 2010). To estimate the possible role of Sld7 associated with Sld2, the linear paths from ORC and MCM to Sld2 were also analyzed. We identified 114 paths starting with Orc1, Orc2, Orc3, Orc6 or Mcm2-7 and involving Sld7. Sld7 was encountered as the fourth protein in all the paths involving seven proteins. The upstream

proteins that interacted with Sld7 were either Srp40, Sld3 or Hek2; the downstream proteins were Sld3 or Mth1. The proteins that interacted with Sld2 were Mis1, Hta2, Ssb1, Hmo1, Cdc28 or Clb5. The other proteins that were encountered in these paths were Ssa1, Pex13, Psf1 or Dpb11. The analyzed paths were confirmed by the information flow reported in Masumoto *et al.*, 2002.

Pol2 (Pol ϵ) is required for the initiation of DNA replication during mitotic DNA synthesis (Araki, 2011). Some of the paths starting with Orc1, Orc2 or Orc6 involved Sld7. The paths of ‘Orc2 – Pol2’ and ‘Orc6 – Pol2’ involved the same proteins in the same order except the first two proteins (Orc and second one), but the proteins were different in the path of ‘Orc1-Pol2’. Sld7 was again encountered as the fourth protein and all the paths involved seven proteins. The upstream and downstream proteins that directly interacted with Sld7 were always Srp40 and Sld3, respectively. The protein interacting with Pol2 was always Psf1, one of the subunits of GINS complex. The other proteins that were encountered in the paths were Hta2, Ssb1 or Hmo1.

Cdc7 is a kinase protein, the catalytic subunit of DDK (Dbf4-dependent kinase) (Aparicio, 2013). It is required for origin firing and replication fork progression in mitotic S phase through phosphorylation of Mcm2-7 complexes and Cdc45 (Labib, 2010). 16 paths starting with Orc1, Orc2, or Orc6 involved Sld7. The Cdc7-interacting proteins in these paths were Mis1, Hta2, Ssb1 or Hmo1. Sld7 was the fourth protein in all these paths, and its upstream proteins (3rd or 4th) were either Srp40 or Hek2; the downstream protein was always Sld3. Rad53 (Sld6) or Mcm4 also existed in these paths.

Sld7-Sld3-Cdc45 complex was reported to bind to Mcm2 and Mcm7 (Bruck and Kaplan, 2015). Therefore, Mcm2-7 were chosen as the starting proteins and the pre-LC proteins, which were Sld2, Pol ϵ and Dpb11, were set as the target proteins. In all the paths involving Sld7, it interacted with Sld3. However, Cdc45-Sld3-Sld7 complex was only encountered in the paths starting with Mcm2 or Mcm7 and extending to Sld2 or Dpb11.

The paths from Mcm2-7 to Cdc28, which is a cyclin dependent kinase (CDK) protein and responsible for the regulation of chromosome dynamics, were also analyzed. The paths starting with Mcm2, Mcm3, Mcm5 and Mcm7 had Sld7. The information flow in these paths

was found to be identical. Sld7 was encountered as 3rd protein in the paths, interacting with Sld3 and Mth1 as upstream and downstream proteins, respectively. Cdc28 phosphorylates Dbp11 and separates pre-LC from the pre-IC. The signalling flow, found here from Mcm- to Cdc28, is supported by the information given in literature (Labib, 2010).

Rad53 has a genetic interaction with Sld7 and it physically interacts with Sld3 and Cdc7 in the analyzed linear paths. Rad53 (Sld6) is an essential protein for inheritance, and it has a regulatory role in nuclear and mitochondrial DNA related processes. DDK (Cdc7-Dfb4) and CDK (Cdc28) inhibit Rad53 activity in dependent pathways. The mutation of *rad53-cdc7* caused synthetic lethality (Dohrmann and Sclafani, 2006). Rad53 phosphorylates Sld3 to inhibit the initiation of replication (Zegerman and Diffley, 2007). The linear paths found here supported the interaction of Rad53 with Sld3 and Cdc7 on computational basis.

The above mentioned results are in agreement with the published mechanisms occurring in cell cycle and DNA replication (Labib, 2010) and confirm that the networks were correctly reconstructed. In the initiation of DNA replication process, 160 different proteins were encountered in the linear paths including starter and target proteins. In total, Sld7 was encountered in 1022 different pathways. Among the proteins in these linear paths of information flow, Dot6, Hta2 and Hmo1 have a role in DNA binding (Zhu *et al.*, 2009; Wullschleger *et al.*, 2006; Bauerle *et al.*, 2006), i.e. they help gene products' non-covalent binding to DNA. Clb5 is responsible for transition from G1 phase to S phase (Schwob and Nasmyth, 1993). Psf1 is one of the subunits of GINS complex (Takayama *et al.*, 2003). Ssa1 and Ssa2 are responsible for ATPase activity (Needham and Masison, 2008; Boorstein *et al.*, 1994). These proteins can also be considered as assist proteins in binding the main proteins to each other, as indicated by the information flow of our linear path analysis. So far an assisting role has not been mentioned in literature for any of these proteins at the initiation of DNA replication.

4.1.4. Reconstruction of CCN4 - S phase proteins with unknown GO terms (P, F, C)

There are 71 proteins responsible for S phase in yeast. The same procedure was followed to reconstruct S phase PPI network in yeast, CCN4.

4.1.4.1. Cluster Identification and GO Enrichment Analysis. The tool MCODE was run to find any cluster, which involves YOR060C. This process is called “top to bottom” approach. There are 33 clusters, yet none of them involves YOR060C, that means there is no clear information about it. On the other hand, SRP40 (YKR092C), HEK2 (YBL032W) and MTH1 (YDR277C) the interactors of YOR060C, were in clusters 6, 7 and 9, respectively. The molecular functions of the clusters that involved these proteins were examined by GO enrichment analysis.

CCN4 - Cluster 6 – YKR092C: Cluster 6 had 98 proteins (Table 4.20) including SRP40 (YKR092C) that is one of the serine rich proteins that has a role in pre-ribosome assembly of transport. It has 7 physical interactions in yeast, one of them is SLD7 (YOR060C). The results of GO enrichment analysis of cluster 6 were given in Table 4.21. The ratio of cluster frequency over background frequency was calculated for each function term to find the statistically meaningful result for the function of cluster properly. By comparing with p-values, the percentage results of GO enrichment analysis indicated that, this cluster was mainly responsible for small molecule binding, binding, heterocyclic compound binding, organic cyclic compound binding, protein binding, RNA polymerase II C-terminal domain phosphoserine binding and purine nucleotide binding. These findings are in agreement with the results of CCN3.

CCN4 - Cluster 7 – YBL032W: Cluster 7 had 98 proteins (Table 4.22) including HEK2 (YBL032W) that is one of the RNA binding proteins that localizes to cytoplasmic mRNA processing body and the telomeric region of nuclear chromosome. It has 25 physical interactions, one of them is SLD7 (YOR060C). The results of GO enrichment analysis of cluster 7 were given in Table 4.23. The ratio of cluster frequency over background frequency was calculated for each function term to find the statistically meaningful result for the function of cluster properly. By comparing with p-values, the percentage results of GO enrichment analysis indicated that, this cluster was mainly responsible for ribonuclease MRP activity, ribonuclease P activity, tRNA-specific ribonuclease activity, endoribonuclease activity, producing 5'-phosphomonoesters, endonuclease activity, active with either ribo- or deoxyribonucleic acids and producing 5'-phosphomonoesters, endoribonuclease activity and endonuclease activity. These findings are in agreement with the results of CCN3.

Table 4.20. List of proteins in cluster 6 (YKR092C) at CCN4

Protein Names						
YGR134W	YGR120C	YNL041C	YLR103C	YGL244W	YKL051W	YPR175W
YDL213C	YGL223C	YML016C	YOL133W	YER095W	YGL227W	YDR127W
YLR430W	YNL207W	YGR173W	YCL037C	YHR107C	YNL288W	YLR196W
YPR105C	YPL010W	YFL039C	YDR025W	YBR279W	YNL051W	YGR179C
YGL178W	YML071C	YMR078C	YBR130C	YLR180W	YCR002C	YOR144C
YOR257W	YGL005C	YDL175C	YDR258C	YDL208W	YKL032C	YGL241W
YOR290C	YGL195W	YGR274C	YBR088C	YOR113W	YPL180W	YNL004W
YOR123C	YBL003C	YNL216W	YMR237W	YCR033W	YCR030C	YDL055C
YFL008W	YPL233W	YMR135C	YDR255C	YKR092C	YOL068C	YPL204W
YNL016W	YGL030W	YIL097W	YIL017C	YDL225W	YML074C	YNL287W
YJL098W	YNL006W	YBL049W	YJL130C	YGL014W	YNR052C	YHL001W
YKR029C	YPL246C	YKR024C	YJR068W	YGL150C	YPL226W	YOR027W
YBR049C	YCR021C	YKL054C	YML067C	YML073C	YDL029W	YOR038C
YLR330W	YBR103W	YPR161C	YGL171W	YDL028C	YDR383C	YCL039W

Table 4.21. The results of GO enrichment analysis belonging to cluster 6 (YKR092C) at CCN4

GOID	GO_term	Cluster frequency	Background frequency	P-value	%
36094	small molecule binding	9 out of 98 genes, 9.2%	91 out of 7165 background genes, 1.3%	0.00043	9,89
1990269	RNA polymerase II C-terminal domain phosphoserine binding	3 out of 98 genes, 3.1%	5 out of 7165 background genes, 0.1%	0.00294	60,00

Table 4.21. The results of GO enrichment analysis belonging to cluster 6 (YKR092C) at CCN4 (cont.).

GOID	GO_term	Cluster frequency	Background frequency	P-value	%
97159	organic cyclic compound binding	34 out of 98 genes, 34.7%	1286 out of 7165 background genes, 17.9%	0.00604	2,64
17076	purine nucleotide binding	6 out of 98 genes, 6.1%	52 out of 7165 background genes, 0.7%	0.00832	11,54

Table 4.22. List of proteins in cluster 7 (YBL032W) at CCN4

Protein Names					
YGL252C	YDR141C	YNL076W	YMR010W	YPR104C	YDR451C
YGR285C	YJL074C	YLR150W	YJL099W	YJR053W	YML013W
YOR198C	YHR152W	YLR148W	YFR027W	YOL090W	YKL203C
YDL149W	YFR031C-A	YDL077C	YJL047C	YER151C	YDR110W
YGR218W	YGR262C	YJR090C	YJR138W	YKL080W	YFR009W
YIL128W	YOR179C	YER177W	YCL030C	YKL157W	YML117W
YKL171W	YNL221C	YGL031C	YDL131W	YDL229W	YJR033C
YKR027W	YOR299W	YAL034W-A	YAL033W	YKL130C	YDR202C
YDL149W	YFR031C-A	YDL077C	YJL047C	YER151C	YDR110W
YGL137W	YPL028W	YKL104C	YJR092W	YDR172W	YGL234W
YNL311C	YGR030C	YBL032W	YBR257W	YGL009C	YGR052W
YDR310C	YDR054C	YBR060C	YCL031C	YGR040W	YKL067W
YDL149W	YFR031C-A	YDL077C	YJL047C	YER151C	YDR110W
YLR005W	YBR167C	YIL026C	YEL056W	YDL003W	YLR022C
YDR311W	YBL018C	YGR234W	YJR017C	YJL187C	YCR093W
YNL311C	YGR030C	YBL032W	YBR257W	YGL009C	YGR052W
YBR109C	YMR231W	YHR061C	YPR025C	YNL183C	YAR019C

Table 4.23. The results of GO enrichment analysis belonging to cluster 7 (YBL032W) at CCN4

GOID	GO_term	Cluster frequency	Background frequency	P-value	%
171	ribonuclease MRP activity	7 out of 98 genes, 7.1%	9 out of 7165 background genes, 0.1%	2.71e-10	77,78
4526	ribonuclease P activity	7 out of 98 genes, 7.1%	12 out of 7165 background genes, 0.2%	5.78e-09	58,33
4549	tRNA-specific ribonuclease activity	7 out of 98 genes, 7.1%	16 out of 7165 background genes, 0.2%	7.98e-08	43,75
16891	endoribonuclease activity, producing 5'-phosphomonoesters	7 out of 98 genes, 7.1%	21 out of 7165 background genes, 0.3%	7.67e-07	33,33
4521	endoribonuclease activity	7 out of 98 genes, 7.1%	33 out of 7165 background genes, 0.5%	2.46e-05	21,21
4842	ubiquitin-protein transferase activity	8 out of 98 genes, 8.2%	86 out of 7165 background genes, 1.2%	0.00217	9,30
4672	protein kinase activity	9 out of 98 genes, 9.2%	131 out of 7165 background genes, 1.8%	0.00739	6,87

CCN4 - Cluster 9 – YDR277C: Cluster 9 had 104 proteins (Table 4.24) including MTH1 (YDR277C) that is one of the ATP binding proteins, which has 15 physical interactions in yeast. It is also an interactor of SLD7 (YOR060C) and involved in cell cycle

network. The results of GO enrichment analysis of cluster 9 were given in Table 4.25. By comparing with p-values, the percentage results of GO enrichment analysis indicated that, this cluster was mainly responsible for protein binding, macromolecular complex binding, poly(A) binding and RNA polymerase II core binding.

Table 4.24. List of proteins in cluster 9 (YDR277C) at CCN4

Protein Names				
YEL055C	YJR117W	YJL050W	YLR398C	YLR418C
YML095C	YLR370C	YJL115W	YAL029C	YER086W
YGL153W	YPL141C	YGL123W	YKR059W	YNL124W
YNL243W	YML042W	YER165W	YDR244W	YKL099C
YBL047C	YBR215W	YER142C	YBL076C	YLR319C
YNL298W	YIL068C	YDL137W	YMR026C	YDL155W
YBL051C	YBR251W	YHR079C	YDR265W	YDR295C
YIR001C	YPL240C	YMR192W	YMR106C	YLR229C
YDR166C	YLR310C	YFL003C	YGR240C	YDL239C
YBR102C	YGR116W	YDL132W	YLR314C	YHL014C
YHR108W	YPL055C	YLR362W	YDR325W	YIL131C

Table 4.25. The results of GO enrichment analysis belonging to cluster 9 (YDR277C) in CCN4

GOID	GO_term	Cluster frequency	Background frequency	P-value	%
5515	protein binding	31 out of 104 genes, 29.8%	622 out of 7165 background genes, 8.7%	4.65e-08	4,98
44877	macromolecular complex binding	15 out of 104 genes, 14.4%	205 out of 7165 background genes, 2.9%	2.58e-05	7,32

Table 4.25. The results of GO enrichment analysis belonging to cluster 9 (YDR277C) in CCN4 (cont.).

GOID	GO_term	Cluster frequency	Background frequency	P-value	%
70717	poly-purine tract binding	4 out of 104 genes, 3.8%	6 out of 7165 background genes, 0.1%	7.43e-05	66,67
8143	poly(A) binding	4 out of 104 genes, 3.8%	6 out of 7165 background genes, 0.1%	7.43e-05	66,67

4.1.4.2. CCN4 Features: Degree and Betweenness Centrality. The top 20 proteins (Figures 4.19 and 4.20) with highest betweenness centrality and degree values were taken as clusters (Cluster-b and Cluster-d) these clusters were examined by GO enrichment analysis for molecular function. The list of the proteins with their degree and betweenness centrality were given in Tables 4.26 and 4.27, respectively. The proteins in cluster-d were mainly responsible for protein binding, ATPase activity, nucleoside-triphosphatase activity, hydrolase activity, acting on acid anhydrides and unfolded protein binding. The proteins in cluster-b were mainly responsible for cyclin-dependent protein serine/threonine kinase activity in addition to cluster-d functions (results not shown). The proteins that were not intersect YFR028C (CDC14), YBL003C (HTA2), YDR224C (HTB1), YHR027C (RPN1), YPL031C (PHO85), YHR114W (BZZ1), YKL049C (CSE4), and YNL030W (HHF2).

4.1.5. Reconstruction of chronological aging network

The chronological aging network was reconstructed to determine the molecular function of YOR060C. Yücel and Ulgen (2011) constructed this network. The followed procedure was the same (Table 4.28). The article was published in 2011 and 10 proteins were added into list through the years. These added proteins increased the GO terms and that caused an increase in the number of interactions. The visualization of this network was done by Cytoscape.

Table 4.26. The list of top 15 proteins with the highest betweenness centrality results at S phase

Standard Name	Systematic Name	Betweenness Centrality Score
SSB1	YDL229W	1714740,61
SSA1	YAL005C	1205205,86
NAB2	YGL122C	648094,00
HEK2	YBL032W	613028,04
HSP82	YPL240C	399959,24
SSB2	YNL209W	281616,80
JSN1	YJR091C	151057,49
SSZ1	YHR064C	125456,07
HSP78	YDR258C	125070,96
TEF1	YPR080W	114197,08
RPN11	YFR004W	106834,61
CDC28	YBR160W	104669,97
HSC82	YMR186W	96215,15
YDJ1	YNL064C	88639,18
BZZ1	YHR114W	88108,26

Table 4.27. The comparison of constructed and reconstructed chronological aging network (CAN)

	Yucel and Ulgen (2011)	Reconstructed Network
Number of Proteins	20	30
GO Term Annotation Number	178	1097
Number of Interacted proteins	2359	3601
Number of Interactions	12314	33772

4.1.5.1. Cluster Identification and GO-Functional Enrichment. The highly interacted proteins were formed as clusters by using MCODE plug-in of Cytoscape software. There were 36 clusters and two of them involve the interactors of YOR060C. These were SLD3 (YGL113W), MTH1 (YDR277C) and HEK2 (YBL032W) in cluster 9, 15 and 20, respectively.

CAN- Cluster 9 – YGL113W : Cluster 9 had 62 proteins (Table 4.29) including SLD3 (YGL113W) that is one of the chromatin binding proteins located in nucleus and has 25 physical interactions in yeast. It is also an interactor of SLD7 (YOR060C) and involved in cell cycle network. The results of GO enrichment analysis of cluster 9 were given in Table 4.29. The ratio of cluster frequency over background frequency was calculated for each function term to determine the function of cluster properly by comparing with p-values. Based on both p-value and percentage, this cluster was mainly responsible for amino acid binding, carboxylic acid binding, organic acid binding, RNA polymerase II C-terminal domain phosphoserine binding, basal RNA polymerase II transcription machinery binding and protein phosphorylated amino acid binding, phosphoserine binding (Table 4.30). These results were similar to the result of and cluster 6 (YGL113W) at CCN2.

Table 4.28. List of proteins in cluster 9 (YGL113W) at CAN

Protein Names			
YGL113W	YDR255C	YLR438C-A	YCR088W
YPL220W	YDR324C	YCL024W	YCR002C
YHR140W	YAL007C	YMR135C	YOR123C
YNL132W	YGR128C	YAR007C	YHR016C
YLR002C	YPL204W	YIL097W	YCL039W
YDR378C	YBL026W	YPL153C	YIL017C
YLR150W	YDR174W	YDL014W	YLR418C
YFR001W	YLR103C	YDR496C	YGR274C
YMR215W	YOL145C	YPR171W	YOR272W
YGL227W	YBR279W	YHR107C	YNL166C
YMR214W	YDR507C	YJR076C	YDL015C

Table 4.29. The results of GO enrichment analysis belonging to Cluster 9 (YGL113W) at CAN

GOID	GO_term	Cluster frequency	Background frequency	P-value	%
16597	amino acid binding	5 out of 48 genes, 10.4%	12 out of 7165 background genes, 0.2%	7.08e-07	41.67
31406	carboxylic acid binding	5 out of 48 genes, 10.4%	12 out of 7165 background genes, 0.2%	7.08e-07	41.67
43177	organic acid binding	5 out of 48 genes, 10.4%	12 out of 7165 background genes, 0.2%	7.08e-07	41.67
1990269	RNA polymerase II C-terminal domain phosphoserine binding	4 out of 48 genes, 8.3%	5 out of 7165 background genes, 0.1%	7.49e-07	80.00
45309	protein phosphorylated amino acid binding	4 out of 48 genes, 8.3%	8 out of 7165 background genes, 0.1%	1.03e-05	50.00
44877	macromolecular complex binding	10 out of 48 genes, 20.8%	204 out of 7165 background genes, 2.8%	6.07e-05	4.90
1098	basal transcription machinery binding	4 out of 48 genes, 8.3%	22 out of 7165 background genes, 0.3%	0.00101	18.18

Table 4.30. The results of GO enrichment analysis belonging to Cluster 9 (YGL113W) at CAN (cont.).

GOID	GO_term	Cluster frequency	Background frequency	P-value	%
1099	basal RNA polymerase II transcription machinery binding	4 out of 48 genes, 8.3%	22 out of 7165 background genes, 0.3%	0.00101	18.18
10314	phosphatidylinositol-5-phosphate binding	3 out of 48 genes, 6.2%	9 out of 7165 background genes, 0.1%	0.00196	33.33
1089	transcription factor activity, TFIIF-class transcription factor binding	2 out of 48 genes, 4.2%	2 out of 7165 background genes, 0.0%	0.00374	100.00

CAN - Cluster 15 – YDR277C: Cluster 15 had 92 proteins (Table 4.31) with MTH1 (YDR277C) that is that is one of the ATP binding proteins, and it has 15 physical interactions in yeast. It is also an interactor of SLD7 (YOR060C) and involved in cell cycle network. The results of GO enrichment analysis of Cluster 15 were given in Table 4.32. The ratio of cluster frequency over background frequency was calculated for each function term to determine the function of cluster properly by comparing with p-values. Based on both p-value and percentage results, this cluster was found to be mainly responsible for ribonuclease P activity, ribonuclease MRP activity, tRNA-specific ribonuclease activity and endoribonuclease activity, producing 5'-phosphomonoesters. These results were similar to the results of cluster 6 (YGL113W) at CCN3 and cluster 7 (YBL032W) at CCN4 and CCN5.

Table 4.30. List of proteins in Cluster 15 (YDR277C) at CAN

Protein Names			
YDR032C	YJR033C	YKR003W	YJR005W
YOR386W	YDR202C	YDR484W	YHL029C
YKL129C	YPL131W	YHR001W	YOR290C
YGR281W	YOR145C	YML109W	YBL007C
YDR012W	YNL099C	YPL065W	YIL121W
YBR052C	YER047C	YBR257W	YNL106C
YKR020W	YPR055W	YAL033W	YOR243C
YJR102C	YIR015W	YLL036C	YGR234W
YPL002C	YKL080W	YER142C	YPR086W
YIL068C	YGR262C	YEL060C	YBL051C
YHR062C	YNL221C	YCR057C	YBR060C
YHR152W	YJL029C	YDR069C	YCR095C
YPL249C-A	YJR058C	YLR413W	YOR284W
YHR216W	YGL049C	YGR030C	YIL095W
YLR432W	YER036C	YDL149W	YGL195W
YPL248C	YGR162W	YBR167C	YOL062C
YDR166C	YNL186W	YDR299W	YNL288W
YBR102C	YNL298W	YDR310C	YDL134C
YKL143W	YOR273C	YKL112W	YNL049C
YGL181W	YER165W	YPL058C	YLR417W
YBL018C	YKL157W	YDR277C	YLR166C
YNL207W	YIL156W	YBL037W	YBR114W
YPR152C	YDR027C	YKL139W	YML124C

Table 4.31. The results of GO enrichment analysis belonging to Cluster 15 (YDR277C) at CAN

GOID	GO_term	Cluster frequency	Background frequency	P-value	%
4526	ribonuclease P activity	8 out of 92 genes, 8.7%	12 out of 7165 background genes, 0.2%	2.78e-11	66.67
171	ribonuclease MRP activity	7 out of 92 genes, 7.6%	9 out of 7165 background genes, 0.1%	1.73e-10	77.78
4549	tRNA-specific ribonuclease activity	8 out of 92 genes, 8.7%	16 out of 7165 background genes, 0.2%	6.93e-10	50.00
16891	endoribonuclease activity, producing 5'-phosphomonoesters	8 out of 92 genes, 8.7%	21 out of 7165 background genes, 0.3%	1.04e-08	38.10
4521	endoribonuclease activity	8 out of 92 genes, 8.7%	33 out of 7165 background genes, 0.5%	6.25e-07	24.24
4519	endonuclease activity	8 out of 92 genes, 8.7%	66 out of 7165 background genes, 0.9%	0.00018	12.12

CAN - Cluster 20 – YBL032W: Cluster 20 had 41 proteins (Table 4.33) with HEK2 (YBL032W) that is RNA binding proteins that localizes to cytoplasmic mRNA processing body and the telomeric region of nuclear chromosome and it has 25 physical interactions in yeast. It is also an interactor of SLD7 (YOR060C) and involved in cell cycle network. The results of GO enrichment analysis of Cluster 20 were given in Table 4.34. The ratio of cluster frequency over background frequency was calculated for each function term to determine

the function of cluster properly by comparing with p-values. Based on both p-value and percentage results, this cluster was found to be mainly responsible for heme-copper terminal oxidase activity, oxidoreductase activity, acting on a heme group of donors, oxidoreductase activity, acting on a heme group of donors, oxygen as acceptor, cytochrome-c oxidase activity, electron carrier activity and macromolecular complex binding.

Table 4.32. List of proteins in Cluster 20 (YBL032W) at CAN

Protein Names			
YKR067W	YDL123W	YJL099W	YPL101W
YGR052W	YGR060W	YLR175W	YAL029C
YPR175W	YLR038C	YKR027W	YLR086W
YJR117W	YER159C	YAL035W	YGR163W
YDR477W	YDR397C	YHR187W	YBL097W
YNL183C	YGL023C	YGL187C	YML121W
YNL052W	YER006W	YDL067C	YOL086C
YDR517W	YDR325W	YGL213C	YDL013W
YLL013C	YMR312W	YBR077C	YBL032W
YMR229C	YPL086C	YML123C	YOR299W
YKR007W			

4.1.5.2. CAN Features: Degree and Betweenness Centrality. The top 20 proteins (Figures 4.25 and 4.26) with highest betweenness centrality and degree values were taken as clusters (Cluster-b and Cluster-d) and these clusters were examined by GO enrichment analysis for molecular function. The list of the proteins with highest degree and betweenness centrality were given in Tables 4.35 and 4.36. The proteins in cluster-d were mainly responsible for protein binding, ATPase activity, nucleoside-triphosphatase activity, hydrolase activity, acting on acid anhydrides and unfolded protein binding. The proteins in cluster-b were mainly responsible for cyclin-dependent protein serine/threonine kinase activity in addition to cluster-d functions (results not shown). The proteins that were not intersect YER022W (SRB4), YER125W (RSP5), YDR224C (HTB1), YBL007C (SLA1), YPL031C (PHO85), YBL003C (HTA2), YDL055C (PSA1), and YNL030W (HHF2).

Table 4.33. The results of GO enrichment analysis belonging to cluster 20 (YBL032W) at CAN

GOID	GO_term	Cluster frequency	Background frequency	P-value	%
15002	heme-copper terminal oxidase activity	4 out of 41 genes, 9.8%	12 out of 7165 background genes, 0.2%	3.22e-05	33.33
16675	oxidoreductase activity, acting on a heme group of donors	4 out of 41 genes, 9.8%	12 out of 7165 background genes, 0.2%	3.22e-05	33.33
16676	oxidoreductase activity, acting on a heme group of donors, oxygen as acceptor	4 out of 41 genes, 9.8%	12 out of 7165 background genes, 0.2%	3.22e-05	33.33
4129	cytochrome-c oxidase activity	4 out of 41 genes, 9.8%	12 out of 7165 background genes, 0.2%	3.22e-05	33.33
9055	electron carrier activity	4 out of 41 genes, 9.8%	22 out of 7165 background genes, 0.3%	0.00046	18.18
44877	macromolecular complex binding	7 out of 41 genes, 17.1%	204 out of 7165 background genes, 2.8%	0.00986	3.43

Table 4.34. The list of top 20 proteins with highest degree of centrality at CAN

Standard Name	Systematic Name	Degree Score
SSB1	YDL229W	1389
HEK2	YBL032W	562
HSP82	YPL240C	552
SSB2	YNL209W	483
HSP78	YDR258C	318
RPN11	YFR004W	290
TEF1	YPR080W	289
HSC82	YMR186W	272
HHF2	YNL030W	265
CDC28	YBR160W	262
YDJ1	YNL064C	252
ACT1	YFL039C	246
HTA2	YBL003C	233
HTB1	YDR224C	224
RVS167	YDR388W	216
HSP104	YLL026W	206
VMA2	YBR127C	201
RPN1	YHR027C	199
CDC14	YFR028C	196
PSA1	YDL055C	194

Table 4.35. The list of top 15 proteins with the highest betweenness centrality results at
CAN

Standard Name	Systematic Name	Betweenness Centrality Score
SSB1	YDL229W	2123562,02
HEK2	YBL032W	760110,25
HSP82	YPL240C	517225,34
SSB2	YNL209W	365238,90
RPN11	YFR004W	148237,90
TEF1	YPR080W	137181,62
HSP78	YDR258C	136900,20
CDC28	YBR160W	117865,74
HSC82	YMR186W	112264,43
YDJ1	YNL064C	107762,09
RVS167	YDR388W	99095,72
ACT1	YFL039C	81608,58
HSP104	YLL026W	80760,22
PHO85	YPL031C	79033,11
RSP5	YER125W	75303,25

5. EXPERIMENTAL RESULTS

5.1. Effect of YNB medium on wild type BY4741 haploid (Sld7: GFP) cells

5.1.1. Experiment 1

The YNB medium including 2% glucose without histidine was used as the nutrient medium for Sld7-GFP tagged wild type BY4741 cells. For the inoculation, the yeast cells were first cultivated in shake flasks containing YNB medium. The growth of yeast cells were observed under microscope and photos taken at 24th, 36th and 43th hours were given in Figure 5.1. The size of the cells and the turbidity of the flasks did not change significantly (Figure 5.2). The optical density (OD) of the loaded yeast-medium mixture should be 0.5, however the OD of the pure yeast culture was measured as 0.08. As a result, yeast cells were loaded into the microbioreactor without being diluted. So the experiment was started at 43th hour of the inoculum (growth phase of cells).

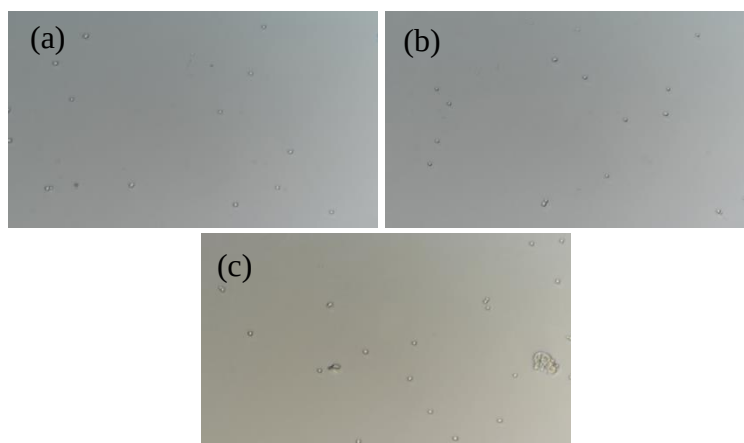


Figure 5.1. (a) The yeast cells at 24 hr (b) yeast cells at 30 hr (c) yeast cells at 43 hr

6 microfluidic devices were used in these experiments. 3 devices were attached to inlet apparatus (made of PMMA) with double-sided tape and other 3 were attached with PDMS instead of double-sided tape (Figure 3a). The setup mechanism compressed the

PMMA inlet apparatus with metal block to prevent leakage (Figure 3b). The flowrates of the side channels and middle channel were adjusted as 3 $\mu\text{l}/\text{min}$ and 5 $\mu\text{l}/\text{min}$, respectively. The diameters of the medium and yeast filled syringes were 30 mm and 15 mm, respectively. The yeast loading flowrate was adjusted as 1 $\mu\text{l}/\text{min}$.

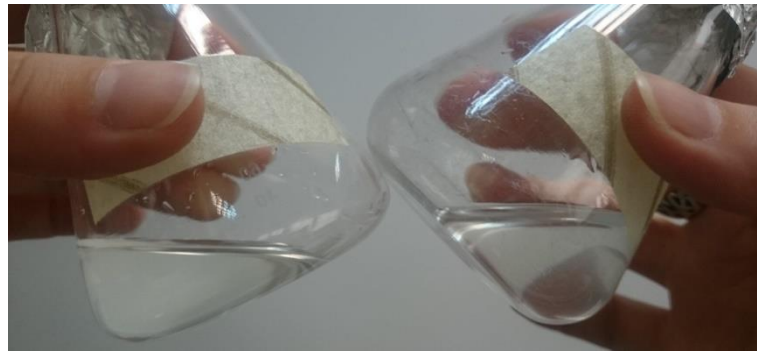


Figure 5.2. The turbidity of medium after 43 hours



Figure 5.3. Experimental set-up system with syringe pumps and Ti inverted microscope

The leakage between double-sided tape and device was observed in all three devices. The double-sided tape resisted at most 5 hours. The cell loading was achieved by only one of these devices out of 3 devices attached with double-sided tape. The cells were trapped in all chambers except chamber 7 (Figure 5.4). However, the system had to be ended because of the leakage problem between the tape and PS device. The medium loading process takes approximately 3-4 hours if there is no leakage problem. The growth phase images (Figure 5.4) indicated that the size and number of the yeast cells did not change; the cells might be already dead.

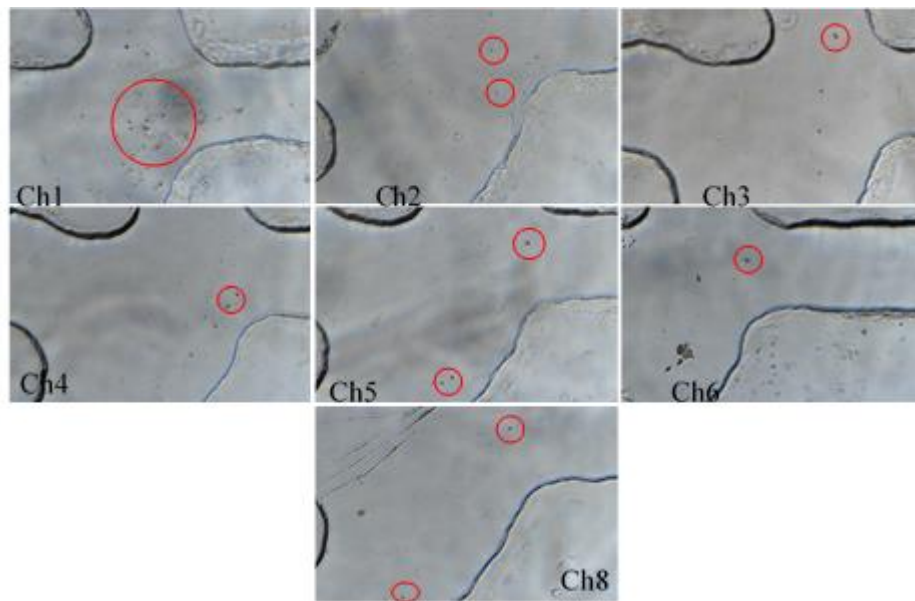


Figure 5.4. The trapped yeast cells in different chambers of the device attached with double-side tape

The yeast cells could be loaded into one device only, which was attached to inlet system via PDMS (Figure 5.6b), however dimensional instability problem at the entrance of the first chamber caused clogging, preventing the mobilization of cells towards the other chambers (Figure 5.5). The leakage problem between PDMS-PMMA and PDMS-PS was also observed in the other two devices (Figure 5.6c).

The dimensional instability problem might be solved by changing the applied pressure during the bonding process. The leakage problem between PMMA-PDMS or PS-PDMS might be solved by increasing the size of the inlet holes to increase the alignment area of the holes in all polymers, which are PMMA, PDMS and PS.

5.1.2. Experiment 2

New plates were prepared from frozen stock. The yeast cultivation was carried out from one of these newly prepared plates. The yeast growth was observed under microscope after 24 hours. The size of the cells and the turbidity of the medium were acceptable to start the experiment (Figure 5.7).

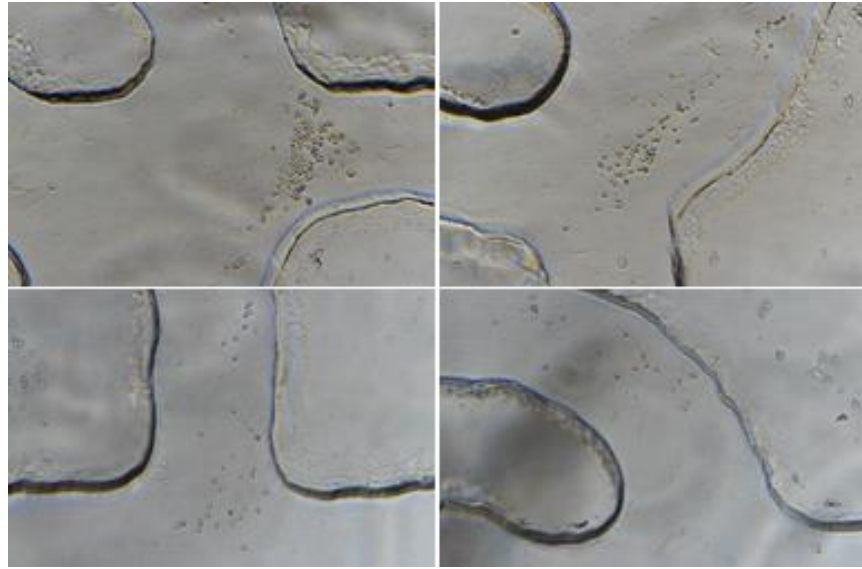


Figure 5.5. The necklace image of yeast cells at inlet channel of chamber 1

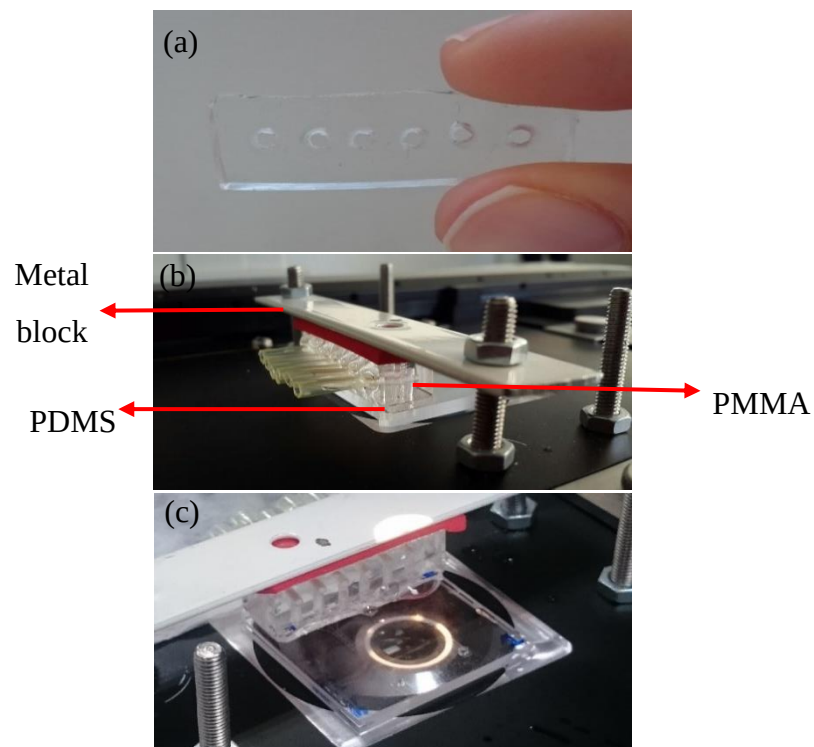


Figure 5.6. (a) The PDMS piece with inlet holes (b) The inlet set up with PDMS-PMMA-PS (c) The leakage between PS and PDMS

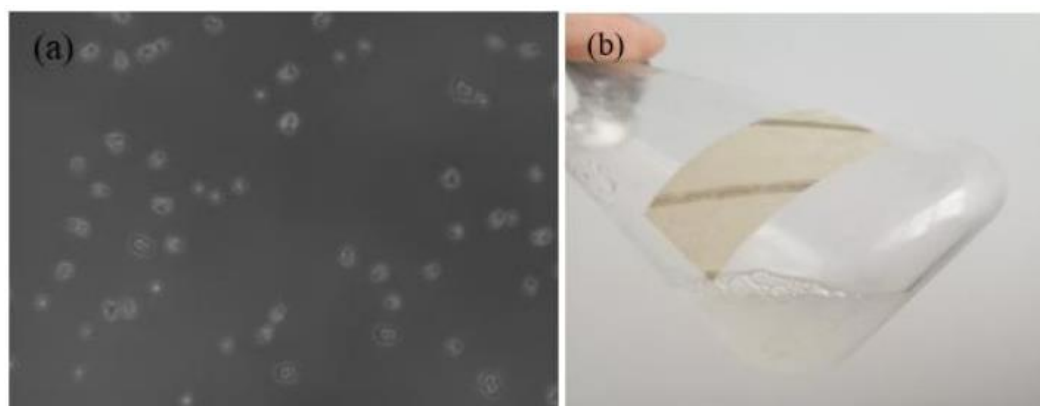


Figure 5.7. (a) The yeast cells under microscope (b) the turbidity of yeast culture after 24 hours

When we compare the growth rate and size of the yeast cells used in experiments 1 and 2, it can be said that the yeast cells were dead in the first experiment. In this experiment, 3 chips were tested, which were bonded under different pressures (10, 11 and 12 bar) but at the same temperature of 93 °C. The flowrates of the side channels and middle channel were adjusted as 3 and 5 $\mu\text{l}/\text{min}$, respectively. The leakage problem between PDMS patch and PS chip caused the experiment to finish (Figure 5.6c). The last chip, which was bonded under 10 bar, allowed medium flow from all inlets through the channels and chambers. The medium filled all the microbioreactor in 4 hours, then the medium in the middle inlet was changed by diluted yeast pre-culture, of which the optical density was measured as 0.45. The OD of the undiluted yeast culture was measured as 1.45. The diluted yeast pre-culture was fed through the middle inlet at a flowrate of 0.5 $\mu\text{l}/\text{min}$. The yeast flow could not be observed in the first 15 hours. The inlet tubes were removed first and then were attached again to be sure that there was a flow into the chip. The diluted yeast culture was out of the inlet, the pumped liquid could not flow into the chip (Figure 5.8). The yeast flow could be observed just after the tube was re-attached. The metal piece prevents us to view the inlet hole (Figure 5.6b), but it was predicted that the yeast cells were accumulated in the inlet hole during 15 hours of the experiment. Therefore, the medium was started to be fed through the middle inlet instead of diluted yeast preculture. The outlet flow was observed, the outlet stream was collected after yeast-trapping process started (Figure 5.9). After 25 hours, yeasts invaded all chambers and blocked outlet and inlets (Figure 5.10). As a result, the experiment was ended at 25th hour because of the excess amount of yeast cells inside the microbioreactor. There

was no leakage between any polymers. The inlet holes were aligned exactly, which might prevent the leakage from this device.



Figure 5.8. The image after removal of inlet tubes

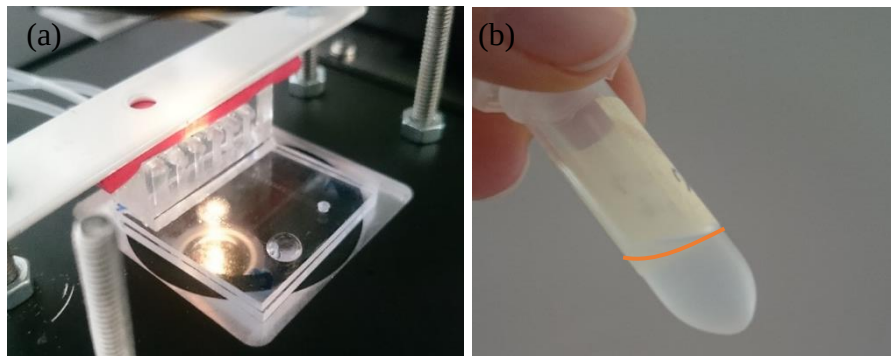


Figure 5.9. (a) The observed outlet flow in chip (b) the collected outlet stream after yeast flow

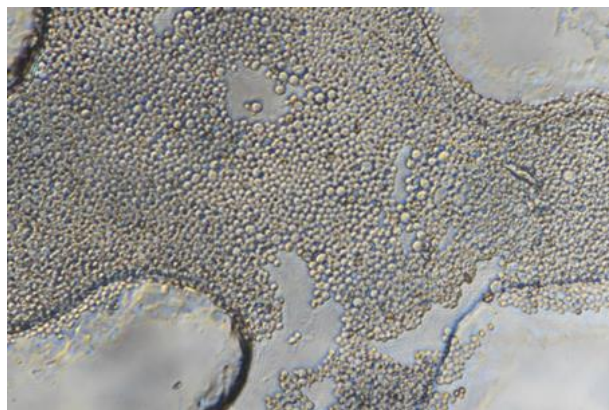


Figure 5.10. The image of chamber 1 after 25 hours

The cells in low autofluorescence YNB medium (without histidine including 2% glucose) were observed under Nikon Ti-inverted microscope for 24 hours. The cells were trapped in all the chambers, however some of them swept away or moved away through the outlet. The images of chamber 1 at specific hours were given to illustrate the number of cells, i.e. changes in a chamber were not caused by growth or reproduction of yeast cells, but due to their moving away (Figure 5.11). The time, t_0 , refers to the start of the first cell trapping. It can be seen that the number of yeast cells were increasing till 6th hour. The number of the cells were decreased between 8th and 17th hour because some of them were swept away from the trapping area. At the 17th hour, yeast cells were moved away from the inlet and hence increased the number of cells in the 1st chamber. The changes in the number of yeast cells were almost same in other chambers, but did not follow the same pattern, i.e. increasing or decreasing in the number of cells occurred at different time intervals. In the end, the cells were swept away again. In brief, the number of cells were fluctuating during the experiment.

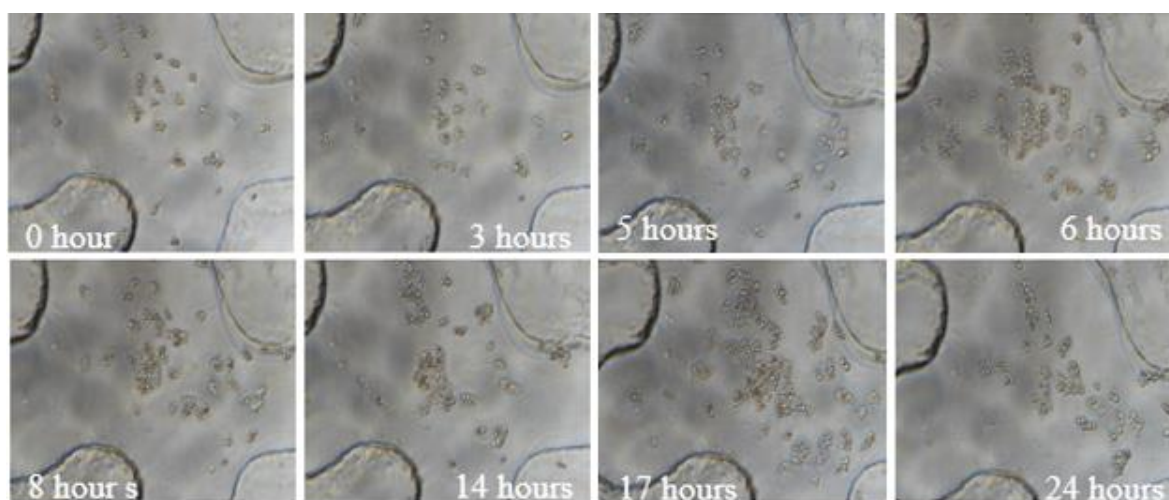


Figure 5.11. The change in the number of yeast cells in chamber 1 at different time points

5.1.2.1. Chamber 1. The cell number fluctuated throughout the experiment. After 240th minutes, the number of cells rose sharply (Figure 5.12). The cell area and cell perimeter showed almost same trend as cell count (Figure 5.13a, b). The ratios of cell area to cell count and cell perimeter to cell count were expected to remain constant. However, these ratios fluctuated till the 360th minutes, then changed slightly. These ratios were slightly higher in the beginning than the ratios at the end of the experiment. The total cell area within the

chamber decreased as the cells were possibly replaced by smaller cells throughout the experiment (Figure 5.13c, d).

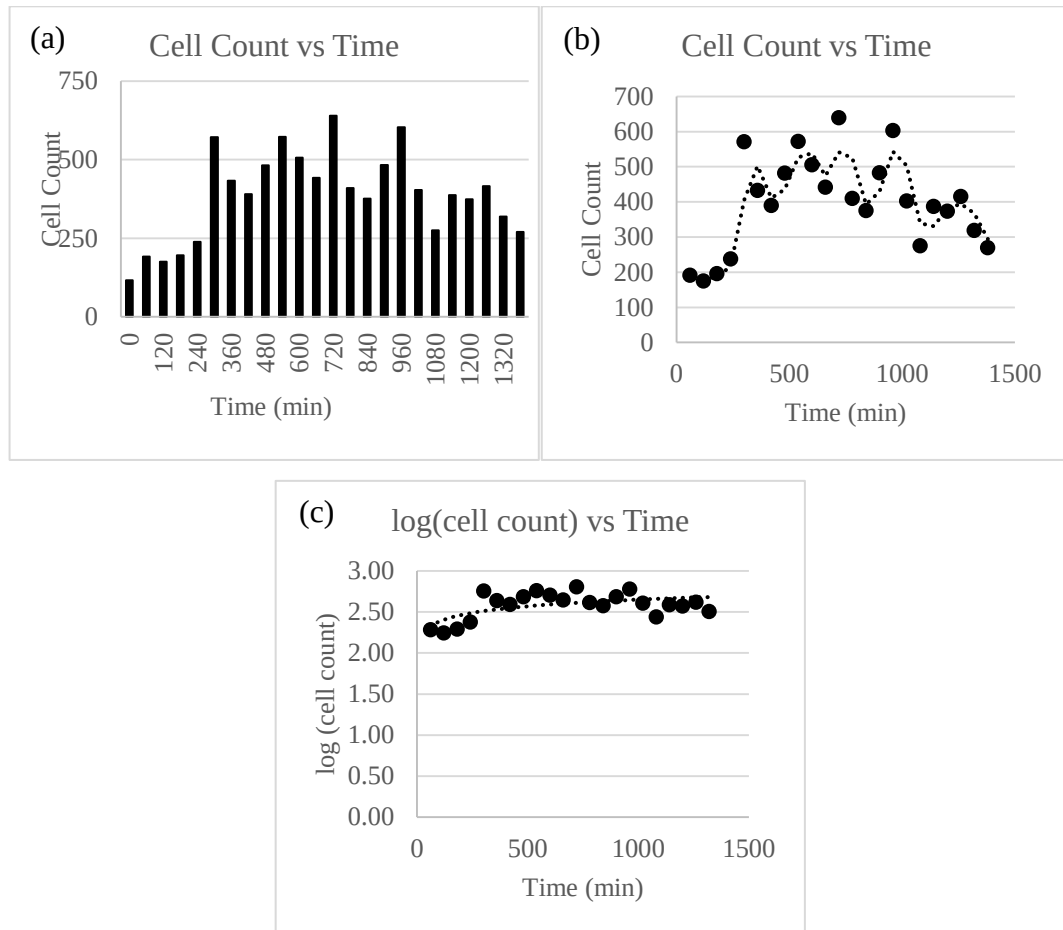


Figure 5.12. Graphical representation of processed data for chamber 1 (a, b) cell count vs time graph (c) log (cell count) vs time graph (brightfield images)–experiment 2.

5.1.2.2. Chamber 2. The number of cells increased gradually till 1080th minutes. After 1080th minutes, a dramatic decrease was observed in cell number (Figure 5.14). The cell area and cell perimeter showed almost same trend as cell count (Figure 5.15a, b). The ratios of cell area to cell count and cell perimeter to cell count decreased slightly throughout the experiment (Figure 5.15c, d).

5.1.2.3. Chamber 3. The cell area and cell perimeter showed almost same trend as cell count (Figure 5.16 and Figure 5.17a, b). The ratios of cell area to cell count and cell perimeter to cell count were slightly increasing till the 900th minutes. These ratios were increasing

significantly between 900th and 1140th minutes. The ratios decreased gradually after 1140th minutes, because cell number along with the cell area and cell perimeter were decreasing. The ratios of cell area to cell count and cell perimeter to cell count fluctuated throughout the experiment (Figure 5.17c, d).

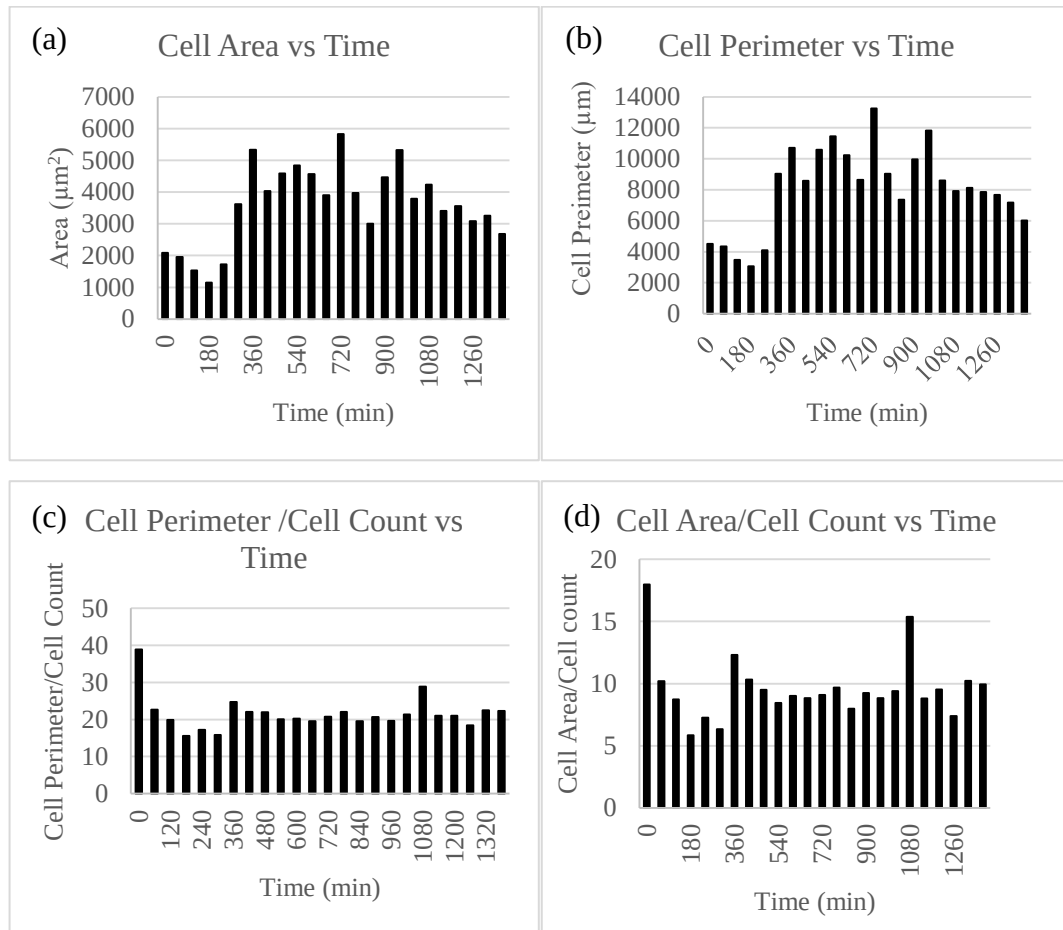


Figure 5.13. Graphical representation of processed data of chamber 1 (a) cell area vs time graph (b) cell perimeter vs time graph (c) cell area/cell count vs time graph (d) cell perimeter/cell count vs time graph (brightfield images)-experiment 2.

5.1.2.4. Chamber 4. In chamber 4, the number of cells changed slightly in the first 360 minutes, then fluctuated (Figure 5.18). The cell area and cell perimeter showed same trend as the cell count (Figure 5.19a, b). The ratios of cell area to cell count and cell perimeter to cell count increased gradually (Figure 5.19c, d). One can speak of the growth of the cells in chamber 4 with respect to the rising trend of the ratios of cell area/cell count and cell perimeter/cell count.

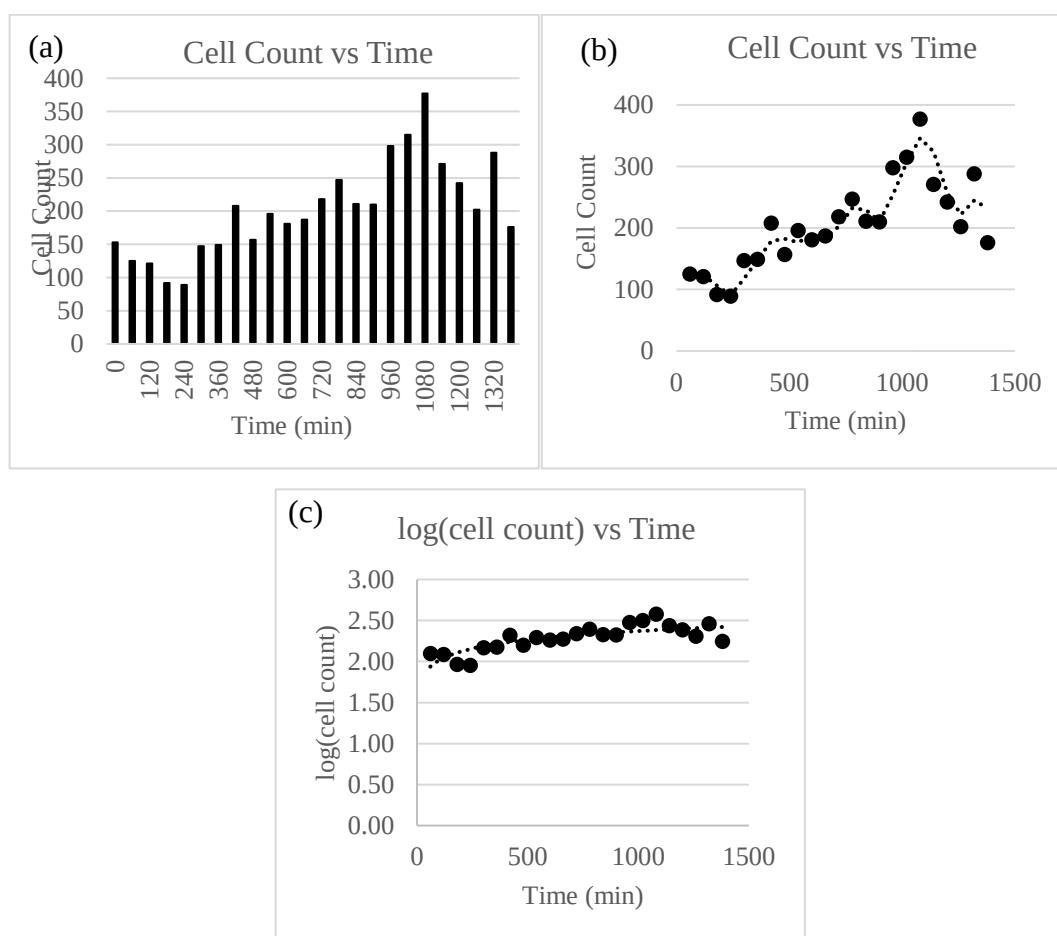


Figure 5.14. Graphical representation of processed data for chamber 2 (a,b) cell count vs time graph (c) log(cell count) vs time graph (brightfield images)–experiment 2.

5.1.2.5. Chamber 5. In the first 360 minutes, the number of cells slightly increased in chamber 5. Then the number of cells dropped sharply at 420th minutes. The cell number fluctuated between 720th and 1380th minutes (Figure 5.20). The cell area and cell perimeter showed same trend as the cell count (Figure 5.21a, b). The ratio of cell area to cell count changed slightly up to 480th minutes and fluctuated between 540th and 1380th minutes (Figure 5.21c). The ratio of cell perimeter to cell count showed almost the same trend as the ratio of cell area to cell count (Figure 5.21d). It was observed that the number of cells at the end of the experiment (1380th minutes) were less than those in the beginning; the ratios of cell area/cell count and cell perimeter/cell count were slightly higher at the end of the experiment than those at the beginning. Even though the cells in the chamber (Chamber 5) were not same (some swept away and replaced by the new ones), the cells were able to grow successfully during the experiment.

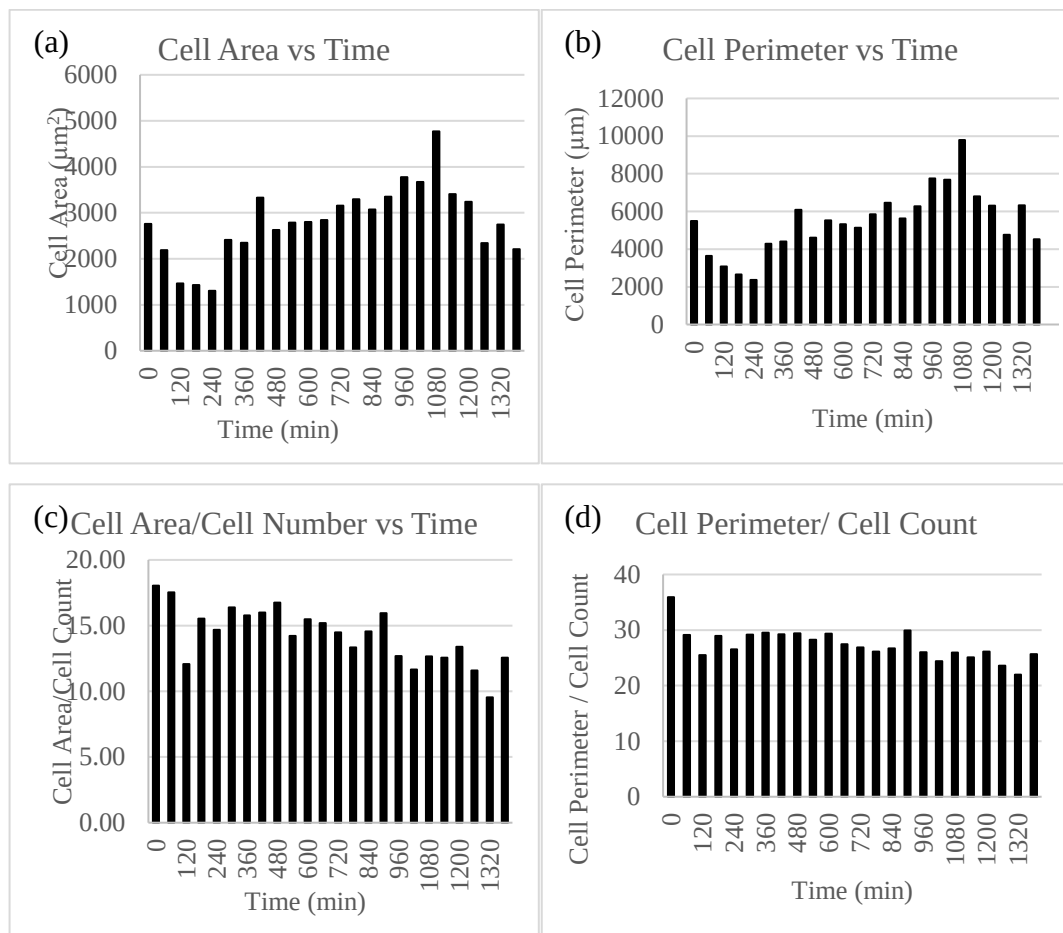


Figure 5.15. Graphical representation of processed data of chamber 2 (a) cell area vs time graph (b) cell perimeter vs time graph (c) cell area/cell count vs time graph (d) cell perimeter/cell count vs time graph (brightfield images)-experiment 2.

5.1.2.6. Chamber 6. The total cell number fluctuated during the experiment (Figure 5.22). The cell area and cell perimeter showed same trend as the cell count (Figure 5.23a, b). The ratios of cell area to cell count and cell perimeter to cell count increased gradually (Figure 5.24c, d). Growth of the yeast cells were clearly observed considering the rising trend of the ratios of cell area/cell count and cell perimeter/cell count in chamber 6.

5.1.2.7. Chamber 7. The total number of cells changed slightly during the experiment in chamber 7 (Figure 5.24). The cell area and cell perimeter showed same trend as the cell count (Figure 5.25a, b). The ratios of cell area to cell count and cell perimeter to cell count did not change considerably (Figure 5.25c, d). Therefore, the ratios of cell area/cell count and cell perimeter/cell count can be considered as constant.

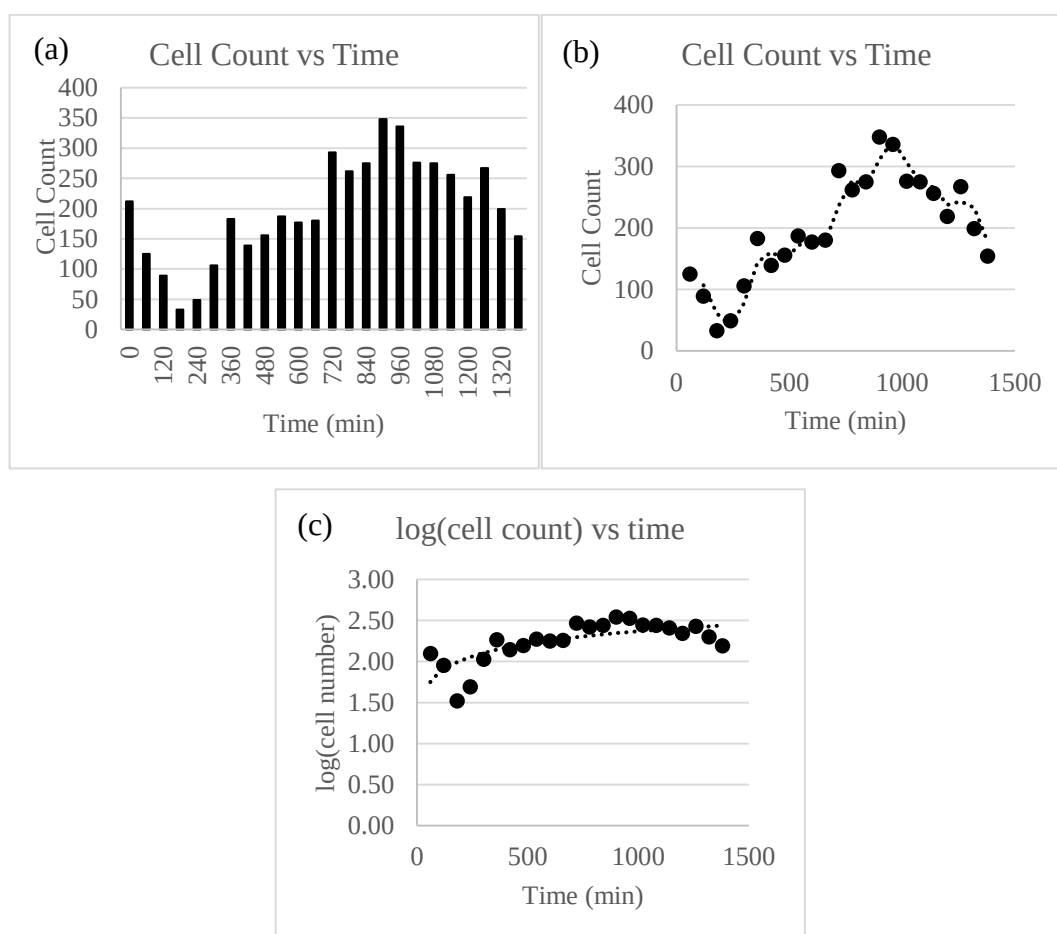


Figure 5.16. Graphical representation of processed data for chamber 3 (a, b) cell count vs time graph (c) log(cell count) vs time graph (brightfield images)–experiment 2.

5.1.2.8. Chamber 8. In the first 720 minutes, the number of cells in chamber 8 changed moderately. Then the number of cells rose suddenly after 720th minutes. The cell number fluctuated between 840th and 1380th minutes (Figure 5.26). The cell area and cell perimeter showed same trend as the cell count (Figure 5.27a, b). The ratios of cell area/cell count and cell perimeter/ cell count showed a peak at 480th minutes (Figure 5.27c, d). Even though the cells in the chamber were not same, the growth of the cells was observed by pictures taken during the experiment.

5.1.2.9. Remarks. The cells were trapped in all the chambers, but have a fluctuating profile because of the high flowrate of the medium, which was 0.5 $\mu\text{L}/\text{min}$. The cells were swept away from one chamber to the other and through the outlet of the microbioreactor. The ones, accumulated at the inlet hole, moved away towards the other chambers. Therefore, the

change in cell number by time might not be caused by growth only. The fluctuations in cell count, cell area and cell perimeter were given in Figure 5.28, 5.29 and 5.30, respectively. The total sizes of the cells were compared in different polymers such as COP and PS (Table 5.1). Even though the number of trapped cells were different, the cell area per cell count and cell perimeter per cell count were similar. In this case, it can be said that both polymeric microbioreactors were appropriate to observe the growth of yeast cells. However, the background fluorescence signals of these two thermoplastics are indeed different. The fluorescence signal of PS is 7.24 times higher than the fluorescence signal of COP bringing a disadvantage for the visualization of green fluorescently tagged protein of the yeast cells. The captured background fluorescence signals of the polymers are shown in Figure 5.31.

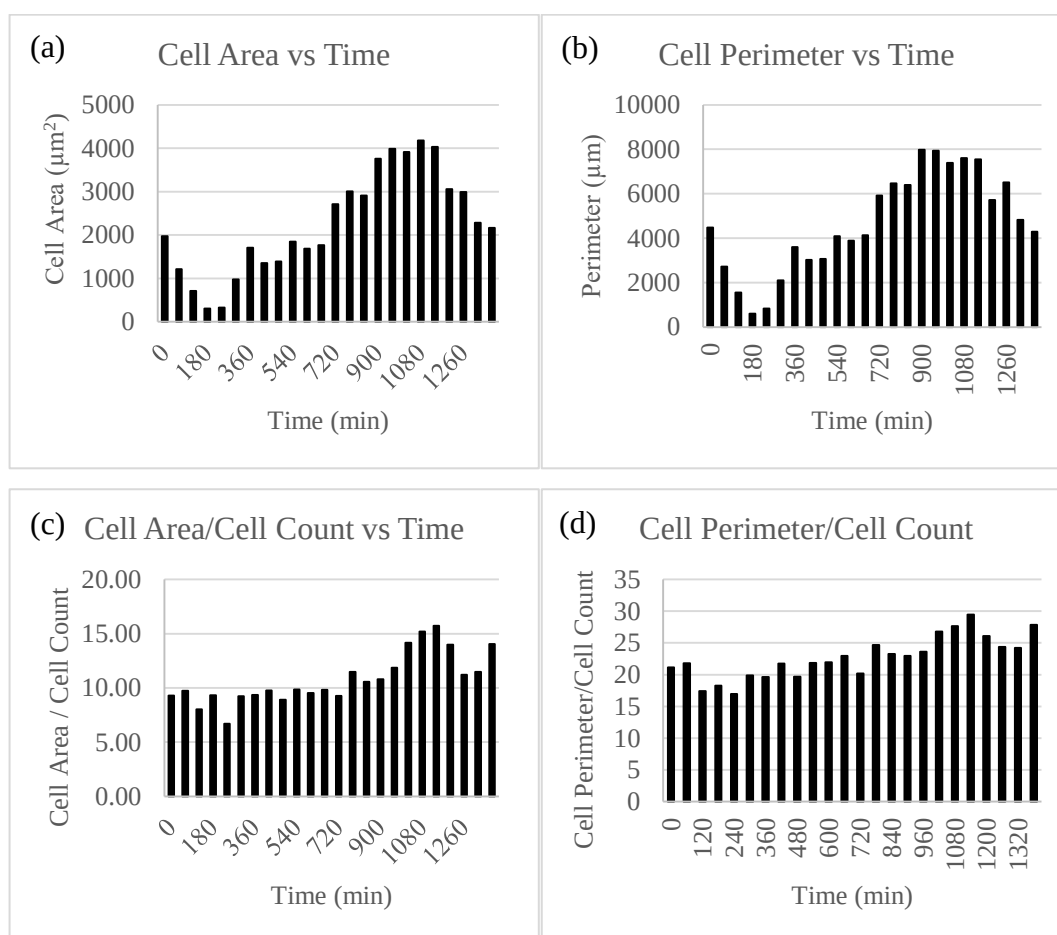


Figure 5.17. Graphical representation of processed data of chamber 3 (a) cell area vs time graph (b) cell perimeter vs time graph (c) cell area/cell count vs time graph (d) cell perimeter/cell count vs time graph (brightfield images)-experiment 2.

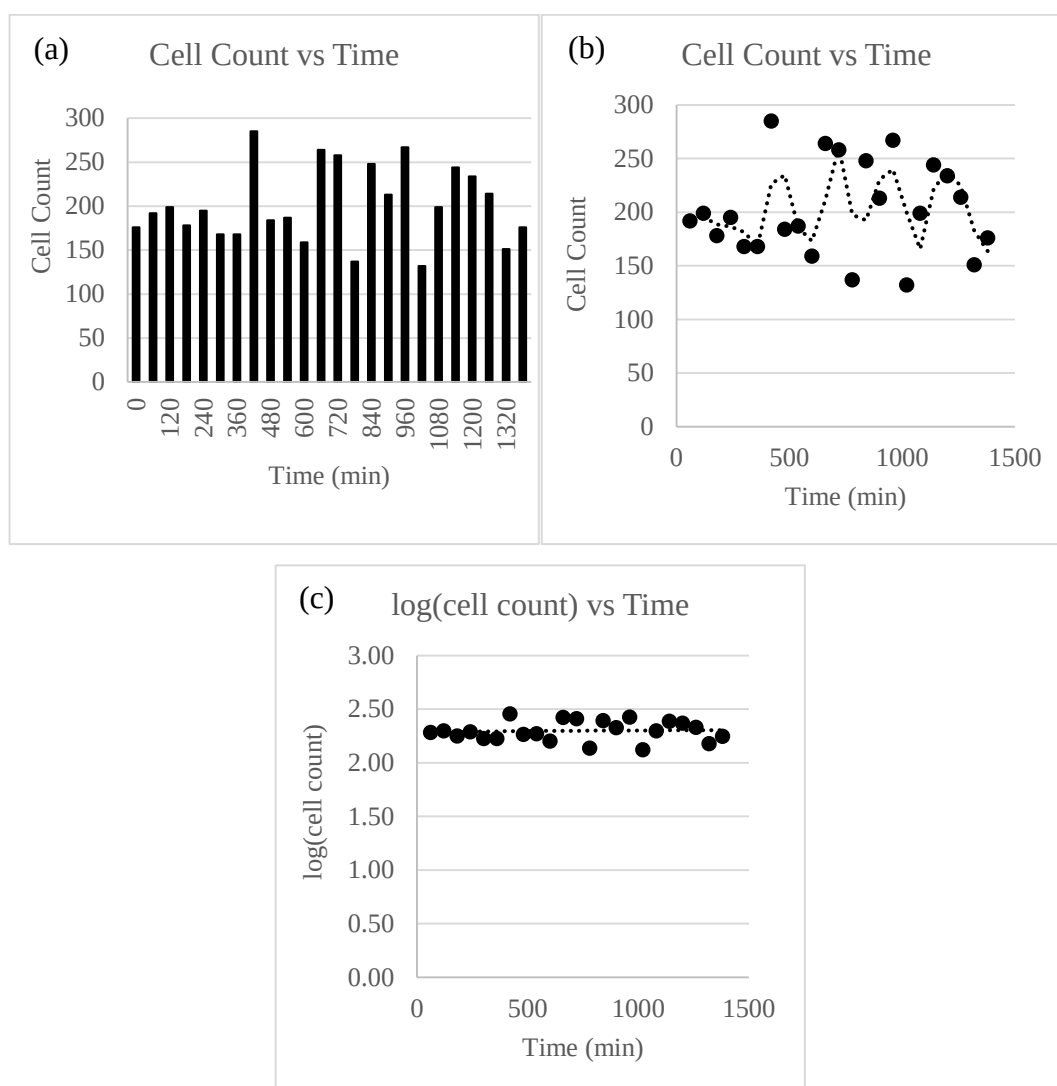


Figure 5.18. Graphical representation of processed data for chamber 4 (a,b) cell count vs time graph (c) log(cell count) vs time graph (brightfield images)–experiment 2.

Table 5.1. The comparison of the cell sizes-BY4741 in different polymers

Polymer Type	Duration of Experiment (h)	Number of Cells	Total Cell Area (μm^2)	Total Cell Perimeter (μm)	Cell Area/Cell Count	Cell Perimeter/Cell Count
COP	15	463	7950	12519	17	27
PS	23	1588	26774	50597	17	32

5.2. Effect of inhibitor on wild type BY4741 haploid (Sld7:GFP) cells in YNB medium (2% glucose)

5.2.1. Experiment 3

The inhibitor (Methyl methanesulfonate) is a DNA alkylating agent that blocks DNA replication elongation by forming a lesion in DNA and preventing DNA polymerases' binding to replication origin (Groth *et al.*, 2010; Tkach *et al.*, 2012). MMS induces genes involved in cellular redox homeostasis in yeast (Tkach *et al.*, 2012), increases the reactive oxygen species (ROS) concentration, and thus prevents cell proliferation, and causes cell death (Martindale *et al.*, 2012).

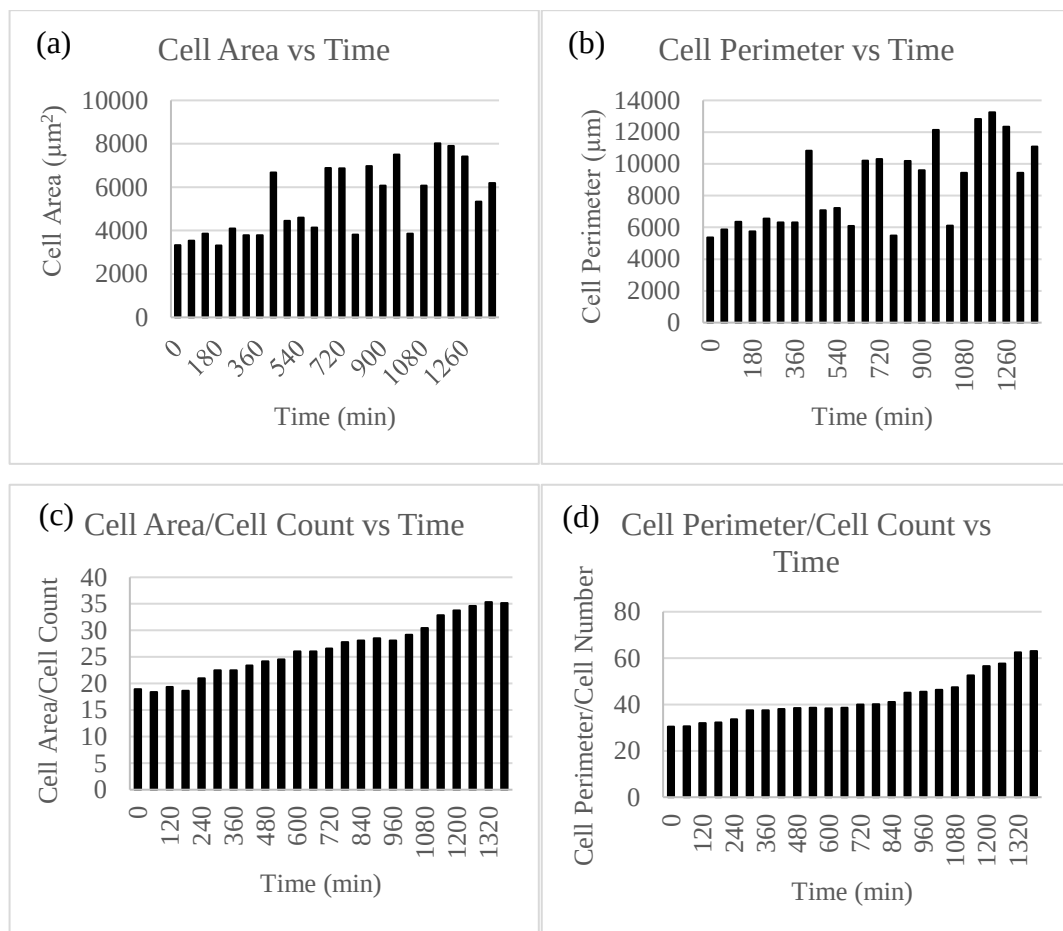


Figure 5.19. Graphical representation of processed data of chamber 4 (a) cell area vs time graph (b) cell perimeter vs time graph (c) cell area/cell count vs time graph (d) cell perimeter/cell count vs time graph (brightfield images)-experiment 2.

The oxidative stress forces cells to autophagic cell death (cells that undergo an extreme amount of stress experience cell death), where cells begin to shrink, bleb and die (Zakeri *et al.*, 1995). Proteins change their location from nucleus to cytoplasm in an environment involving MMS (Tkach *et al.*, 2012). The response time of the cells to MMS depends on the amount of MMS (Groth *et al.*, 2010).

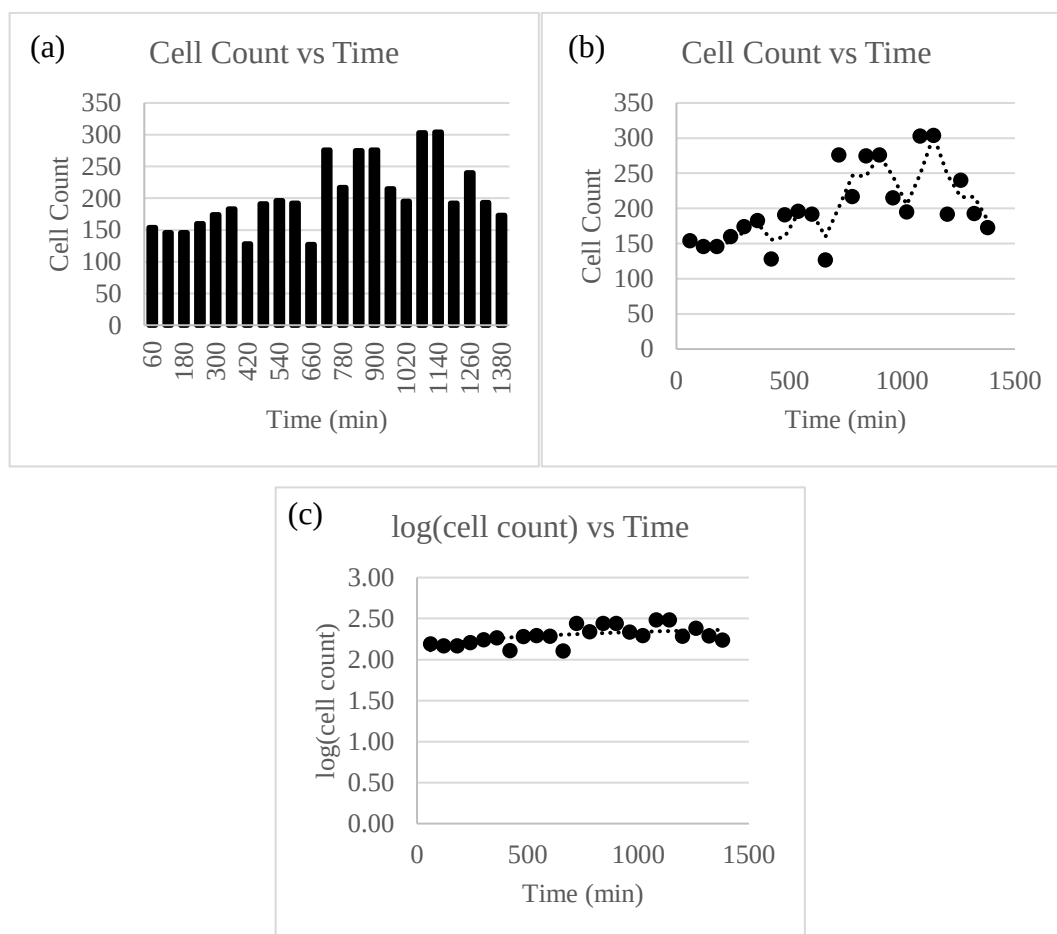


Figure 5.20. Graphical representation of processed data for chamber 5 (a, b) cell count vs time graph (c) log(cell count) vs time graph (brightfield images)—experiment 2.

YNB medium without histidine including 2% glucose (N1) was used as the culture medium for Sld7-GFP tagged wild type BY4741 haploid yeast cells. The frozen stock cells were used to prepare the preculture. The cells were let to grow in an incubator for 36 hours and then diluted by N1 medium, and prepared (OD: 0.49) for inoculation into microbioreactor.

The syringes were connected to the inlet block by using PEEK tubing (IDEX Health and Science – Product Code: 1538L). However, the hardness of the PEEK prevented the movements of the lab-on-a-chip platform and deformed the inlet mechanism, which may cause leakage (Figure 5.32). In addition, the opacity of PEEK prevented the visualization of the medium through the tubing. Hence, instead of PEEK tubings, polyethylene tubings were used to connect syringes to the microbio reactor, just as in the previous experiments. Plastic syringes were replaced by glass syringes in order to prevent volume extension due to pressure, and 5 mL, 10 mL and 1 mL glass syringes (Hamilton Company) were used to load yeast, N1 medium and N2 medium into the microbio reactor, respectively. The flowrate of the syringes at the inlets were adjusted to 3 $\mu\text{L}/\text{min}$. The air bubble, formed in the right channel, filled all the chambers. Then, the system was closed and the chip was changed.

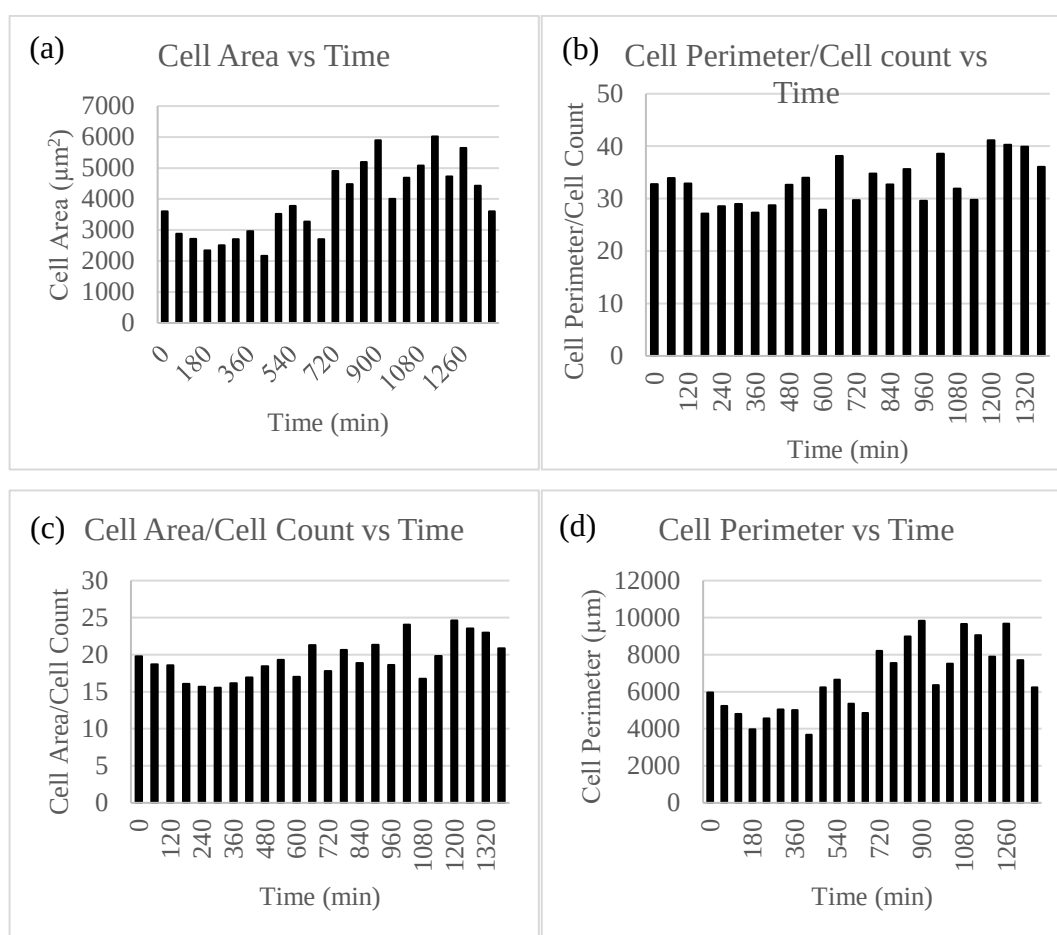


Figure 5.21. Graphical representation of processed data of chamber 5 (a) cell area vs time graph (b) cell perimeter vs time graph (c) cell area/cell count vs time graph (d) cell perimeter/cell count vs time graph (brightfield images)-experiment 2.

The N1 medium was loaded into the new microbio reactor through the middle inlet only (to prevent air bubble formation, which may be caused due to the leakage between PDMS-PMMA-PS connection in the right channel) at a rate of 1 $\mu\text{L}/\text{min}$ until all the chambers and channels were filled (10 hours). Then, the loading of yeast cells was initiated at a rate of 0.1 $\mu\text{L}/\text{min}$. After 2 hours of yeast cell loading, the N1 feeding at a rate of 0.1 $\mu\text{L}/\text{min}$ was started and continued till the system reached at steady state (it was 8-9 hours based on previous experiment). The time, t_0 , refers to the start of the first cell trapping. The N1 medium was changed with N2 medium (YNB medium without histidine including 2% glucose and 0.01% MMS) at 510th minutes (Table 5.2). The N2 was fed for 10 hours at a rate of 0.1 $\mu\text{L}/\text{min}$. The brightfield and fluorescence microscopic images of the chambers were taken under 40X and 90X magnification for 17 hours during N1 and N2 media loading. The terms “first part of the experiment” and “the second part of the experiment” refer to the microbio reactor, either full of N1 or N2 medium, respectively.

The yeast cells started to be trapped after 2 hours of N1 loading. The yeast cells were trapped in the first, fourth, sixth and seventh chamber in the first part of the experiment. However, the cells in the first and sixth chambers were swept away in the second part of the experiment. Therefore, it was not possible to analyse the effect of MMS on cells in the chambers, specifically. All in all, the effect of MMS on yeast cells that were trapped in the c-shape of the fourth and seventh chambers were observed with respect to the changes in area, perimeter and volume. The trapped cells were not seen as a colony. Therefore, it was easier to keep track of changes in the cell count, area, perimeter and volume. The integrated density cannot be measured because of the high auto-fluorescence background of PS, which prevents the visualization of the signal coming from GFP tagged protein. All processing results were based on brightfield images taken under 40X.

5.2.1.1. Chamber 1. The cells were trapped at 240th minutes of the experiment. However, cells cannot be trapped permanently, which means they were swept away after 180 minutes at the 420th minutes (t_0 refers to the start of the first cell trapping). There were 4 cells at 240th minutes (Figure 5.33a), one with the bud (left). Two buds appeared from different cells at 300th minutes and 330th minutes (Figure 5.33c, d). Yet, one of the cells with a bud was swept away and the cell number decreased to four, including the buds (Figure 5.33e).

The cell area (Figure 5.34a) and cell perimeter (Figure 5.34b) increased and decreased because of the bud formation and sweeping away, respectively. However, area, perimeter and volume of the both mother and daughter cells increased significantly as expected (Figure 5.34 – 5.40). The trend change of the area, perimeter and volume of the all cells was similar.

The cell area of M1, D1, M2, D2 and M3 increased by 60%, 130%, 50%, 80% and 20%, respectively (Table 5.3). In addition to, the cell perimeter of M1, D1, M2, D2 and M3 increased by 30%, 50%, 20%, 30% and 10%, respectively (Tables 5.3, 5.4 and 5.5).

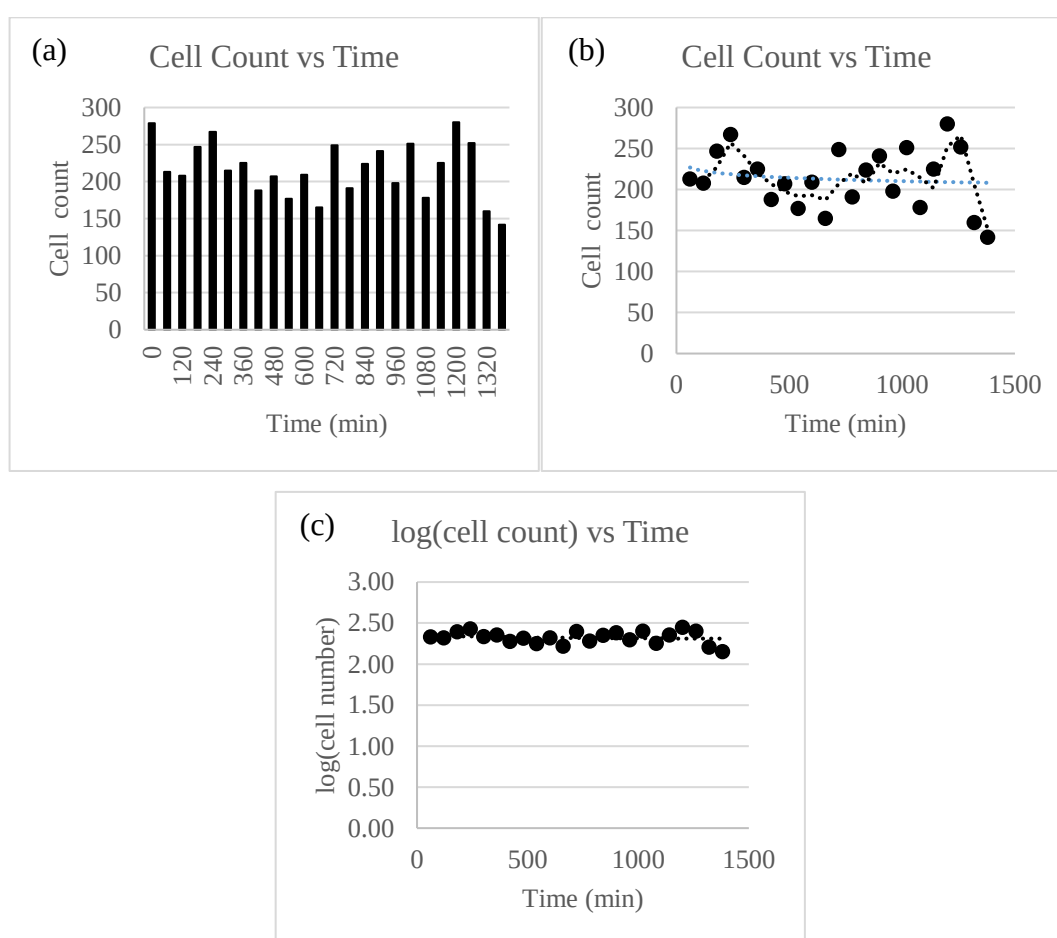


Figure 5.22. Graphical representation of processed data for chamber 6 (a,b) cell count vs time graph (c) log(cell count) vs time graph (brightfield images) (d) integrated density vs time graph (fluorescence image)—experiment 2.

5.2.1.2. Chamber 2. The cells were trapped in the second chamber in the second part of the experiment only, not in the first part. The change in the size of the cells, that were trapped

in the second chamber, was given in Figure 5.41. There were only 3 cells in this chamber. The area, perimeter and volume of the cells decreased slightly with time, then became stable (Figures 5.42-5.46). The trend of the change of the area, perimeter and volume for all the cells was similar.

The cell area of M1, D1 and M2 decreased 62%, 69% and 70%, respectively (Table 5.4). In addition, the cell perimeter of M1, D1 and M2 decreased 38%, 45% and 45%, respectively (Tables 5.6 and 5.7).

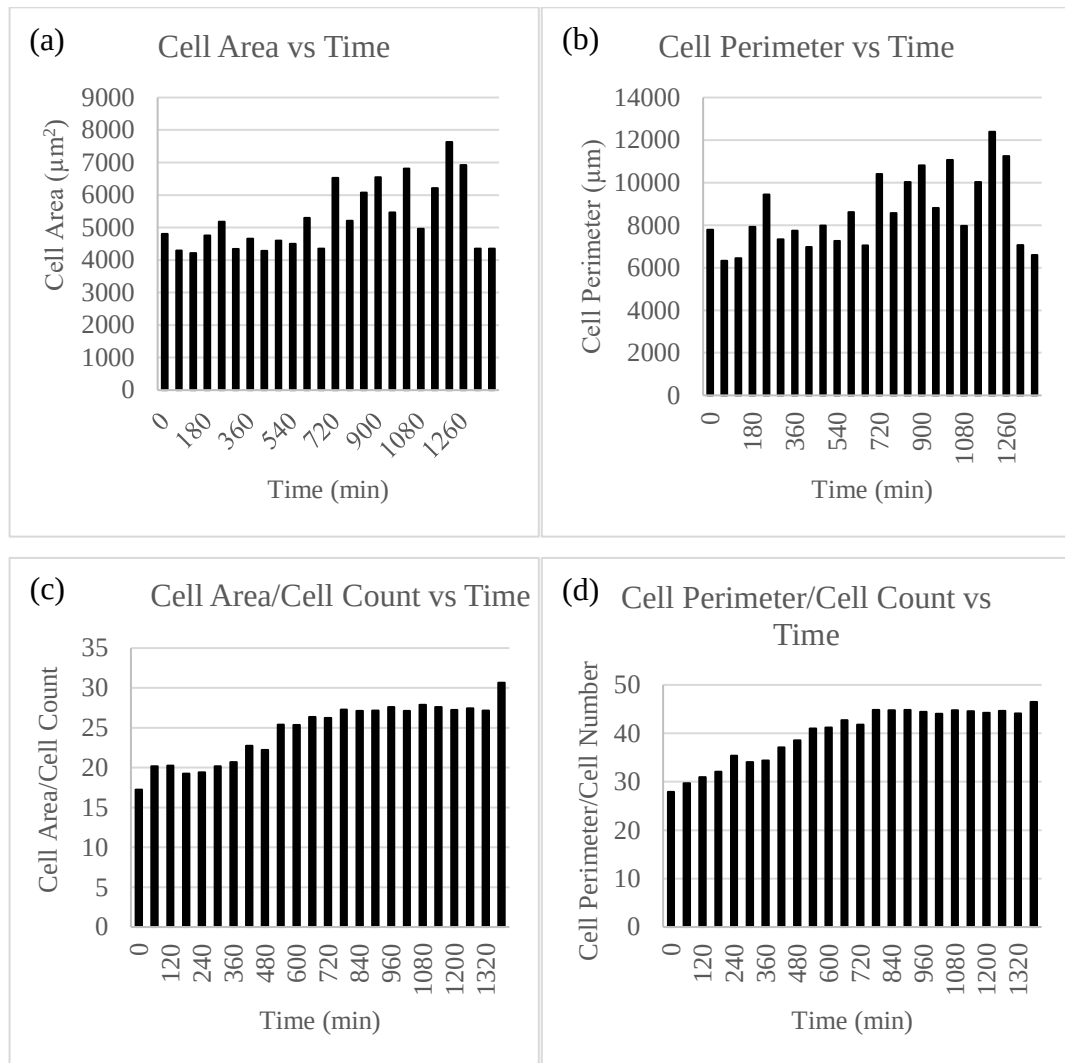


Figure 5.23. Graphical representation of processed data of chamber 6 (a) cell area vs time graph (b) cell perimeter vs time graph (c) cell area/cell count vs time graph (d) cell perimeter/cell count vs time graph (brightfield images)-experiment 2.

The cell area of M1, D1 and M2 decreased 62%, 69% and 70%, respectively (Table 5.4). In addition, the cell perimeter of M1, D1 and M2 decreased 38%, 45% and 45%, respectively (Tables 5.6 and 5.7).

5.2.1.3. Chamber 4. The yeast cells were trapped efficiently at the C-shaped region of the fourth chamber (Figure 5.47). The cells were observed from 270th minutes (t_0 refers to the start of the first cell trapping) to the end of the experiment for 12 hours. There were two buds appeared between 300th and 330th minutes (Figure 5.48a, b).

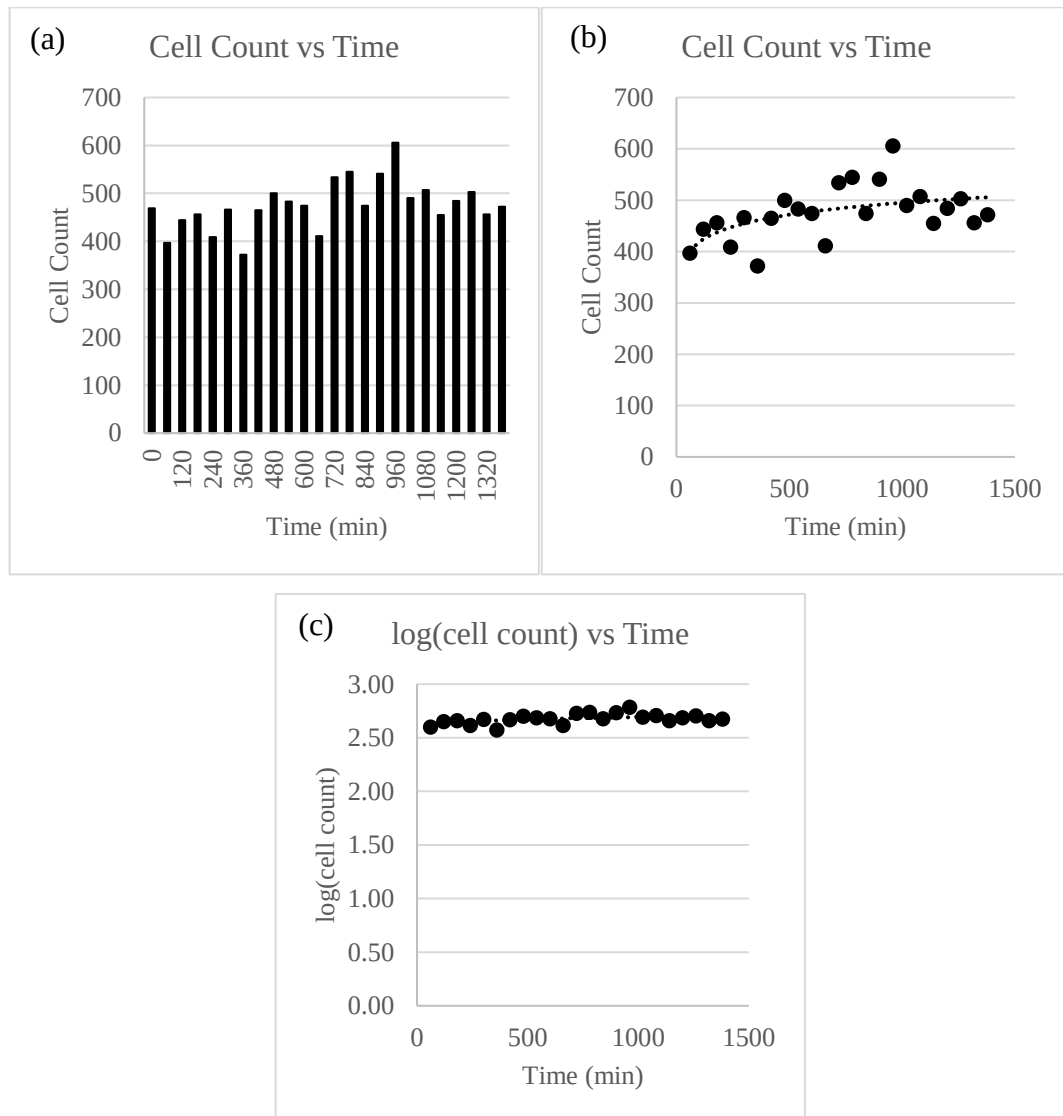


Figure 5.24. Graphical representation of processed data for chamber 7 (a, b) cell count vs time graph (c) log(cell count) vs time graph (brightfield images - experiment 2).

There were 5 cells in chamber 4 (Figure 5.49). The area, perimeter and volume of both mother and daughter cells increased gradually till 510th minutes before feeding N2. These changes support that cells grow in glucose rich YNB media without histidine (N1). At the end of the 510 minutes, the cell area of M1, D1, M2, D3 and M3 increased by 22%, 161%, 26%, 48% and 35%, respectively (Table 5.8). The cell perimeter of M1, D1, M2, D3 and M3 increased by 14%, 84%, 13%, 41% and 17%, respectively (Table 5.8). The total cell area and total cell perimeter increased by 50 % and 28 %, respectively at the end of the 510th minutes.

At 510th minute, N1 medium was changed by N2 medium, with MMS. After 610th minutes, area, perimeter and volume of the cells decreased significantly, then changed slightly for 295 minutes (till 905th minutes), and became stable (Figures 5.50-5.55). The cell area of M1, D1, M2, D3 and M3 decreased by 66%, 75%, 56%, 63% and 65% after 510th minutes, respectively (Table 5.8). The cell perimeter of M1, D1, M2, D3 and M3 decreased by 43%, 51%, 32%, 40% and 42% after 510th minutes, respectively (Tables 5.8 and 5.9). The total area, perimeter and volume values were reduced by more than 60 %, 40 % and 40%, respectively between 610th and 935th minutes. The change in area, perimeter and volume of all the cells were shown in Figures 5.50-5.55. The trend in change of the area, perimeter and volume of all the cells was similar in both N1 and N2 media.

5.2.1.4. Chamber 6. A budding yeast was trapped efficiently in the sixth chamber in the first part of the experiment. The change in the size of the trapped budding yeast in N1 media was shown in Figure 5.56. The cells were swept away after 330th minute. Therefore, it was not possible to observe the effect of MMS on these cells.

There were only 2 cells in chamber 6 (Figure 5.56). The cell count was constant, yet area, perimeter and volume of both mother and daughter cells increased as expected (Figures 5.57 and 5.58). The area of mother and daughter cells increased by 76% and 132%, respectively. The perimeter of mother and daughter cells increased by 33% and 53%, respectively. The increase in total cell area and total cell perimeter were greater than 90 % and 40 %, respectively, at the end of the 330th minutes (Table 5.10). The change in area, perimeter and volume in all the cells were shown in Figure 5.59.

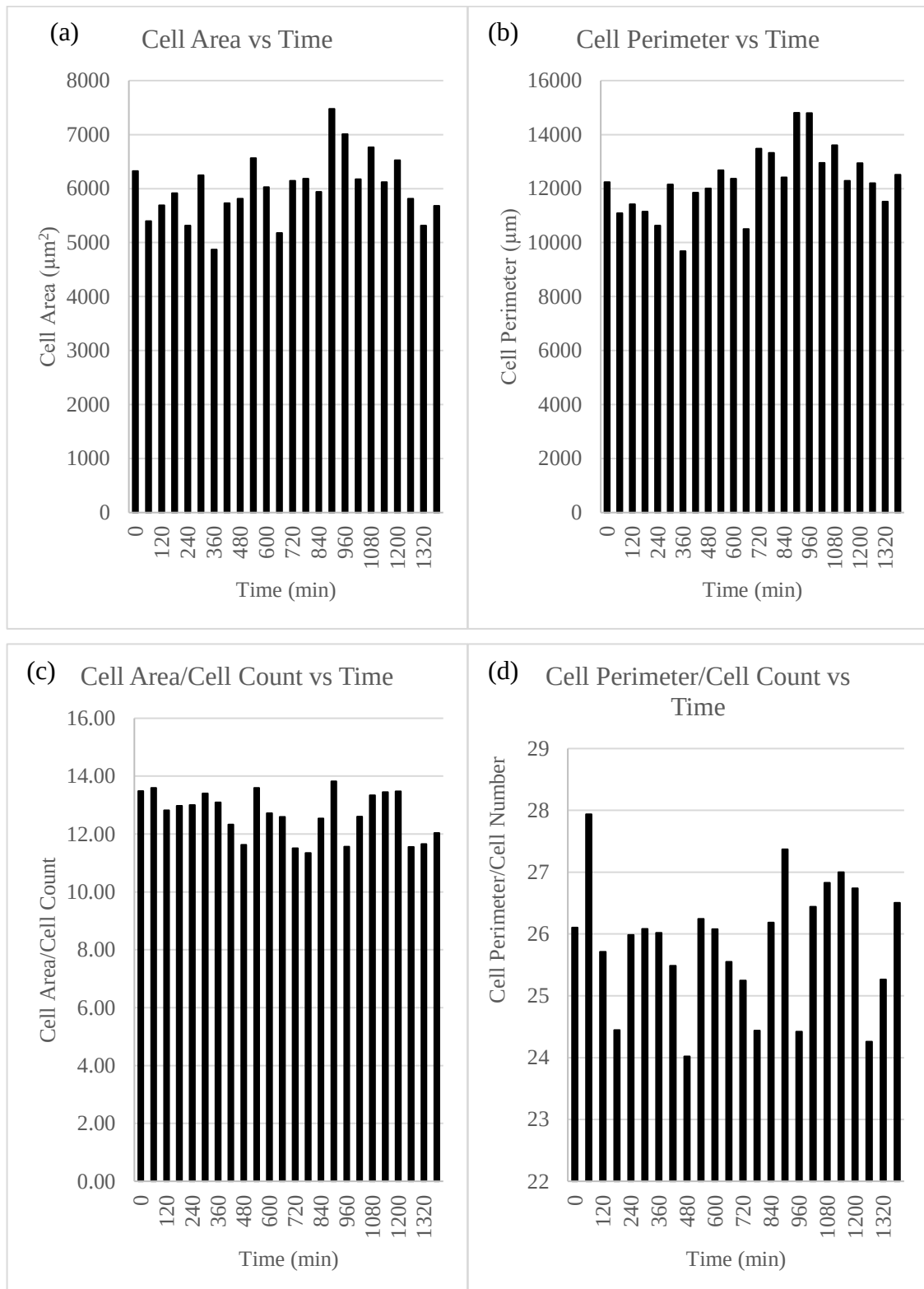


Figure 5.25. Graphical representation of processed data of chamber 7 (a) cell area vs time graph (b) cell perimeter vs time graph (c) cell area/cell count vs time graph (d) cell perimeter/cell count vs time graph (brightfield images)-experiment 2.

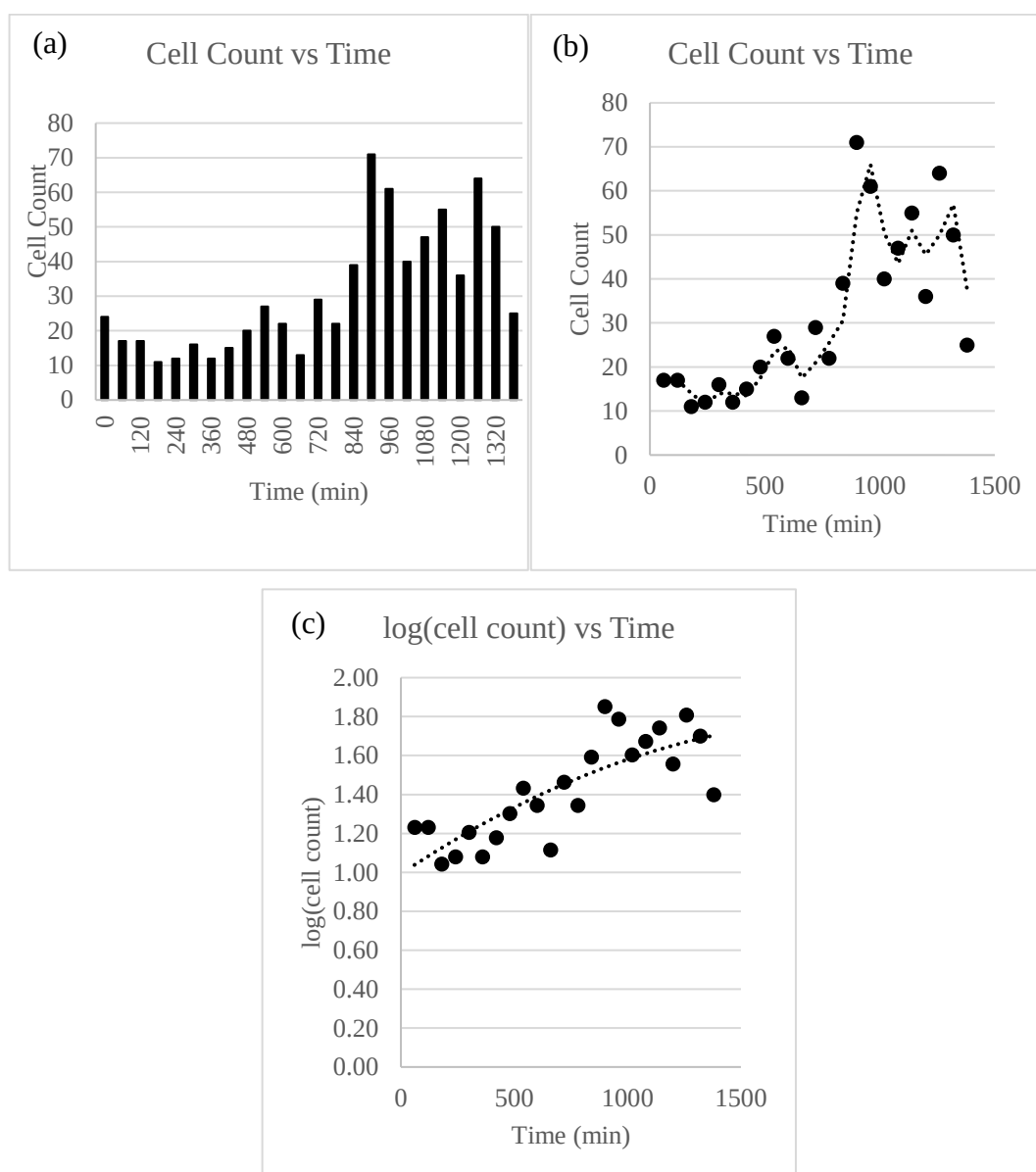


Figure 5.26. Graphical representation of processed data for chamber 8 (a,b) cell count vs time graph (c) log(cell count) vs time graph (brightfield images) –experiment 2.

5.2.1.5. Chamber 7. The single cell was efficiently trapped at the seventh c-shape region (Figure 5.60). The budding phase of the cell was observed for 300 minutes in N1 (Figure 5.61) and 415 minutes in N2 (Figure 5.62). The bud appeared at 270th minutes (Figure 5.61c). The area, perimeter and volume increased gradually between 210th and 610th minutes in N1 as expected (Figure 5.63 and Figure 5.64). The area, perimeter and volume decreased significantly between 510th and 610th minutes, then became constant in N2. The cells may have stopped growing due to MMS.

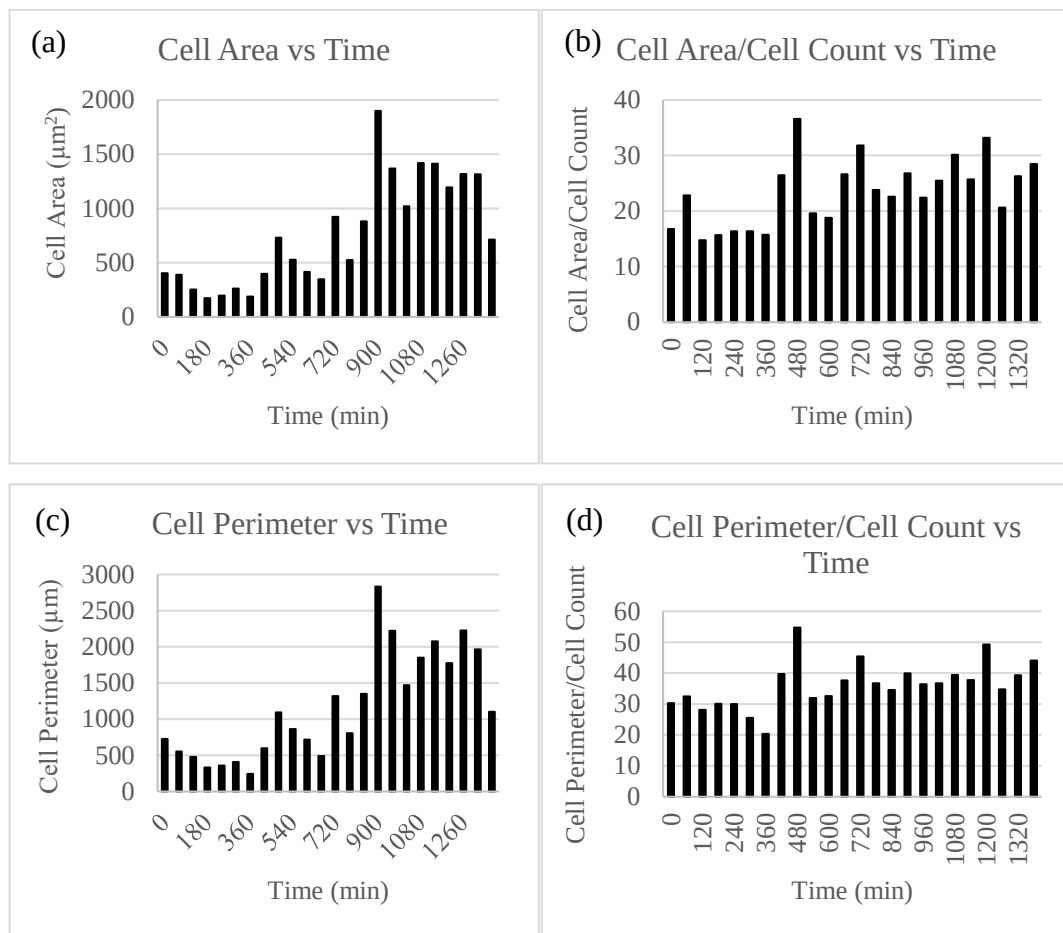


Figure 5.27. Graphical representation of processed data of chamber 8 (a) cell area vs time graph (b) cell perimeter vs time graph (c) cell area/cell count vs time graph (d) cell perimeter/cell count vs time graph (brightfield images)-experiment 2.

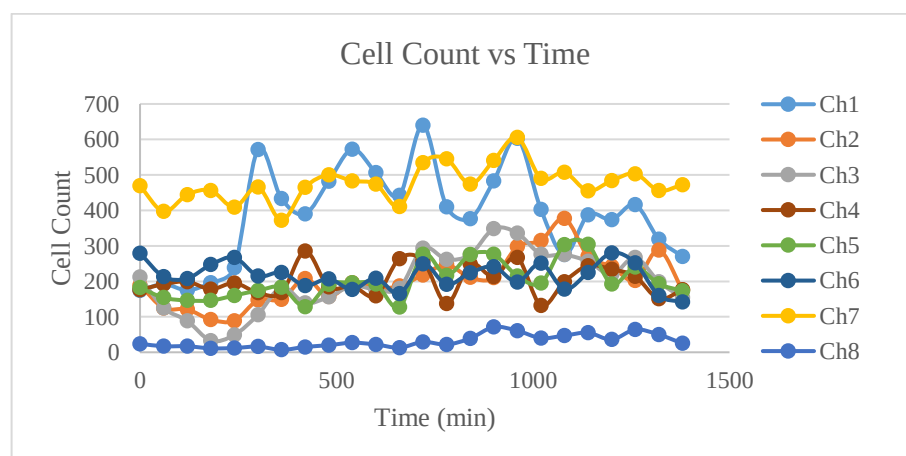


Figure 5.28. Comparison of the change in cell count in all chambers

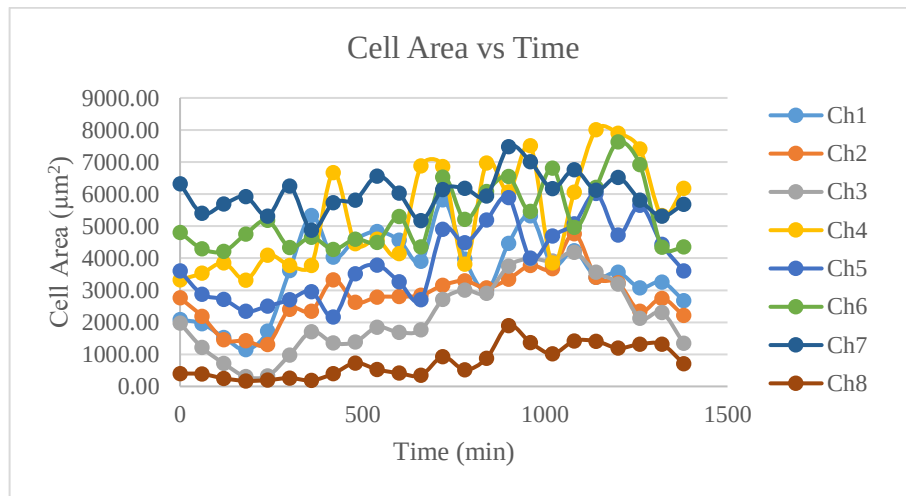


Figure 5.29. Comparison of the change in cell area in all chambers

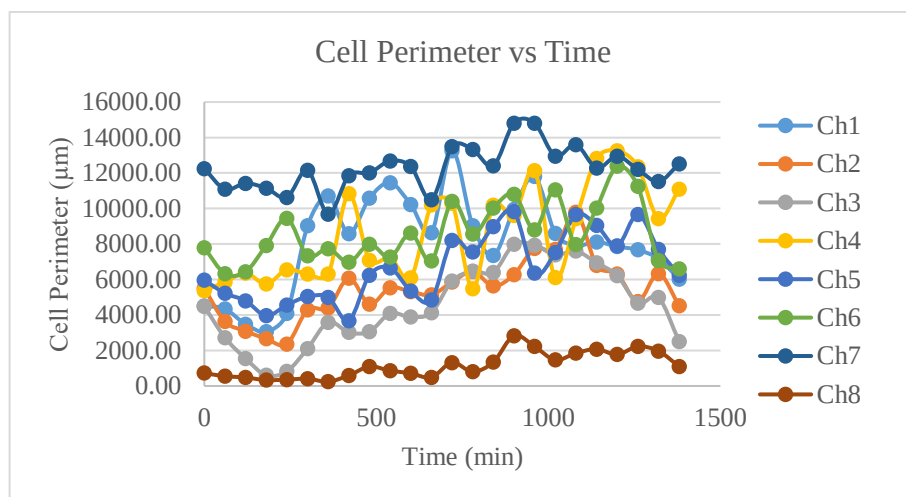


Figure 5.30. Comparison of the change in cell perimeter in all chambers

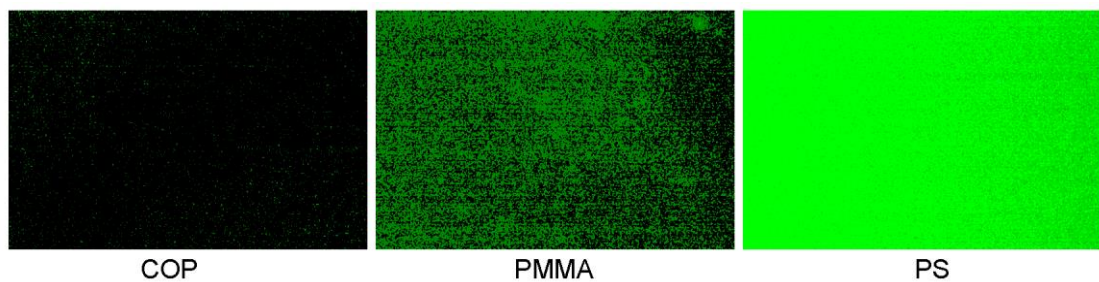


Figure 5.31. Captured background fluorescence signal of the polymers using the same recording parameters of the microscope and its detector.

At the end of the 510th minutes, the area of mother and daughter cells increased by 30% and 79%, respectively. The perimeters of mother and daughter cells increased by 13% and 37%, respectively. After MMS treatment the cell area of mother and daughter cells decreased by 60% and 59%, respectively. In addition, the cell perimeters of mother and daughter cells decreased by 55% and 61%, respectively (Table 5.11). The change in area, perimeter and volume in all cells were shown in Figure 5.63 and 5.64.

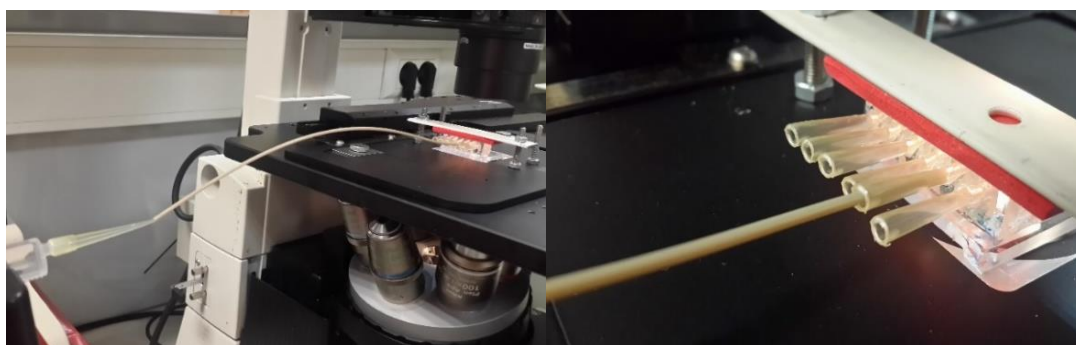


Figure 5.32. Images of system with PEEK tubing

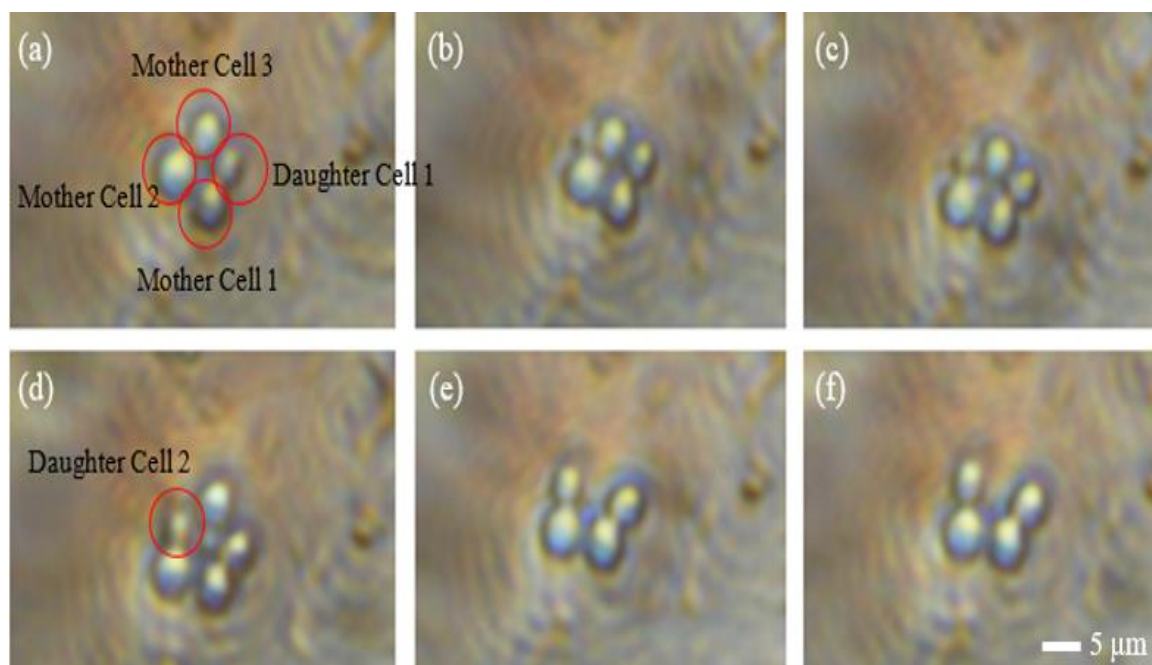


Figure 5.33. The trapped cells in chamber 1 under 90X in a glucose rich YNB media without MMS (a) 240th minutes (b) 270th minutes (c) 300th minutes (d) 330th minutes (e) 375th minutes (f) 420th minutes

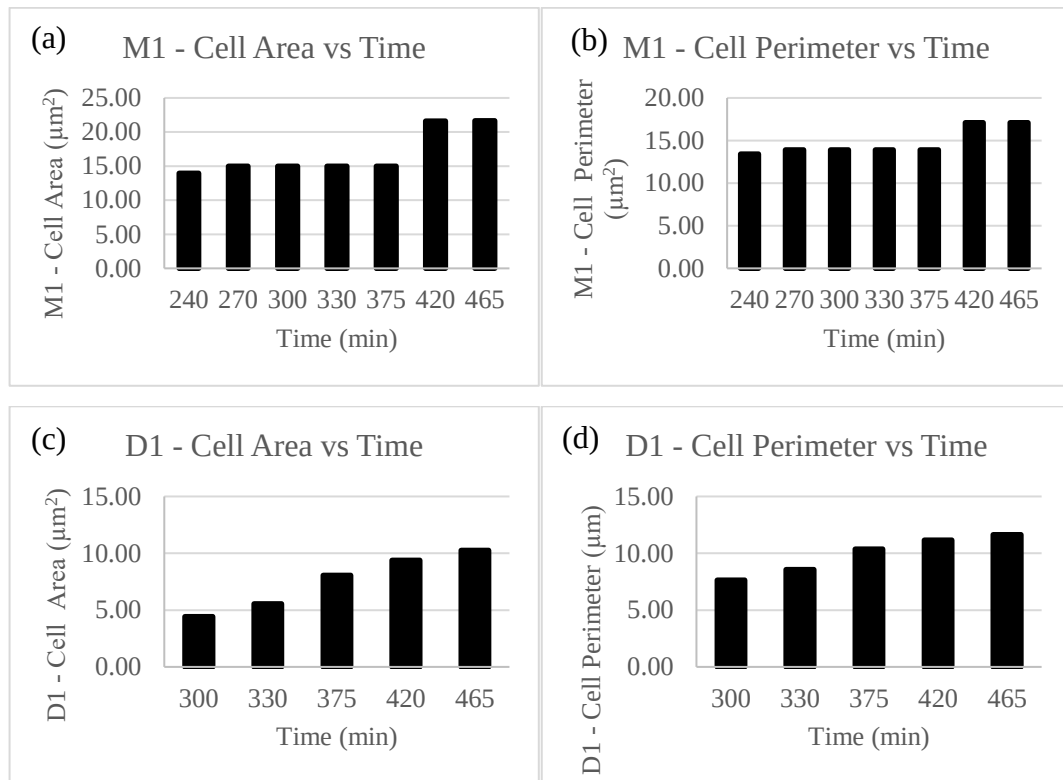


Figure 5.34. Graphical representation of processed data of chamber 1 in an environment without MMS (a) M1 area vs time graph (b) M1 perimeter vs time graph (c) D1 area vs time graph (d) D1 perimeter vs time graph (brightfield images)-EXP3.

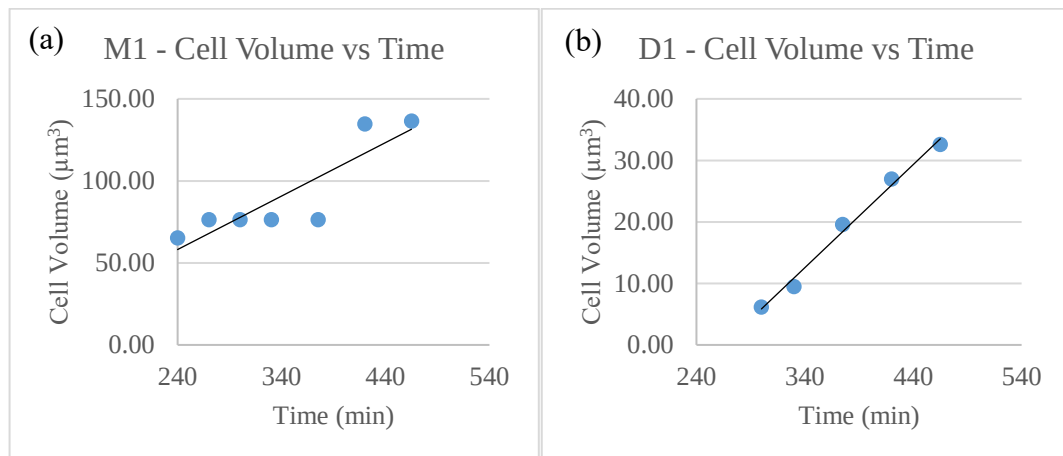


Figure 5.35. Graphical representation of processed data of chamber 1 (a) M1 volume vs time graph (b) D1 volume vs time graph - EXP 3

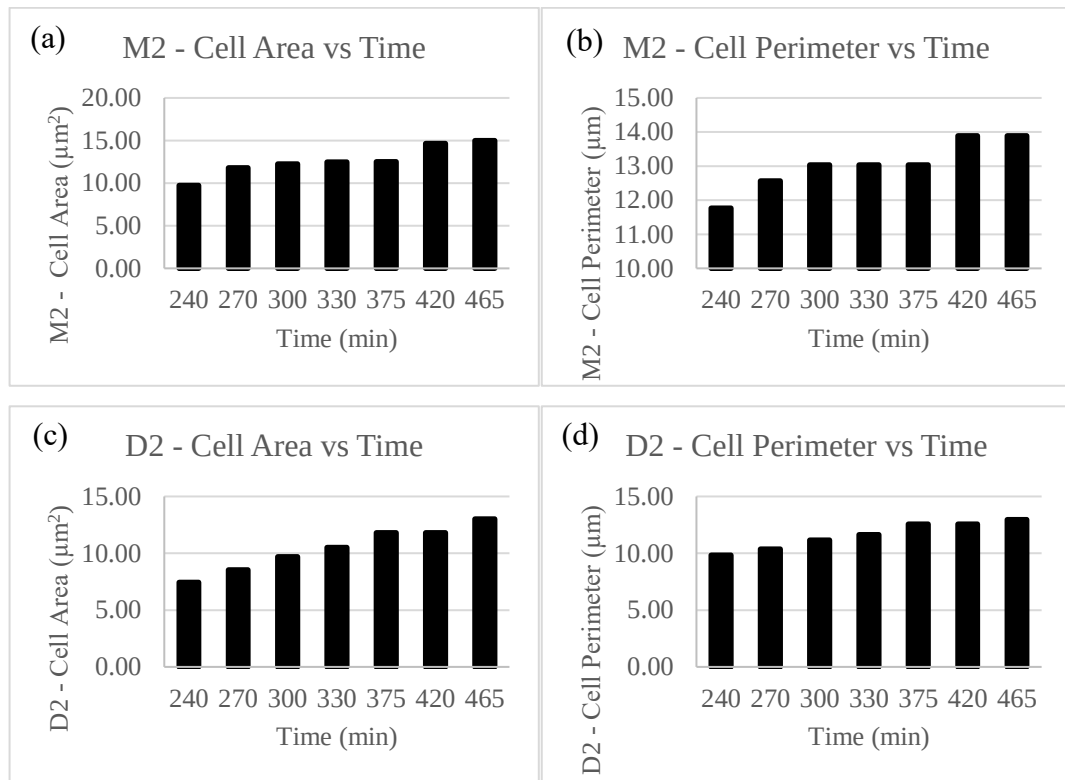


Figure 5.36. Graphical representation of processed data of chamber 1 in an environment without MMS (a) M2 area vs time graph (b) M2 perimeter vs time graph (c) D2 area vs time graph (d) D2 perimeter vs time graph (brightfield images)-EXP3

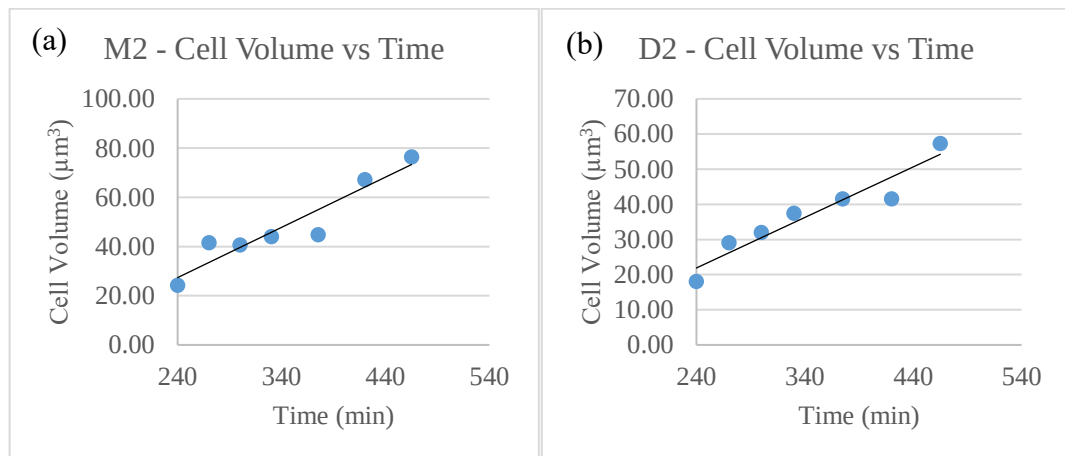


Figure 5.37. Graphical representation of processed data of chamber 1 (a) M2 volume vs time graph (b) D2 volume vs time graph - EXP 3

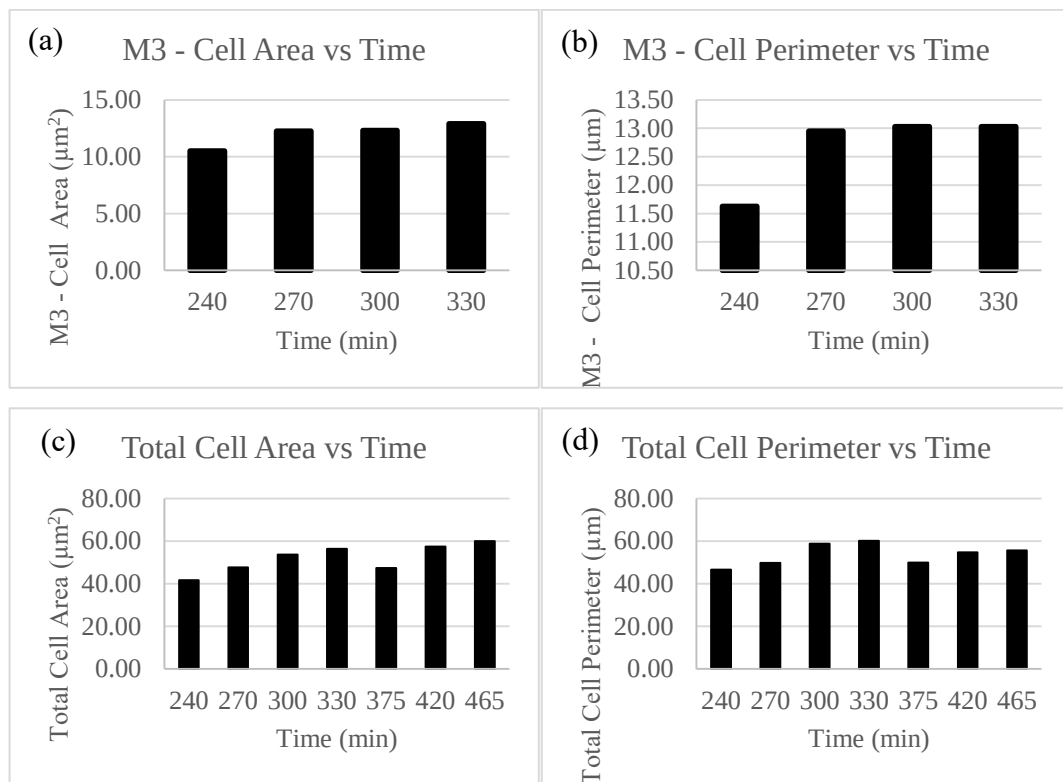


Figure 5.38. Graphical representation of processed data of chamber 1 in an environment without MMS (a) M3 area vs time graph (b) M3 perimeter vs time graph (c) total cell area vs time graph (d) total cell perimeter vs time graph (brightfield images)-EXP3.

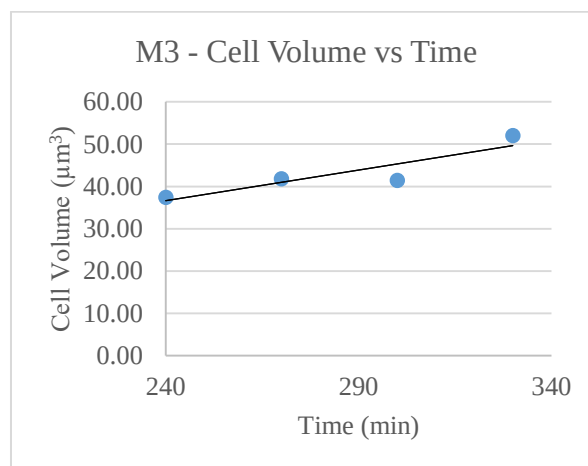


Figure 5.39. Graphical representation of processed data of chamber 1, M3 volume vs time graph - EXP 3

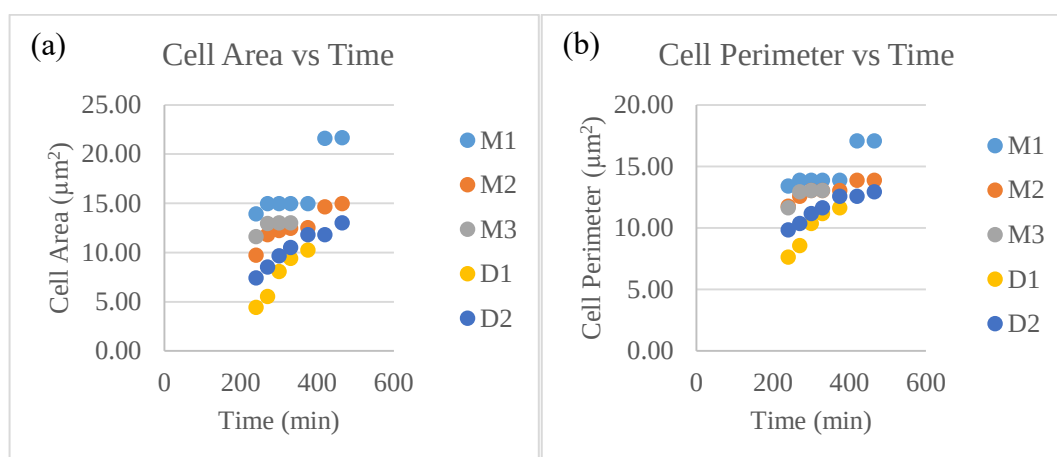


Figure 5.40. Graphical representation of changes in both mother and daughter cells (a) cell area (b) cell perimeter

Table 5.2. The rate of change in area, perimeter and volume of M1 and D1 cells in chamber 1

Time (min)	M1-Cell Area Fold Change	M1-Cell Perimeter Fold Change	M1-Cell Volume Fold Change	D1-Cell Area Fold Change	D1-Cell Perimeter Fold Change	D1-Cell Volume Fold Change
240	1.00	1.00	1.00	-	-	-
270	1.07	1.03	1.17	-	-	-
300	1.07	1.03	1.17	1.00	1.00	1.00
330	1.07	1.03	1.17	1.25	1.12	1.54
375	1.07	1.03	1.17	1.82	1.36	3.18
420	1.55	1.27	2.07	2.12	1.46	4.38
465	1.55	1.27	2.09	2.31	1.52	5.30

5.2.1.6. Remarks. In the previous experiment, the cells were trapped as a colony and some of the chambers were clogged because of the high number of cells. Some of these cells were swept away because of the high flowrate of the medium. Therefore, flowrate of medium was decreased by 10 fold from 1 $\mu\text{L}/\text{min}$ to 0.1 $\mu\text{L}/\text{min}$. Consequently, the number of trapped cells, were decreased, which makes the observation easier. Yet, some of the cells were swept

away anyway. The cells grew efficiently in N1 media, and then began to shrink after loading MMS into microbioreactor just as expected. The cell area and perimeter did not change considerably after 6.5 and 5.5 hours of the MMS application in the fourth and seventh chamber, respectively (Figures 5.66 and 5.67). It was not possible to understand the viability of cells absolutely, because of the unmeasurable fluorescence signal caused by the high auto-fluorescence background of PS. If the integrated density could be measured, the fluorescence signal was expected to disappear when cells died. All in all, it can be said that MMS affect all cells in the same way by shrinking the volume (Figure 5.68).

Table 5.3. The rate of change in area, perimeter and volume of M2 and D2 cells in chamber 1

Time (min)	M2-Cell Area Fold Change	M2-Cell Perimeter Fold Change	M2-Cell Volume Fold Change	D2-Cell Area Fold Change	D2-Cell Perimeter Fold Change	D2-Cell Volume Fold Change
240	1.00	1.00	1.00	1.00	1.00	1.00
270	1.21	1.07	1.72	1.15	1.05	1.62
300	1.26	1.11	1.68	1.30	1.13	1.77
330	1.28	1.11	1.81	1.41	1.18	2.08
375	1.29	1.11	1.85	1.59	1.28	2.31
420	1.51	1.18	2.77	1.59	1.28	2.31
465	1.54	1.18	3.16	1.75	1.32	3.18

Table 5.4. The rate of change in area, perimeter and volume of M3 cell in chamber 1

Time (min)	M3-Cell Area Fold Change	M3-Cell Perimeter Fold Change	M3-Cell Volume Fold Change
240	1.00	1.00	1.00
270	1.17	1.11	1.12
300	1.17	1.12	1.11
330	1.23	1.12	1.39

5.3. Effect of YPD medium (2% glucose) on *sld7*Δ mutants -diploid cells

5.3.1. Experiment 4

The *sld7*Δ yeast cells were used to understand the role of Sld7 in budding yeast. Geneticin (G-418) is an antibiotic that was used for the selection of eukaryotic cells and it is important that the media does not contain ammonium sulfate as the primary nitrogen source. Ammonium sulfate will impede the function of G418 (Cheng *et al.*, 2000). In this case, monosodium glutamate (MSG) can be used as the nitrogen source (Cheng *et al.*, 2000) instead of ammonium sulfate in YNB media with G418. Unfortunately, MSG was not readily available in our laboratory, then glucose rich YPD medium was prepared. 100 mg G418 was added to 500 mL YPD media after sterilization, just before the preparation of pre-culture. The *sld7*Δ cells from frozen stock were used to prepare the preculture. The cells were let to grow in an incubator for 18 hours at 28 °C and then diluted by YPD medium, prepared (OD: 0.545) for inoculation into the microbio reactor. The medium was loaded into the microbio reactor at a rate of 1 μL/min through the middle inlet only. This first microbio reactor was filled in 5 hours, until all the chambers and channels were filled with YPD medium. However, the system had to be closed because of the leakage problem between PDMS and PMMA (the inlet section).

In the second microbio reactor, after medium loading, the loading of yeast cells was initiated at a rate of 0.1 μL/min (10 fold lower rate compared to first one). After 2 hours of yeast cell loading, the YPD medium feeding at a rate of 0.1 μL/min was started. After 5 hours of medium feeding, there were no trapped yeast cells. Therefore, the chip system was

stopped. The sizes of these deletion mutants ($> 5 \mu\text{m}$) were bigger than the previous ones ($\leq 5 \mu\text{m}$), which were shown in Figure 5.69. The bonding pressure might be the reason that the heights of the channels were smaller than the yeast sizes. Therefore, the bonding pressure has to be decreased to prevent the collapse of channels or the decrease in the height of the channels. In addition to, the cells had to be grown in an incubator for less than 18 hours to load into the microbio reactor easily and then to observe the budding phases.

5.3.2. Experiment 5

The medium and cell type were same as those in the previous experiment (EXP4). The *sld7 Δ* cells were let to grow in an incubator for 14 hours, then put into the refrigerator ($+4^\circ\text{C}$) to stop the growth phase. The size of the cells were smaller than $5 \mu\text{m}$. The bonding pressure of the microbio reactors were decreased from 10 bar to 8 bar to reduce collapsing around the c-shape. The medium was loaded into the microbio reactor at a rate of $5 \mu\text{L}/\text{min}$ through the middle inlet only. The first chip (microbio reactor) was filled in 2 hours until all the chambers and channels were filled. However, the system was stopped because of the leakage problem between PDMS and PMMA.

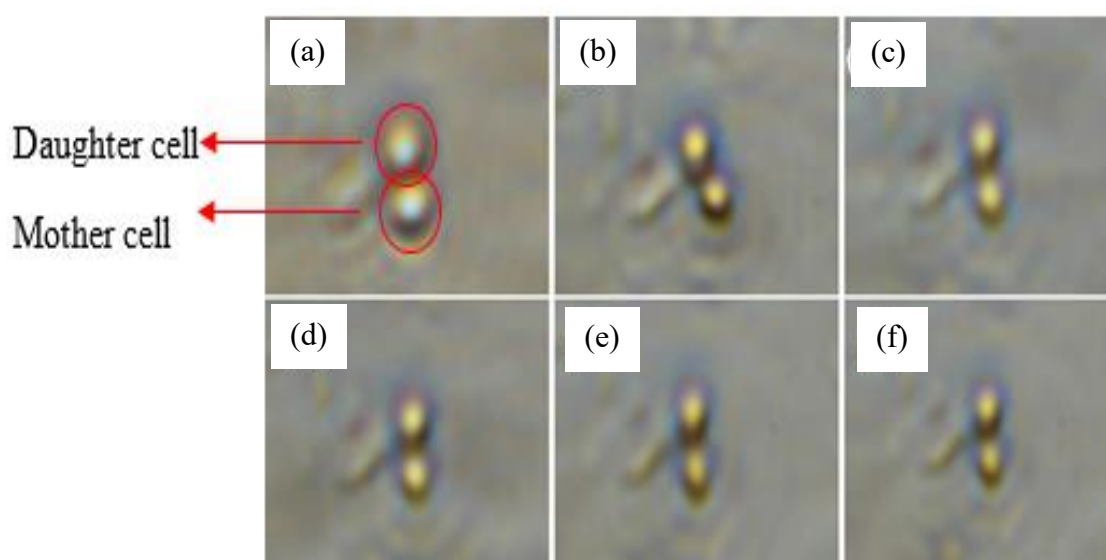


Figure 5.41. The trapped cells in the trapping region of chamber 2 in an environment with MMS (a) 610th minutes (b) 685th minutes (c) 700th minutes (d) 775th minutes (e) 865th minutes (f) 905th minutes under 90X

Next, the third chip (microbioreactor) was filled with medium until all the chambers and channels were filled (1 hour). The yeast cells, diluted by YPD medium, prepared (OD: 0.50) for inoculation into microbioreactor, were loaded for 20 minutes at a rate of 3 $\mu\text{L}/\text{min}$ till they were observed in the chambers. The cells were trapped in all chambers and these cells were observed for 8 hours. The experiment was ended because of the yeast invasion, i.e. high amount of yeast cells filled the channels and chambers as shown in Figure 5.70.

5.3.2.1. Chamber 1. The yeast cells were fed through the middle channel. The C-shaped area was collapsed and the cells were trapped at channels instead of chambers. It was not possible to observe a specific cell in chamber 1 during the experiment because of sweeping away of the cells from chamber 1 and trapping of new cells at the chamber inlet coming from the chip inlet (Figure 5.70 and Figure 5.95a).

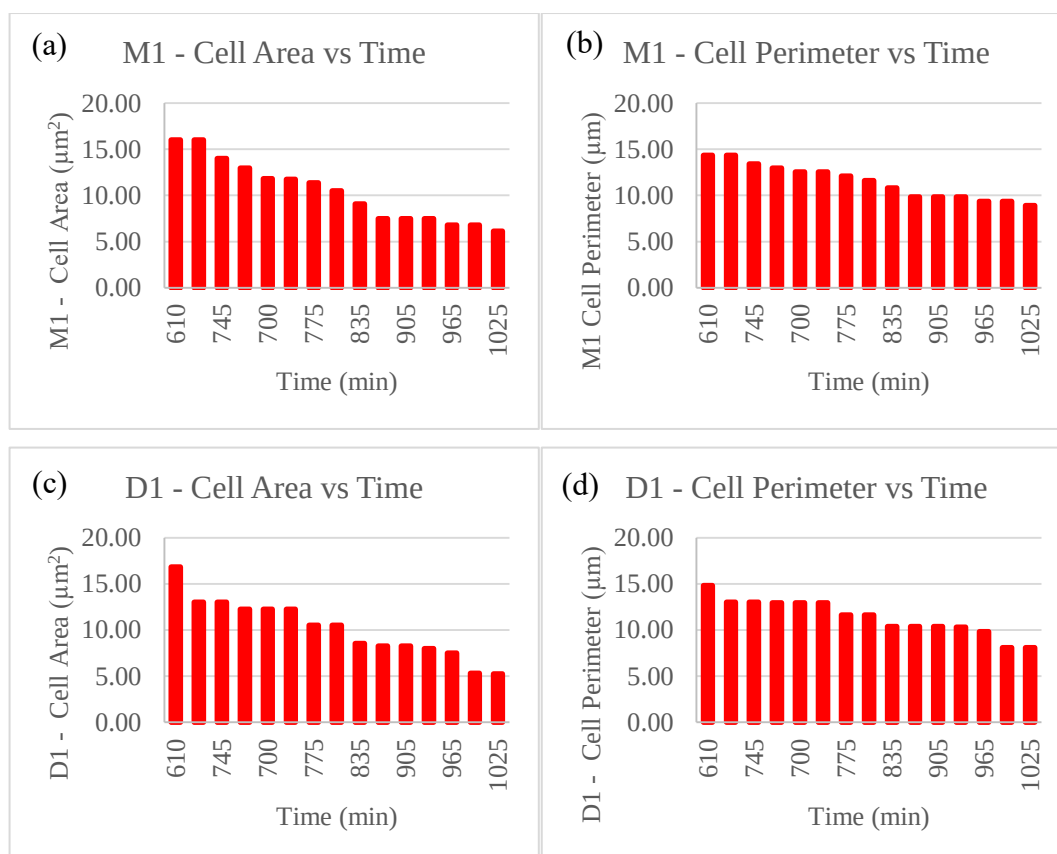


Figure 5.42. Graphical representation of processed data of chamber 2 in an environment with MMS (a) M1 area vs time graph (b) M1 perimeter vs time graph (c) D1 area vs time graph (d) D1 perimeter vs time graph (brightfield images)-EXP3.

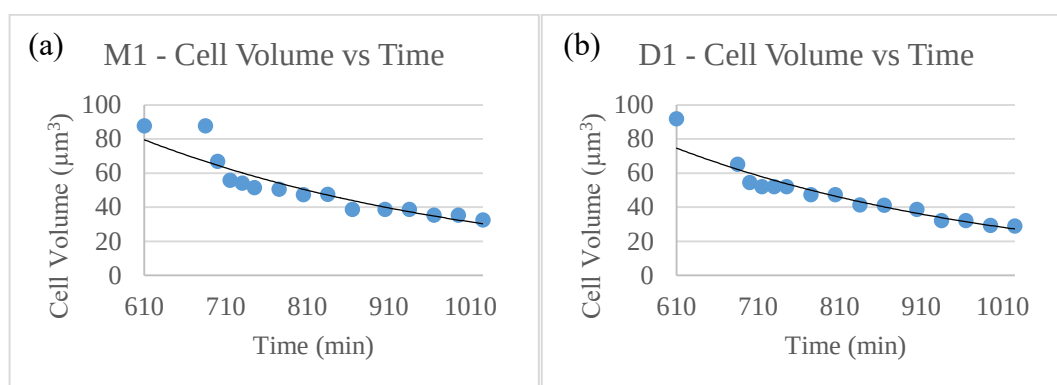


Figure 5.43. Graphical representation of processed data of chamber 2 (a) mother cell 1 volume vs time graph (b) daughter cell 1 volume vs time graph - EXP 3

5.3.2.2. Chamber 2. The cells were first trapped at the middle channel and then spread within chamber 2 (Figure 5.95b). The number of cells were increasing due to cell proliferation and new moved cells from the inlet. The life span of the selected cell was analyzed (Figure 5.71). The time, T_0 , refers to the start of the first cell trapping, which is two hours after yeast cell loading.

The analysed cell formed four buds/daughter cells (Table 5.12). The area, perimeter and volume of the cell increased during budding phases. The fold change of the area, perimeter and volume of the cells were given in Table 5.13. It was observed that the volume of the cell reached 5 times higher than before dividing. After division, the cell area, cell perimeter and cell volume decreased (Figures 5.72 and 5.73).

5.3.2.3. Chamber 3. The cells were trapped first at channels and then spread within the chamber, though not inside the c-shaped region (Figure 5.95c). The number of cells increased caused by both proliferation and new moved cells. The life span of the selected cells were analyzed (Figure 5.74). The change in area, perimeter and volume of the cells were given in Figure 5.75-5.77.

The mother cell 1 (M1), mother cell 2 (M2) and daughter cell 1 (D1) formed five, one and one bud/daughter cells, respectively (Table 5.14). The fold change of the area, perimeter and volume of the cells were given in Tables 5.15 and 5.16. The area, perimeter and volume of the cells increased during the budding phases. After division, the cell area,

cell perimeter and cell volume decreased as expected. It was observed that the volume of the M1 cell reached 1.5 or 2.5 times higher than before dividing. The area, perimeter and volume of M2 decreased after 140th minute, then did not change (Table 5.16). In addition to, the color of M2 changed to yellowish while its size decreased. Consequently, M2 cell might be dead.

5.3.2.4. Chamber 4. The cells were trapped at channels (Figure 5.95d). The number of cells increased, caused by both proliferation and new moved cells. The life span of the selected cells was analyzed (Figure 5.78).

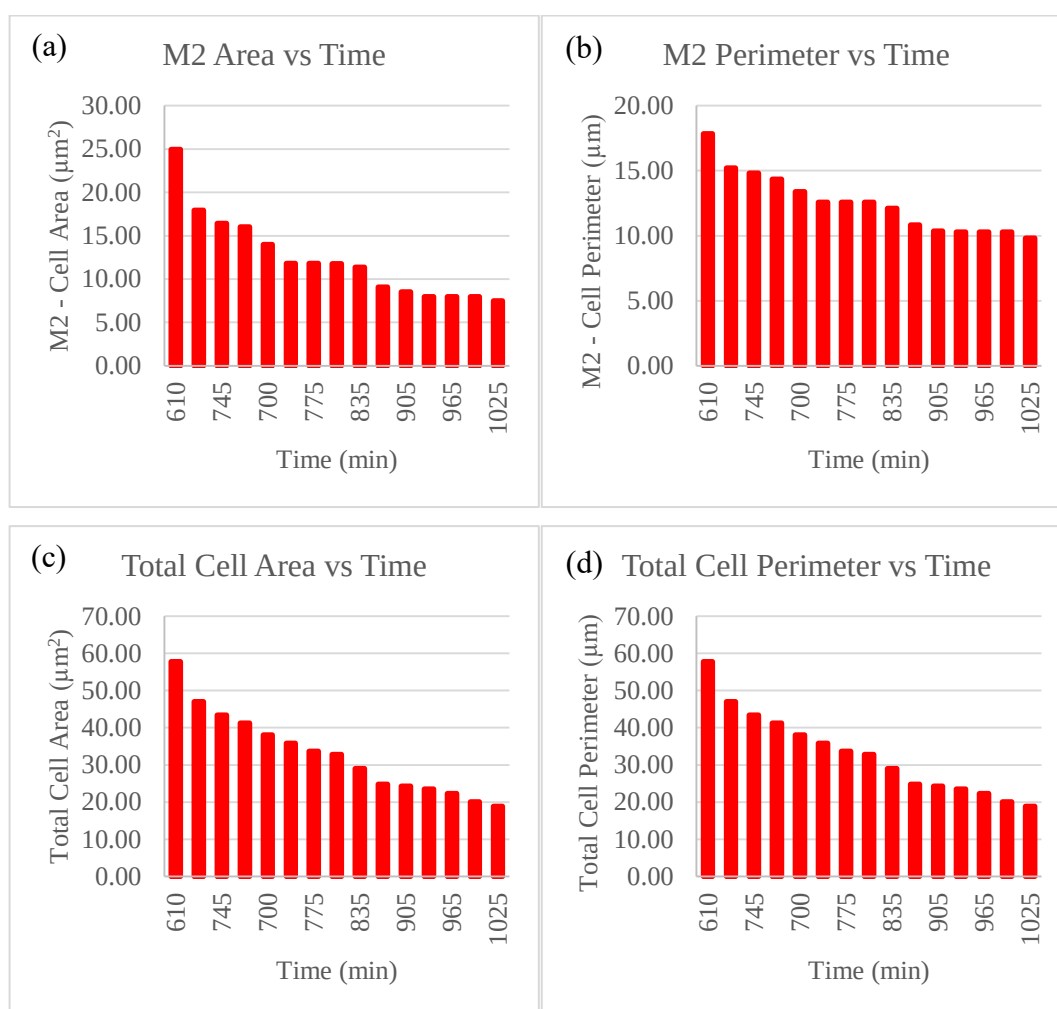


Figure 5.44. Graphical representation of processed data of chamber 2 in an environment with MMS (a) M2 area vs time graph (b) M2 perimeter vs time graph (c) total cell area vs time graph (d) total cell perimeter vs time graph (brightfield images)-EXP3.

The mother cell 1 (M1), mother cell 2 (M2), mother cell 3 (M3) and mother cell 4 (M4) formed five, two, two and one buds/daughter cells, respectively (Table 5.17). The fold change of the area, perimeter and volume of the cells were given in Tables 5.18 and 5.19. The volume, area and perimeter of cell increased during budding phases. After division, the cell area, cell perimeter and cell volume decreased (Figures 5.79, 5.80 and Figure 5.81). It was observed that the volume of the M1 cell reached 2.5 or 3.5 times higher than before dividing (Table 5.18), whereas the volume of the M2 and M4 cells reached almost 2.5 times higher than before dividing (Table 5.19).

5.3.2.5. Chamber 5. The cells were trapped at the entrance of chamber 5 (Figure 5.96a). The number of cells increased due to both proliferation and new moved cells. The life span of the selected cell was analysed (Figure 5.83).

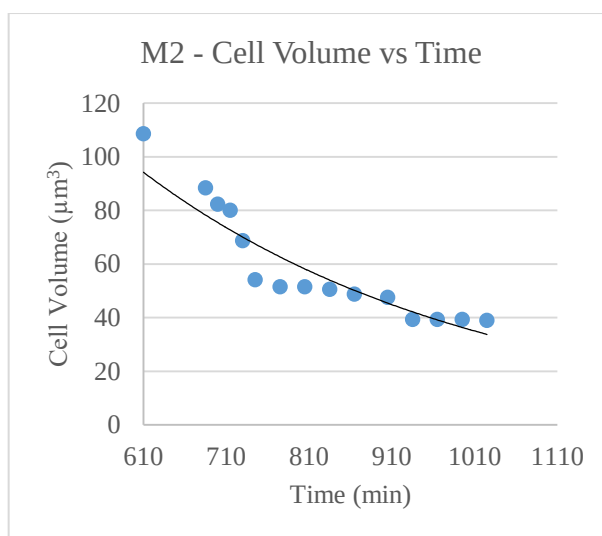


Figure 5.45. Graphical representation of processed data of chamber 2, mother cell 1 volume vs time graph - EXP 3

The selected mother cell formed five buds. The duration of G1 and S/G2/M phases were given in Table 5.20. The fold change of the area, perimeter and volume of the cell were given in Table 5.21. The cell area, cell perimeter and cell volume of M1 increased during budding phases. After division, the cell area, cell perimeter and cell volume decreased (Figures 5.84 and 5.85). It was observed that the volume of the M1 cell reached 2 or 2.5 times higher than before dividing. The budding phases lasted between 40 and 65 minutes.

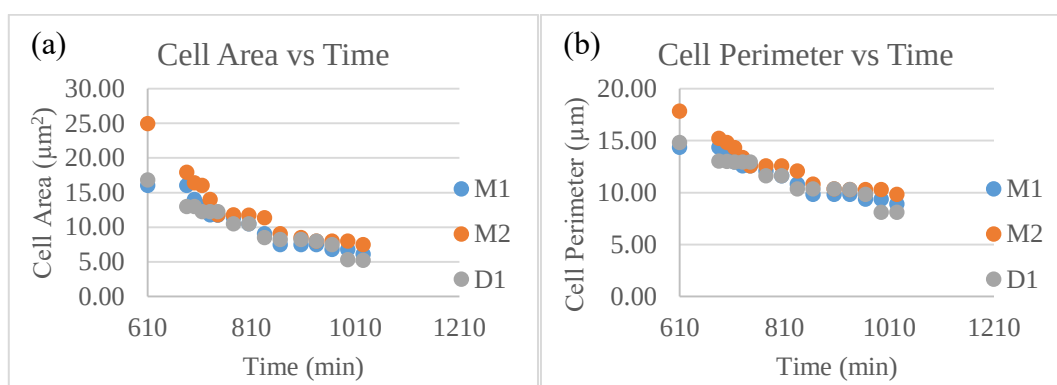


Figure 5.46. Graphical representation of changes in both mother and daughter cells (a) cell area (b) cell perimeter

5.3.2.6. Chamber 6. The cells were trapped at the entrance of the c-shaped area (Figure 5.96b). The number of cells increased, due to both proliferation and new moved cells. The life span of the selected cell was analysed (Figure 5.86).

The mother cell 1 (M1) formed one bud/daughter cell, whereas mother cell 2 (M2) did not form any bud. The fold change of the area, perimeter and volume of the cells were given in Table 5.22. The cell area, cell perimeter and cell volume of M1 cell increased during budding phase (Figure 5.87a, b and Figure 5.88a). The cell area, cell perimeter and cell volume of M2 decreased by time; its color changed, it took a yellowish color, which means it might be dead (Figure 5.87c, d and Figure 5.88b).

Table 5.5. The rate of change in area, perimeter and volume of M1 and D1 cells in chamber 2

Time (min)	M1-Cell Area Fold Change	M1-Cell Perimeter Fold Change	M1-Cell Volume Fold Change	D1-Cell Area Fold Change	D1-Cell Perimeter Fold Change	D1-Cell Volume Fold Change
610	1.00	1.00	1.00	1.00	1.00	1.00
685	1.00	1.00	1.00	0.77	0.88	0.71
700	0.87	0.93	0.76	0.77	0.88	0.59

Table 5.5. The rate of change in area, perimeter and volume of M1 and D1 cells in chamber 2 (cont.).

Time (min)	M1-Cell Area Fold Change	M1-Cell Perimeter Fold Change	M1-Cell Volume Fold Change	D1-Cell Area Fold Change	D1-Cell Perimeter Fold Change	D1-Cell Volume Fold Change
715	0.81	0.90	0.64	0.73	0.87	0.57
730	0.74	0.88	0.62	0.73	0.87	0.57
745	0.73	0.88	0.59	0.73	0.87	0.57
775	0.71	0.84	0.58	0.62	0.79	0.52
805	0.66	0.81	0.54	0.62	0.79	0.52
835	0.57	0.75	0.54	0.51	0.70	0.45
865	0.47	0.69	0.44	0.49	0.70	0.45
905	0.47	0.69	0.44	0.49	0.70	0.42
935	0.47	0.69	0.44	0.47	0.70	0.35

5.3.2.7. Chamber 7. The cells were trapped both at channel and c-shaped area (Figure 5.96c). The number of cells increased, caused by both proliferation and new moved cells. The life spans of the selected mother cells were analysed (Figure 5.89).

The mother cell 1 (M1) and mother cell 2 (M2) formed two and three buds/daughter cells. The duration of bud formation and budding phase times of both mother cells were close to each other (Table 5.23). The fold change of the area, perimeter and volume of the cells were given in Table 5.24. The cell area, cell perimeter and cell volume increased during budding phases. The volume of the M1 and M2 cells reached 2.5 and 3.5 times of their initial volume, respectively. After division, the cell area, cell perimeter and cell volume decreased (Figure 5.90 and 5.91).

5.3.2.8. Chamber 8. The cells were trapped at c-shaped area (Figure 5.96d). The number of cells increased, caused by both proliferation and new moved cells. The life spans of the selected mother cells were analyzed (Figure 5.92).

The mother cell formed three buds/daughter cells. The duration of bud formation and budding phase were given in Table 5.25. The fold change of the area, perimeter and volume of the cell were given in Table 5.26. The cell area, cell perimeter and cell volume increased during budding phases. The volume of the cell reached almost 2.5 times of its initial volume, respectively. After division, the cell area, cell perimeter and cell volume decreased (Figures 5.93 and 5.94).

5.3.2.9. Remarks. The cell area, cell perimeter and cell volume of the mother cells were considered with their buds. The cell area, cell perimeter and cell volume of the daughter cells were calculated after they divided from their mother. The cell area changed between 30 and 85 μm^2 (Figure 5.97), the cell perimeter changed between 20 and 56 μm (Figure 5.98). The cell size almost doubled just before daughter cell separated from the mother cell (Figure 5.99). In each generation following the previous one, the area, perimeter and volume of the mother cell increased after the separation of the bud with respect to their initial sizes, whereas the size of the each daughter cell decreased with the number of generations. The cell volume changed between 1.5 and 5.5 of their initial sizes before dividing. Some of the mother cells did not form bud and the area, perimeter and volume of these cells decreased by time. In addition to, the color of the cells turned into yellow while cell size was decreasing.

Table 5.6. The rate of change in area, perimeter and volume of M2 cell in chamber 2

Time (min)	M2-Cell Area Fold Change	M2-Cell Perimeter Fold Change	M2-Cell Volume Fold Change
610	1.00	1.00	1.00
685	0.90	0.85	0.99
700	0.82	0.83	0.92
715	0.80	0.80	0.89
730	0.70	0.75	0.77
745	0.59	0.70	0.60
775	0.59	0.70	0.58
805	0.59	0.70	0.58

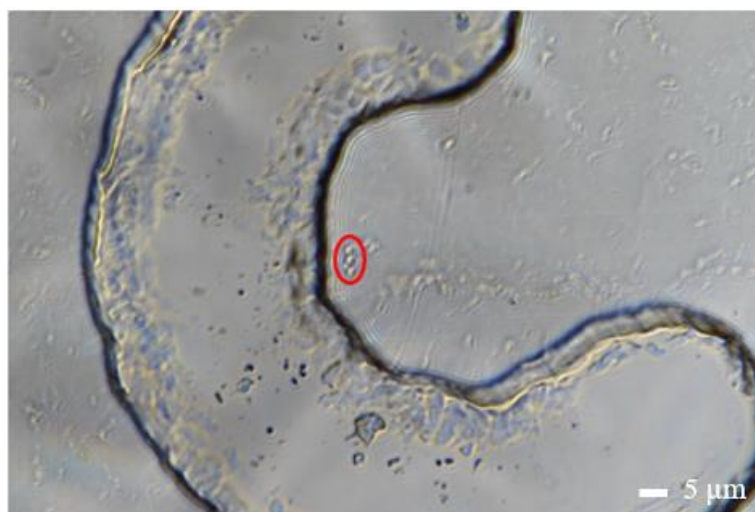


Figure 5.47. The trapped cells in the C-shape of the fourth chamber

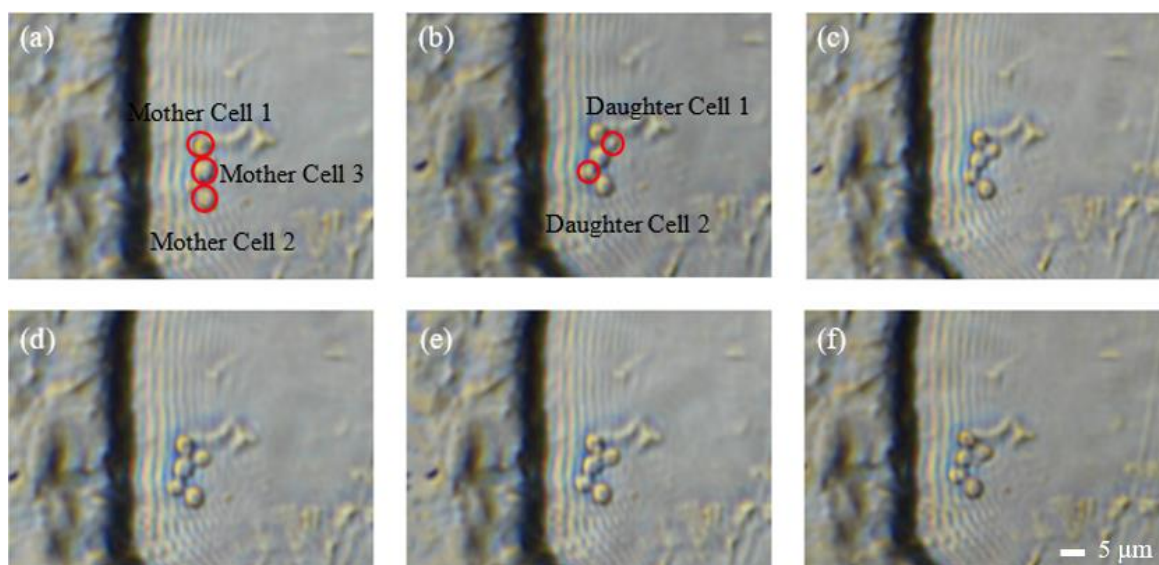


Figure 5.48. The trapped cells in the trapping region of chamber 4 under 90X (a) 300th minutes (b) 330th minutes (c) 375th minutes (d) 420th minutes (e) 465th minutes (f) 510th minutes

The molecular function of Sld7 is unknown. To understand the role of Sld7 in DNA synthesis, the growth of the *sld7Δ* yeast cells in YPD media was investigated. We observed that the buds formed as early as approximately in 15 minutes (G1 phase) as late as 85 minutes (Figure 5.100a). The budding phase (S/G₂/M) continued between 30 and 95 minutes (Figure 5.100b). The cell cycle length of the cells change between 55 minutes and 155 minutes

(Figure 5.100c). These cells might be dead. The volume of the cells changed between 1.5 or 2 fold while the cell was doubling. Some of these cells increased their volume as 5 times higher than their initial sizes (Figure 5.101). It was observed that the duration of the G_1 and S/ G_2 /M phases of the cells were different (Figure 5.103). Most of the cells completed their cell cycle in 105 minutes. It can be said that the G_1 phase in *sld7 Δ* cells is longer than those of the wild type cells. In that case, lack of Sld7p causes delay in the start of S phase. Tanaka *et al.* (2011) found the doubling time of *sld7 Δ* cells and wild type cells (W303) in liquid YPD media at 30 °C as 86.2 min and 77.5 min, respectively. Brewer *et al.* (1984) measured that the duration of G_1 phase and S/ G_2 /M phases of the diploid wild type (A364A D5) cells as 22 minutes and 59 minutes in YPD medium, respectively. The z-score of the area, and perimeter of the cells were given in Figure 5.102.

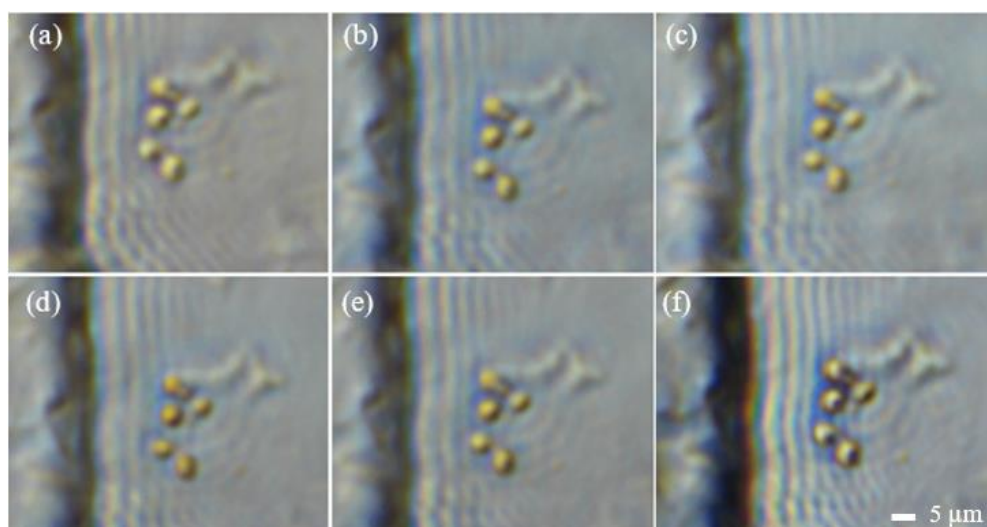


Figure 5.49. The trapped cells in the trapping region of chamber 4 in an environment with MMS under 90X (a) 610th minutes (b) 685th minutes (c) 700th minutes (d) 775th minutes (e) 865th minutes (f) 905th minutes

The duration of cell cycle with its phases were compared between mother and daughter cells. The duration of the G_1 phase of daughter cells was longer than in mother cells (Brewer *et al.*, 1984). In our case, the opposite situation was observed (Table 5.27). Yet, this comparison was completed between 1 daughter cell and 13 mother cells. Therefore, this comparison may not reflect the precise results.

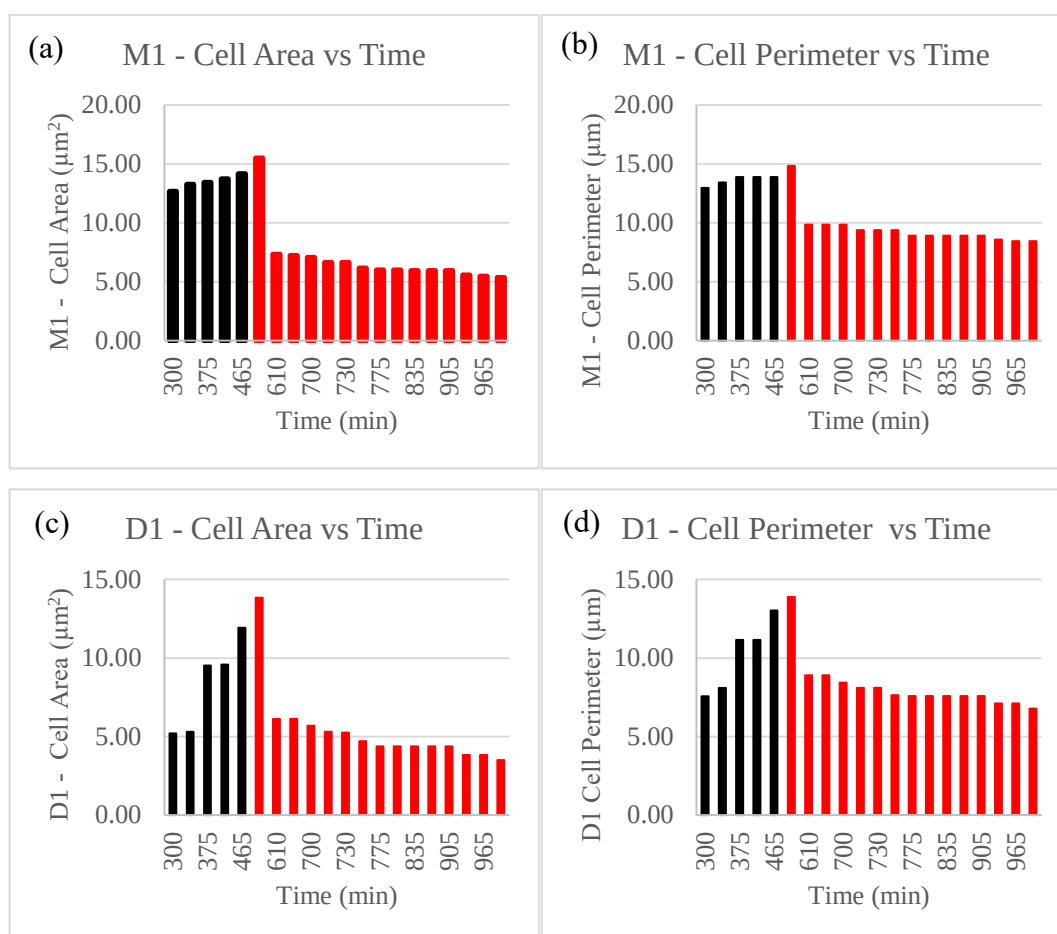


Figure 5.50. Graphical representation of processed data of chamber 4 in an environment with and without MMS (a) mother cell 1 area vs time graph (b) mother cell 1 perimeter vs time graph (c) daughter cell 1 area vs time graph (d) daughter cell 1 perimeter vs time graph (brightfield images)-EXP3.

5.4. Effect of inhibitor on *sld7* Δ mutants in YPD medium (2 % glucose)

5.4.1. Experiment 6

The medium and cell type were same as those in the previous experiments (EXP4 and EXP5). The *sld7* Δ cells were let to grow in an incubator for 12 hours, then put into the refrigerator (+4 °C) to stop the growth phase. The size of the cells were smaller than 5 μm . The medium was loaded into the microbioreactor at a rate of 5 $\mu\text{L}/\text{min}$ through the middle inlet only. The first chip (microbioreactor) was filled in 2 hours until all the chambers and channels were filled with medium. The yeast cell culture was diluted with YPD medium,

prepared (OD: 0.50) for inoculation into the microbioreactor and loaded at a rate of 3 $\mu\text{L}/\text{min}$, till the cells were observed in the chambers (15 minutes). However, the system was closed because the cells did not get trapped in the c-shaped area.

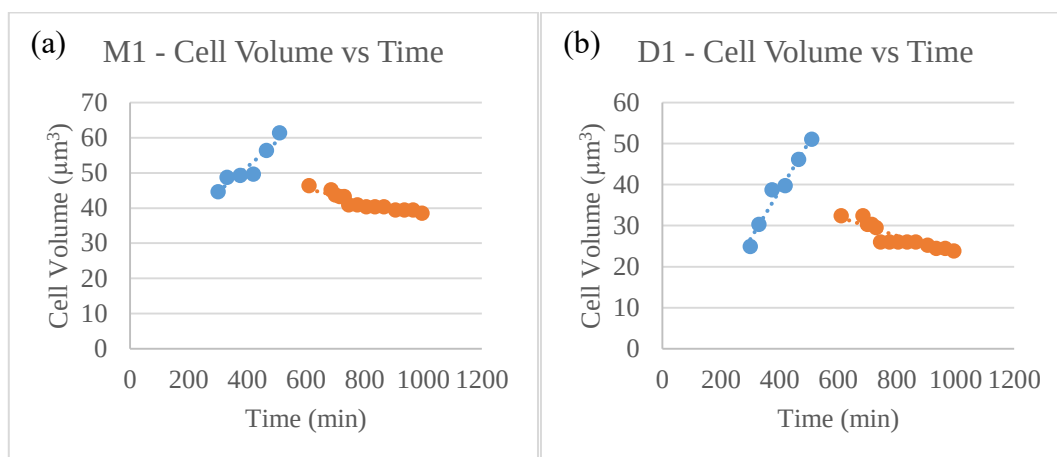


Figure 5.51. Graphical representation of processed data of chamber 4 (a) mother cell 1 volume vs time graph (b) daughter cell 1 volume vs time graph – EXP 3.

The second microbioreactor was filled in 1 hour until all the chambers and channels were filled. The yeast cell cultures, diluted with YPD medium, prepared (OD: 0.50) for inoculation into the microbioreactor, and loaded at a rate of 1 $\mu\text{L}/\text{min}$, till the cells were observed in the chambers (10 minutes). Nevertheless, the cells did not get trapped and went through the outlet.

Next, the third chip (microbioreactor) was filled with medium until all the chambers and channels were filled (1 hour). The loading rate of cells was high in the previous trials. Therefore, the loading rate of the yeast cells was decreased to 0.5 $\mu\text{L}/\text{min}$. After 15 minutes of yeast cell loading, yeast cells were trapped in chambers, and the YPD medium containing 2 % glucose (N1) feeding at a rate of 0.1 $\mu\text{L}/\text{min}$ was started ($t=0$ or 15 min) and continued for 5 hours. The N1 medium was changed with N2 medium (YPD medium with 0.01% MMS) at 280th minutes. The N2 medium was fed for 2.5 hours at a rate of 0.1 $\mu\text{L}/\text{min}$. Then, N2 medium was changed with N1R fresh media for 5 hours to observe the effect of fresh medium on cells. The brightfield microscopic images of the chambers were taken under 40X and 90X magnification for 12 hours during N1 and N2 media loading. The experiment was

ended because of the yeast invasion, i.e. high amount of yeast cells filled the channels and chambers as shown in Figure 5.104.

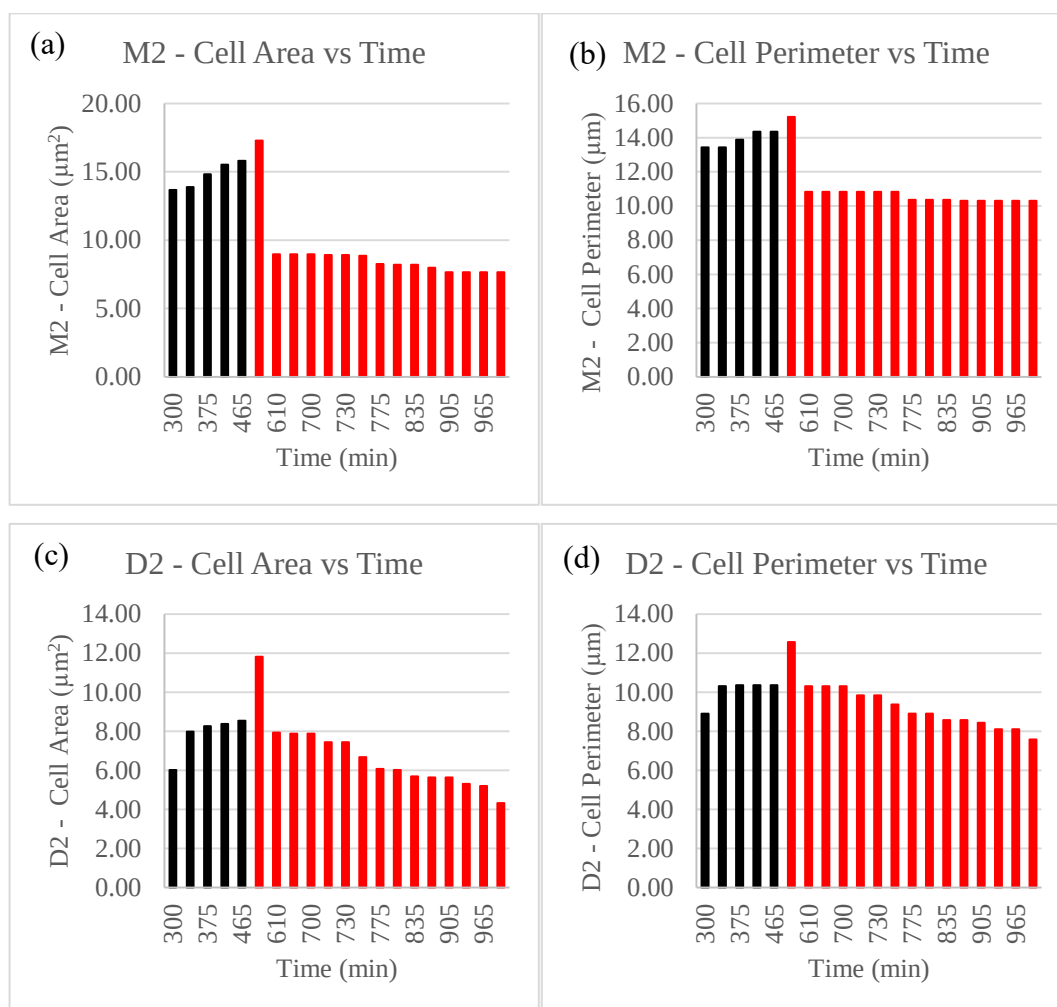


Figure 5.52. Graphical representation of processed data of chamber 4 in an environment with and without MMS (a) mother cell 2 area vs time graph (b) mother cell 2 perimeter vs time graph (c) daughter cell 2 area vs time graph (d) daughter cell 2 perimeter vs time graph (brightfield images)-EXP3.

5.4.1.1. Chamber 1. The yeast cells were fed through the middle channel. The C-shaped area was collapsed and the cells were trapped at channels instead of chambers. It was not possible to observe a specific cell in chamber 1 during the experiment because of sweeping away of the cells from chamber 1 and trapping of new cells at the chamber inlet coming from the chip inlet.

5.4.1.2. Chamber 2. The numbers of cells were increasing due to cell proliferation as well as due to new moving cells from the inlet (Figure 5.105). It was not possible to track the cells after feeding N2 medium because of the colonies. The cells, that were trapped in the form of a single cell, were swept away for a while, which makes tracking of these cells impossible. Therefore, the life span of the cell (M1) that was trapped at time zero was analyzed in chamber 2. The analyzed mother cell formed four buds/daughter cells (Table 5.28). The area and perimeter of this cell increased during the budding phases.

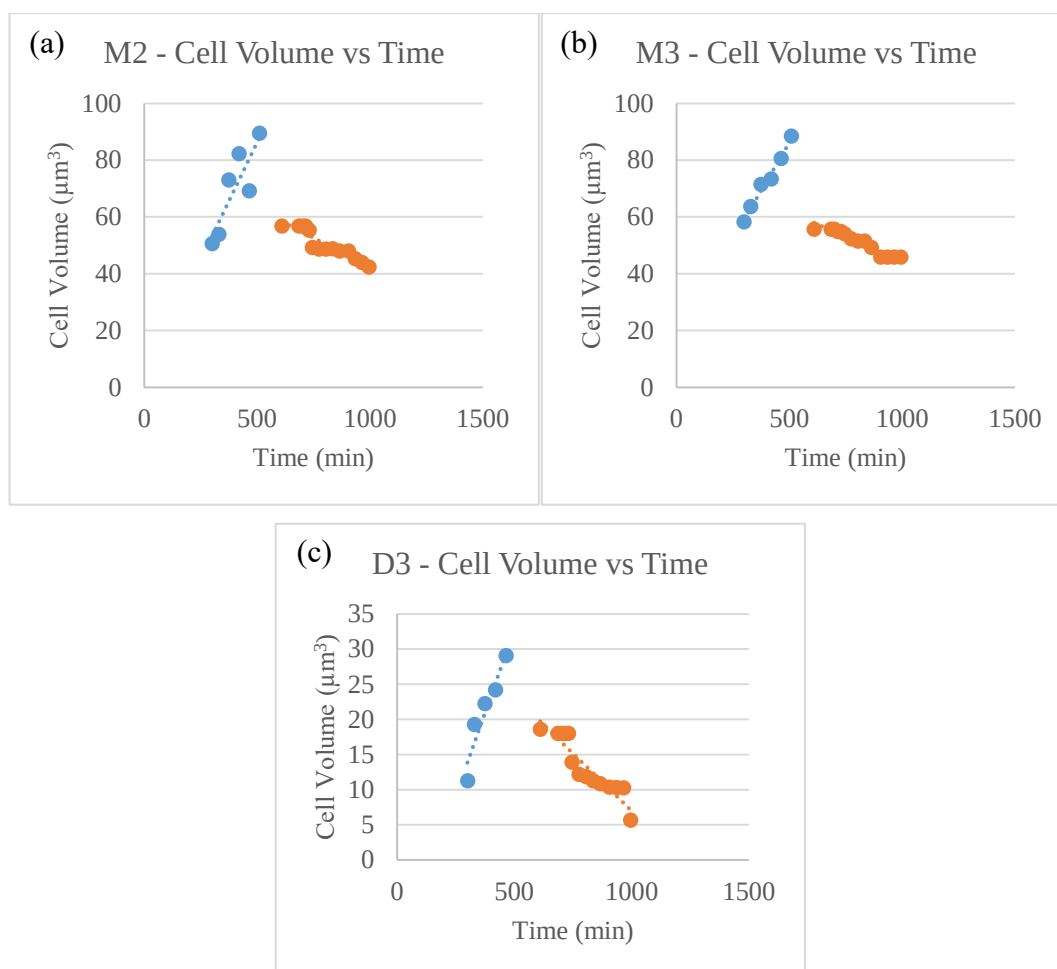


Figure 5.53. Graphical representation of processed data of chamber 4 (a) mother cell 2 volume vs time graph (b) mother cell 3 volume vs time graph (c) daughter cell 3 volume vs time graph - EXP 3.

After division, the cell area, cell perimeter and cell volume decreased (Figures 5.106 and 5.107). The first daughter cell of this mother cell was also analyzed. This daughter cell

further formed 3 buds. The fold change of the area, perimeter and volume of the cells were given in Table 5.29. It was observed that the volumes of the cells were almost 2 times higher than before dividing.

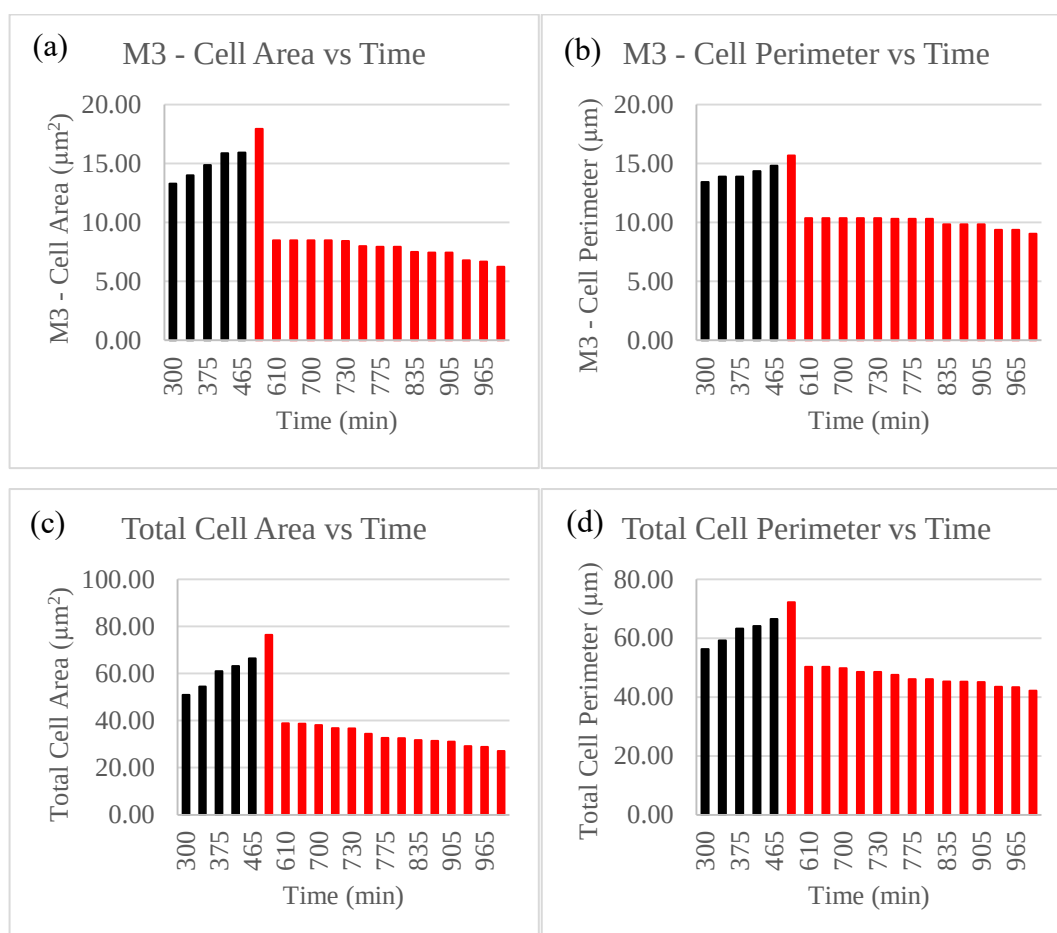


Figure 5.54. Graphical representation of processed data of chamber 4 in an environment with and without MMS (a) mother cell 3 area vs time graph (b) mother cell 3 perimeter vs time graph (c) total cell area vs time graph (d) total cell perimeter vs time graph (brightfield images)-EXP3.

5.4.1.3. Chamber 3. The number of cells were increasing due to cell proliferation and new moved cells from the inlet (Figure 5.108). The cells that were trapped as single cell were swept away for a while, which makes the tracking of these cells impossible. Therefore, the life span of any cell that was trapped before feeding N2 medium, cannot be analyzed in chamber 3. The life span of the cell that was trapped at 435th minutes was analyzed till the end of the experiment (Figures 5.109 and 5.110). The cell formed three buds during the

experiment, the duration of the cell cycle and its phases for each bud were given in Table 5.30. The fold change of the area, perimeter and volume of the cells were given in Table 5.31. It was observed that the volume of the cells were almost 2 times higher than before dividing.

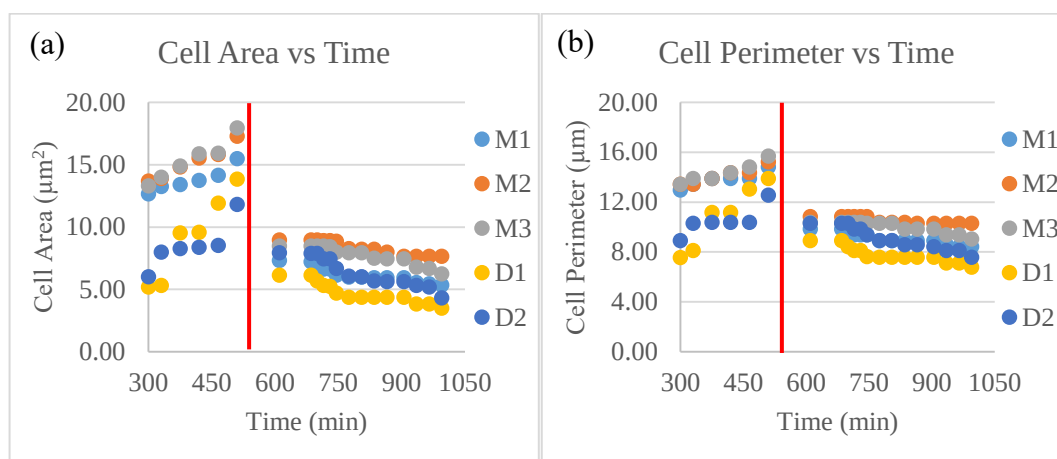


Figure 5.55. Graphical representation of changes in both mother and daughter cells (a) cell area (b) cell perimeter

5.4.1.4. Chamber 4. The life span of 3 cells that were trapped before and/or after feeding N2 medium were analyzed (Figures 5.111-5.115). The mother cell 1 (M1), mother cell 2 (M2) and mother cell 3 (M3) formed three, five and three buds, respectively. The duration of the cell cycle and its phases for each bud were given in Table 5.32. The fold change of the area, perimeter and volume of the cells were given in Tables 5.33 and 5.34. It was observed that the volume of the cells were almost 2 times higher than before dividing under effect of MMS. After feeding fresh medium (N1R), the volume of these cells were almost 3 times higher than before division.

5.4.1.5. Chamber 5. The numbers of cells were increasing due to cell proliferation and new moved cells from the inlet (Figure 5.116). The cells that were trapped as single cell were swept away for a while, which makes the tracking of these cells impossible. Therefore, the life span of 3 cells that were trapped before and/or after feeding N2 medium were analyzed (Figures 5.117 – 5.120). The mother cell 1 (M1), mother cell 2 (M2) and mother cell 3 (M3) formed two, three and two buds, respectively.

Table 5.7. The rate of change in area, perimeter and volume of M1 and D1 cells in chamber 4

Time (min)	M1-Cell Area Fold Change	M1-Cell Perimeter Fold Change	M1-Cell Volume Fold Change	D1-Cell Area Fold Change	D1-Cell Perimeter Fold Change	D1-Cell Volume Fold Change
300	1.00	1.00	1.00	1.00	1.00	1.00
330	1.05	1.04	1.09	1.24	1.07	1.21
375	1.06	1.07	1.10	2.27	1.48	1.55
420	1.08	1.07	1.11	2.28	1.48	1.59
465	1.12	1.07	1.26	2.84	1.72	1.85
510	1.22	1.14	1.37	3.30	1.84	2.04
610	0.58	0.76	1.04	1.46	1.18	1.30
685	0.57	0.76	1.01	1.46	1.18	1.30
700	0.56	0.76	0.98	1.35	1.16	1.21
715	0.52	0.72	0.97	1.24	1.07	1.21
730	0.52	0.72	0.97	1.24	1.07	1.18
745	0.49	0.72	0.91	1.02	1.01	1.04
775	0.47	0.69	0.91	1.04	1.00	1.04
805	0.47	0.69	0.90	1.04	1.00	1.04
835	0.47	0.69	0.90	1.04	1.00	1.04
865	0.47	0.69	0.90	1.04	1.00	1.04
905	0.47	0.69	0.88	1.04	1.00	1.01
935	0.44	0.66	0.88	0.91	0.94	0.98
965	0.43	0.65	0.88	0.91	0.94	0.98
995	0.42	0.65	0.86	0.83	0.90	0.95

Table 5.8. The rate of change in area, perimeter and volume of M2 and M3 cells in chamber 4

Time (min)	M2-Cell Area Fold Change	M2-Cell Perimeter Fold Change	M2-Cell Volume Fold Change	M3-Cell Area Fold Change	M3-Cell Perimeter Fold Change	M3-Cell Volume Fold Change
300	1.00	1.00	1.00	1.00	1.00	1.00
330	1.02	1.00	1.09	1.33	1.16	1.71
375	1.08	1.03	1.23	1.37	1.16	1.97
420	1.14	1.07	1.26	1.39	1.16	2.14
465	1.16	1.07	1.38	1.42	1.16	2.58
510	1.26	1.13	1.52	1.96	1.41	3.68
610	0.66	0.81	0.96	1.32	1.16	1.65
685	0.66	0.81	0.96	1.31	1.16	1.60
700	0.66	0.81	0.96	1.31	1.16	1.60
715	0.65	0.81	0.94	1.24	1.11	1.60
730	0.65	0.81	0.94	1.24	1.11	1.60
745	0.65	0.81	0.93	1.11	1.05	1.23
775	0.60	0.77	0.90	1.01	1.00	1.08
805	0.60	0.77	0.88	1.00	1.00	1.05
835	0.60	0.77	0.88	0.95	0.96	1.00
865	0.58	0.77	0.85	0.94	0.96	0.96
905	0.56	0.77	0.79	0.94	0.95	0.92
935	0.56	0.77	0.79	0.86	0.91	0.91
965	0.56	0.77	0.79	0.86	0.91	0.91
995	0.56	0.77	0.79	0.72	0.85	0.50

The duration of cell cycle and its phases for each bud were given in Table 5.35. The fold change of the area, perimeter and volume of the cells were given in Tables 5.36 and 5.37. It was observed that the volumes of the cells were almost 2 times higher than before

dividing. After feeding fresh medium (N1R), the volumes of these cells were almost 3 times higher than before division.

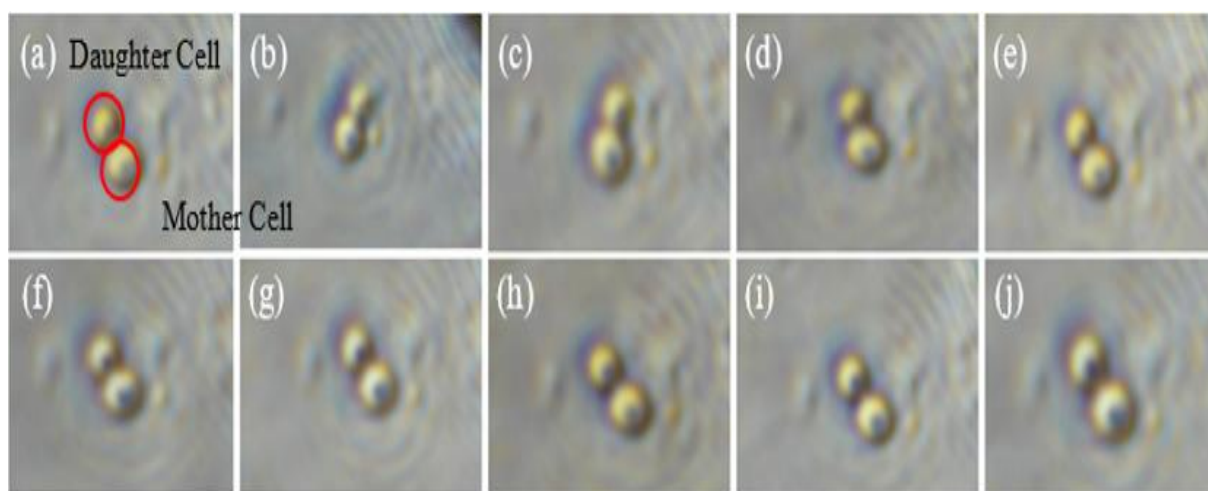


Figure 5.56. The trapped cells in the trapping region of chamber 6 (a) 0th minutes (b) 15th minutes (c) 45th minutes (d) 60th minutes (e) 75th minutes (f) 105th minutes (g) 120th minutes (h) 135th minutes (i) 150th minutes (j) 180th minutes.

5.4.1.6. Chamber 6. It was not possible to observe a specific cell in chamber 6 during the experiment because of sweeping away of the cells from chamber 6 and trapping of new cells at the chamber inlet coming from the chip inlet (Figure 5.121).

5.4.1.7. Chamber 7. The number of cells were increasing due to cell proliferation and new moved cells from the inlet (Figure 5.122). Therefore, the life span of 2 cells that were trapped before and/or after feeding N2 medium were analyzed (Figures 5.123 and 5.124). The mother cell 1 (M1) and mother cell 2 (M2) formed two and three buds, respectively. The duration of cell cycle and its phases for each bud were given in Table 5.38. The fold change of the area, perimeter and volume of the cells were given in Table 5.39. It was observed that the volume of the cells were almost 3 times higher than before dividing. After feeding N2 and N1R medium, the volume of these cells were almost 1.5 and 2 times higher than before division, respectively.

5.4.1.8. Chamber 8. The cells were trapped in chamber 8 at 280th minute, just before feeding N2. Therefore, the cell behavior cannot be observed in N1 media. The number of cells were

increasing due to cell proliferation and new moved cells from the inlet (Figure 5.125). The cells that were trapped as single cell were swept away for a while, which makes the tracking of the cells impossible. Therefore, the life span of a cell that was trapped after feeding N2 medium, was analyzed (Figures 5.126 and 5.127).

Table 5.9. The rate of change in area, perimeter and volume of M1 and D1 cells in chamber 6

Time (min)	M1-Cell Area Fold Change	M1-Cell Perimeter Fold Change	M1-Cell Volume Fold Change	D1-Cell Area Fold Change	D1-Cell Perimeter Fold Change	D1-Cell Volume Fold Change
0	1.00	1.00	1.00	1.00	1.00	1.00
15	1.08	1.07	1.03	1.18	1.10	1.26
45	1.10	1.08	1.03	1.26	1.11	1.90
60	1.15	1.08	1.34	1.26	1.11	1.90
75	1.23	1.11	1.53	1.26	1.11	1.90
105	1.32	1.15	1.79	1.34	1.14	2.13
120	1.32	1.15	1.79	1.55	1.24	2.45
135	1.38	1.19	1.82	1.68	1.29	2.89
150	1.38	1.19	1.85	1.74	1.34	2.72
180	1.38	1.19	1.85	1.74	1.34	2.72
210	1.40	1.19	1.87	1.81	1.39	2.71
240	1.44	1.23	1.98	2.06	1.43	4.27
270	1.51	1.26	2.01	2.06	1.43	4.38
300	1.52	1.26	2.01	2.06	1.43	4.38
330	1.76	1.33	3.04	2.32	1.53	5.18

The mother cell formed 3 buds during the experiment. The duration of cell cycle and its phases for each bud were given in Table 5.40. The fold change of the area, perimeter and volume of the cells were given in Table 5.41. It was observed that the volume of the cells were almost 2 times higher than before dividing both in N2 and N1R medium.

5.4.1.9. Remarks. The cell growth of 12 *sld7* Δ mutants was analyzed. The effect of inhibitor can only be observed on 7 of these cells. The area, perimeter and volume of the mother cells were considered with their buds. The area, perimeter and volume of the daughter cells were considered after they were separated from their mother cells. The cell area changed between 25 and 65 μm^2 (Figure 5.128), the cell perimeter changed between 20 and 65 μm (Figure 5.129). The cell volume changed between 1.5 and 3 of their initial sizes just before daughter cell separated from the mother cell (Figure 5.130). The total area and perimeter of the mother cell together with its bud decreased after they were exposed to inhibitor (Table 5.42). The division volume of some of these cells decreased after they were exposed to inhibitor, whereas some of them did not change.

In experiment 3, it was observed that the size of the wild type BY4741 cells decreased as a response to inhibitor in YNB media. Yet, in experiment 6 the sizes of the total cell (new mother and bud) that were grown in fresh medium N1R were bigger than the cells grown in N2 medium, i.e. the cells recovered after exposure to the inhibitor. The z-score for area changed between -2 and 2 (Figure 5.131), and z-score for perimeter changed between -2 and 2.5 (Figure 5.132).

Tanaka *et al.* (2011) observed that the viability of *sld7* Δ cells decreased more severely than wild type cells when they were exposed to MMS. The durations of maximum, minimum and average durations of cell cycle phases are given in Table 16. The duration of cell cycle increased when the cells were exposed to inhibitor. Yet, the longest cell cycle was observed during transition from N2 medium to N1R medium. In that case, the DNA was alkylated by MMS in G₁ phase and it caused a delay at the start of S phase of the cell cycle. As the DNA of the cells was damaged, that extended the duration of G₁ phase and also cell cycle. The duration of the cell cycle became shorter after feeding fresh N1R medium but not as short as the cells that were not exposed to inhibitor (Table 5.43). As a result, the inhibitor damaged DNA of the *sld7* Δ cells temporarily.

The duration of cell cycle with its phases were compared between mother and daughter cells (Table 5.44). The duration of the G₁ phase of daughter cells was reported to be longer than that of mother cells (Brewer *et al.*, 1984). In our case, the duration of both G₁ phase and cell cycle of the mother cells were longer than those of the daughter cells. Yet,

this comparison was done between 1 daughter cell and 11 mother cells in transition between N1 and N2, and N2 medium. Therefore, this comparison may not reflect the precise results. Otherwise, this may also be due to *sld7Δ* mutant cells. The volumetric fold change of the cells were compared in each media (Figure 5.133). The size of the cells increased as almost two-fold of their initial size (average=1.74) in glucose rich YPD media and YPD media containing MMS (average=1.74), whereas the the change in the cell sizes after feeding fresh medium were increased to 1.77. In this case, the volume change of the cells was not significant.

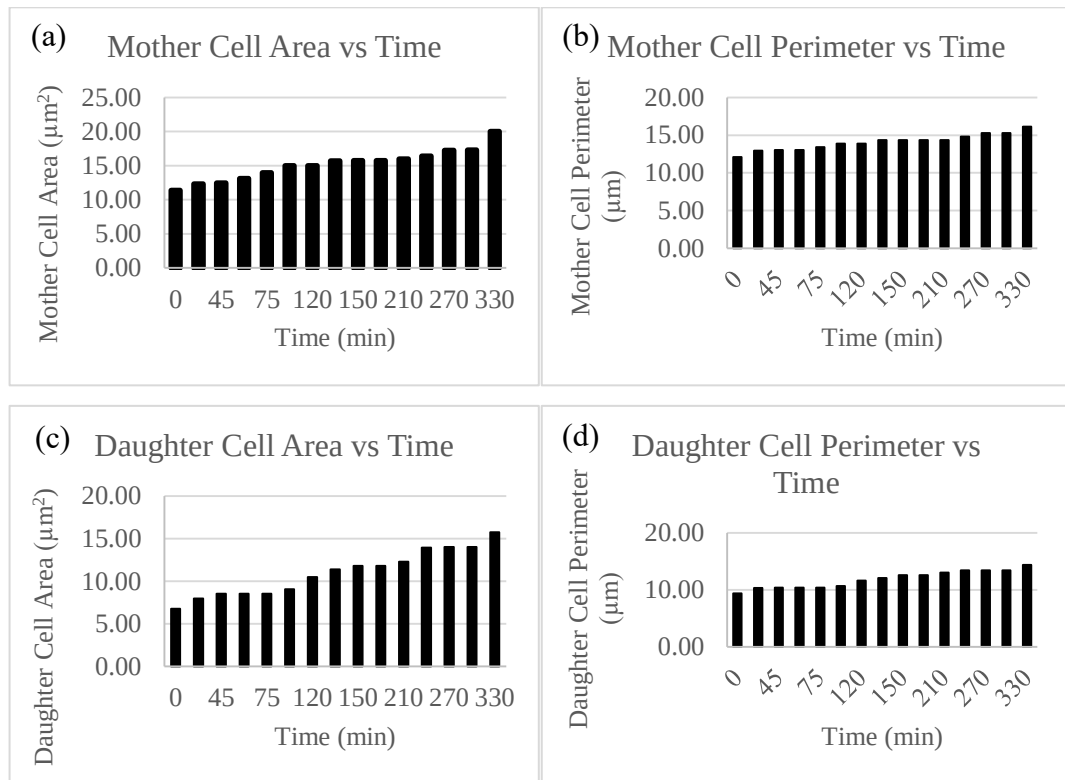


Figure 5.57. Graphical representation of processed data of chamber 6 in an environment without MMS (a) M1 area vs time graph (b) M1 perimeter vs time graph (c) D1 area vs time graph (d) D1 perimeter vs time graph-EXP3.

The duration of the cell cycle of all the cells were compared in each medium (Figures 5.134 and Figure 5.135). The durations of G_1 and $S/G_2/M$ phases were reported as 22 and 59 min for wild type cells in glucose rich YPD medium (Brewer *et al.*, 1984). The duration of the cell cycle of most of these cells were longer than those of the wild type (AD364) cells in each medium.

5.5. Effect of Caloric Restriction on *sld7Δ* mutants in YPD medium with 0.2 % glucose

CR leads to a decreased incidence of breast, skin, intestinal, ovarian, hepatic and brain cancers. CR modulates many biochemical and metabolic processes on different levels. CR modulates the level of metabolites altering the adenosine triphosphate/adenosine diphosphate (ATP/ADP) ratio, acetyl-coenzyme A (acetyl-CoA)/CoA ratio, the ratios of reduced and oxidized nicotinamide adenine dinucleotide NADH/NAD⁺ and NAD phosphate NADPH/NADP⁺ (Kopeina *et al.*, 2016). It promotes decreasing the level of growth factors (GF) essential for cell growth and division (Fontana *et al.*, 2016). Moreover, it can regulate apoptosis and autophagy (Lee *et al.*, 2012; Raffaghello *et al.*, 2008). Finally, nutrient restriction regulates hormonal status.

Another benefit of CR in the context of anti-tumor therapy is an ability to promote adaptive mechanisms of normal cells in response to stress caused by treatment with anti-cancer drugs. Many anti-tumorigenic agents are able to introduce DNA damage and activate various mechanisms of programmed cell death; however, they have significant toxic effects on healthy cells (Kopeina *et al.*, 2016).

Accumulating evidence suggests that the combination of stress stimuli, such as DNA damage and CR, has a synergistic effect on cancer cell death, while the negative side effects on normal cells can be reduced (Kopeina *et al.*, 2016).

The combination of CR or CRMs with anti-cancer therapy can not only improve chemo- and radiotherapy but also might enhance the efficiency of targeted drugs. E.g. in an experimental mouse model of prostate cancer, the treatment of xenografts with ganitumab (anti-IGF-1R antibody) under food restriction resulted in an improved metabolic profile and a significant decrease of tumor weight (Kopeina *et al.*, 2016).

Collectively, the combination of caloric and nutrient restriction or CRMs with the targeted drugs has displayed promising synergistic anti-cancer activity that brings hope for the development of new effective strategies for clinical application (Kopeina *et al.*, 2016).

5.5.1. Experiment 7

The cell type was same as those used in previous experiments (EXP5 and EXP6). The 2% (w/v) and 0.2% (w/v) glucose containing YPD media were prepared to grow cells in the incubator and in the microbioreactor, respectively. The *sld7Δ* cells were let to grow in an incubator for 12 hours, then put into the refrigerator (+4 °C) to stop the growth phase. The size of the cells were smaller than 5 μm. The medium was loaded into the microbioreactor at a rate of 5 μL/min through the middle inlet only. The first chip (microbioreactor) was filled in 2 hours until all the chambers and channels were filled with medium. The yeast cell culture was diluted with YPD medium, prepared (OD: 0.50) for inoculation into microbioreactor and loaded at a rate of 0.5 μL/min, till the cells were observed in the chambers (1 hour). However, the cells clogged the inlet of the first chamber, then the system was closed.

The second microbioreactor was filled in 1 hour until all the chambers and channels were filled. The yeast cell cultures diluted with YPD medium, prepared (OD: 0.50) for inoculation into the microbioreactor loaded at a rate of 0.5 μL/min. After 20 minutes of yeast cell loading, yeast cells were trapped in chambers, and the 0.2 % (w/v) YPD medium (N1) feeding at a rate of 0.1 μL/min was started (t=0 or 20 min) and continued for 7 hours. The N1 medium was changed with N2 medium (YPD medium with 0.01% MMS) at 425th minutes. The N2 medium was fed for 3 hours at a rate of 0.1 μL/min. Then, the N2 medium was changed with N1R fresh media for 3 hours to observe the effect of fresh medium on cells. The brightfield microscopic images of the chambers were taken under 40X and 90X magnification for 13 hours during N1, N2 and N1R media loading. The experiment was ended because of the yeast invasion, i.e. high amount of yeast cells filled the channels and chambers as shown in Figure 5.136.

5.5.1.1. Chamber 1. The yeast cells were fed through the middle channel. The cells were trapped in the C-shaped area. The responses of some of these cells were observed during the experiment (Figure 5.136). The changes in the area and perimeter of the cells were given in Figure 5.137. The duration of G₁ and S/G₂/M phases under different environmental conditions was given in Table 5.45. The volume of the cells were doubled while budding (Figure 5.138).

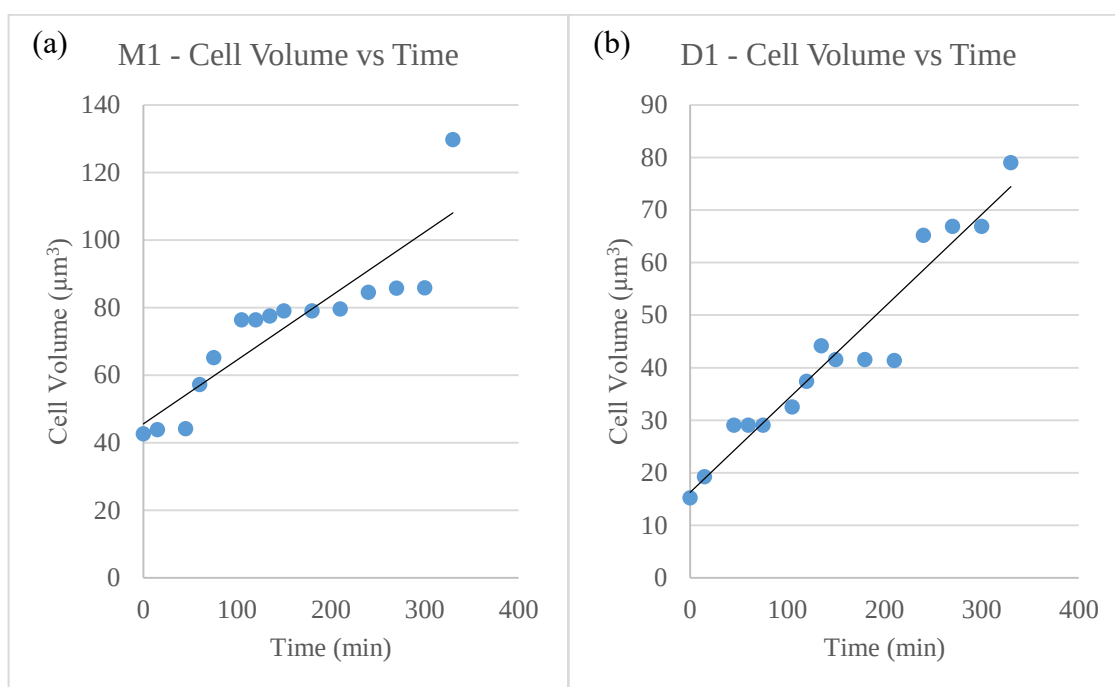


Figure 5.58. Graphical representation of processed data of chamber 6 (a) mother cell 1 volume vs time graph (b) daughter cell 1 volume vs time graph - EXP 3

5.5.1.2. Chamber 2. The cells that were trapped in C-shaped area in N1 medium were observed in Figure 5.139. Then, these cells were swept away from chamber 2. The changes in area, perimeter and volume were given in Figure 5.140 and Figure 5.141, respectively. The response of the cells to the N1 medium is given in Table 5.47. The fold changes of the area, perimeter and volume of the cells were given in Table 5.48. It was observed that the volumes of the cells were 2 times higher than before dividing.

5.5.1.3. Outlet. The yeast cells were trapped at the outlet of the chip/microbioreactor. The responses of 14 cells were processed. However, some of these cells could not be observed throughout the entire experiment, because some cells were swept away while changing medium from N1 to N2 or N2 to N1R. The area, perimeter and volume of the cells were given in Figures 5.144-5.159. The duration of the G₁ and S/G₂/M phases of these cells were given in Tables 5.49, 5.51, 5.53, 5.55, 5.57, 5.59 and 5.61. The fold change of the area, perimeter and volume of the cells were given in Tables 5.50, 5.52, 5.54, 5.56, 5.58, 5.60 and 5.62.

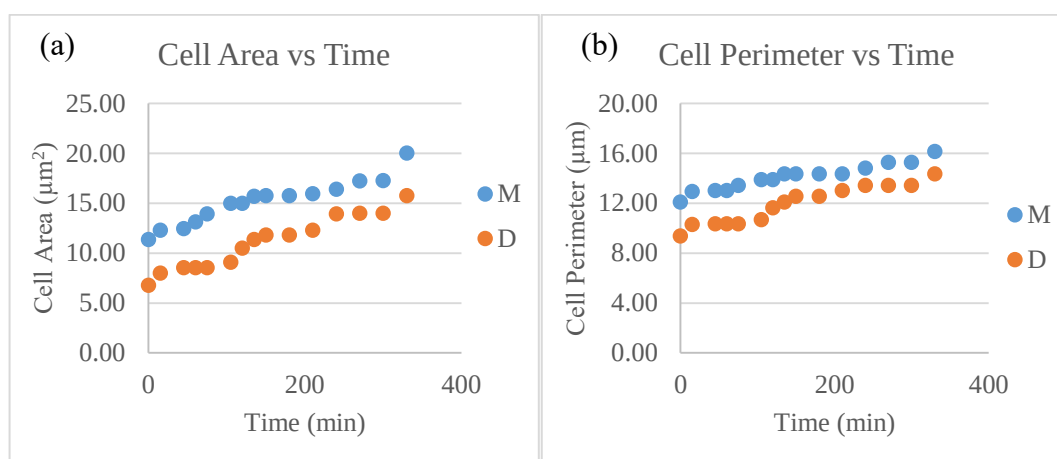


Figure 5.59. Graphical representation of changes in both mother and daughter cells (a) cell area (b) cell perimeter



Figure 5.60. A trapped yeast cell at C-shaped region in the seventh chamber

5.5.1.4. Remarks. The cell growth data of 18 *sld7Δ* cells were analyzed. The effect of inhibitor can only be observed on 7 of these cells. The durations of maximum, minimum and average cell cycle phases were given in Table 5.60. The duration of cell cycle increased when the cells were exposed to inhibitor. The longest cell cycle was observed during transition from N2 medium to N1R medium. In that case, the DNA was alkylated by MMS in G₁ phase and it caused a delay in budding phase of the cell cycle. As the DNA of the cells was damaged, that extended the duration of G₁ phase and also cell cycle. The duration of the cell cycle became shorter after feeding fresh N1R medium (Table 5.63). A similar experiment was not reported in literature. Therefore, it was not possible to compare the results with the literature findings.

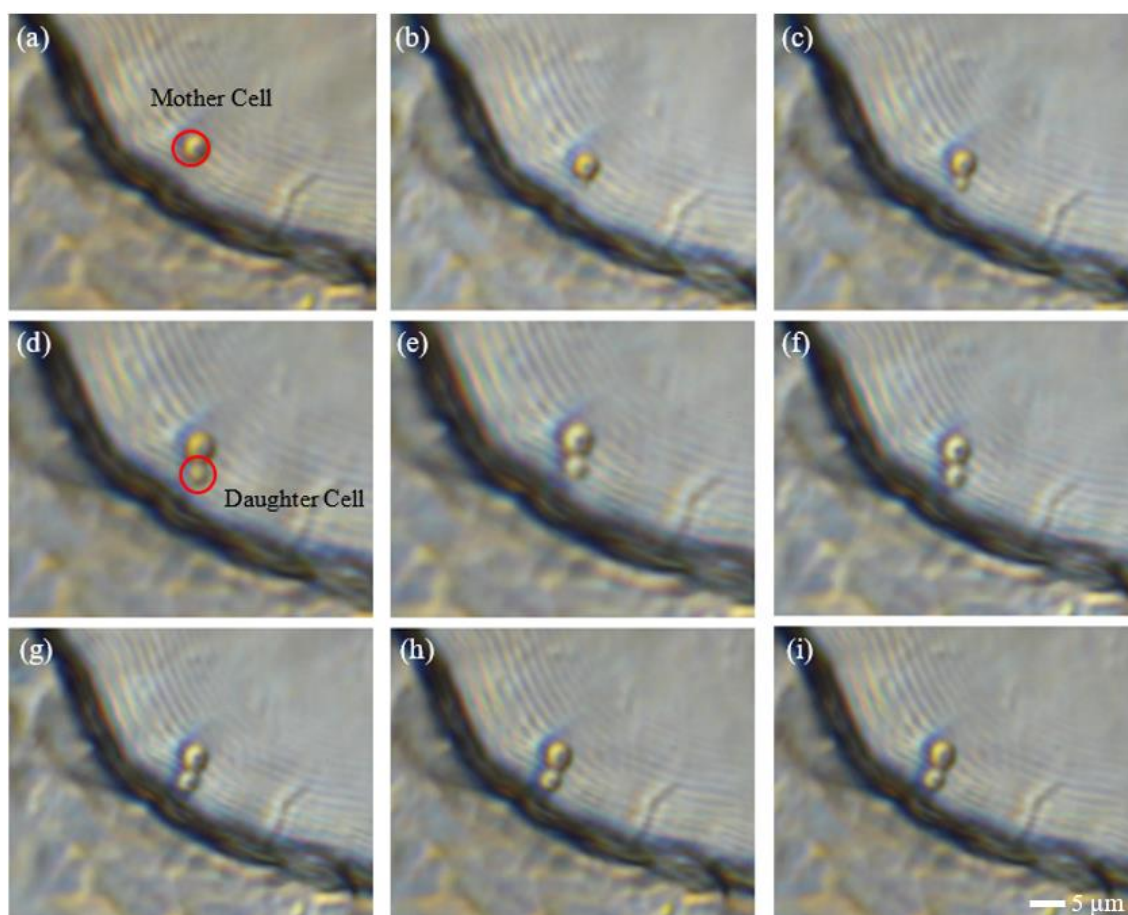


Figure 5.61. The budding period of a trapped cell in the trapping region of chamber 7 (a) 210th minutes (b) 240th minutes (c) 270th minutes (d) 300th minutes (e) 330th minutes (f) 375th minutes (g) 420th minutes (h) 465th minutes (i) 510th minutes

The duration of G_1 phase is 5 minutes longer, whereas $S/G_2/M$ phases are approximately 5 min shorter in caloric restricted environment compared to glucose rich medium (Table 5.64). After the cells were exposed to N2 medium, it was observed that the duration of G_1 and $S/G_2/M$ phases became shorter in the case of caloric restriction. The duration of these phases became even shorter after the cells were exposed to N1R medium. It was observed that the duration of G_1 phase did not change in 2% and 0.2% glucose (n1 and N1R). Although the G_1 phase was restored, it was observed that the $S/G_2/M$ phase was longer than before the DNA alkylation. The response of the mutant cells to the glucose rich and caloric restricted environment were similar.

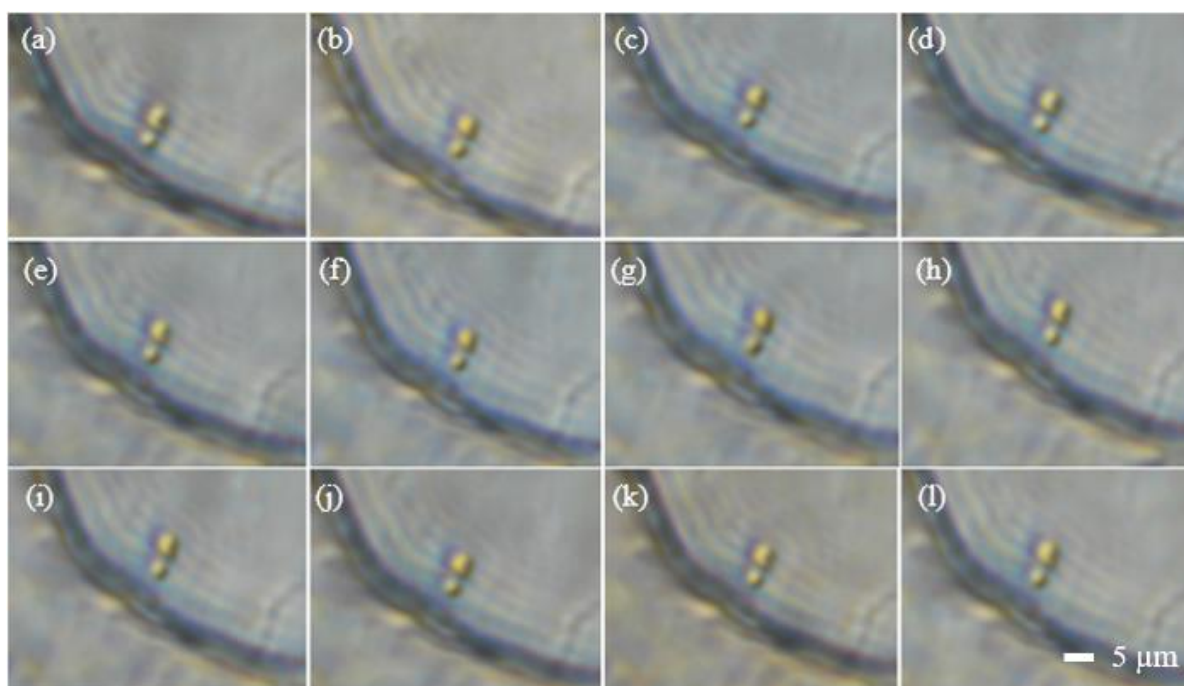


Figure 5.62. The budding period of a trapped cell in the trapping region of chamber 7 in an environment involving MMS (a) 610th minute (b) 685th minutes (c) 715th minutes (d) 730th minutes (e) 745th minutes (f) 775th minutes (g) 805th minutes (h) 835th minutes (i) 865th minutes (j) 905th minutes (k) 935th minutes (l) 965th minutes

The duration of cell cycle with its phases were compared between mother and daughter cells (Table 5.65). The duration of the G₁ phase of daughter cells was reported to be longer than that of mother cells (Brewer *et al.*, 1984). In our case, the duration of both G₁ phase and cell cycle of the daughter cells were longer than those of the mother cells.

The duration of the cell cycle of all the cells was compared in each medium (Figure 5.160). The duration of G₁ and S/G₂/M phases are different for each cell in each medium. The duration of G₁ and S/G₂/M phases were given as 22 and 59 min for wild type cells in glucose rich YPD medium (Brewer *et al.*, 1984). The duration of the G₁ and S/G₂/M phases of all cells were longer than that of the wild type cells in each medium. The duration of the cell cycle of the mother and its daughter cells were compared (Figure 5.161). The duration of G₁ phases were same but the duration of S/G₂/M phases of daughter cell was longer than the S/G₂/M phases of mother cell.

The cell area and cell perimeter of the mother cells were considered with their buds. The area and perimeter of the daughter cells were considered after they were separated from mother cells. The cell area changed between 23 and 73 μm^2 (Figure 5.162), the cell perimeter changed between 20 and 62 μm (Figure 5.163). The volume of the cells changed between 35 and 400 μm^3 . Before daughter cell separated from the mother cell, the cell sizes increased between 1.5 and 3.5 times of their initial sizes, just (Figure 5.164). The total area and perimeter of the mother cell together with its bud were decreased after they were exposed to inhibitor.

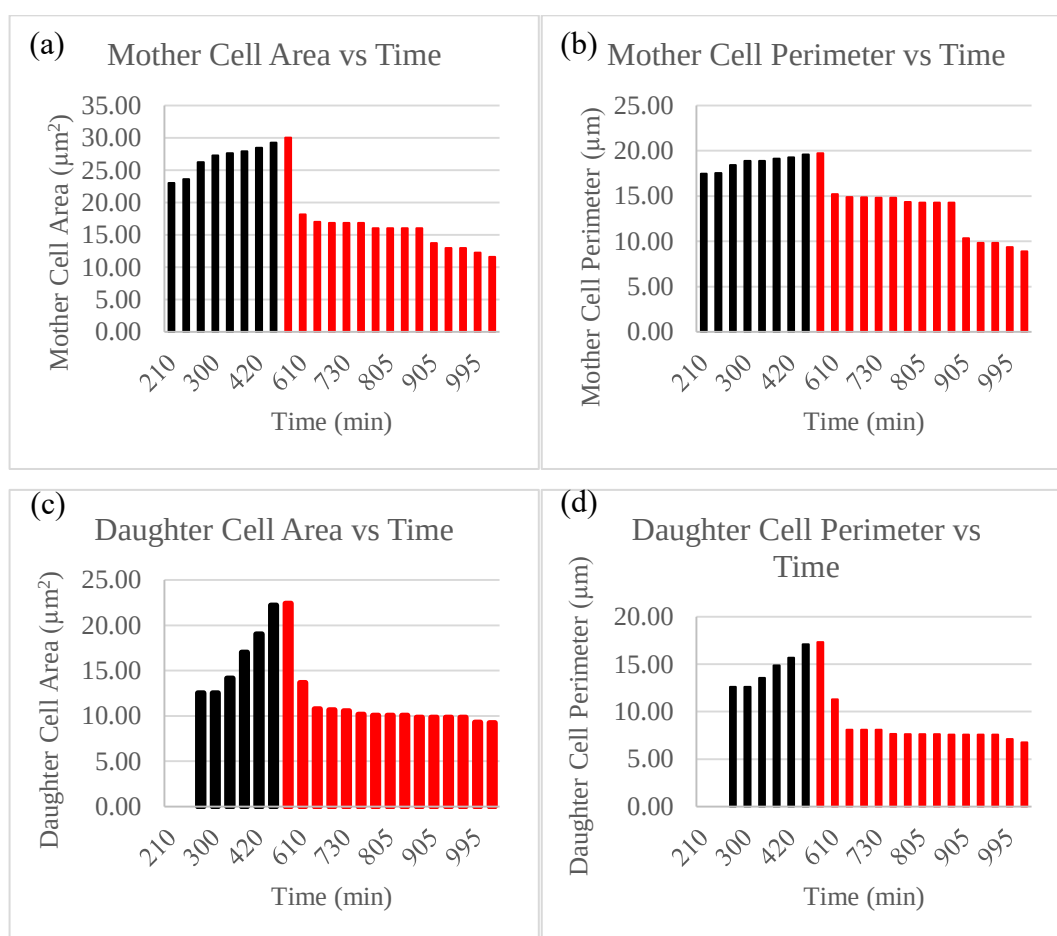


Figure 5.63. Graphical representation of processed data of chamber 7 in an environment with and without MMS (a) mother cell area vs time graph (b) mother cell perimeter vs time graph (c) daughter cell area vs time graph (d) daughter cell perimeter vs time graph (brightfield images)-EXP3.

The volumetric fold change of the cells were compared in each media (Figure 5.165). The size of the cells increased as almost two-fold of their initial size (average=1.88) in each media. In this case, the MMS did not have any effect on cell size of the *sld7* mutant cells. The average fold change was similar in glucose rich media. It can be said that, the caloric restriction did not have any effect on cell size as well.

The z-score for area, perimeter and volume are represented in Figures 5.166, 5.167 and 5.168 respectively. The z-score for area, perimeter and volume for the cells that were exposed to inhibitor were represented in Figure 5.169, 5.170 and 5.171, respectively.

The duration of cell cycle with its phases were compared between mother and daughter cells (Table 5.65). The duration of the G₁ phase of daughter cells was reported to be longer than that of mother cells (Brewer *et al.*, 1984). In our case, the duration of both G₁ phase and cell cycle of the daughter cells were longer than those of the mother cells.

The duration of the cell cycle of all the cells was compared in each medium (Figure 5.160). The duration of G₁ and S/G₂/M phases are different for each cell in each medium. The duration of G₁ and S/G₂/M phases were given as 22 and 59 min for wild type cells in glucose rich YPD medium (Brewer *et al.*, 1984).

The duration of the G₁ and S/G₂/M phases of all cells were longer than that of the wild type cells in each medium. The duration of the cell cycle of the mother and its daughter cells were compared (Figure 5.161). The duration of G₁ phases were same but the duration of S/G₂/M phases of daughter cell was longer than the S/G₂/M phases of mother cell.

The cell area and cell perimeter of the mother cells were considered with their buds. The area and perimeter of the daughter cells were considered after they were separated from mother cells. The cell area changed between 23 and 73 μm^2 (Figure 5.162), the cell perimeter changed between 20 and 62 μm (Figure 5.163). The volume of the cells changed between 35 and 400 μm^3 . Before daughter cell separated from the mother cell, the cell sizes increased between 1.5 and 3.5 times of their initial sizes, just (Figure 5.164). The total area and perimeter of the mother cell together with its bud were decreased after they were exposed to inhibitor.

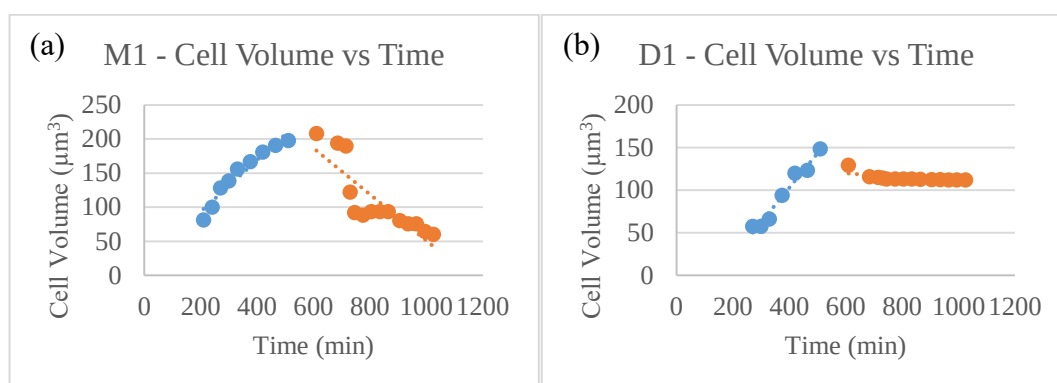


Figure 5.64. Graphical representation of processed data of chamber 7 (a) mother cell 1 volume vs time graph (b) daughter cell 1 volume vs time graph - EXP 3

The volumetric fold change of the cells were compared in each media (Figure 5.165). The size of the cells increased as almost two-fold of their initial size (average=1.88) in each media. In this case, the MMS did not have any effect on cell size of the *sld7* mutant cells. The average fold change was similar in glucose rich media. It can be said that, the caloric restriction did not have any effect on cell size as well.

The z-score for area, perimeter and volume are represented in Figures 5.166, 5.167 and 5.168 respectively. The z-score for area, perimeter and volume for the cells that were exposed to inhibitor were represented in Figure 5.169, 5.170 and 5.171, respectively.

5.6. Effect of the inhibitor on wild type BY4743 diploid cells in YPD medium (2% glucose)

5.6.1. Experiment 8

The wild type diploid cells (BY4743) were used to compare the response of the wild type cells with the *sld7Δ* mutant cells (diploid). The 2% (w/v) glucose containing YPD medium was prepared to grow cells in the incubator and in the microbioreactor. The *sld7Δ* cells were let to grow in the incubator for 8 hours, then put into the refrigerator (+4 °C) to stop the growth phase. The sizes of the cells were smaller than 5 μm. The medium was loaded into the microbioreactor at a rate of 5 μL/min through the middle inlet only. The first chip (microbioreactor) was filled in 2 hours until all the chambers and channels were filled with medium. However, the leakage between epoxy and pipette tips on the PMMA inlet

apparatus was observed. Then, the chip was changed with the new one. The same problem was encountered for 3 more times. The reason was that the inlet apparatus was prepared in less than 24 hours, and the epoxy did not dry sufficiently. At last, one of the prepared inlet apparatus was used for a new chip.

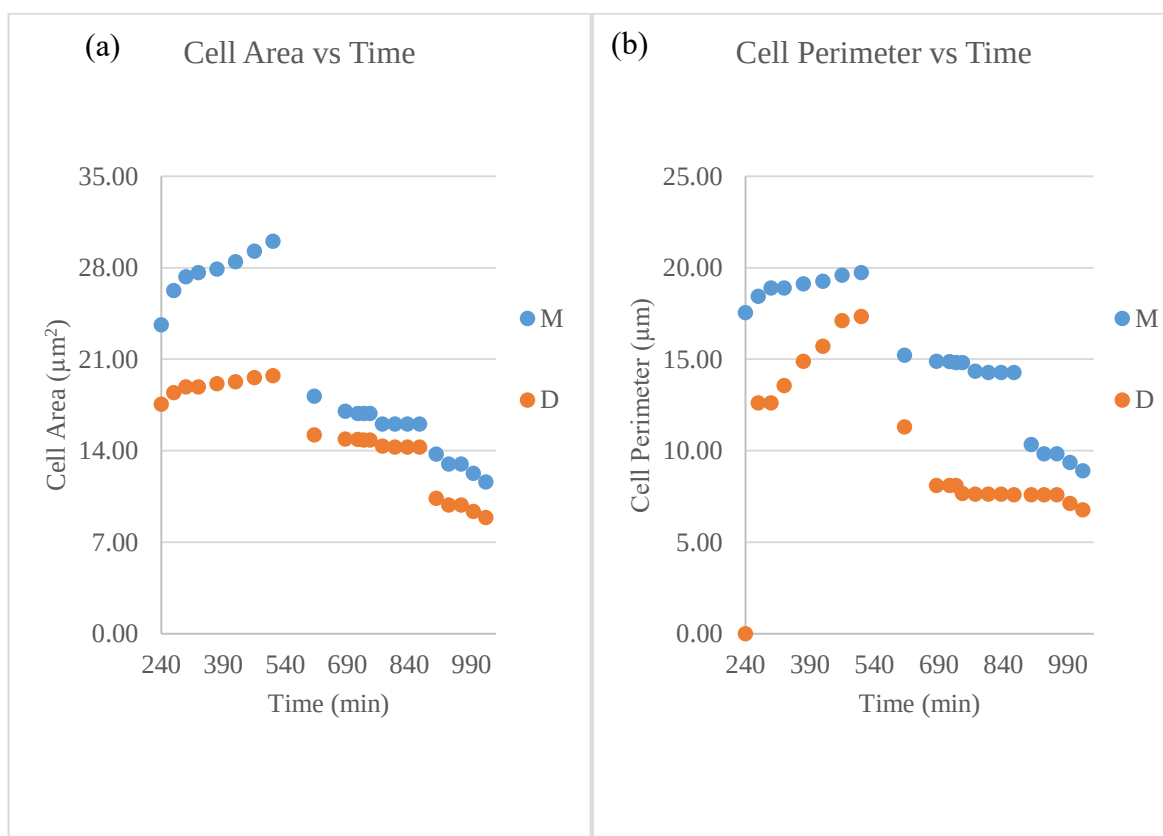


Figure 5.65. Graphical representation of changes in both mother and daughter cells (a) cell area (b) cell perimeter

The medium was loaded into the microbioreactor at a rate of 5 $\mu\text{L}/\text{min}$ through the middle inlet only. The flowrate was decreased to 0.5 $\mu\text{L}/\text{min}$ 1 hour before loading yeast cells to prevent vortex in the microbioreactor. The yeast cell culture was diluted with YPD medium, prepared (OD: 0.50) for inoculation into the microbioreactor, and loaded at a rate of 0.5 $\mu\text{L}/\text{min}$. After 20 minutes of yeast cell loading, yeast cells were trapped in chambers, and the 2 % (w/v) YPD medium (N1) feeding at a rate of 0.1 $\mu\text{L}/\text{min}$ was started ($t=0$ or 60 min) and continued for 6 hours.

Table 5.10. The rate of change in area, perimeter and volume of M1 and D1 cells in chamber 7

Time (min)	M1-Cell Area Fold Change	M1-Cell Perimeter Fold Change	M1-Cell Volume Fold Change	D1-Cell Area Fold Change	D1-Cell Perimeter Fold Change	D1-Cell Volume Fold Change
210	1.00	1.00	1.00	-	-	-
240	1.03	1.00	1.23	-	-	-
270	1.14	1.05	1.58	1.00	1.00	1.00
300	1.19	1.08	1.71	1.00	1.00	1.00
330	1.20	1.08	1.92	1.13	1.07	1.16
375	1.21	1.09	2.05	1.36	1.18	1.63
420	1.24	1.10	2.23	1.52	1.24	2.09
465	1.27	1.12	2.35	1.77	1.36	2.15
510	1.30	1.13	2.44	1.79	1.37	2.58
610	0.79	0.87	2.56	1.09	1.29	2.25
685	0.74	0.85	2.39	0.86	1.33	2.02
715	0.73	0.85	2.34	0.85	1.20	2.01
730	0.73	0.85	1.50	0.84	1.20	1.99
745	0.73	0.85	1.13	0.81	1.24	1.97
775	0.70	0.82	1.09	0.80	1.24	1.97
805	0.70	0.82	1.15	0.80	1.24	1.97
835	0.70	0.82	1.15	0.80	1.24	1.97
865	0.70	0.82	1.15	0.79	1.16	1.96
905	0.42	0.71	0.99	0.79	1.16	1.96
935	0.39	0.62	0.93	0.79	1.16	1.96
965	0.39	0.62	0.93	0.79	1.16	1.95
995	0.36	0.65	0.79	0.74	1.04	1.95
1025	0.37	0.62	0.74	0.74	1.09	1.95

The N1 medium was changed with N2 medium (YPD medium 2 % glucose with 0.01% MMS) at 376th minutes. The N2 medium was fed for 3 hours at a rate of 0.1 $\mu\text{L}/\text{min}$. Then, the N2 medium was changed with N1R fresh media for 3 hours to observe the effect of fresh medium on cells. The brightfield microscopic images of the chambers were taken under 40X and 90X magnification for 12 hours during N1, N2 and N1R media loading.

5.6.1.1. Chamber 1. The cells that were trapped in C-shaped area in N1 medium were observed in Figure 5.172. The cells that were coming from inlet hole of the microbioreactor prevented tracking of these mother cells (Figure 5.172b). The growth of two mother cells were processed (Figure 5.173). The changes in area, perimeter and volume were given in Figure 5.174 and Figure 5.175, respectively. The duration of the cell cycle phases of these cells with respect to medium changes were given in Table 5.66. It was observed that the area, perimeter and volume of the cells before division were different in each division/separation (Table 5.67).

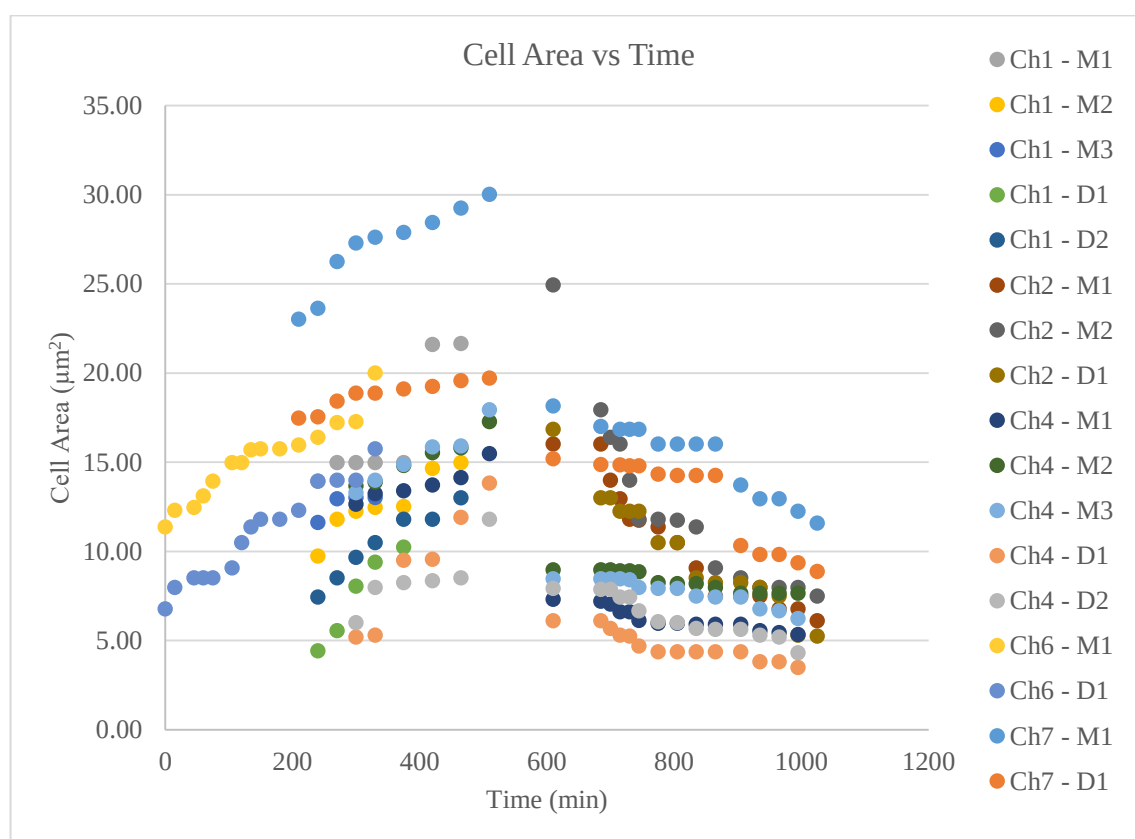


Figure 5.66. The comparison of cell area of the processed cells

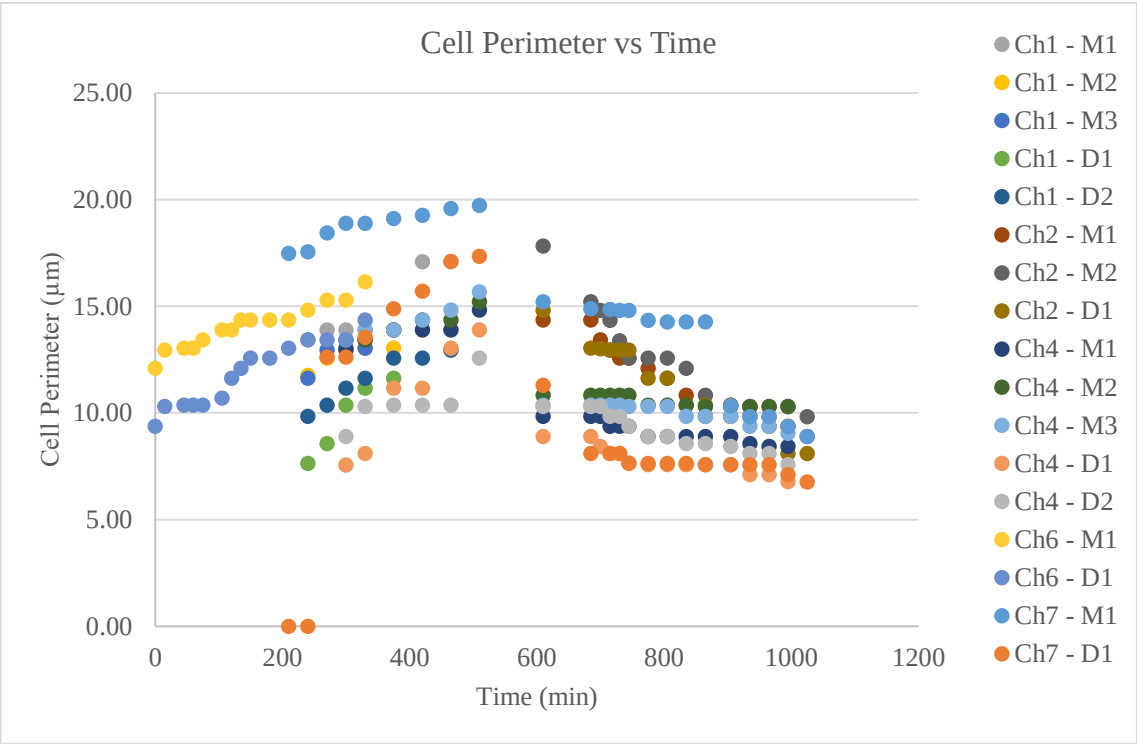


Figure 5.67. The comparison of cell perimeter of the processed cells

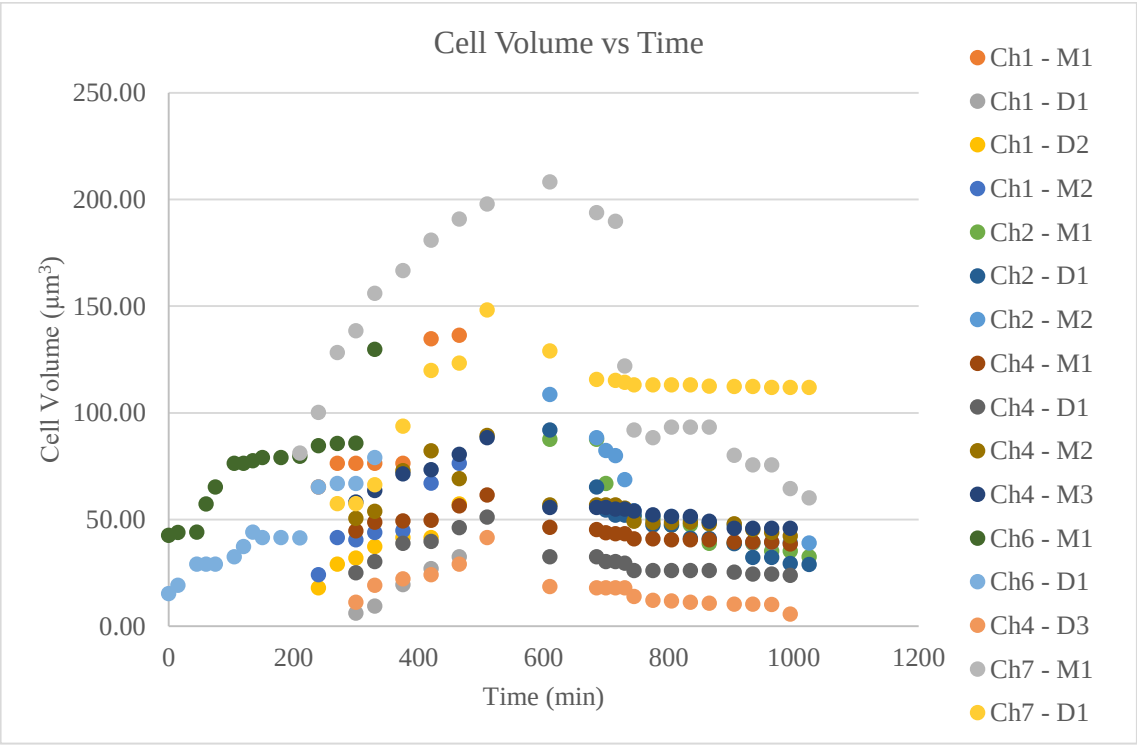


Figure 5.68. The comparison of cell volume of the processed cells

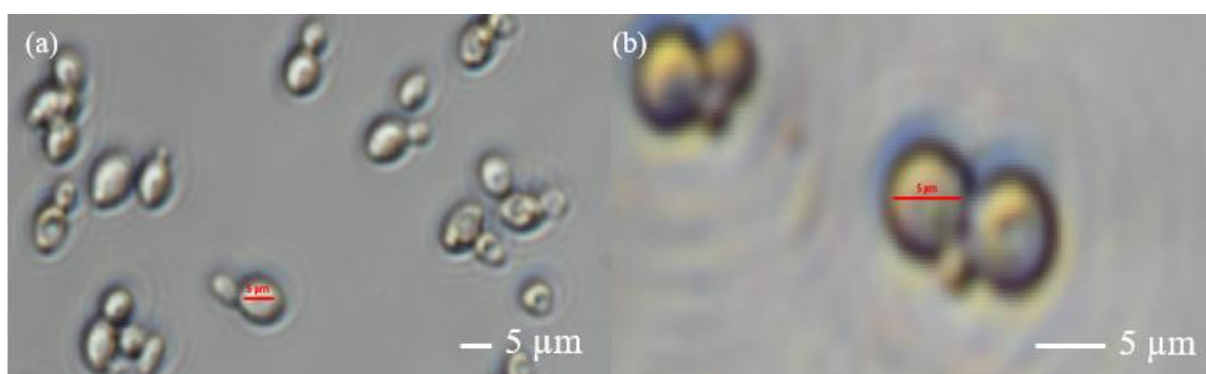


Figure 5.69. The size of yeast cells (a) in experiment 4 deletion, the mutant strain *sld7Δ* (b) in experiment 3, GFP tagged wild-type BY4741 strain

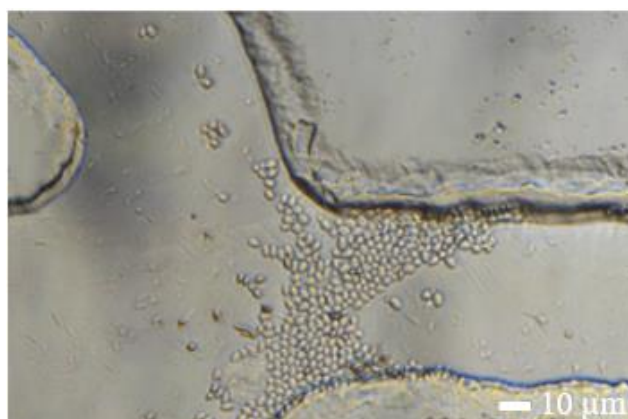


Figure 5.70. The trapped cells in chamber 1 under 40X

5.6.1.2. Chamber 2. The cells that were trapped in C-shaped area in N1 medium were first observed in Figure 5.176a. Then, some of the cells were swept away from inlet hole of the microbioreactor through chamber 2 (Figure 5.176b). The growth of two mother cells were processed (Figure 5.177). The changes in area, perimeter and volume were given in Figure 5.178 and Figure 5.179, respectively. The response of the cells to the N2 medium is given in Table 5.68. It was observed that the area, perimeter and volume of the cells before dividing was different in each division/separation (Table 5.69). Before dividing, the cell size increased between 1.5 and 2.5 times of its initial size.

5.6.1.3. Chamber 3. The cells that were trapped in C-shaped area were first observed in N2 medium in Figure 9a. Then, some of the cells were swept away and some new cells moved

from inlet hole of the microbio reactor towards chamber 3 (Figure 5.180b). The growth of two mother cells were processed (Figure 5.181). The changes in area, perimeter and volume were given in Figure 5.182 and Figure 5.183, respectively. It was observed that the area, perimeter and volume of the cells before dividing was different in each division/separation (Table 5.70). Before dividing, the cell size increased between 1.5 and 2 times of its initial size.

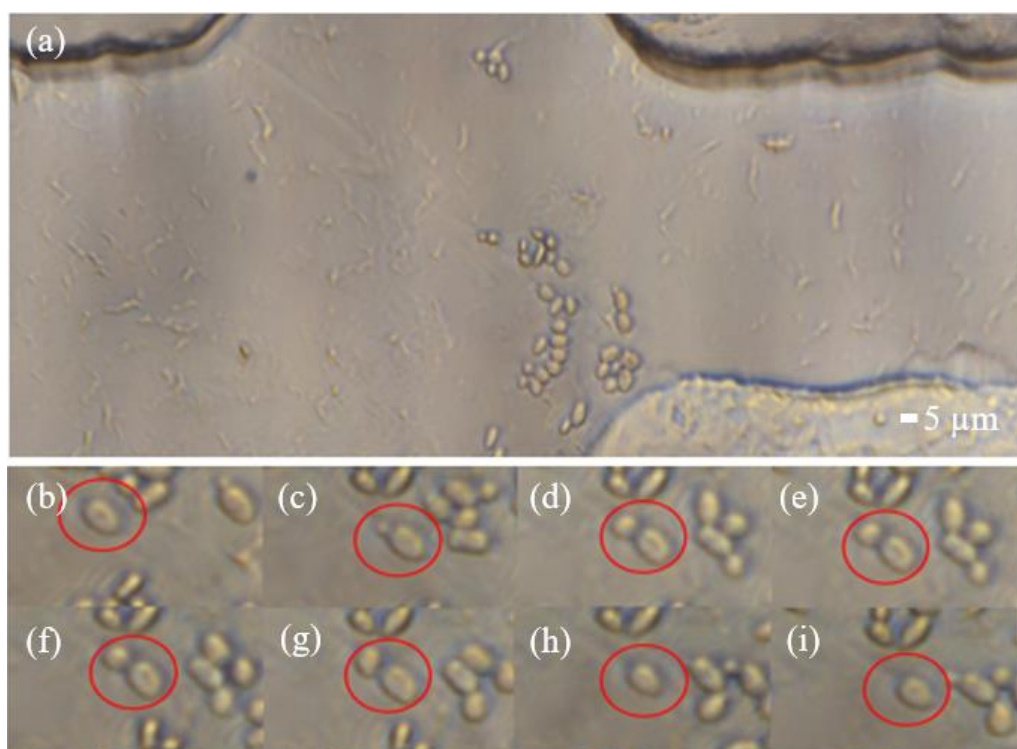


Figure 5.71. The trapped cells in chamber 2 under 40X magnification (a) 0th minute (b) 50th minute (c) 85th minute (d) 110th minute (e) 120th minute (f) 140th minute (g) 155th minute (h) 180th minute (i) 195th minute

5.6.1.4. Chamber 4. The cells, that were trapped in C-shaped area in N1 medium, were observed in Figure 5.184. The response of the two mother cells were observed in N2 and N1 medium (Figure 5.185). The changes in area, perimeter and volume were given in Figure 5.186 and Figure 5.187, respectively. The response of the cells to the N2 medium is given in Table 5.71. It was observed that the area, perimeter and volume of the cells before dividing was different in each division/separation (Table 5.72). Before dividing, the cell size increased between 1.5 and 2 times of its initial size.

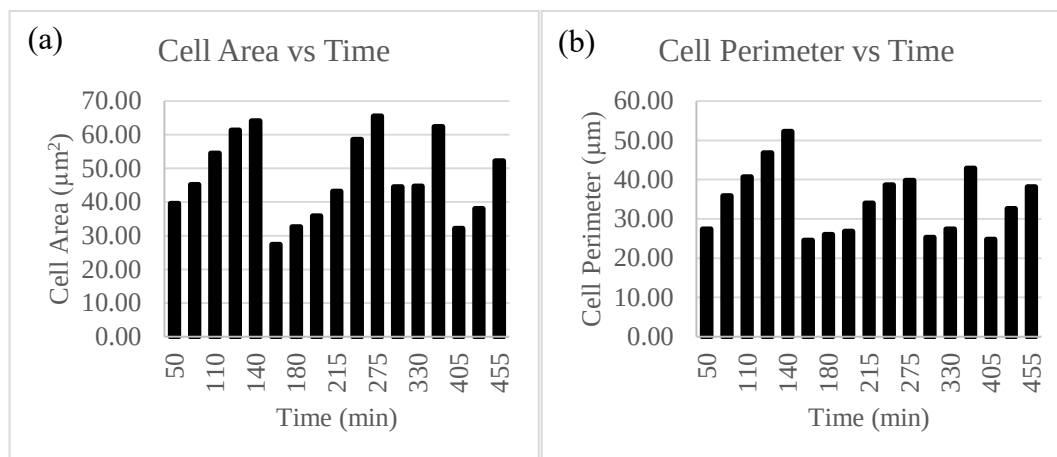


Figure 5.72. Graphical representation of processed data of chamber 2 (a) cell area vs time graph (b) cell perimeter vs time graph

Table 5.11. Duration of G_1 phase and duration of S/ G_2 /M phases of each buds in chamber 2

Bud Number	G_1 phase (min)	S/ G_2 /M phases (min)	Cell Cycle (min)	Area (μm^2)
1	65	70	135	40-65
2	60	75	135	30-65
3	40	75	115	45-65
4	30	50	80	30-55

5.6.1.5. Chamber 5. The cells that were trapped in C-shaped area in N1 medium were observed in Figure 5.188. The response of the two mother cells and a daughter cell were observed in N1, N2 and N1R medium (Figure 5.189). The changes in area, perimeter and volume were given in Figure 5.190, Figure 5.191 and Figure 5.192, Figure 5.193, respectively. The duration of the cell cycle phases of these cells with respect to medium changes were given in Table 5.73. It was observed that the area, perimeter and volume of the cells before dividing was different in each division/separation (Tables 5.74 and 5.75). Before dividing, the cell size increased between 1.5 and 2 times of its initial size.

5.6.1.6. Chamber 6. The cells that were trapped in C-shaped area in N1 and N2 medium were observed in Figure 5.194. The responses of two mother cells were observed in N1, N2 and N1R medium (Figure 5.195). The changes in area, perimeter and volume were given in Figure 5.196 and Figure 5.197, respectively. The duration of the cell cycle phases of these cells with respect to medium changes were given in Table 5.76. It was observed that the area, perimeter and volume of the cells before dividing was different in each division/separation (Table 5.77). Before dividing, the cell size increased between 1.5 and 2 times of its initial size.

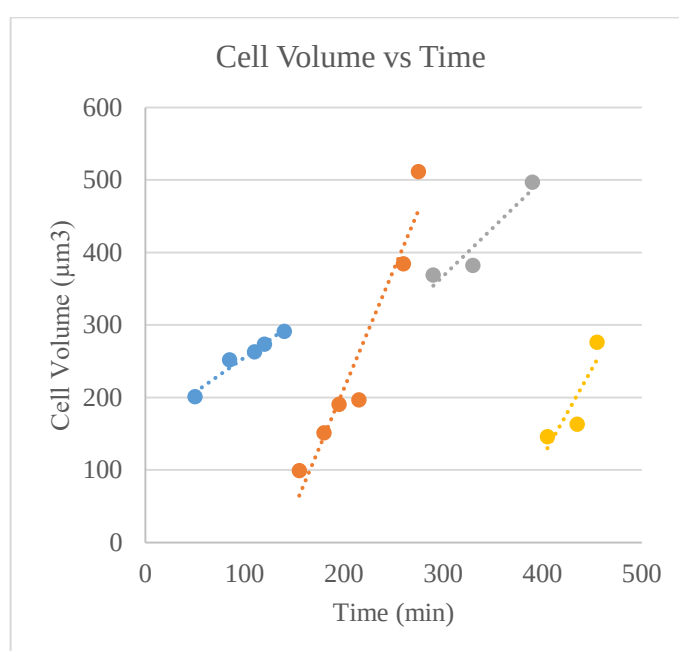


Figure 5.73. Graphical representation of processed data of chamber 2, cell volume vs time graph - EXP 5

5.6.1.7. Chamber 7. The cells, that were trapped in C-shaped area in N1 medium were observed in Figure 5.198. The response of a mother cell and a daughter cell were observed in N1, N2 and N1R medium (Figure 5.199). The changes in area, perimeter and volume were given in Figure 5.200 and Figure 5.201, respectively. The duration of the cell cycle phases of these cells with respect to medium changes were given in Table 5.78. It was observed that the area, perimeter and volume of the cells before dividing was different in each division/separation (Table 5.79). Before dividing, the cell size increased between 1.5 and 2.5 times of its initial size.

Table 5.12. The fold change in area, perimeter and volume of the cell in chamber 2

Time (min)	Cell Area Fold Change	Cell Perimeter Fold Change	Volume Fold Change
50	1.00	1.00	1.00
85	1.14	1.31	1.25
110	1.38	1.49	1.31
120	1.55	1.71	1.36
140	1.62	1.91	1.45
155	1.00	1.00	1.00
180	1.19	1.06	1.53
195	1.31	1.09	1.92
215	1.58	1.38	1.98
260	2.14	1.57	3.87
275	2.40	1.62	5.16
290	1.00	1.00	1.00
330	1.00	1.08	1.04
390	1.40	1.70	1.35
405	1.00	1.00	1.00
435	1.19	1.32	1.12
455	1.63	1.54	1.89

5.6.1.8. Outlet. Some cells were trapped in outlet area in N1 medium.. The response of a mother cell and a daughter cell was observed in N1, N2 and N1R medium (Figure 5.202). The changes in area, perimeter and volume were given in Figure 5.203 and Figure 5.204, respectively. The duration of the cell cycle phases of these cells with respect to medium changes were given in Table 5.80. It was observed that the area, perimeter and volume of the cells before dividing was different in each division/separation (Table 5.81). Before dividing, the cell size increased almost 1.5 times of its initial size.

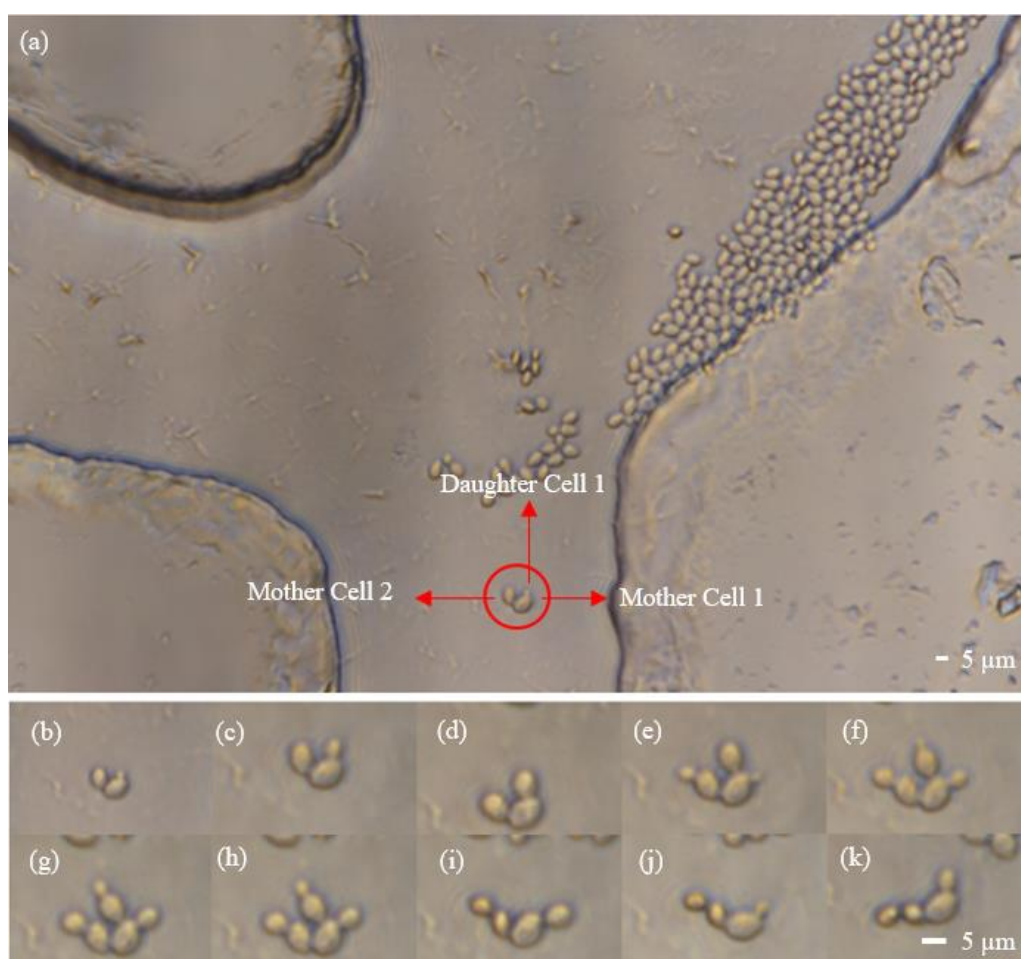


Figure 5.74. The trapped cells in chamber 3 (a) 0th minute under 40X (b) 0th minute (c) 15th minute (d) 50th minute (e) 85th minute (f) 110th minute (g) 120th minute (h) 140th minute (i) 155th minute (j) 180th minute (k) 195th minute under 90X

5.6.1.9. Remarks. The cell growth of 15 wild type BY4743 strain were analyzed. The effect of inhibitor can only be observed on 13 of these cells. The area and perimeter of the mother cells were considered with their buds. The area and perimeter of the daughter cells were considered after they were separated from mother cells. The cell area changed between 30 and 60 μm^2 (Figure 5.207), the cell perimeter changed between 20 and 45 μm (Figure 5.208). The volume of the diploid cells (SC288c background) are approximately 82 μm^3 (Jorgensen *et al.*, 2002), whereas the volume of the present cells (BY4743) changed between 100 and 370 μm^3 (Figure 5.209). Before dividing, the cell size increased between 1.5 and 2 times of its initial size. The total area, perimeter and volume of the mother cell together with its bud were decreased between 5 %, 10 % and 10 % after they were exposed to inhibitor. The

inhibitor was estimated to be effective in the chambers after approximately 1-2 hours. After feeding fresh medium (N1R), it was observed that 12 cells out of 13 cells could not complete their G₁ phases. The area, perimeter and volume of the 12 out of 13 cells slightly increased, they could not complete their G₁ phases after they were exposed to inhibitor.

In experiment 3, it was observed that the size of the wild type BY4741 haploid cells decreased as a response to inhibitor in YNB media. The area, perimeter and volume of the wild type BY4741 cells were smaller than BY4743 cells. The area and volume of the BY4743 cells were 7 times higher than the BY4741 cells and perimeter of BY4743 cells were 3.5 times higher than the BY4741 cells.

Table 5.13. Duration of G₁ phase and duration of S/G₂/M phases of each buds in chamber

3

Cell Name	Bud Number	G ₁ phase (min)	S/G ₂ /M phases (min)	Cell Cycle (min)	Area (μm ²)
M1	1	-	50	>50	50-75
M1	2	35	55	90	60-75
M1	3	40	35	75	50-80
M1	4	45	30	75	50-60
M1	5	40	60	100	50-70
D1	1	25	55	80	40-55
M2	1	85	≥130	>215	30-70

The doubling time of these BY4743 cells were estimated as 60 min in glucose rich YPD medium. Brewer *et al.* (1984) reported the doubling time of the diploid yeast cells with A364A D5 background as 80 min. The time difference may be caused by the strain type. The durations of maximum, minimum and average cell cycle phases were given in Table 5.82. The duration of cell cycle increased when the cells were exposed to MMS.

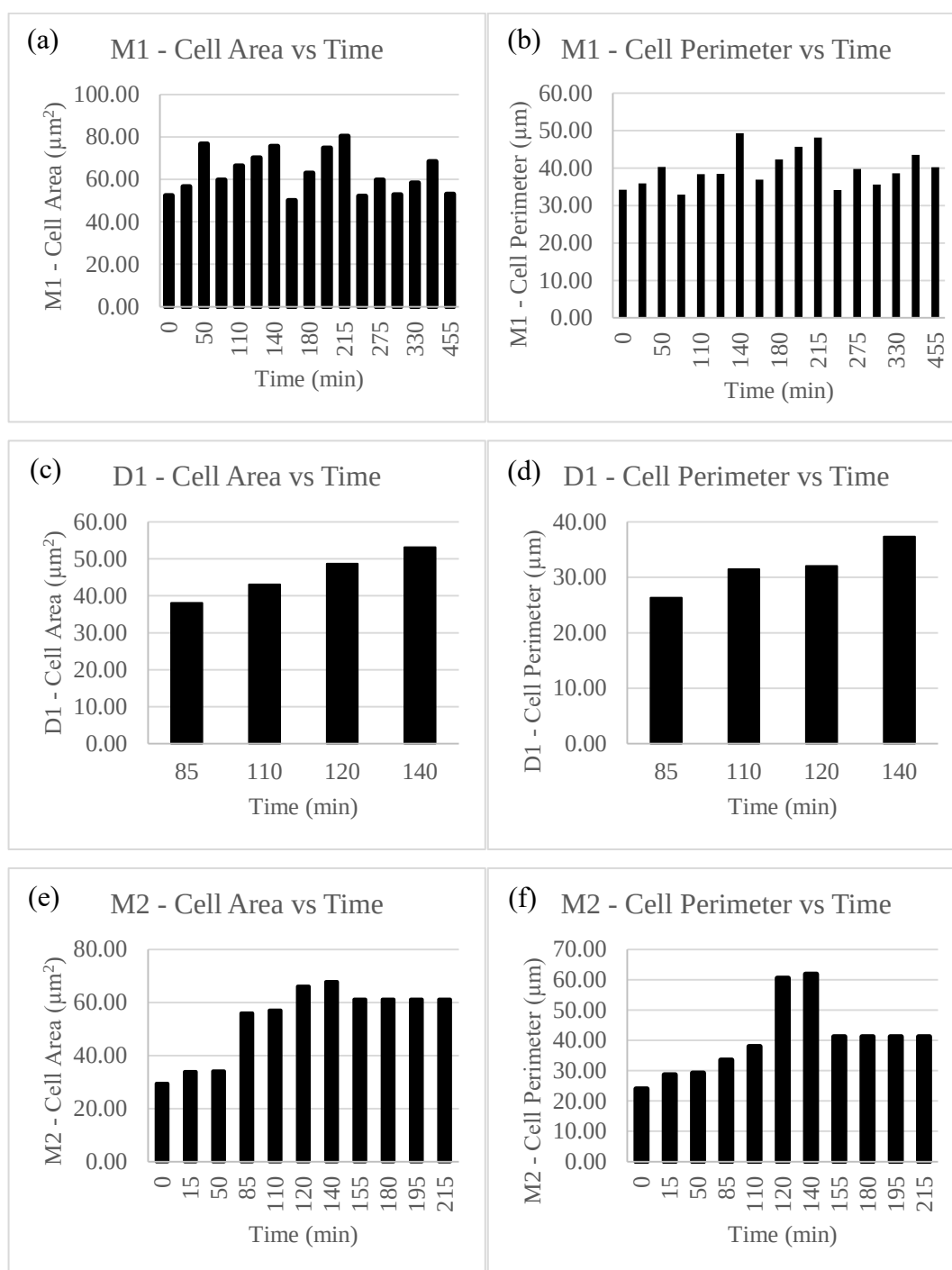


Figure 5.75. Graphical representation of processed data of chamber 3 (a) mother cell 1 area vs time graph (b) mother cell 1 perimeter vs time graph (c) daughter cell 1 area vs time graph (d) daughter cell 1 perimeter vs time graph (e) mother cell 2 area vs time graph (f) mother cell 2 perimeter vs time graph – EXP5

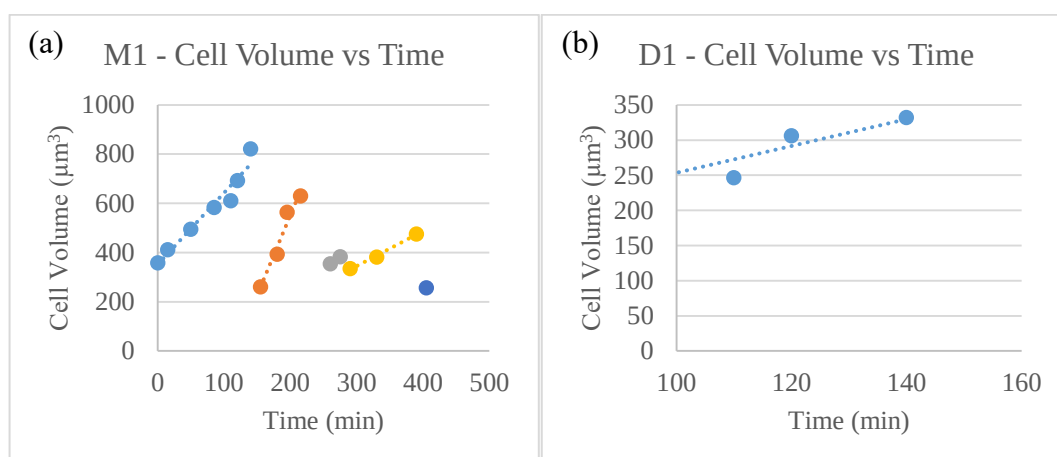


Figure 5.76. Graphical representation of processed data of chamber 3 (a) mother cell 1 volume vs time graph (b) daughter cell 1 volume vs time graph - EXP 5

The longest cell cycle was observed after the cells were exposed to MMS. In that case, the DNA was alkylated by MMS in G_1 phase and it caused a delay in the start of S phase of the cell cycle. As the DNA of the cells were damaged, that extended the duration of G_1 phase and also cell cycle. The duration of the cell cycle seemed to be longer after feeding fresh N1R medium. The reason was that the cells could not complete their cell cycle. The effect of the inhibitor was still continuing in the chambers. In that case, it might be said that the inhibitor damaged the DNA of the wild type cells permanently. The duration of the cell cycle of all the cells were compared in each medium (Figures 5.211 and 5.212). The duration of G_1 and S/ G_2 /M phases is different in each cell in each medium.

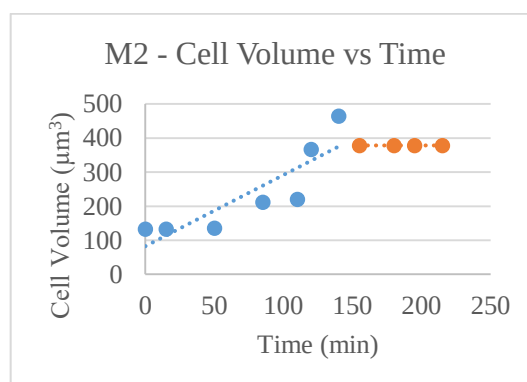


Figure 5.77. Graphical representation of processed data of chamber 3, mother cell 2 - EXP 5

Table 5.14. The fold change in area, perimeter and volume of the M1 and D1 cells in chamber 3

Time (min)	M1-Cell Area Fold Change	M1-Cell Perimeter Fold Change	M1- Volume Fold Change	D1-Cell Area Fold Change	D1-Cell Perimeter Fold Change	D1- Volume Fold Change
0	1.00	1.00	1.00	-	-	-
15	1.08	1.05	1.15	-	-	-
50	1.46	1.18	1.38	-	-	-
85	1.14	0.96	1.63	-	-	-
110	1.27	1.12	1.70	-	-	-
120	1.34	1.12	1.94	-	-	
140	1.45	1.44	2.29	1.00	1.00	1.00
155	1.00	1.00	1.00	1.13	1.20	1.05
180	1.26	1.15	1.51	1.28	1.22	1.31
195	1.49	1.24	2.16	1.40	1.42	1.42
215	1.61	1.30	2.42	-	-	-
260	1.00	1.00	1.00	-	-	-
275	1.15	1.16	1.06	-	-	-
290	1.01	1.04	1.00	-	-	-
330	1.12	1.13	1.15	-	-	-
390	1.31	1.27	1.42	-	-	-
405	1.00	1.00	1.00	-	-	-

The duration of cell cycle with its phases were compared between mother and daughter cells (Table 5.83). This comparison was completed between 1 daughter cell and 14 mother cells in each medium except N1 medium. The duration of the G₁ phase of daughter cells was longer than in mother cells (Brewer *et al.*, 1984). In our case, the duration of phases and cell cycle of both mother and daughter cells were similar in each medium except fresh N1R medium. The only cell that formed a bud after exposed to MMS was the daughter cell. None of these mother cell completed their G₁ phases after they were exposed to MMS even

they were fed with fresh medium after all. Therefore, this comparison may not reflect the precise results. Otherwise, this may also be due to *sld7Δ* mutant cells.

Table 5.15. The fold change in area, perimeter and volume of the M2 cell in chamber 3

Time (min)	M2-Cell Area Fold Change	M2-Cell Perimeter Fold Change	M2-Volume Fold Change
0	1.00	1.00	1.00
15	1.15	1.19	1.00
50	1.16	1.22	1.02
85	1.90	1.40	1.60
110	1.94	1.58	1.66
120	2.24	2.52	2.77
140	2.30	2.57	3.50
155	1.00	1.00	1.00
180	1.00	1.00	1.00
195	1.00	1.00	1.00
215	1.00	1.00	1.00

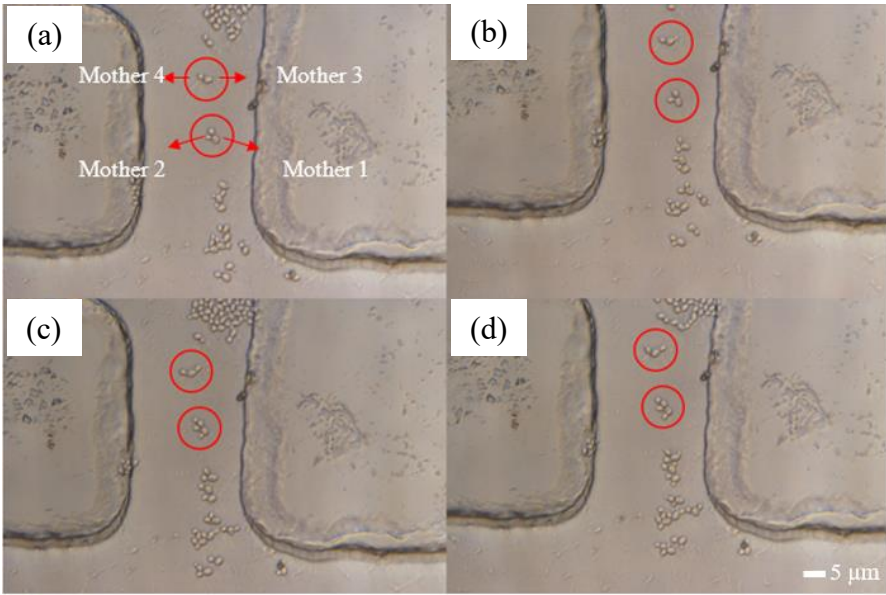


Figure 5.78. The trapped cells in chamber 4 under 40X (a) 15th minute (b) 50th minute (c) 85th minute (d) 110th minute

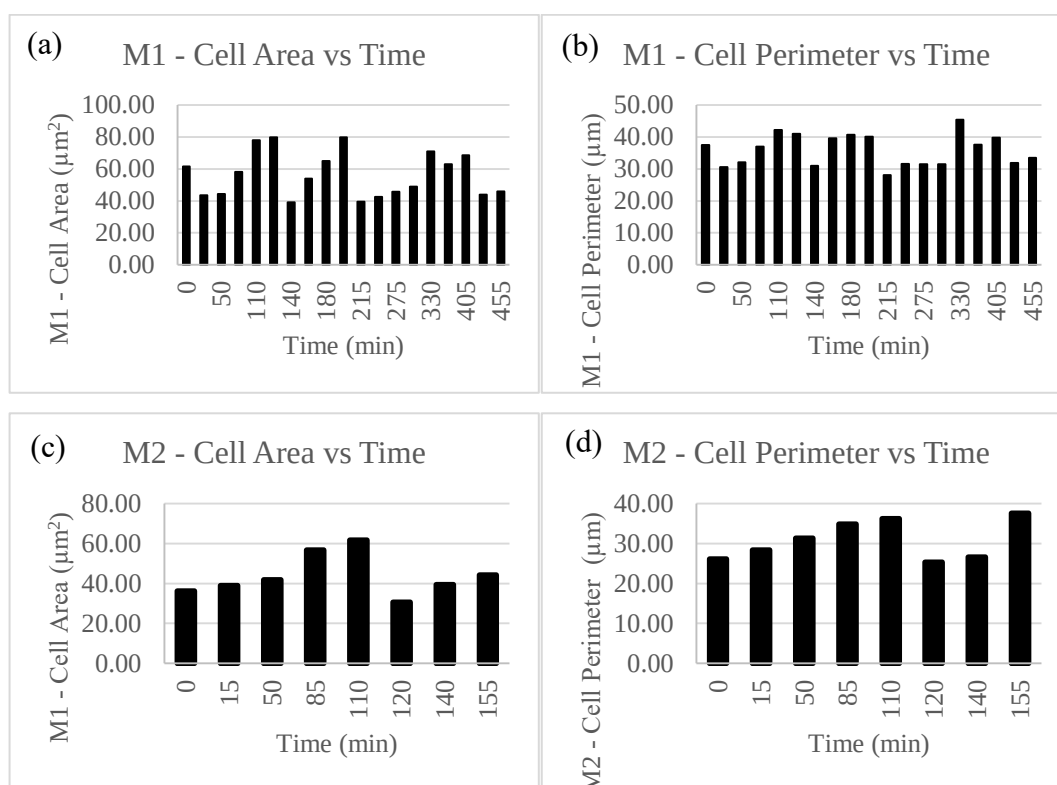


Figure 5.79. Graphical representation of processed data of chamber 4 (a) M1 area vs time graph (b) M1 perimeter vs time graph (c) M2 area vs time graph (d) M2 perimeter vs time graph – EXP5

The duration of the cell cycle of the all cells were compared in each medium (Figures 5.210 and 5.211). The duration of G_1 and $S/G_2/M$ phases were given as 22 and 59 min for wild type cells in glucose rich YPD medium (Brewer *et al.*, 1984). The duration of the G_1 phases a little bit longer but $S/G_2/M$ phases are generally shorter than the given values. The duration of $S/G_2/M$ phases increased after the cells were exposed to MMS.

In experiment 6, the response of the *sld7 Δ* mutant cells were observed in YPD medium containing 2% glucose and inhibitor. In that case, it was observed that the damaged DNA was repaired in *sld7 Δ* mutant cells after feeding with fresh medium (N1R). Yet, the repair mechanism could not be observed for the wild type cells. The comparison of the results of the durations of the G_1 and $S/G_2/M$ phases of *sld7* deletion mutant (W303 background) and wild type (BY4743) cells show that cells are able to survive without Sld7 (repairing the damaged DNA), while Sld7 is present, they cannot repair the DNA. The

duration of the loading time of fresh medium might not enough to repair the DNA itself. (Table 5.84).

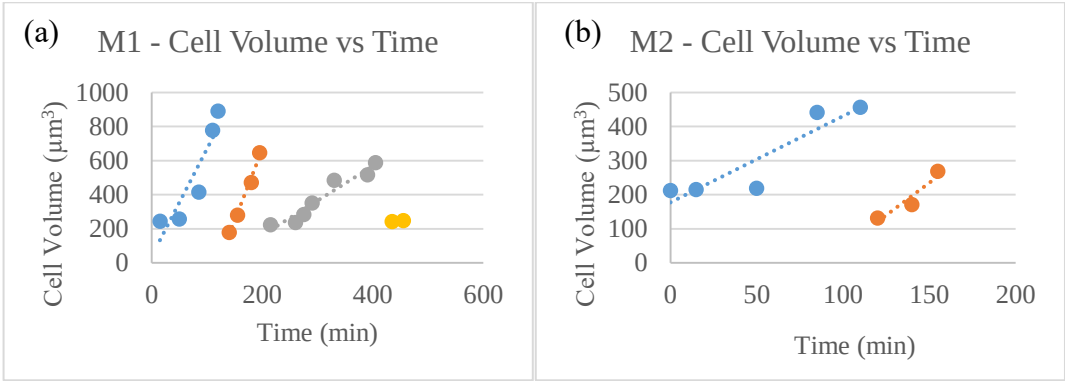


Figure 5.80. Graphical representation of processed data of chamber 4 (a) mother cell 1 volume vs time graph (b) mother cell 1 volume vs time graph - EXP 5

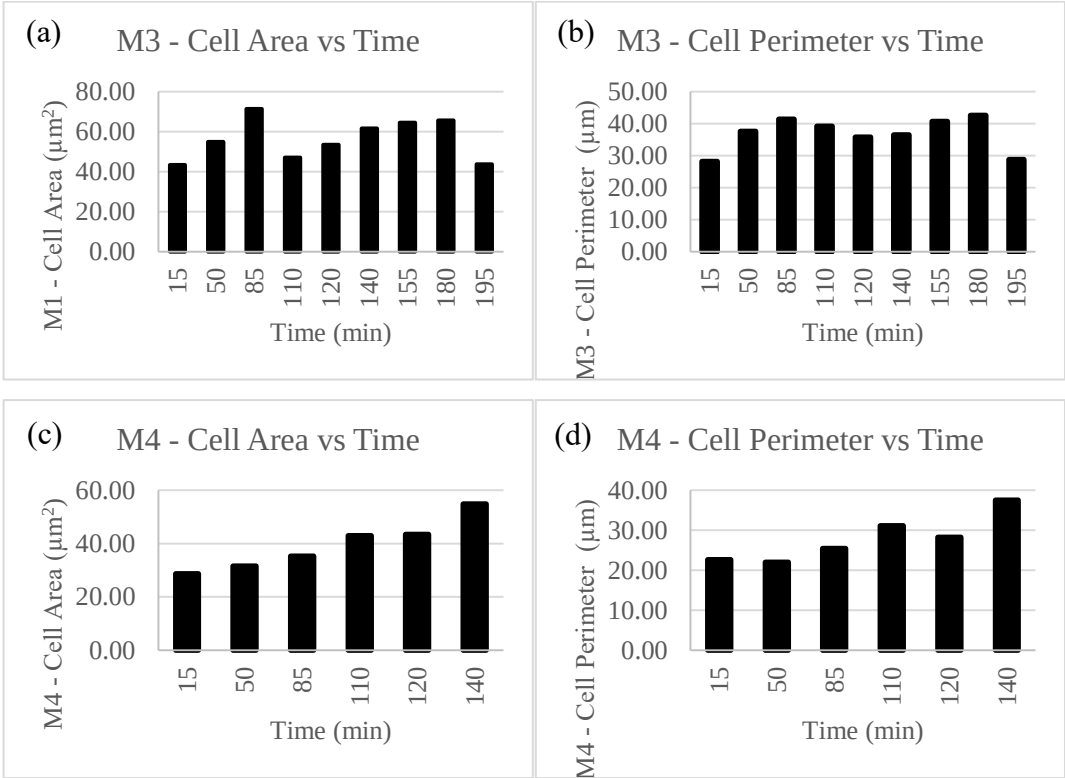


Figure 5.81. Graphical representation of processed data of chamber 4 (a) mother cell 3 area vs time graph (b) mother cell 3 perimeter vs time graph (c) mother cell 4 area vs time graph (d) mother cell 4 perimeter vs time graph – EXP5

Table 5.16. Duration of G1 phase and duration of S/G2/M phases of each buds in chamber

4

Cell Name	Bud Number	G1 phase (min)	S/G2/M phases (min)	Cell Cycle (min)	Area (μm^2)
M1	1	Unknown	≥ 15	> 15	40-80
M1	2	35	70	105	40-80
M1	3	20	75	95	40-70
M1	4	75	40	115	60-70
M1	5	60	45	105	40-50
M2	1	35	70	105	35-65
M2	2	15	≥ 15	> 30	30-45
M3	1	35	70	105	40-70
M3	2	20	40	60	45-65
M4	1	100	Unknown	> 100	35-55

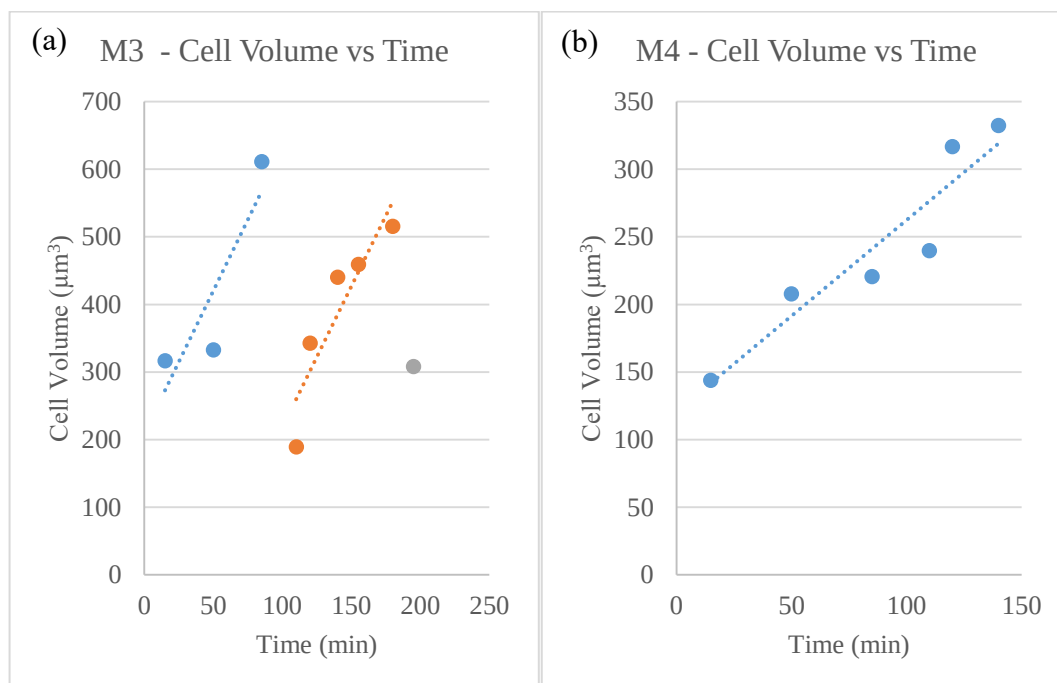


Figure 5.82. Graphical representation of processed data of chamber 4 (a) mother cell 3 volume (b) mother cell 4 volume – EXP5

Table 5.17. The fold change in area, perimeter and volume of the M1 and M2 cells in chamber 4

Time (min)	M1-Cell Area Fold Change	M1-Cell Perimeter Fold Change	M1- Volume Fold Change	M2-Cell Area Fold Change	M2-Cell Perimeter Fold Change	M2- Volume Fold Change
0	1.00	1.00	1.00	1.00	1.00	1.00
15	1.00	1.00	1.00	1.08	1.09	1.01
50	1.02	1.05	1.06	1.16	1.20	1.03
85	1.34	1.21	1.70	1.56	1.33	2.08
110	1.79	1.38	3.18	1.70	1.39	2.15
120	1.83	1.34	3.64	1.00	1.00	1.00
140	1.00	1.00	1.00	1.29	1.05	1.29
155	1.38	1.28	1.56	1.45	1.48	2.04
180	1.66	1.31	2.64	-	-	-
195	2.03	1.29	3.60	-	-	-
215	1.00	1.00	1.00	-	-	-
260	1.08	1.13	1.06	-	-	-
275	1.15	1.12	1.26	-	-	-
290	1.23	1.12	1.56	-	-	-
330	1.79	1.62	2.16	-	-	-
390	1.59	1.34	2.31	-	-	-
405	1.73	1.42	2.62	-	-	-
435	1.00	1.00	1.00	-	-	-
455	1.05	1.05	1.03	-	-	-

Table 5.18. The fold change in area, perimeter and volume of the M3 and M4 cells in chamber 4

Time (min)	M3-Cell Area Fold Change	M3-Cell Perimeter Fold Change	M3-Volume Fold Change	M4-Cell Area Fold Change	M4-Cell Perimeter Fold Change	M4-Volume Fold Change
15	1.00	1.00	1.00	1.00	1.00	1.00
50	1.26	1.33	1.05	1.10	1.05	1.44
85	1.64	1.47	1.93	1.23	1.21	1.53
110	1.00	1.00	1.00	1.50	1.48	1.67
120	1.13	1.23	1.01	1.51	1.35	2.20
140	1.31	1.25	1.30	1.91	1.79	2.31
155	1.37	1.40	1.36	-	-	-
180	1.39	1.46	1.52	-	-	-
195	1.00	1.00	1.00	-	-	-

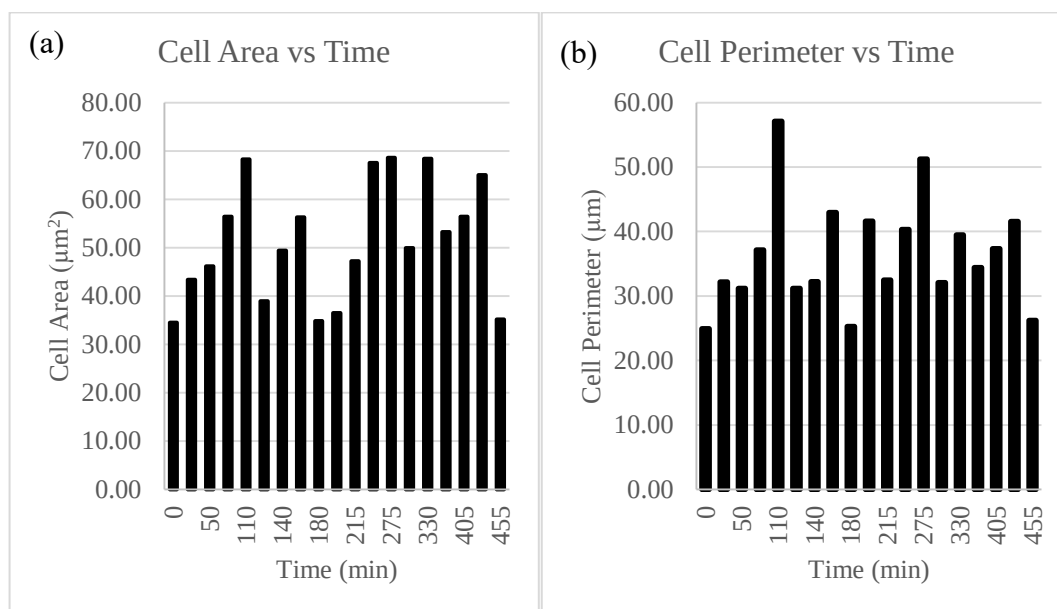


Figure 5.83. Graphical representation of processed data of chamber 5 (a) cell area vs time graph (b) cell perimeter vs time graph – EXP5

Table 5.19. Duration of G1 phase and duration of S/G2/M phases of each buds in chamber

5

Bud Number	G1 phase (min)	S/G2/M phases (min)	Cell Cycle (min)	Area (μm^2)
1	50	60	110	35-70
2	10	60	70	35-60
3	35	60	95	35-70
4	15	40	55	45-70
5	60	65	125	50-65

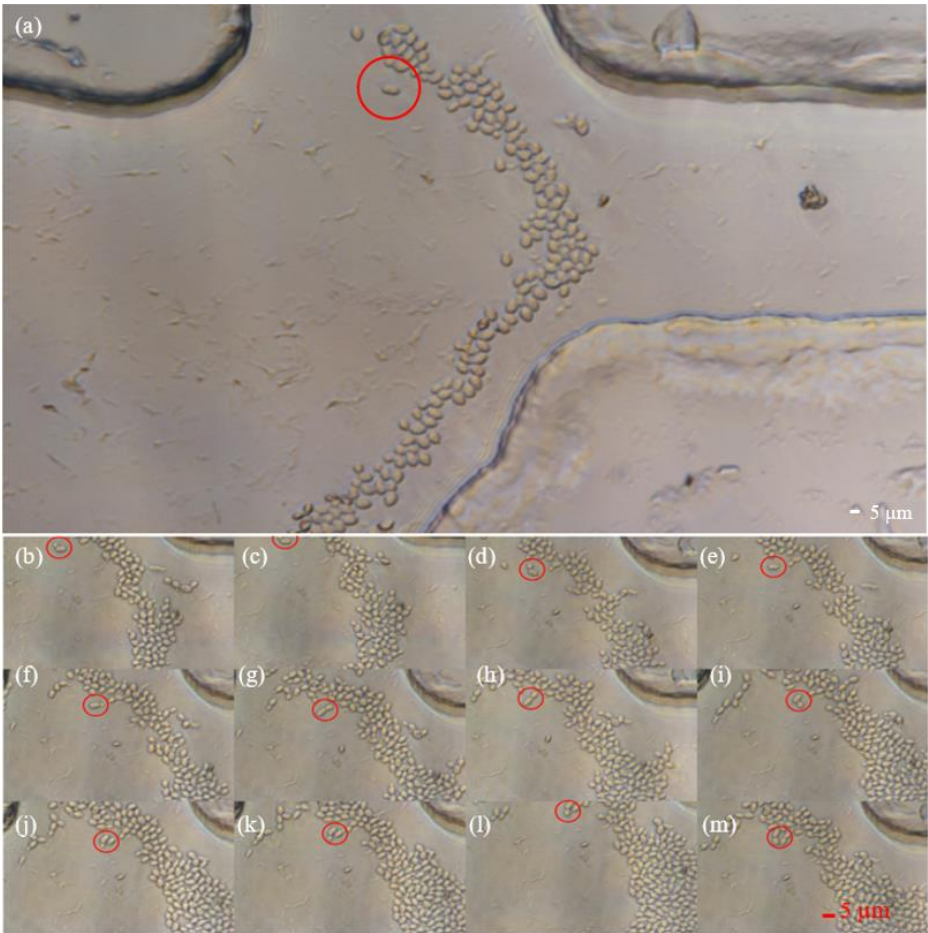


Figure 5.84. The trapped cells in chamber 5 under 90X (a) 0th minute (b) 140th minute (c) 155th minute (d) 180th minute (e) 195th minute (f) 215th minute (g) 260th minute (h) 275th minute (i) 290th minute (j) 330th minute (k) 345th minute (l) 360th minute (m) 380th minute

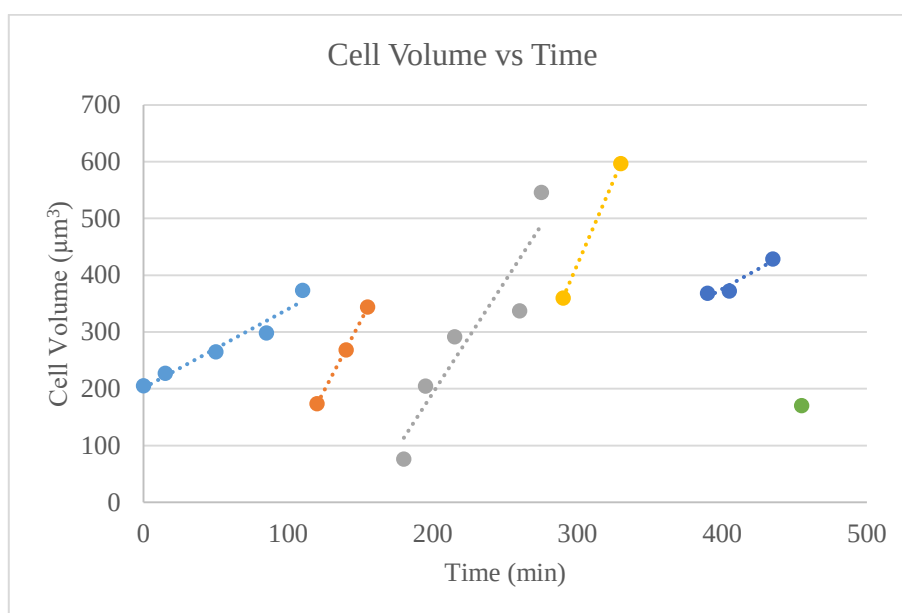


Figure 5.85. Graphical representation of processed data of chamber 5, cell volume vs time graph - EXP 5

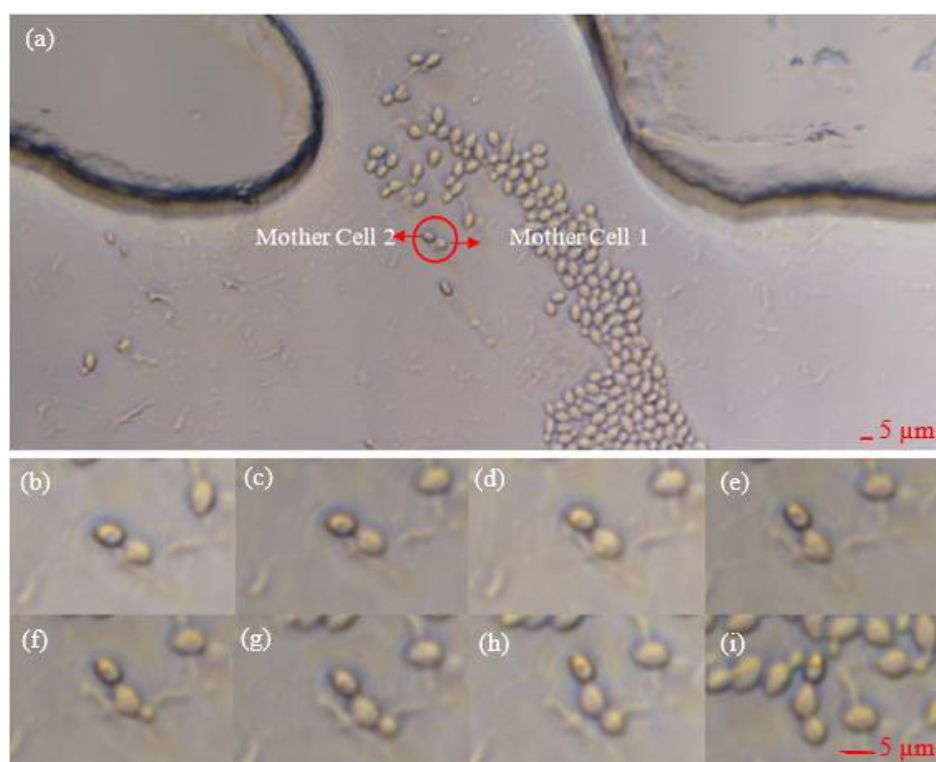


Figure 5.86. The trapped cells in chamber 6 (a) 0th minute under 40X (b) 0th minute (c) 15th minute (d) 50th minute (e) 85th minute (f) 110th minute (g) 120th minute (h) 140th minute (i) 155th minute under 90X

Table 5.20. The fold change in area, perimeter and volume of the cell in chamber 5

Time (min)	Cell Area Fold change	Cell Perimeter Fold Change	Cell Volume Fold Change
0	1.00	1.00	1.00
15	1.26	1.29	1.16
50	1.34	1.25	1.44
85	1.63	1.49	1.91
110	1.98	2.29	2.24
120	1.00	1.00	1.00
140	1.27	1.03	2.01
155	1.45	1.38	2.57
180	1.00	1.00	1.00
195	1.05	1.65	1.16
215	1.35	1.28	1.66
260	1.94	1.60	1.91
275	1.97	2.03	1.96
290	1.00	1.00	1.00
330	1.37	1.23	1.85
390	1.07	1.07	1.00
405	1.13	1.16	1.70
435	1.30	1.29	2.05
455	1.00	1.00	1.00

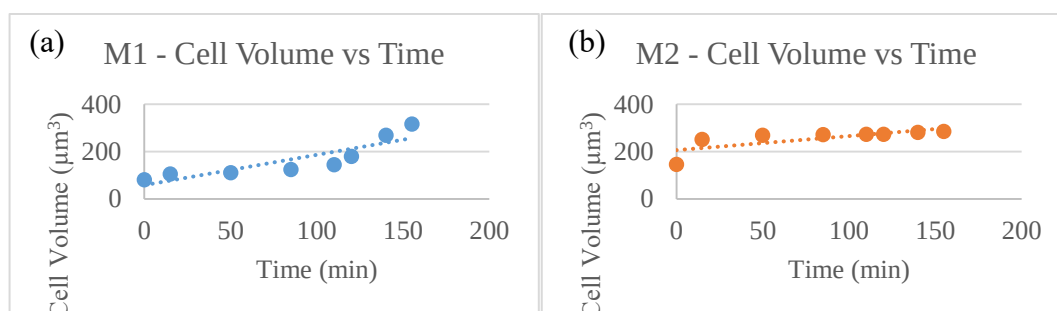


Figure 5.87. Graphical representation of processed data of chamber 6 (a) mother cell 1 volume vs time graph (b) mother cell 2 volume vs time graph - EXP 5

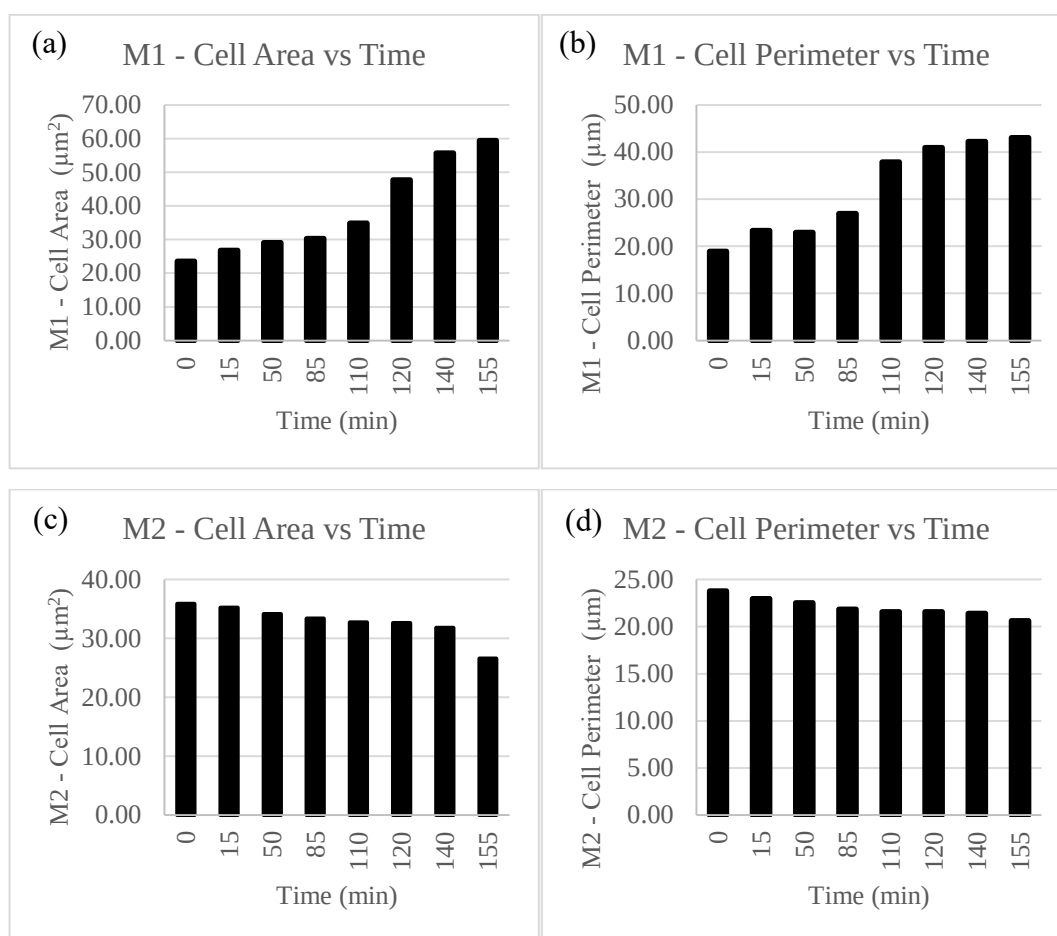


Figure 5.88. Graphical representation of processed data of chamber 6 (a) cell area vs time graph (b) cell perimeter vs time graph (c) mother cell 2 area vs time graph (d) mother cell 2 perimeter vs time graph – EXP5

Table 5.21. The fold change in area, perimeter and volume of the M1 and M2 cells in chamber 6

Time (min)	M1-Cell Area Fold Change	M1-Cell Perimeter Fold Change	M1-Cell Volume Fold change	M2-Cell Area Fold Change	M2-Cell Perimeter Fold Change	M2-Cell Volume Fold change
0	1.00	1.00	1.00	1.00	1.00	1.00
15	1.14	1.23	1.30	0.98	0.97	0.98

Table 5.22. The fold change in area, perimeter and volume of the M1 and M2 cells in chamber 6 (cont.).

Time (min)	M1-Cell Area Fold Change	M1-Cell Perimeter Fold Change	M1-Cell Volume Fold change	M2-Cell Area Fold Change	M2-Cell Perimeter Fold Change	M2-Cell Volume Fold change
50	1.23	1.21	1.38	0.95	0.95	0.96
85	1.29	1.42	1.54	0.93	0.92	0.95
110	1.48	2.00	1.80	0.91	0.91	0.95

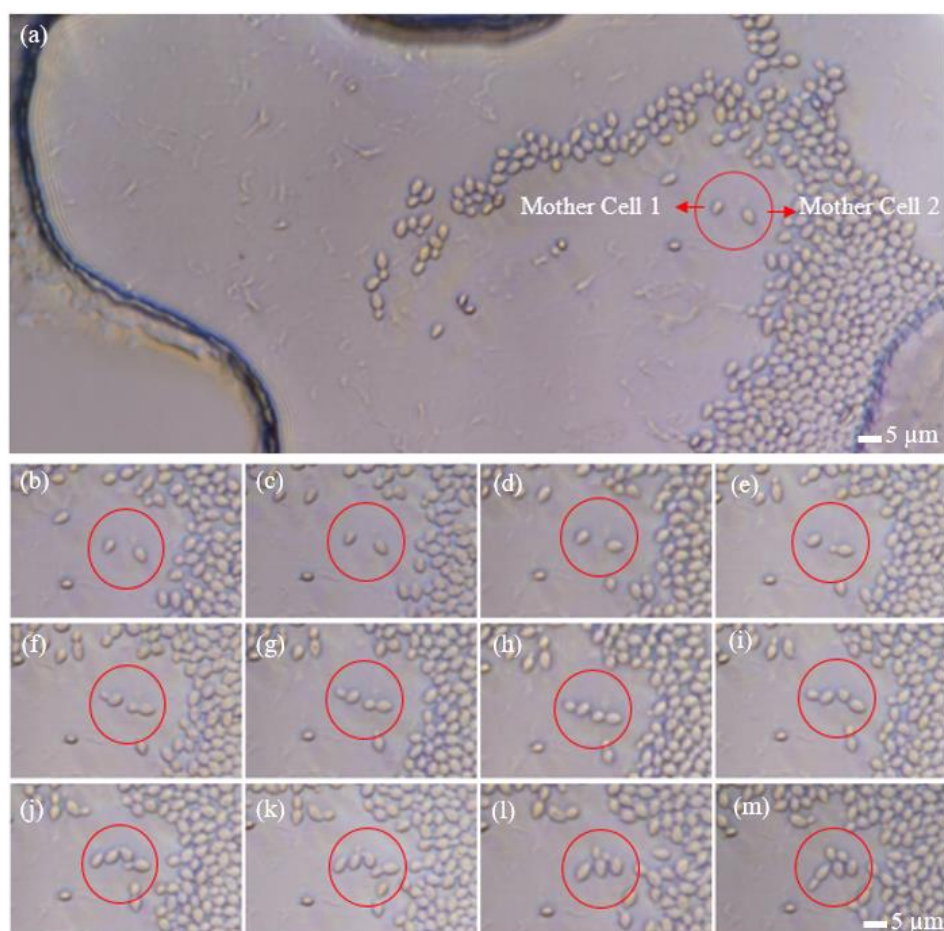


Figure 5.89. The trapped cells in chamber 7 under 40X (a) 0th minute (b) 0th minute (c) 15th minute (d) 50th minute (e) 85th minute (f) 110th minute (g) 120th minute (h) 140th minute (i) 155th minute (j) 180th minute (k) 195th minute (l) 215th minute (m) 260th minute

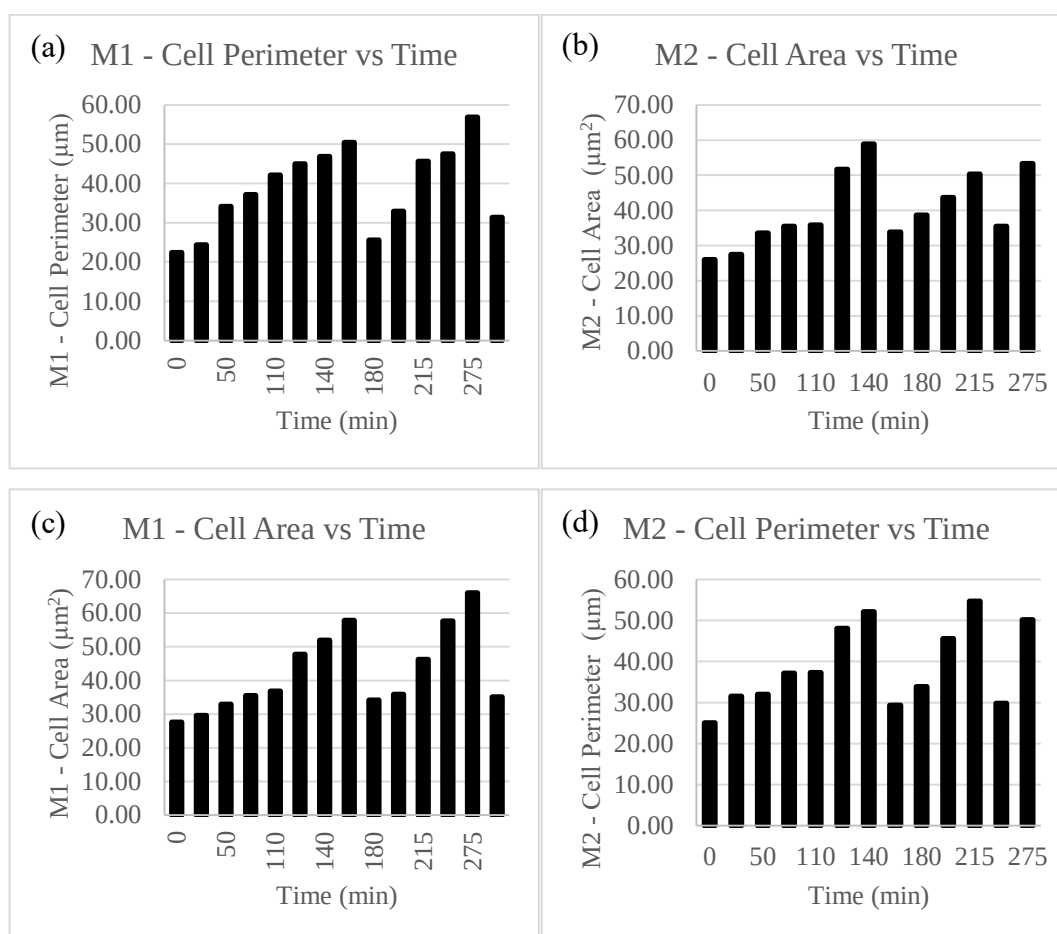


Figure 5.90. Graphical representation of processed data of chamber 7 (a) mother cell 1 area vs time graph (b) mother cell 1 perimeter vs time graph (c) mother cell 2 area vs time graph (d) mother cell 2 perimeter vs time graph – EXP5

Table 5.22. Duration of G1 phase and duration of S/G2/M phases of each buds in chamber

7

Cell Name	Bud Number	G1 phase (min)	S/G2/M phases (min)	Cell Cycle (min)	Area (μm^2)
M1	1	85	70	145	25-60
M1	2	25	80	105	35-65
M2	1	85	70	145	25-60
M2	2	25	95	120	35-50
M2	3	15	≥ 55	> 70	35-55

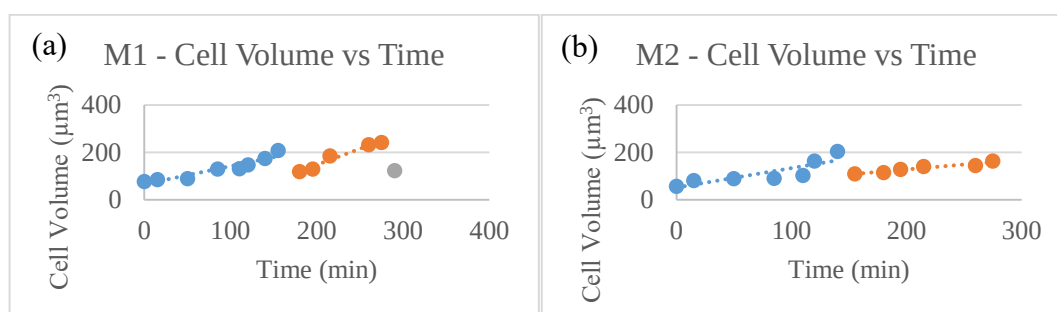


Figure 5.91. Graphical representation of processed data of chamber 7 (a) mother cell 1 volume vs time graph (b) mother cell 2 volume vs time graph - EXP 5

Table 5.23. The fold change in area, perimeter and volume of the M1 and M2 cells in chamber 7

Time (min)	M1-Cell Area Fold Change	M1-Cell Perimeter Fold Change	M1-Cell Volume Fold Change	M2-Cell Area Fold Change	M2-Cell Perimeter Fold Change	M2-Cell Volume Fold Change
0	1.00	1.00	1.00	1.00	1.00	1.00
15	1.07	1.09	1.10	1.05	1.26	1.41
50	1.19	1.53	1.15	1.29	1.28	1.55
85	1.29	1.66	1.69	1.36	1.48	1.59
110	1.34	1.88	1.71	1.38	1.49	1.81
120	1.73	2.01	1.92	1.99	1.92	2.86
140	1.88	2.09	2.27	2.27	2.08	3.57
155	2.10	2.25	2.70	1.00	1.00	1.00
180	1.00	1.00	1.00	1.14	1.15	1.05
195	1.05	1.28	1.09	1.29	1.55	1.18
215	1.35	1.78	1.55	1.49	1.86	1.29
260	1.69	1.86	1.96	1.00	1.00	1.32
275	1.93	2.22	2.03	1.50	1.68	1.34
290	1.00	1.00	1.00	-	-	-

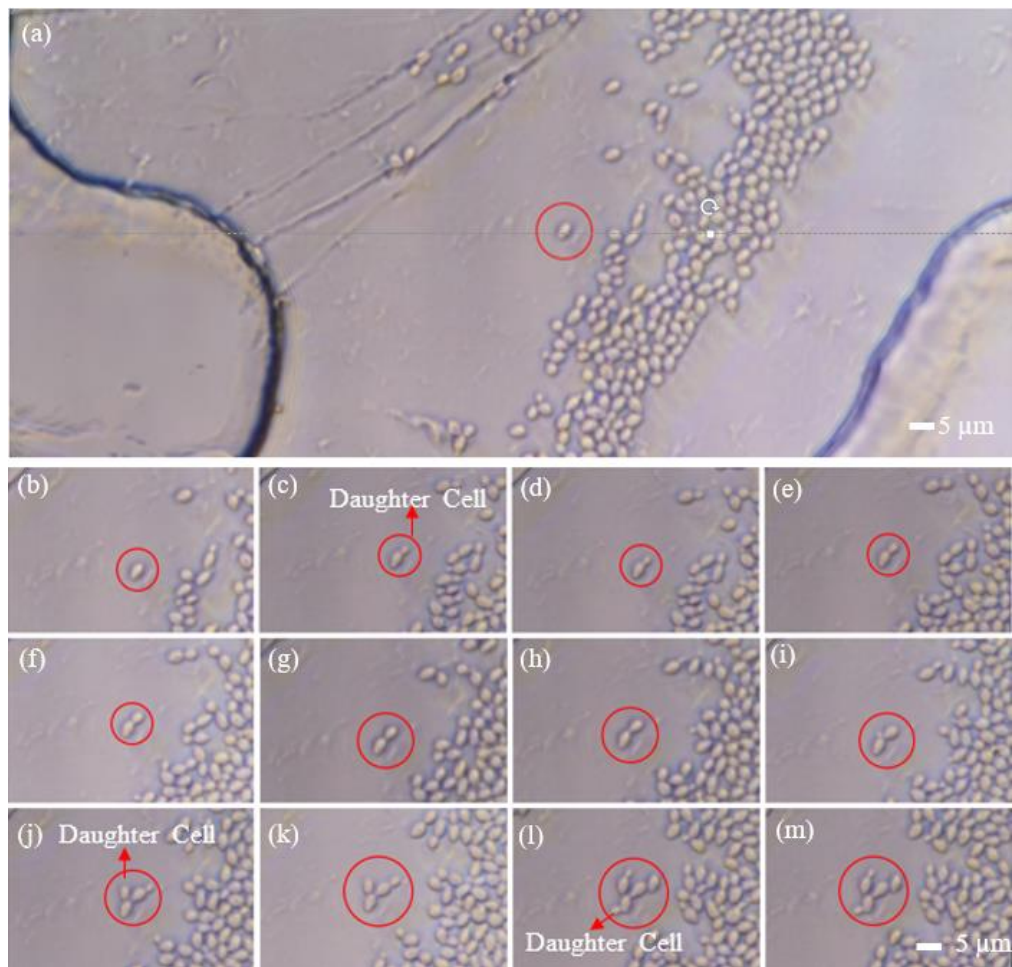


Figure 5.92. The trapped cells in chamber 8 under 40X (a) 85th minute (b) 85th minute (c) 110th minute (d) 120th minute (e) 140th minute (f) 155th minute (g) 180th minute (h) 195th minute (i) 215th minute (j) 260th minute (k) 275th minute (l) 290th minute (m) 330th minute

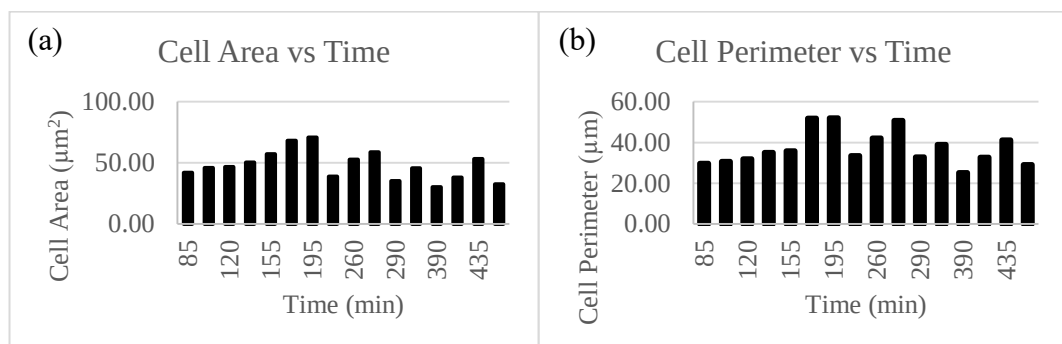


Figure 5.93. Graphical representation of processed data of chamber 8 (a) cell area vs time graph (b) cell perimeter vs time graph – EXP5

Table 5.24. Duration of G1 phase and duration of S/G2/M phases of each buds in chamber

8

Bud Number	G1 phase (min)	S/G2/M phases (min)	Cell Cycle (min)	Area (μm^2)
1	25	85	110	40-70
2	20	60	80	35-60
3	15	60	75	35-45
4	45	20	65	30-55

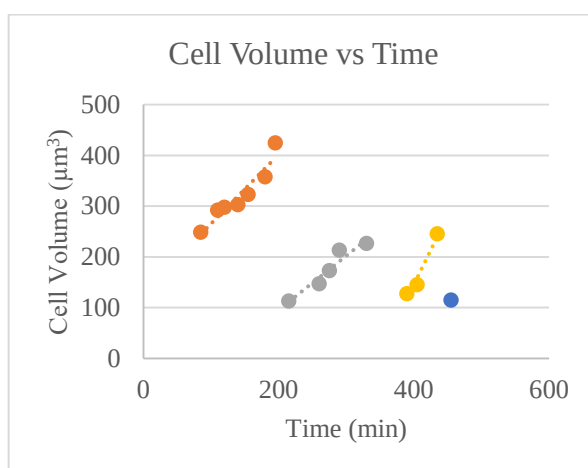


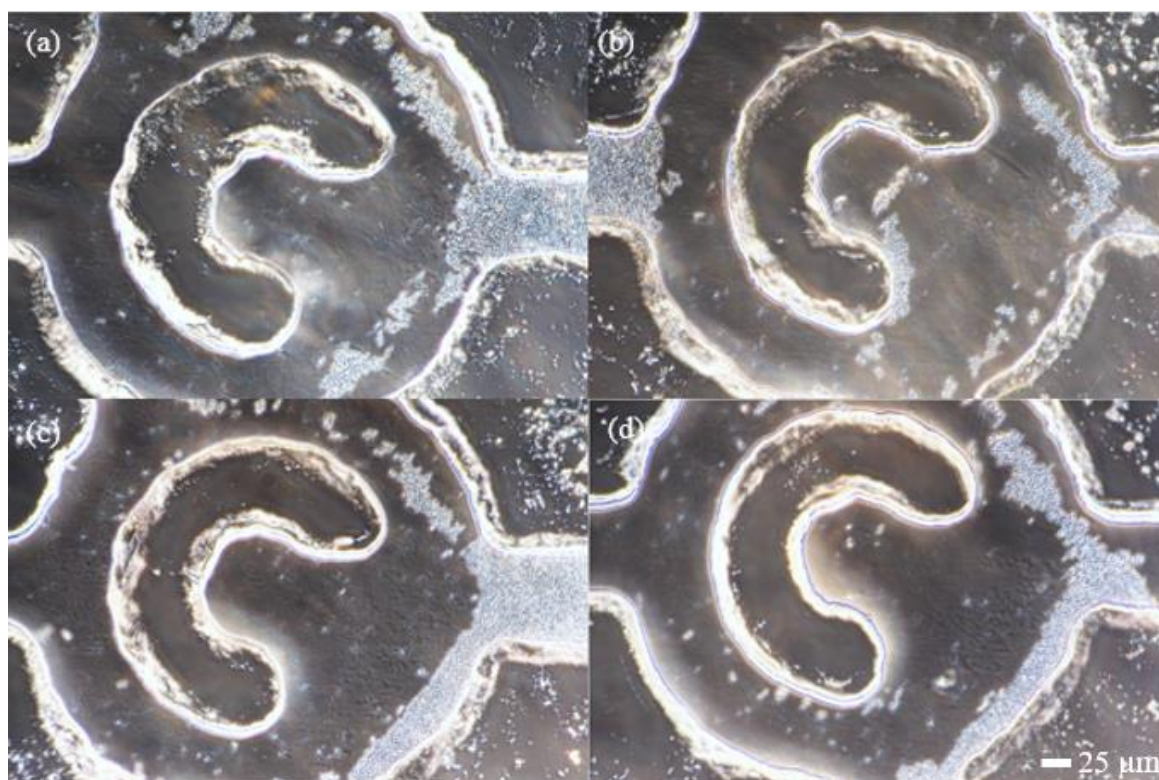
Figure 5.94. Graphical representation of processed data of chamber 8, cell volume vs time graph – EXP5

Table 5.25. The fold change in area, perimeter and volume of the cell in chamber 8

Time (min)	Cell Area Fold Change	Cell Perimeter Fold Change	Cell Volume Fold Change
85	1.00	1.00	1.00
110	1.09	1.04	1.37
120	1.11	1.08	1.70
140	1.20	1.18	2.01
155	1.36	1.21	2.19

Table 5.26. The fold change in area, perimeter and volume of the cell in chamber 8 (cont.).

Time (min)	Cell Area Fold Change	Cell Perimeter Fold Change	Cell Volume Fold Change
180	1.63	1.75	2.25
195	1.69	1.76	2.41
215	1.00	1.00	1.00
260	1.35	1.26	1.12
275	1.51	1.51	1.56
290	1.00	1.00	1.80
330	1.30	1.19	2.13
390	1.00	1.00	1.00
405	1.27	1.30	1.14

Figure 5.95. Images of (a) chamber 1 (b) chamber 2 (c) chamber 3 (d) chamber 4 at 455th minute

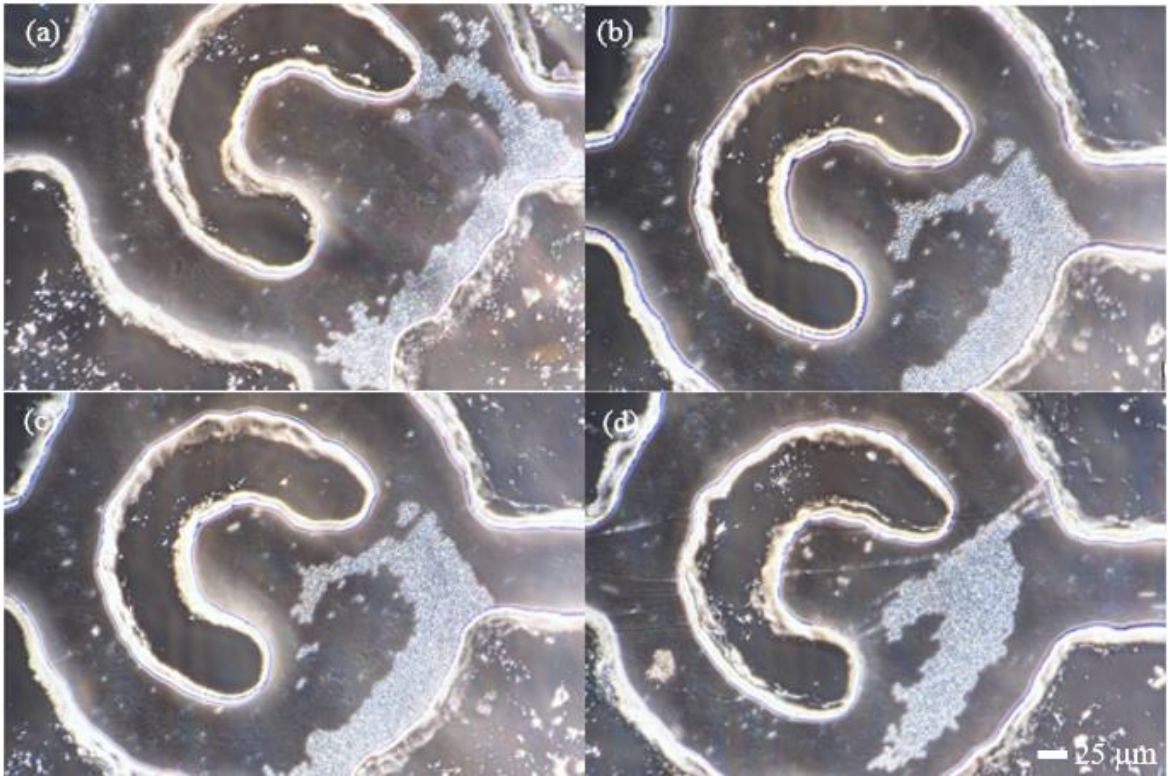


Figure 5.96. Images of (a) chamber 5 (b) chamber 6 (c) chamber 7 (d) chamber 8 at 455th minute

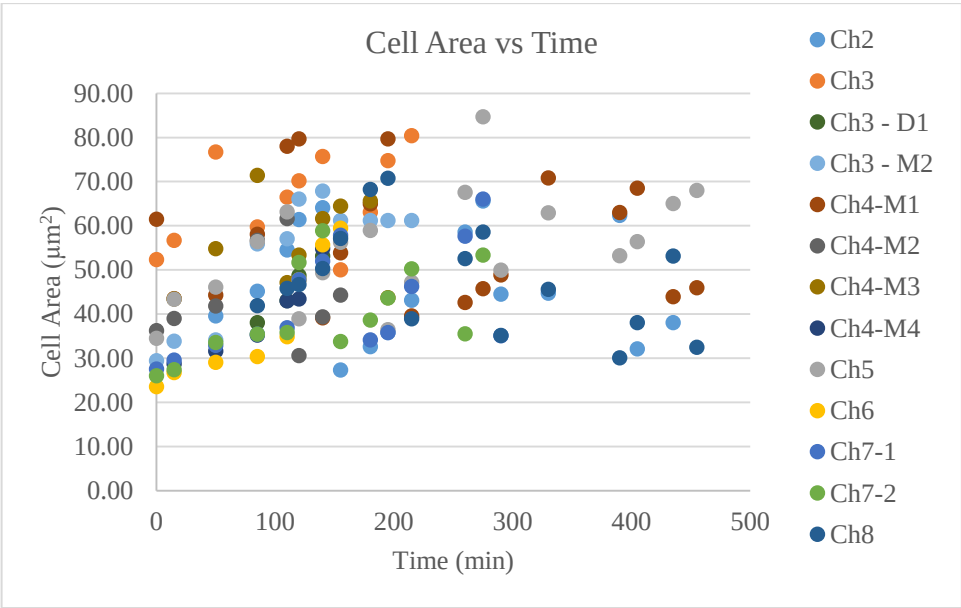


Figure 5.97. Comparison of the area of the cells at different chambers

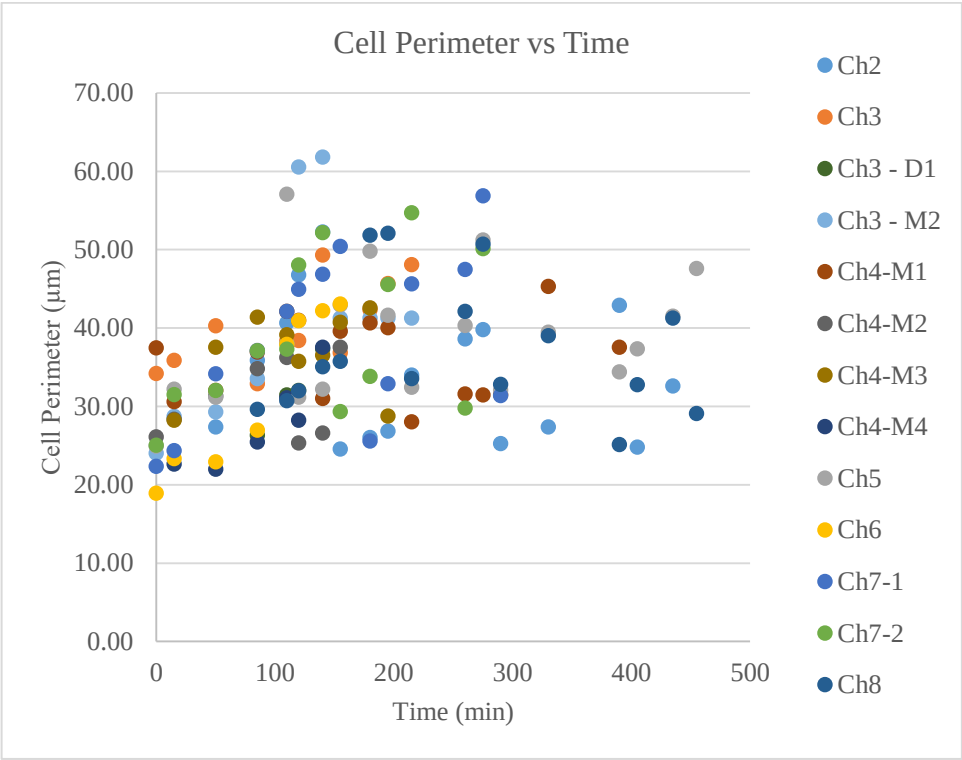


Figure 5.98. Comparison of the perimeter of the cells at different chambers

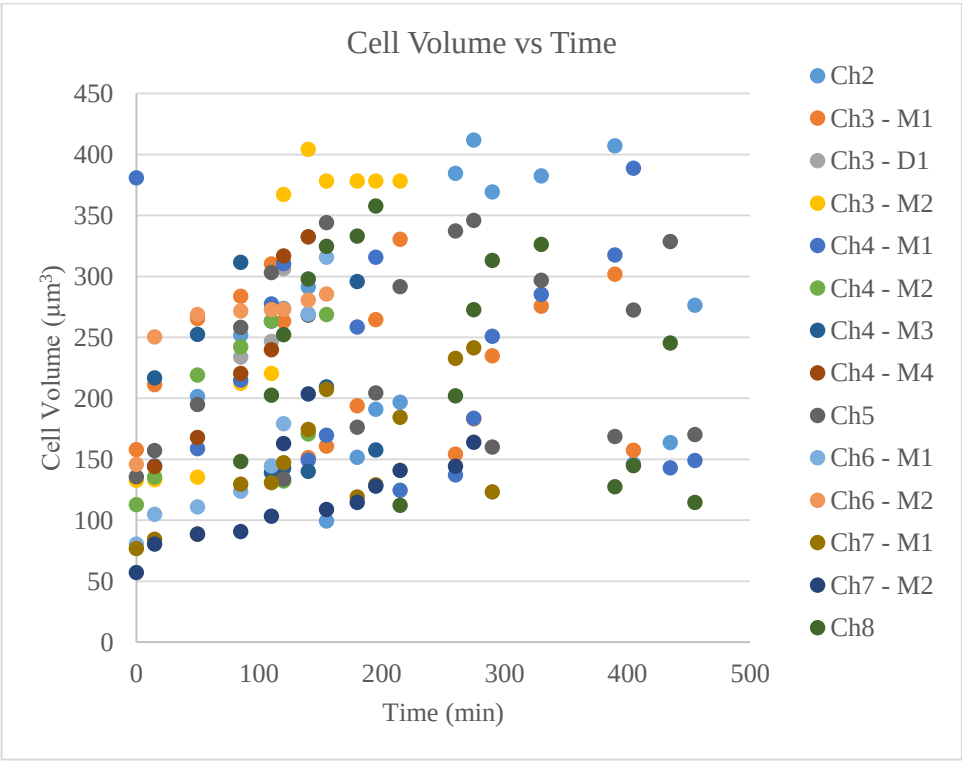


Figure 5.99. Comparison of the volume of the cells at different chambers

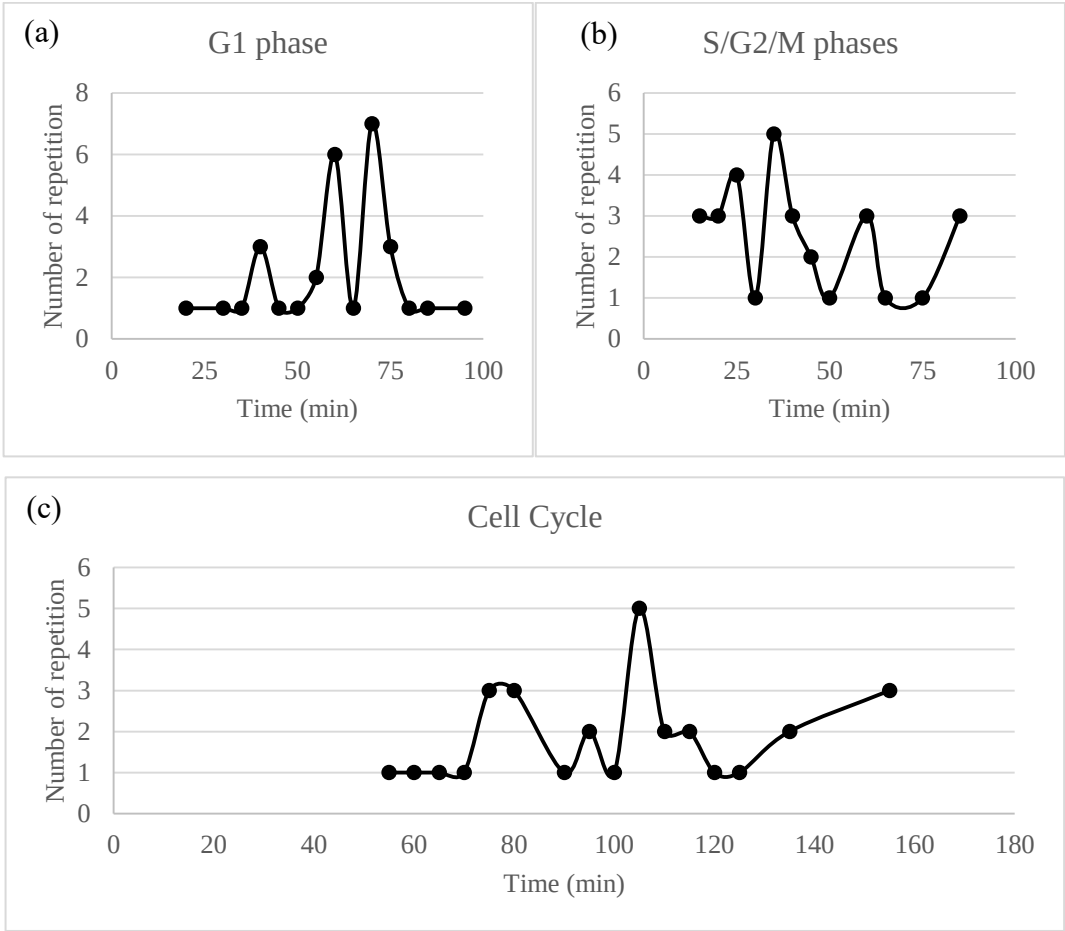


Figure 5.100. (a) Duration of G_1 phase (b) duration of S/G2/M phases (c) duration of cell cycle

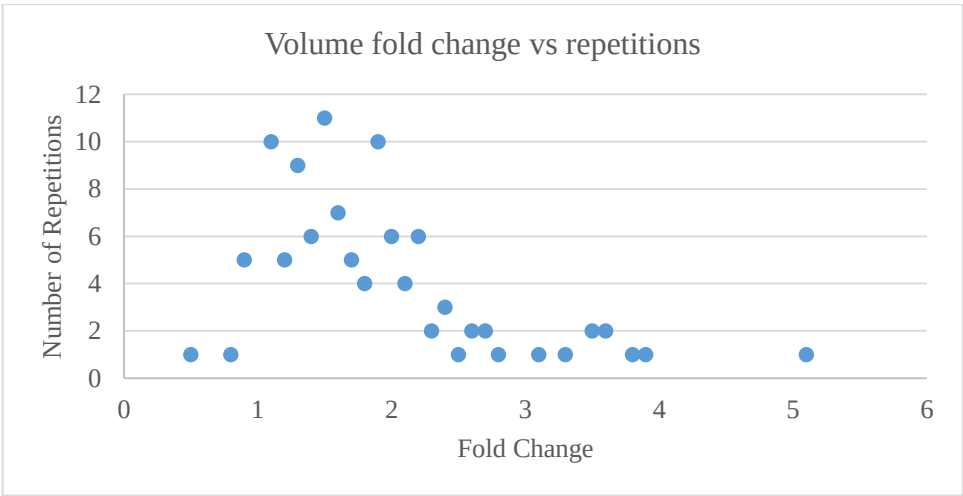


Figure 5.101. Volumetric fold change vs repetitions

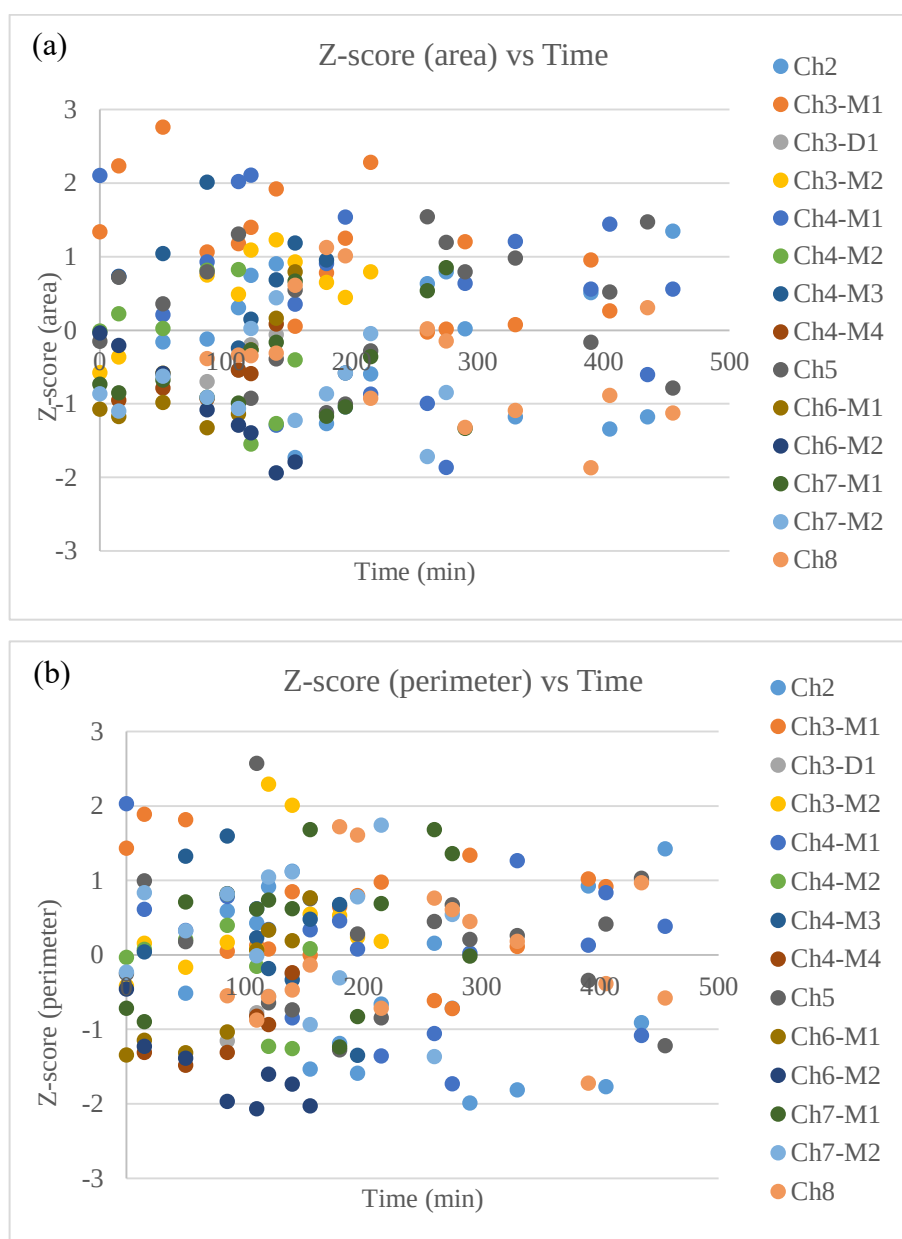


Figure 5.102. Graphical demonstration of Z-score for (a) area (b) perimeter

Table 5.26. The comparison of the duration of cell cycle phases between daughter and mother cells

Cell Name	Ave. G1 (min)	Ave. S/G2/M (min)	Ave. Cell Cycle (min)
Mother	40	60	105
Daughter	25	55	80

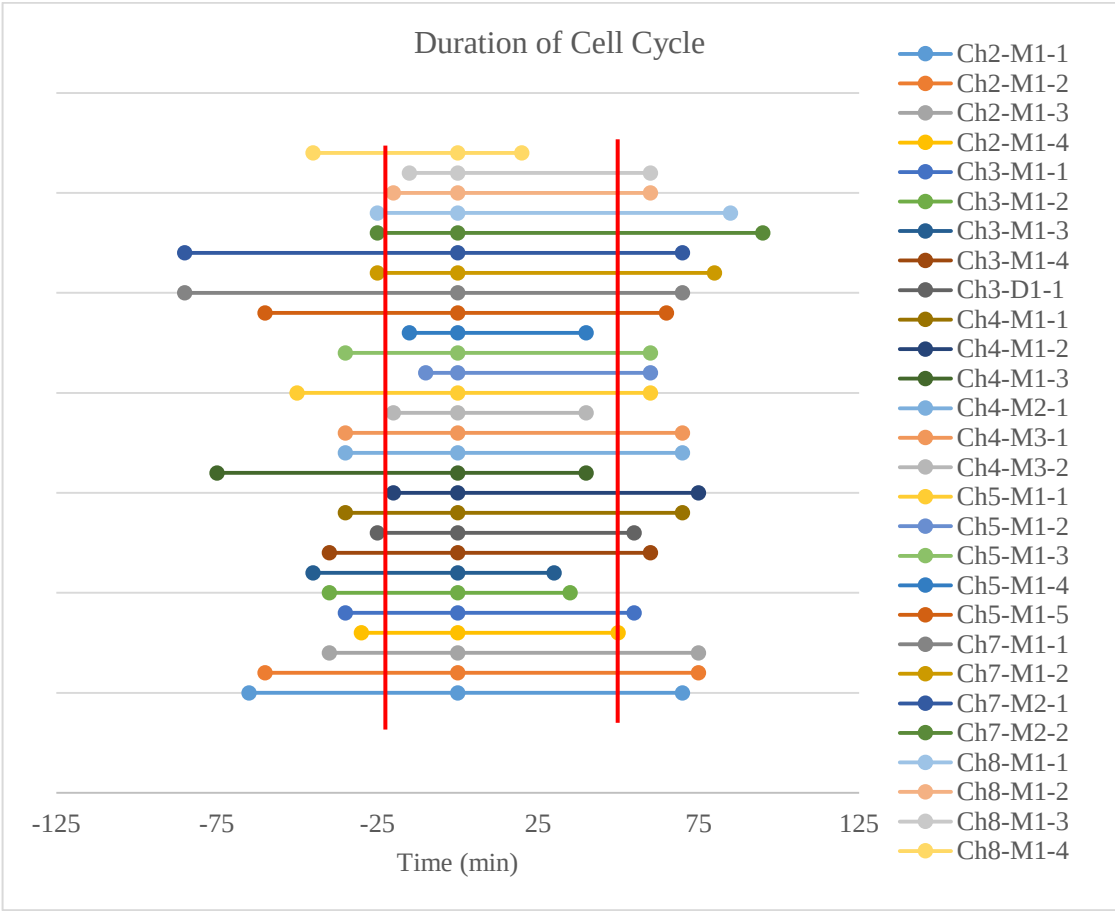


Figure 5.103. The comparison of the duration of cell cycle of the analyzed cells

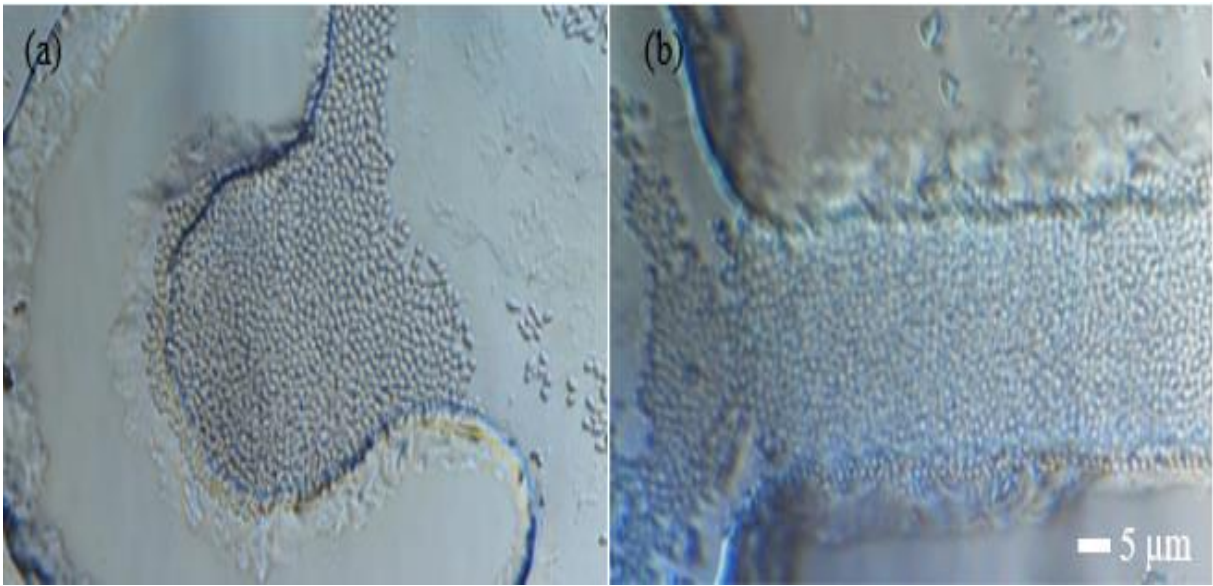


Figure 5.104. Images of (a) chamber 1 (b) middle inlet at the end of the experiment

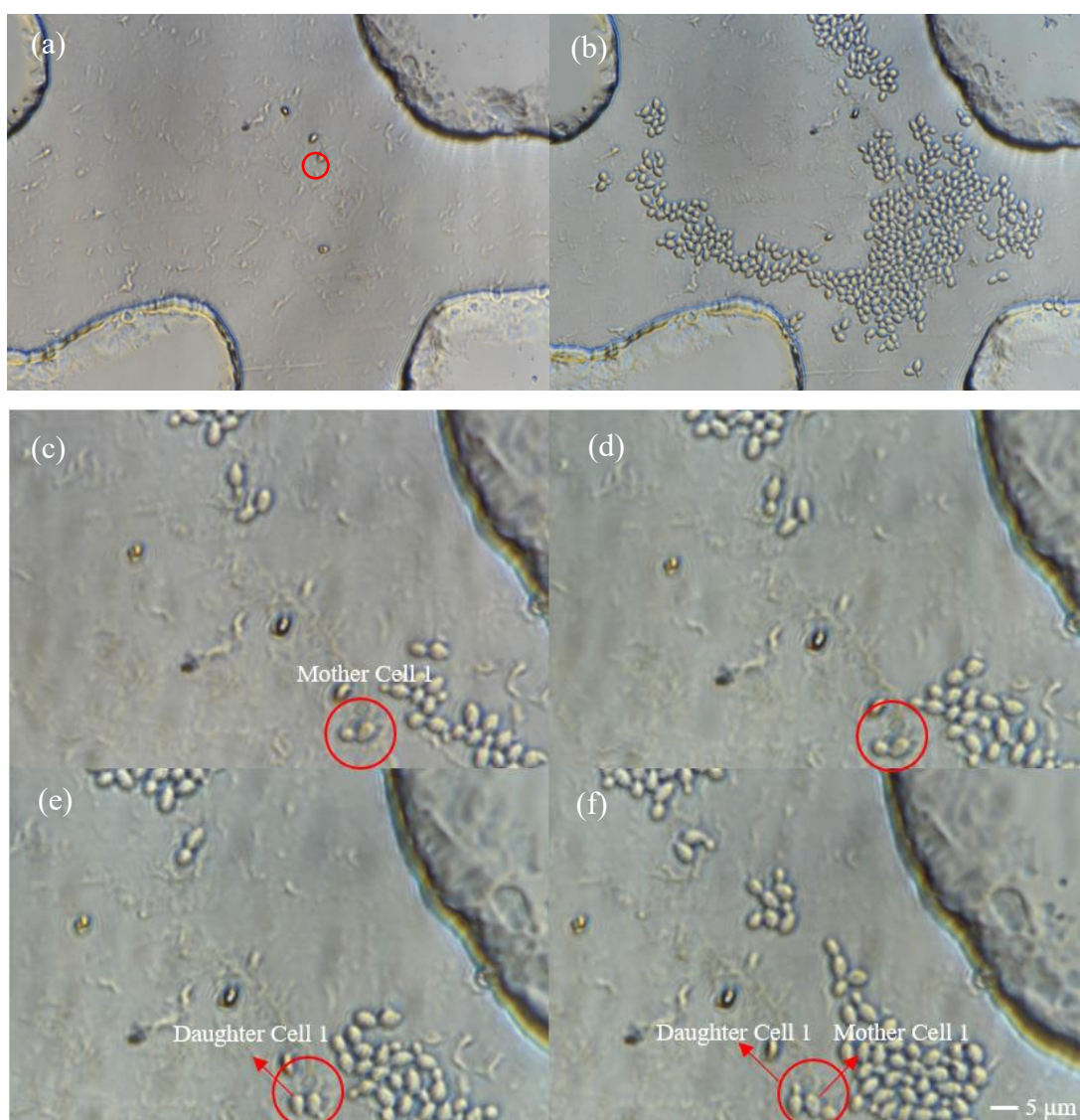


Figure 5.105. Images of chamber 2 (a) 0th minute (b) 740th minute under 40X magnification (c) 195th minute (d) 210th minute (e) 235th minute (f) 280th minute under 90X magnification

Table 5.27. Duration of cell cycle phases of the analyzed cells in chamber 2

Cell Name	Bud Number	Medium Type	G ₁ phase (min)	S/G ₂ /M phases (min)	Cell Cycle (min)	Area (μm ²)
M1	1	N1	85	20	105	30-60
M1	2	N1	20	85	105	40-60

Table 5.27. Duration of cell cycle phases of the analyzed cells in chamber 2 (cont.).

Cell Name	Bud Number	Medium Type	G ₁ phase (min)	S/G ₂ /M phases (min)	Cell Cycle (min)	Area (μm ²)
M1	3	N1-N2	35	125	160	40-60
M1	4	N2-N1R	60	100	160	45-60
D1	1	N1-N2	45	35	80	30-60

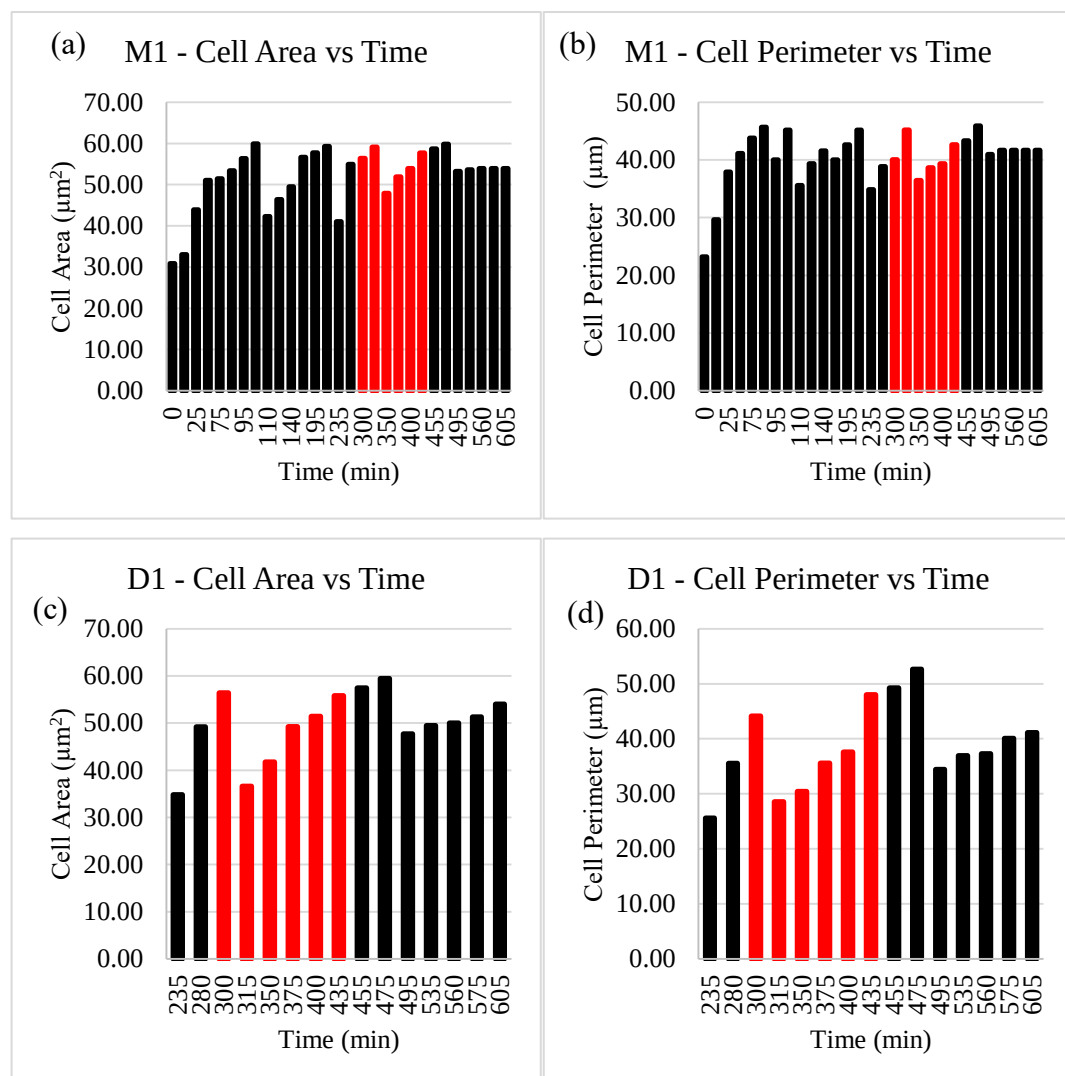


Figure 5.106. Graphical representation of processed data of chamber 2 (a) mother cell 1 area vs time graph (b) mother cell 1 perimeter vs time graph (c) daughter cell 1 area vs time graph (d) daughter cell 1 perimeter vs time graph – EXP 6

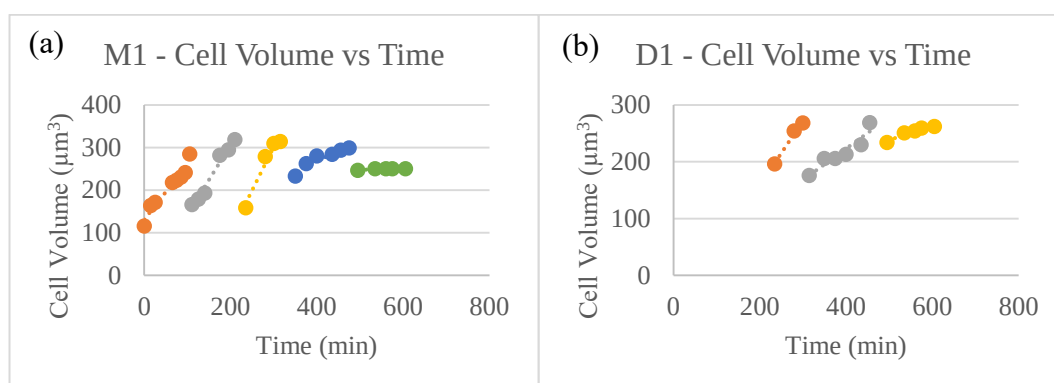


Figure 5.107. Graphical representation of processed data of chamber 2 (a) mother cell 1 volume vs time graph (b) daughter cell 1 volume vs time graph - EXP 6

Table 5.28. The fold change in area, perimeter and volume of the cell in chamber 2

Time	M1-Cell Area Fold Change	M1-Cell Perimeter Fold Change	M1- Volume Fold Change	D1-Cell Area Fold Change	D1-Cell Perimeter Fold Change	D1- Volume Fold Change
0	1.00	1.00	1.00	-	-	-
15	1.07	1.28	1.41	-	-	-
25	1.42	1.63	1.48	-	-	-
65	1.65	1.72	1.88	-	-	-
75	1.67	1.77	1.92	-	-	-
85	1.73	1.89	1.99	-	-	-
95	1.83	1.95	2.08	-	-	-
105	1.94	1.97	2.45	-	-	-
110	1.00	1.00	1.00	-	-	-
125	1.10	1.11	1.07	-	-	-
140	1.17	1.17	1.16	-	-	-
175	1.34	1.13	1.69	-	-	-
195	1.37	1.20	1.77	-	-	-
210	1.40	1.27	1.91	-	-	-

Table 5.28. The fold change in area, perimeter and volume of the cell in chamber 2 (cont.).

Time	M1-Cell Area Fold Change	M1-Cell Perimeter Fold Change	M1- Volume Fold Change	D1-Cell Area Fold Change	D1-Cell Perimeter Fold Change	D1- Volume Fold Change
235	1.00	1.00	1.00	1.00	1.00	1.00
280	1.34	1.11	1.76	1.39	1.42	1.39
300	1.37	1.15	1.95	1.72	1.62	1.72
315	1.44	1.30	1.98	1.00	1.00	1.00
350	1.00	1.00	1.00	1.07	1.14	1.07
375	1.26	1.11	1.65	1.25	1.35	1.25
400	1.31	1.13	1.76	1.32	1.41	1.32
435	1.41	1.22	1.79	1.68	1.52	1.68
455	1.43	1.24	1.85	1.72	1.57	1.72
475	1.46	1.32	1.88	1.84	1.62	1.84
495	1.00	1.00	1.00	1.00	1.00	1.00
535	1.01	1.02	1.01	1.07	1.04	1.07

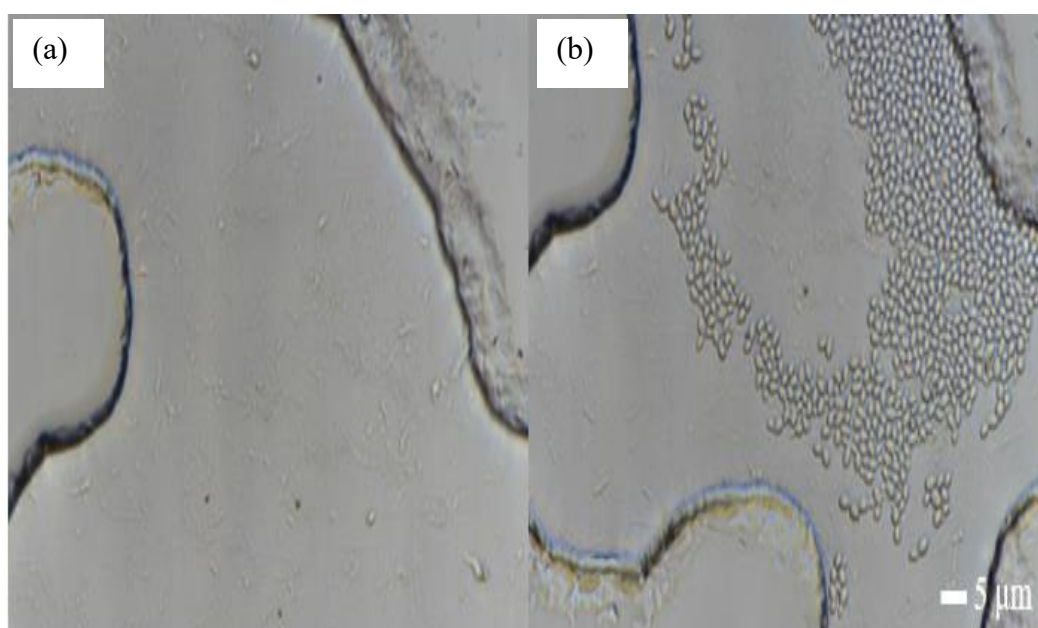
Figure 5.108. Images of chamber 3 (a) 0th minute (b) 740th minute

Table 5.29. Duration of cell cycle phases of the analyzed cells in chamber 3

Cell Name	Bud Number	Medium Type	G ₁ phase (min)	S/G ₂ /M phases (min)	Cell Cycle (min)	Area (μm ²)
M1	1	N2-N1R	-	100	≥100	35-65
M1	2	N1R	40	45	85	40-60
M1	3	N1R	30	-	≥90	40-45

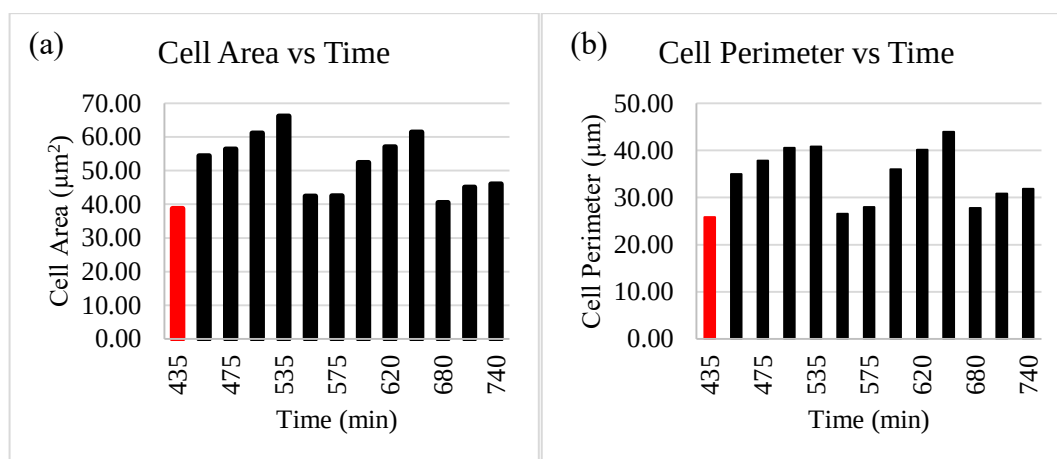


Figure 5.109. Graphical representation of processed data of chamber 3 (a) cell area vs time graph (b) cell perimeter vs time graph– EXP 6

Table 5.30. The fold change in area, perimeter and volume of the cell in chamber 3

Time	Cell Area Fold Change	Cell Perimeter Fold Change	Volume Fold Change
435	1.00	1.00	1.00
455	1.40	1.35	1.04
475	1.46	1.46	1.12
495	1.58	1.57	1.18
535	1.71	1.58	1.40
560	1.00	1.00	1.00

Table 5.30. The fold change in area, perimeter and volume of the cell in chamber 3

Time	Cell Area Fold Change	Cell Perimeter Fold Change	Volume Fold Change
575	1.00	1.06	1.55
605	1.24	1.36	1.60
620	1.35	1.51	1.90
650	1.45	1.66	1.97
680	1.00	1.00	1.00

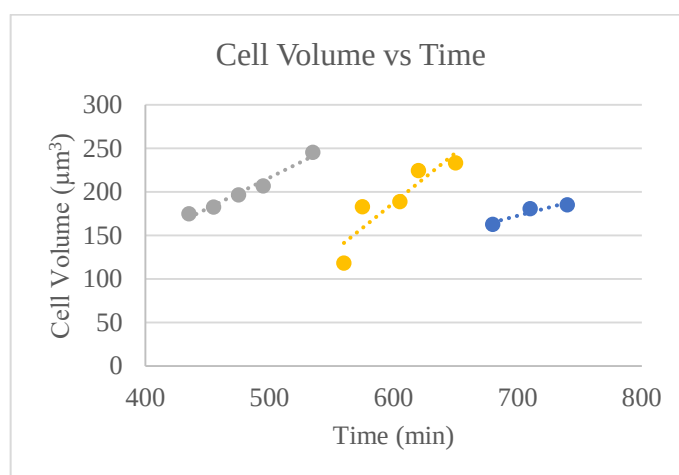


Figure 5.110. Graphical representation of processed data of chamber 3, mother cell 1 volume vs time graph - EXP 6

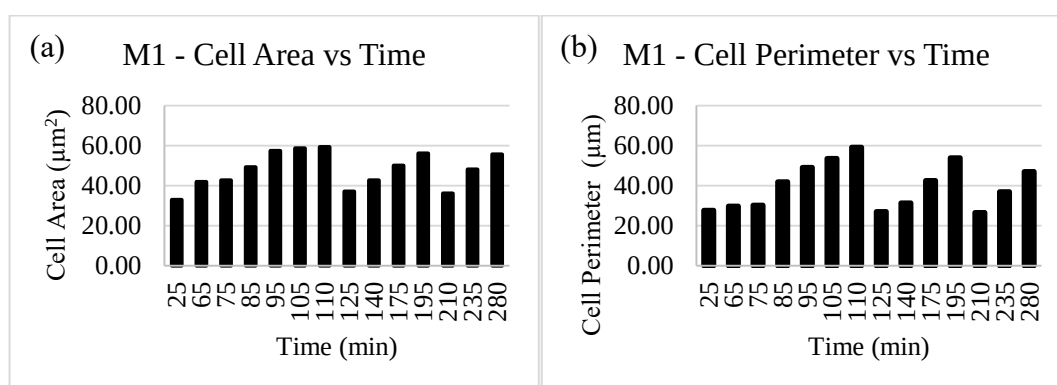


Figure 5.111. Graphical representation of processed data of chamber 4 (a) mother cell 1 area vs time graph (b) mother cell 1 perimeter vs time graph – EXP 6

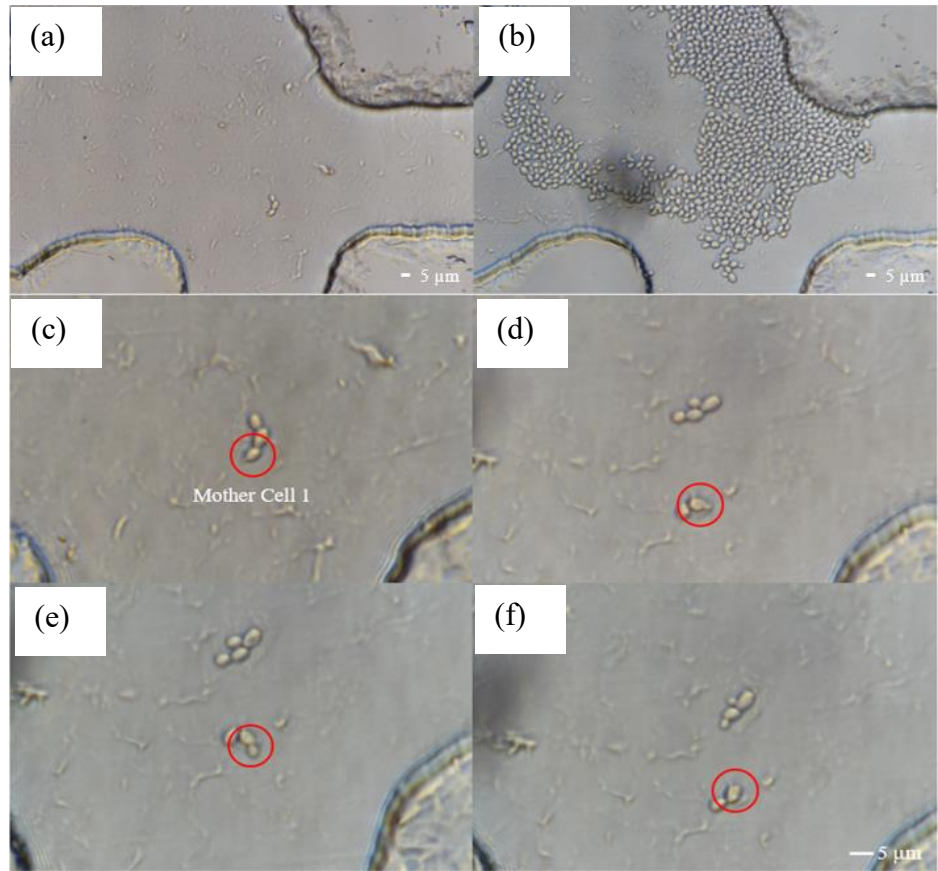


Figure 5.112. Images of chamber 4 (a) 0th minute (b) 740th minute under 20X magnification (c) at 25th minute (d) 75th minute (e) 95th minute (f) 120th minute

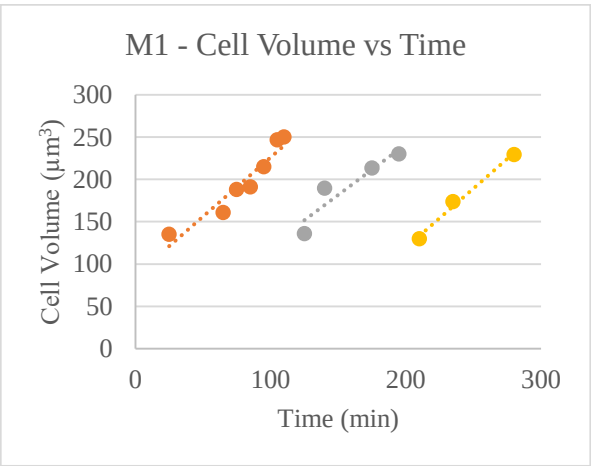


Figure 5.113. Graphical representation of processed data of chamber 4, mother cell 1 volume vs time graph - EXP 6

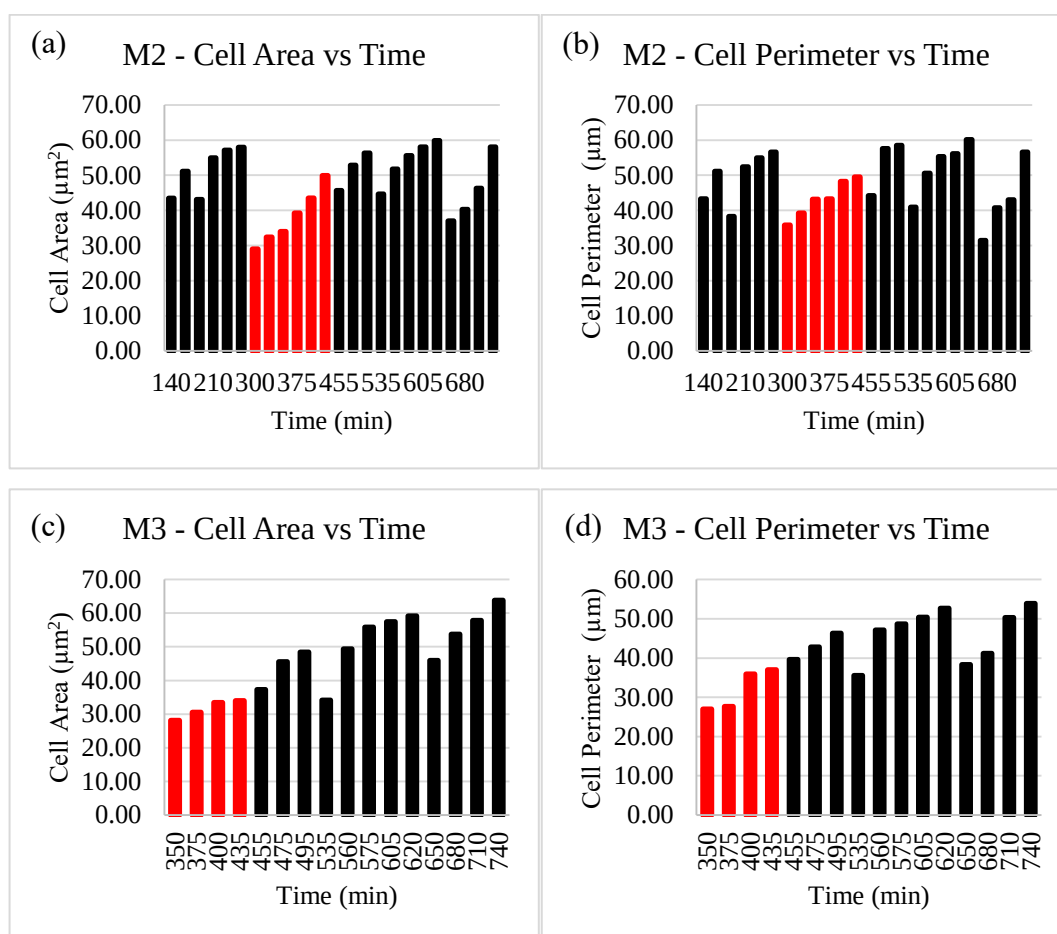


Figure 5.114. Graphical representation of processed data of chamber 4 (a) mother cell 2 area vs time graph (b) mother cell 2 perimeter vs time graph (c) mother cell 3 area vs time graph (d) mother cell 3 perimeter vs time graph – EXP 6

Table 5.31. Duration of cell cycle phases of the analyzed cells in chamber 4

Cell Name	Bud Number	Medium Type	G ₁ phase (min)	S/G ₂ /M phases (min)	Cell Cycle (min)	Area (μm^2)
M1	1	N1	40	45	85	30-60
M1	2	N1	30	55	85	35-60
M1	3	N1	40	45	85	35-55
M2	1	N1	-	≥ 35	≥ 35	40-50
M2	2	N1	20	85	105	40-60

Table 5.31. Duration of cell cycle phases of the analyzed cells in chamber 4 (cont.).

Cell Name	Bud Number	Medium Type	G ₁ phase (min)	S/G ₂ /M phases (min)	Cell Cycle (min)	Area (μm ²)
M2	3	N2-N1R	70	135	205	30-50
M2	4	N1R	40	85	125	45-60
M2	5	N1R	30	≥60	≥90	40-60
M3	1	N2	50	95	145	25-50

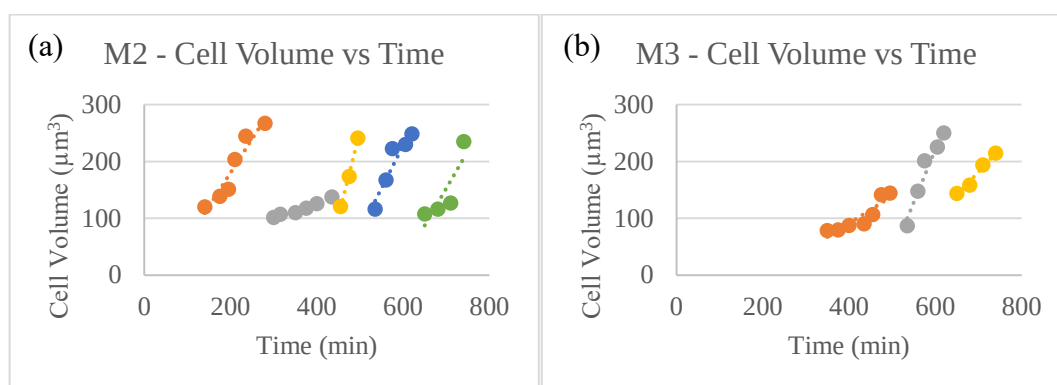


Figure 5.115. Graphical representation of processed data of chamber 4 (a) mother cell 2 volume vs time graph (b) mother cell 3 volume vs time graph - EXP 6

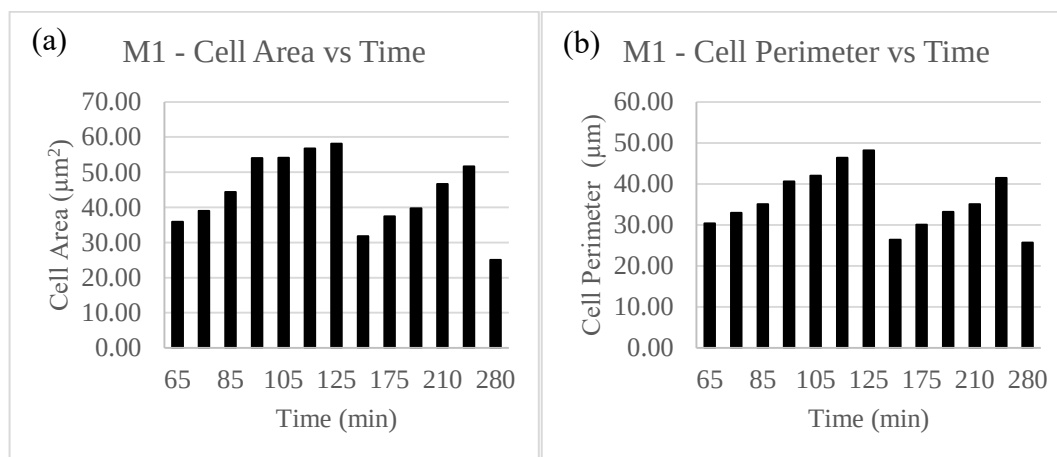


Figure 5.116. Graphical representation of processed data of chamber 5 (a) mother cell 1 area vs time graph (b) mother cell 1 perimeter vs time graph – EXP 6

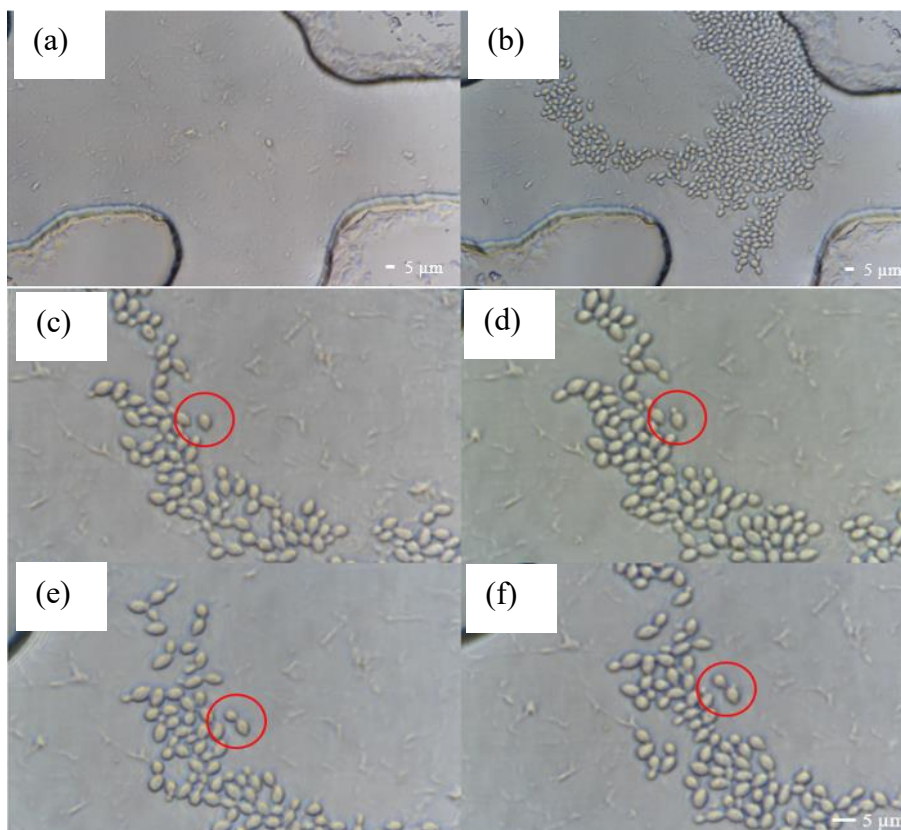


Figure 5.117. Images of chamber 5 (a) 0th minute (b) 740th minute (c) 300th minute (d) 350th minute (e) 455th minute (f) 495th minute

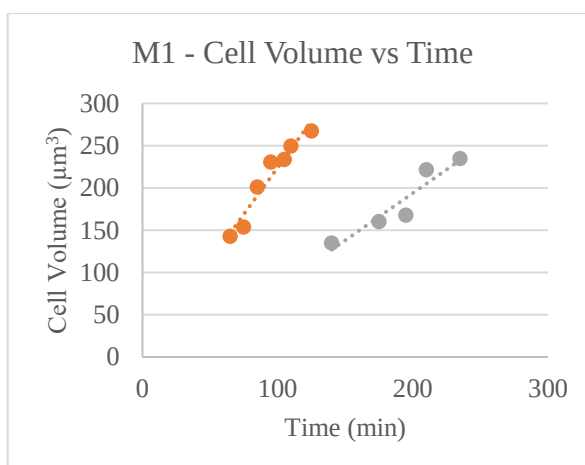


Figure 5.118. Graphical representation of processed data of chamber 5, mother cell 1 volume vs time graph - EXP 6

Table 5.32. The fold change in area, perimeter and volume of the M1 cell in chamber 4

Time	M1-Cell Area Fold Change	M1-Cell Perimeter Fold Change	M1-Volume Fold Change
25	1.00	1.00	1.00
65	1.27	1.08	1.19
75	1.30	1.09	1.39
85	1.50	1.52	1.41
95	1.75	1.78	1.59
105	1.79	1.94	1.83
110	1.81	2.14	1.85
125	1.00	1.00	1.00
140	1.15	1.16	1.40
175	1.35	1.58	1.57
195	1.51	2.00	1.70
210	1.00	1.00	1.00
235	1.34	1.40	1.34
280	1.54	1.78	1.77

Table 5.33. The fold change in area, perimeter and volume of the M2 and M3 cells in chamber 4

Time	M2-Cell Area Fold Change	M2-Cell Perimeter Fold Change	M2- Volume Fold Change	M3-Cell Area Fold Change	M3-Cell Perimeter Fold Change	M3- Volume Fold Change
140	1.00	1.00	1.00	-	-	-
175	1.18	1.18	1.16	-	-	-
195	0.99	0.88	1.26	-	-	-
210	1.26	1.21	1.70	-	-	-
235	1.31	1.27	2.04	-	-	-

Table 5.33. The fold change in area, perimeter and volume of the M2 and M3 cells in chamber 4 (cont.).

Time	M2-Cell Area Fold Change	M2-Cell Perimeter Fold Change	M2- Volume Fold Change	M3-Cell Area Fold Change	M3-Cell Perimeter Fold Change	M3- Volume Fold Change
280	1.33	1.31	2.23	-	-	-
300	1.00	1.00	1.00	-	-	-
315	1.12	1.10	1.06	-	-	-
350	1.17	1.21	1.09	1.00	1.00	1.00
375	1.35	1.21	1.16	1.09	1.02	1.01
400	1.50	1.35	1.24	1.19	1.33	1.11
435	1.72	1.38	1.36	1.21	1.37	1.15
455	1.00	1.00	1.00	1.33	1.47	1.36
475	1.16	1.30	1.44	1.62	1.59	1.80
495	1.23	1.32	2.00	1.72	1.72	1.84
535	1.00	1.00	1.00	1.00	1.00	1.00
560	1.16	1.23	1.45	1.45	1.33	1.70
575	1.25	1.35	1.92	1.64	1.37	2.32
605	1.30	1.37	1.99	1.68	1.42	2.60
620	1.34	1.47	2.15	1.73	1.48	2.89
650	1.00	1.00	1.00	1.00	1.00	1.00

Table 5.34. Duration of cell cycle phases of the analyzed cells in chamber 5

Cell Name	Bud Number	Medium Type	G₁ phase (min)	S/G₂/M phases (min)	Cell Cycle (min)	Area (μm^2)
M1	1	N1	≥ 10	50	≥ 60	35-60
M1	2	N1	50	50	100	30-55
M2	1	N2	70	85	135	25-50

Table 5.35. Duration of cell cycle phases of the analyzed cells in chamber 5 (cont.).

Cell Name	Bud Number	Medium Type	G ₁ phase (min)	S/G ₂ /M phases (min)	Cell Cycle (min)	Area (μm ²)
M2	2	N1R	40	85	125	35-65
M3	1	N2-N1R	55	120	175	25-55

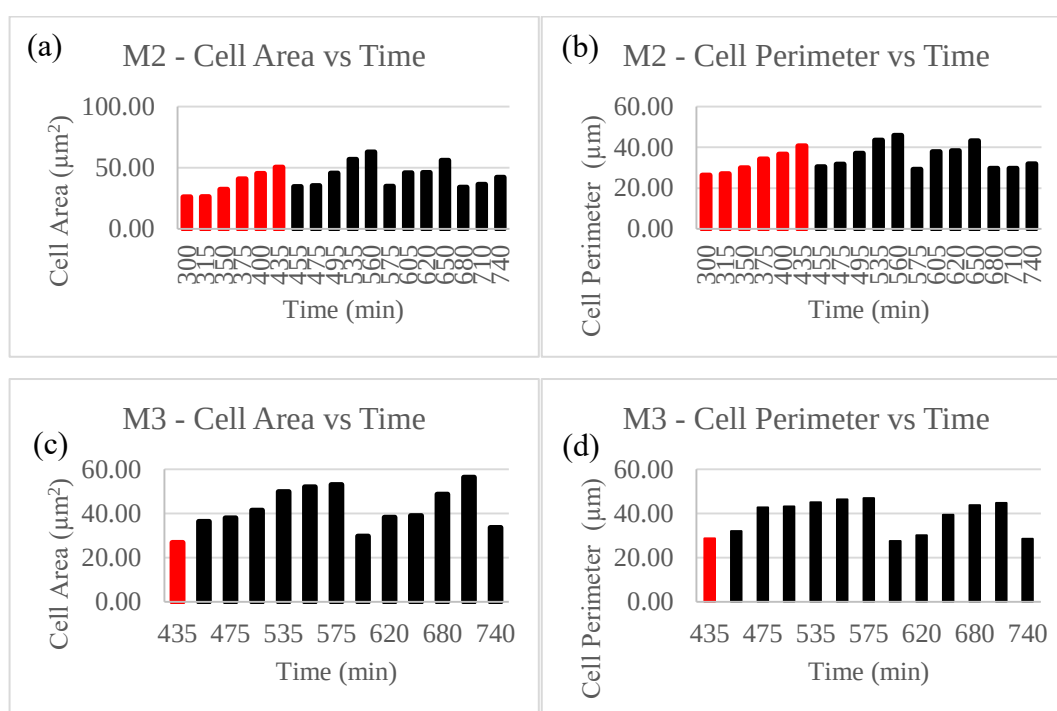


Figure 5.119. Graphical representation of processed data of chamber 5 (a) mother cell 2 area vs time graph (b) mother cell 2 perimeter vs time graph (c) mother cell 3 area vs time graph (d) mother cell 3 perimeter vs time graph – EXP 6

Table 5.35. The fold change in area, perimeter and volume of the M1 cell in chamber 5

Time	M1-Cell Area Fold Change	M1-Cell Perimeter Fold Change	M1-Volume Fold Change
65	1.00	1.00	1.00
75	1.09	1.09	1.08

Table 5.35. The fold change in area, perimeter and volume of the M1 cell in chamber 5
(cont.).

Time	M1-Cell Area Fold Change	M1-Cell Perimeter Fold Change	M1-Volume Fold Change
85	1.24	1.15	1.41
95	1.51	1.34	1.62
105	1.51	1.38	1.64
110	1.58	1.53	1.75
125	1.62	1.59	1.87
140	1.00	1.00	1.00
175	1.18	1.14	1.19
195	1.25	1.26	1.25
210	1.47	1.33	1.64

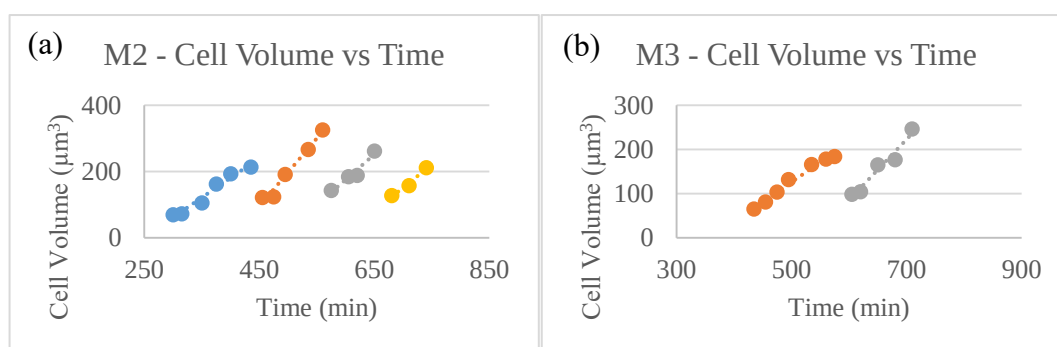


Figure 5.120. Graphical representation of processed data of chamber 5 (a) mother cell 2 volume vs time graph (b) mother cell 3 volume vs time graph - EXP 6

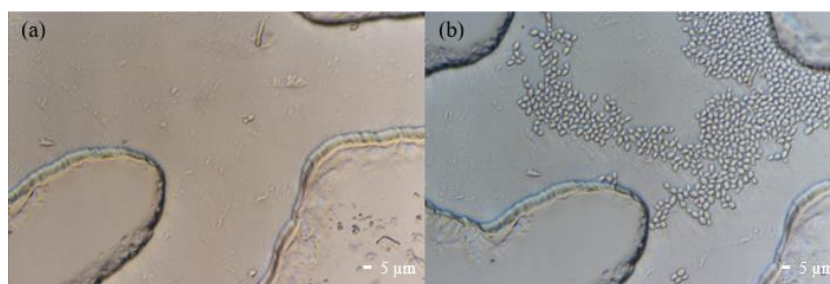


Figure 5.121. Images of chamber 6 (a) 0th minute (b) 740th minute

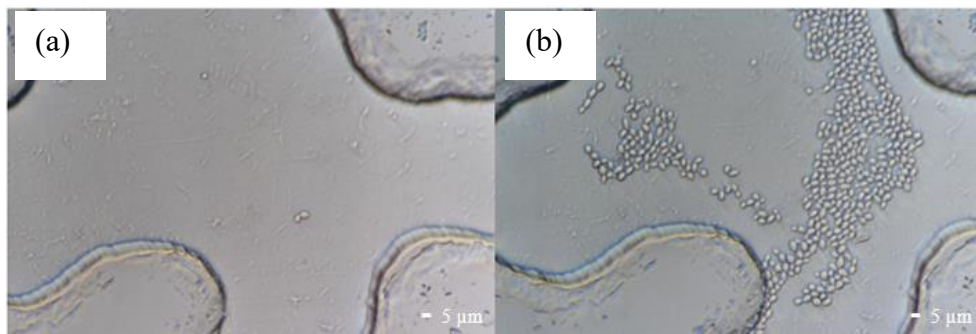


Figure 5.122. Images of chamber 7 (a) 0th minute (b) 740th minute

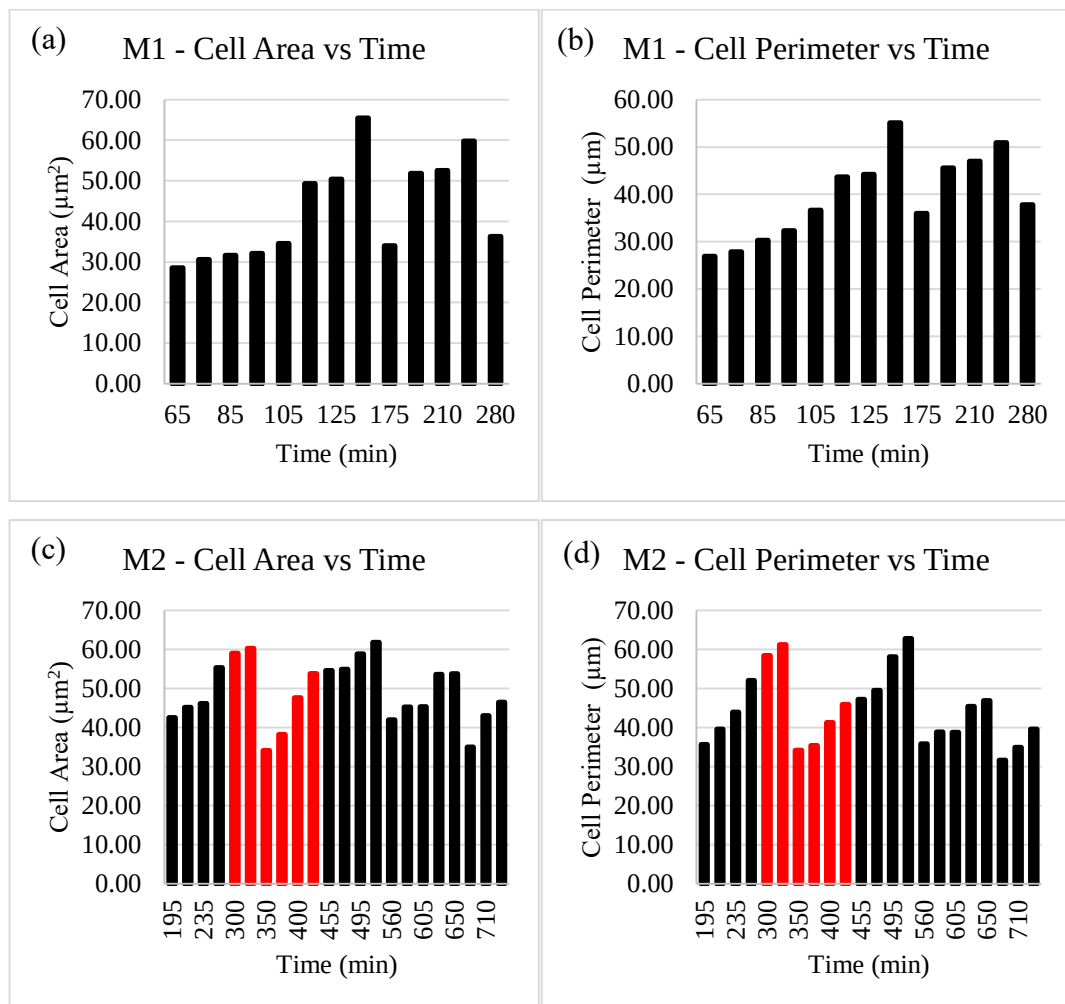


Figure 5.123. Graphical representation of processed data of chamber 7 (a) mother cell 1 area vs time graph (b) mother cell 1 perimeter vs time graph (c) mother cell 2 area vs time graph (d) mother cell 2 perimeter vs time graph – EXP 6

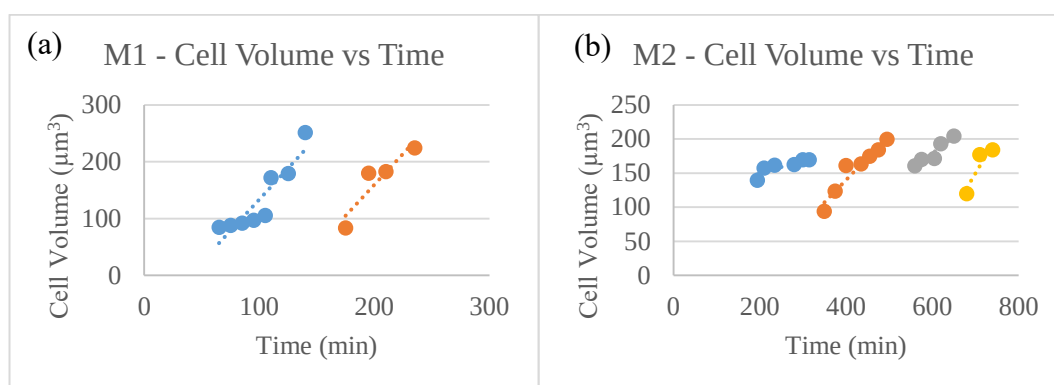


Figure 5.124. Graphical representation of processed data of chamber 7 (a) mother cell 1 volume vs time graph (b) mother cell 2 volume vs time graph - EXP 6

Table 5.36. Duration of cell cycle phases of the analyzed cells in chamber 7

Cell Name	Bud Number	Medium Type	G ₁ phase (min)	S/G ₂ /M phases (min)	Cell Cycle (min)	Area (μm ²)
M1	1	N1	20	55	75	30-65
M1	2	N1	35	60	95	35-60
M2	1	N1	20	100	120	40-60
M2	2	N2-N1R	35	185	220	35-60
M2	3	N1R	25	90	115	40-55

Table 5.37. The fold change in area, perimeter and volume of the M1 and M2 cells in chamber 7

Time (min)	M1-Cell Area Fold Change	M1-Cell Perimeter Fold Change	M1-Cell Volume Fold Change	M2-Cell Area Fold Change	M2-Cell Perimeter Fold Change	M2-Cell Volume Fold Change
65	1.00	1.00	1.00	-	-	-
75	1.07	1.03	1.04	-	-	-
85	1.11	1.13	1.09	-	-	-

Table 5.37. The fold change in area, perimeter and volume of the M1 and M2 cells in chamber 7 (cont.).

Time (min)	M1-Cell Area Fold Change	M1-Cell Perimeter Fold Change	M1- Volume Fold Change	M2-Cell Area Fold Change	M2-Cell Perimeter Fold Change	M2- Volume Fold Change
95	1.12	1.20	1.15	-	-	-
105	1.21	1.36	1.25	-	-	-
110	1.73	1.62	2.04	-	-	-
125	1.76	1.64	2.12	-	-	-
140	2.29	2.05	2.98	-	-	-
175	1.00	1.00	1.00	-	-	-
195	1.52	1.27	2.15	1.00	1.00	1.00
210	1.55	1.31	2.19	1.06	1.11	1.13
235	1.76	1.42	2.45	1.08	1.23	1.16
280	1.00	1.00	1.00	1.30	1.46	1.16
300	-	-	-	1.39	1.64	1.21
315	-	-	-	1.42	1.72	1.22
350	-	-	-	1.00	1.00	1.00
375	-	-	-	1.12	1.04	1.32
400	-	-	-	1.40	1.21	1.72
435	-	-	-	1.58	1.34	1.74
455	-	-	-	1.60	1.38	1.86
475	-	-	-	1.61	1.45	1.96
495	-	-	-	1.73	1.70	2.13
535	-	-	-	1.81	1.84	2.16
560	-	-	-	1.00	1.00	1.00
575	-	-	-	1.08	1.08	1.06
605	-	-	-	1.08	1.08	1.07
620	-	-	-	1.28	1.27	1.20
650	-	-	-	1.28	1.31	1.27

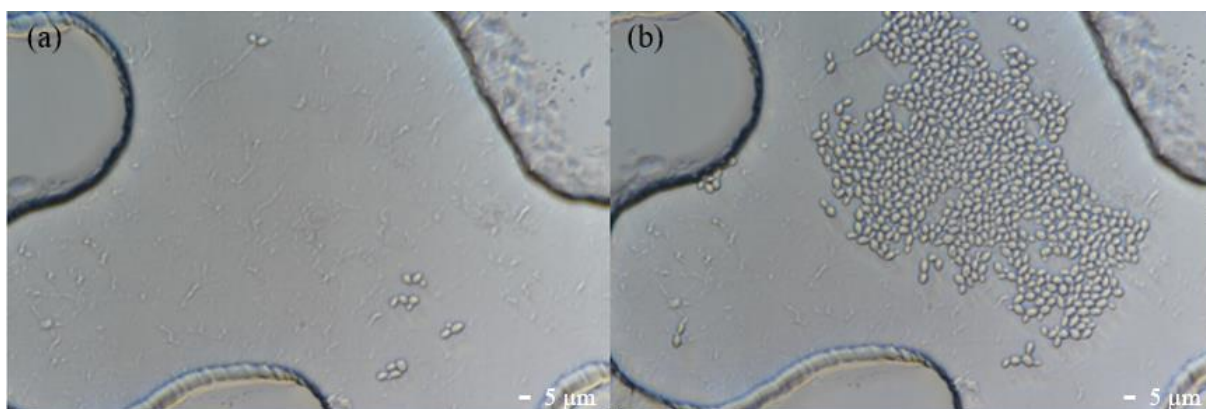


Figure 5.125. Images of chamber 8 (a) 280th minute (b) 740th minute

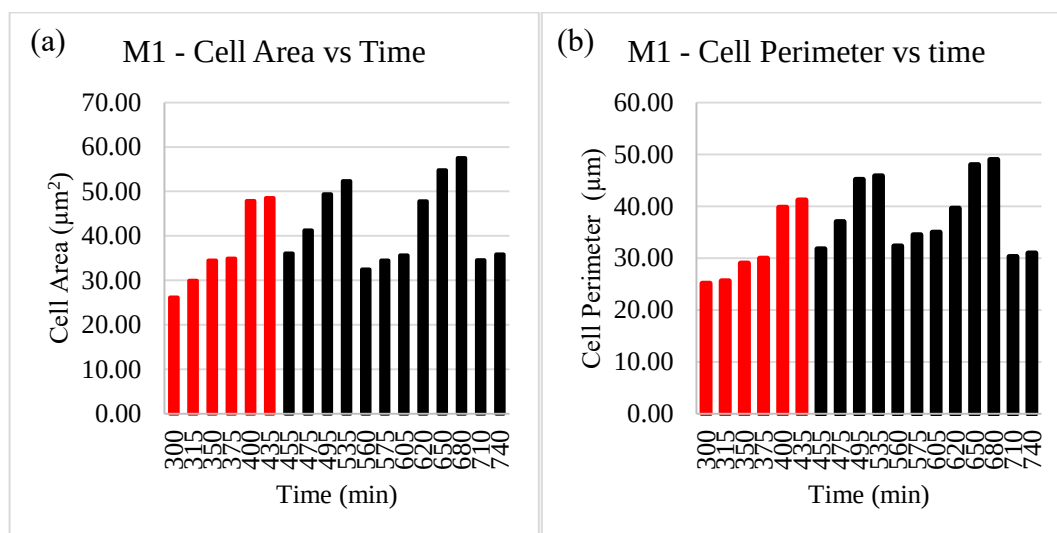


Figure 5.126. Graphical representation of processed data of chamber 8 (a) cell area vs time graph (b) cell 2 perimeter vs time graph – EXP 6

Table 5.38. Duration of cell cycle phases of the analyzed cells in chamber 8

Cell Name	Bud Number	Medium Type	G ₁ phase (min)	S/G ₂ /M phases (min)	Cell Cycle (min)	Area (µm ²)
M1	1	N2	40	95	135	25-50
M1	2	N1R	20	80	100	35-55
M1	3	N1R	15	105	120	30-60

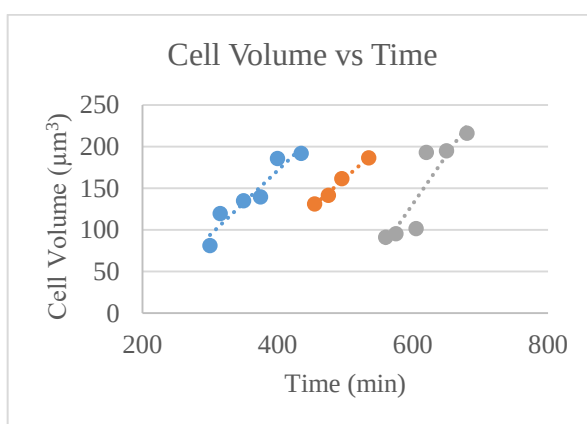


Figure 5.127. Graphical representation of processed data of chamber 8, cell volume vs time graph - EXP 6

Table 5.39. The fold change in area, perimeter and volume of the cell in chamber 8

Time	Cell Area Fold Change	Cell Perimeter Fold Change	Volume Fold Change	Volume Rate
300	1.00	1.00	1.00	0.77
315	1.14	1.02	1.47	
350	1.32	1.15	1.66	
375	1.33	1.19	1.71	
400	1.83	1.58	2.28	
435	1.86	1.64	2.36	
455	1.00	1.00	1.00	0.71
475	1.14	1.17	1.08	
495	1.37	1.42	1.23	
535	1.45	1.44	1.42	
560	1.00	1.00	1.00	1.17
575	1.06	1.07	1.05	
605	1.10	1.08	1.12	
620	1.47	1.22	2.12	
650	1.69	1.48	2.14	
680	1.77	1.52	2.37	

Table 5.40. The comparison of the area and perimeter of the cells in N1 and N2 medium

Chamber #	Cell Name	Medium Type	Cell Area	Cell Perimeter
2	M1	N1	30-60	25-45
		N2	50-60	35-45
4	M2	N1	40-60	35-60
		N2	30-50	35-50
7	M2	N1	40-60	35-60
		N2	35-55	35-45

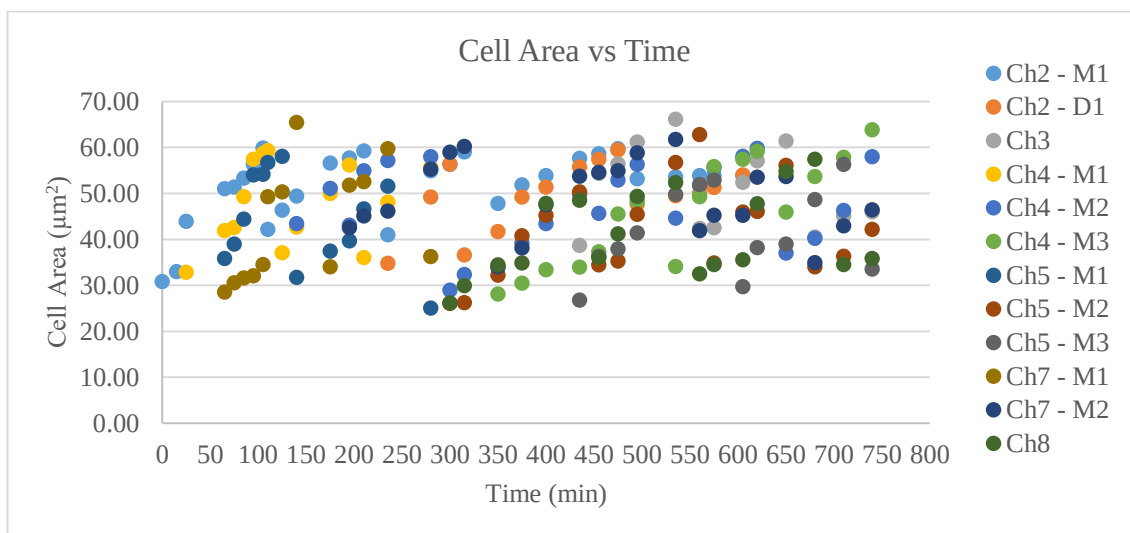


Figure 5.128. The comparison of cell area

Table 5.41. The cell cycle and its phases in different environments

Medium	G ₁ phase (min)	Ave. G ₁ phase (min)	S/G ₂ /M phases (min)	Ave. S/G ₂ /M phases (min)	Cell Cycle (min)	Ave. Cell Cycle (min)
N1	15-85	35	20-100	65	75-125	100
N1-N2	35-55	45	35-125	95	80-175	140

Table 5.41. The cell cycle and its phases in different environments (cont.).

Medium	G1 phase (min)	Ave. G1 phase (min)	S/G2/M phases (min)	Ave. S/G2/M phases (min)	Cell Cycle (min)	Ave. Cell Cycle (min)
N2	40-70	55	85-95	95	135-155	145
N2-N1R	35-70	55	75-185	125	125-220	180
N1R	20-40	35	45-105	85	85-135	120

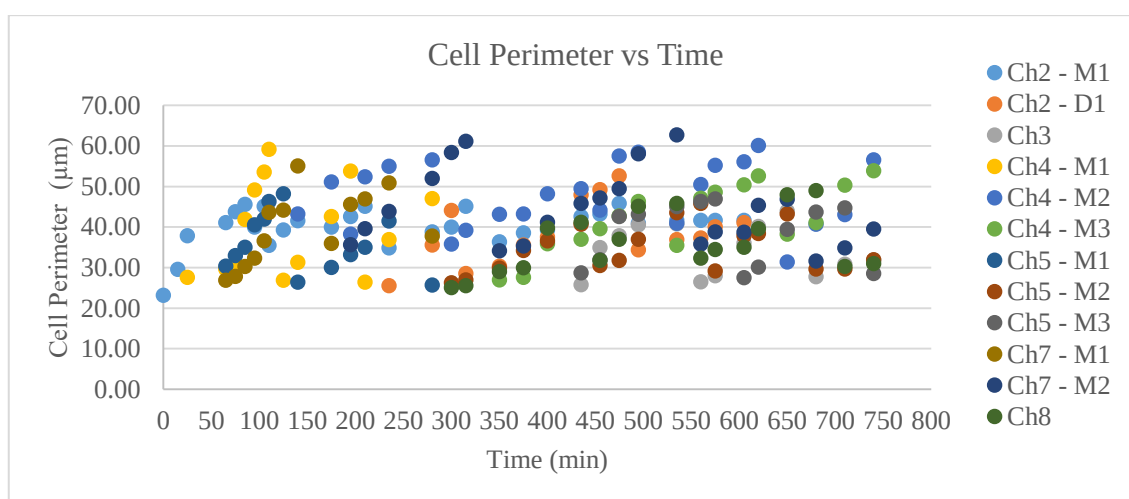


Figure 5.129. The comparison of cell perimeter of the analyzed cells

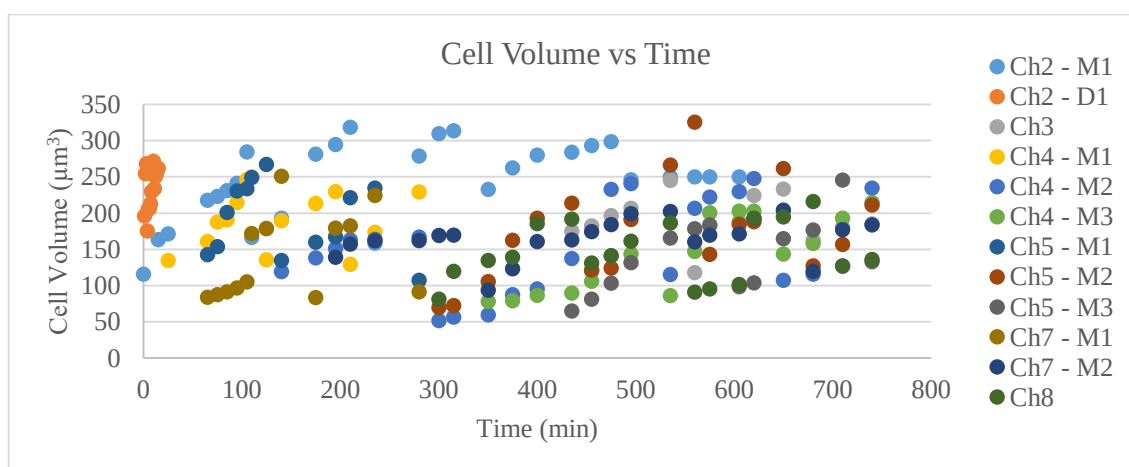


Figure 5.130. The comparison of the changes in cell volume

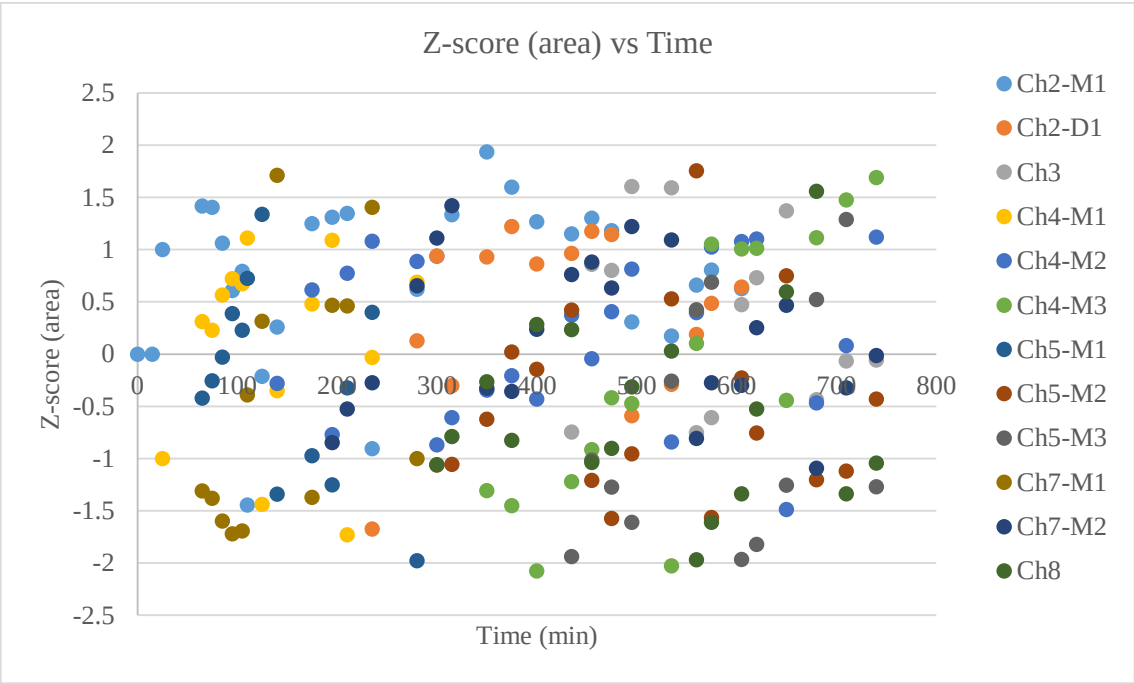


Figure 5.131. Graphical demonstration of Z-score for cell area

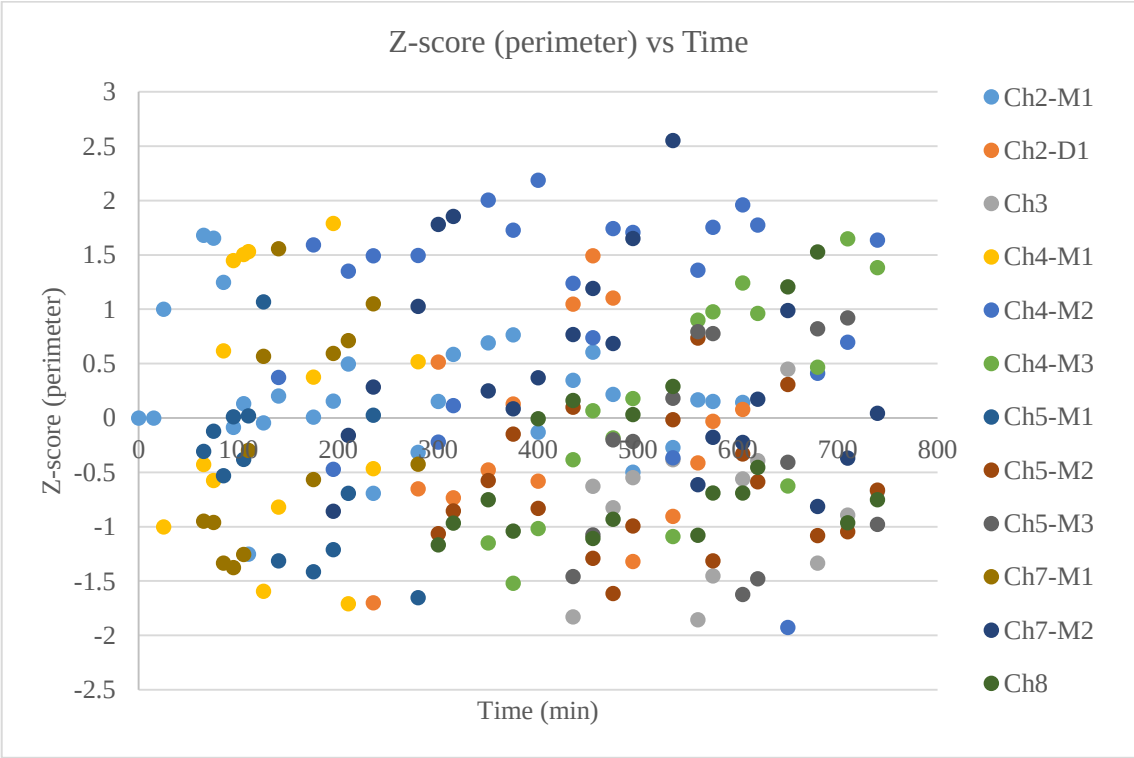


Figure 5.132. Graphical demonstration of Z-score for cell perimeter

Table 5.42. The comparison of the duration of cell cycle phases between daughter and mother cells

Cell Name	Medium Type	Ave. G1 (min)	Ave. S/G2/M (min)	Ave. Cell Cycle (min)
Mother	N1-N2	45	123	167
	N2	53	92	145
Daughter	N1-N2	25	55	80
	N2	50	75	125

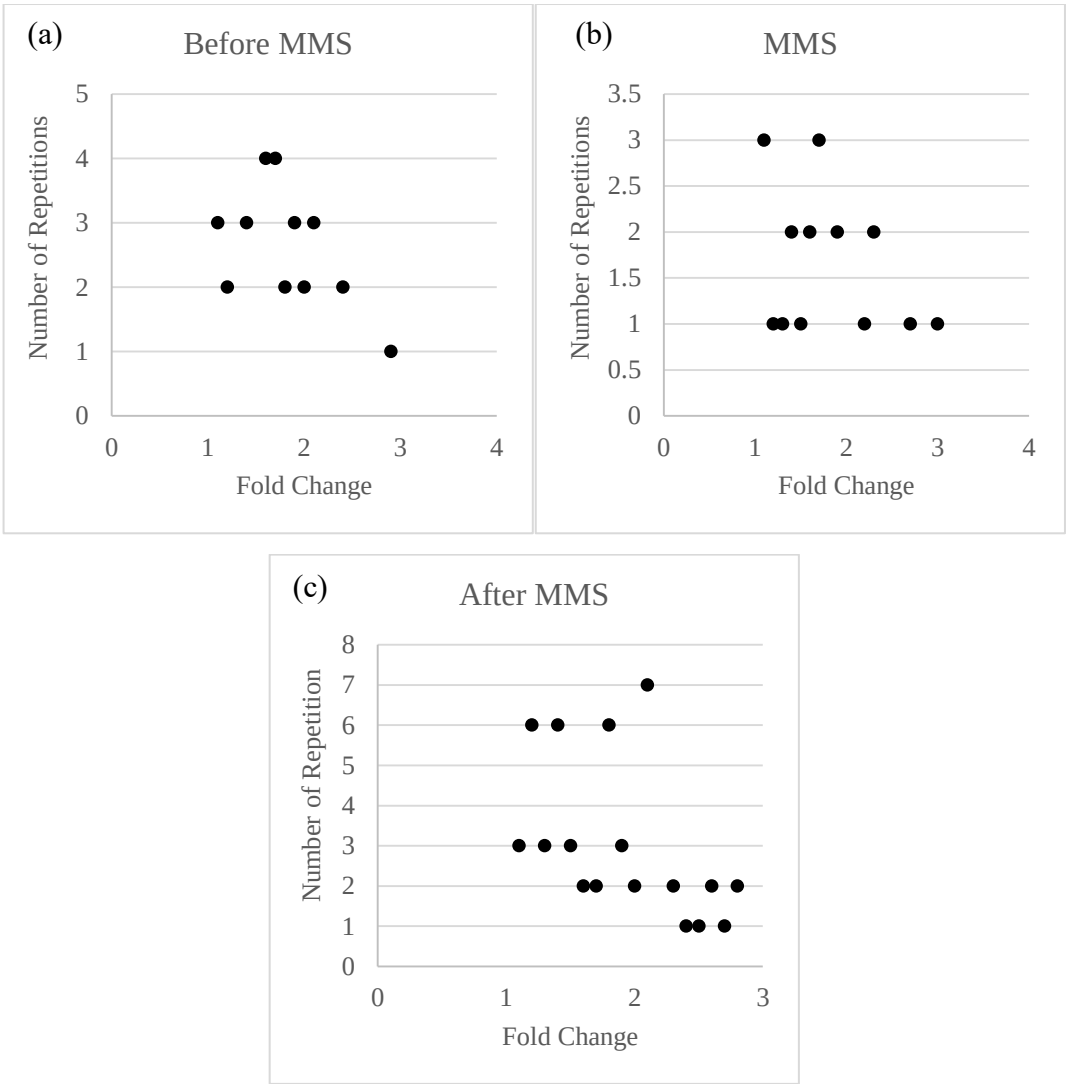


Figure 5.133. The volumetric fold change of the cells (a) in glucose rich YPD media (b) in glucose rich YPD media containing 0.01 MMS (c) in fresh glucose rich YPD medium

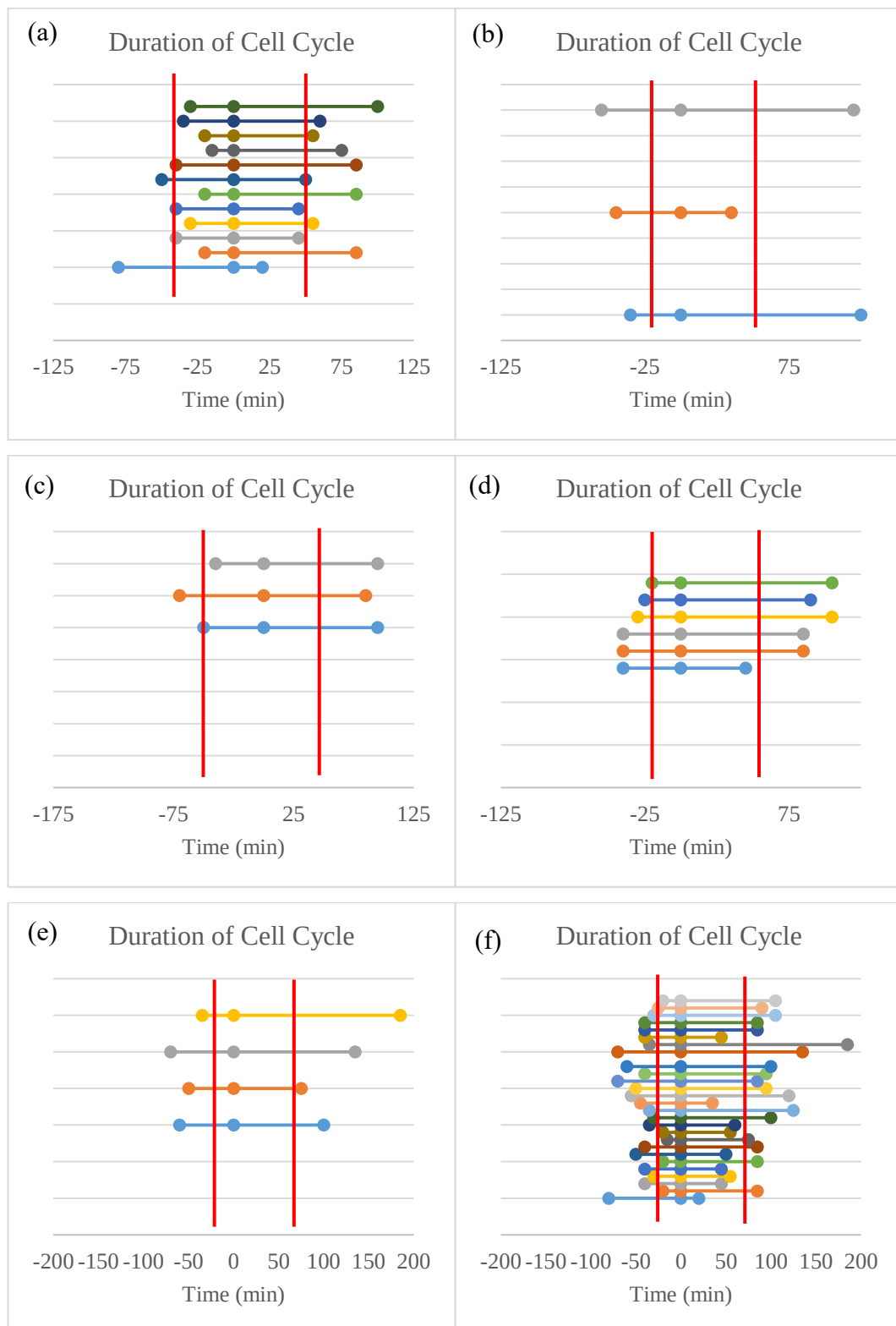


Figure 5.134. The duration of cell cycle (a) in N1 medium (b) in N1-N2 medium (c) in N2 medium (d) in N2-N1R medium (e) N1R medium (f) in all media

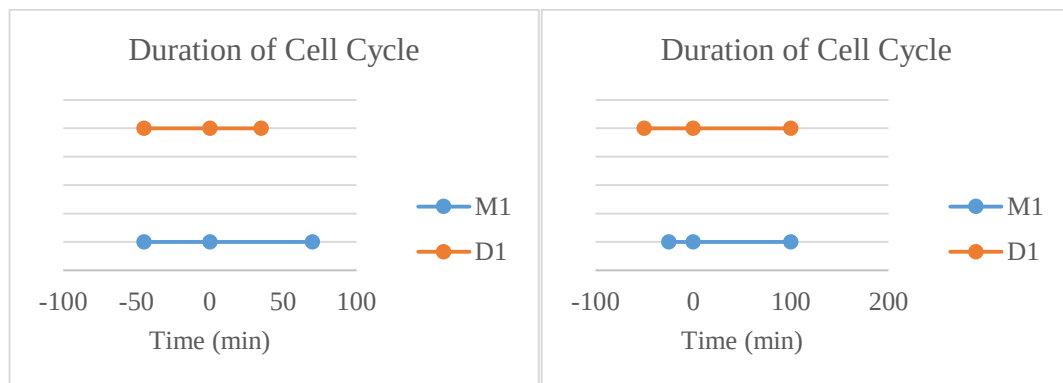


Figure 5.135. Daughter mother comparison

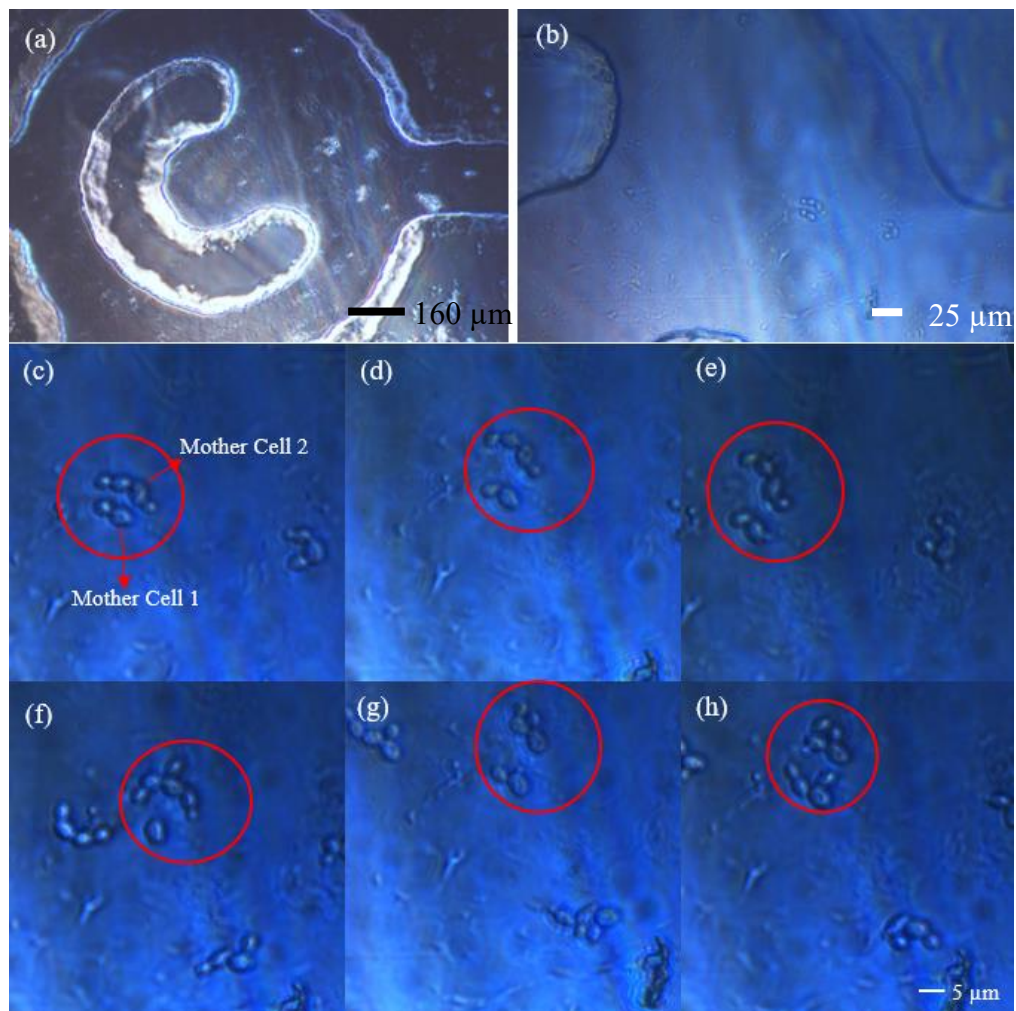


Figure 5.136. The image of chamber 1 (a) 20X (b) 40X at 0th minute, (c) at 0th minute (d) at 25th minute (e) 45th minute (f) at 175th minute (g) 240th minute (h) 290th minute

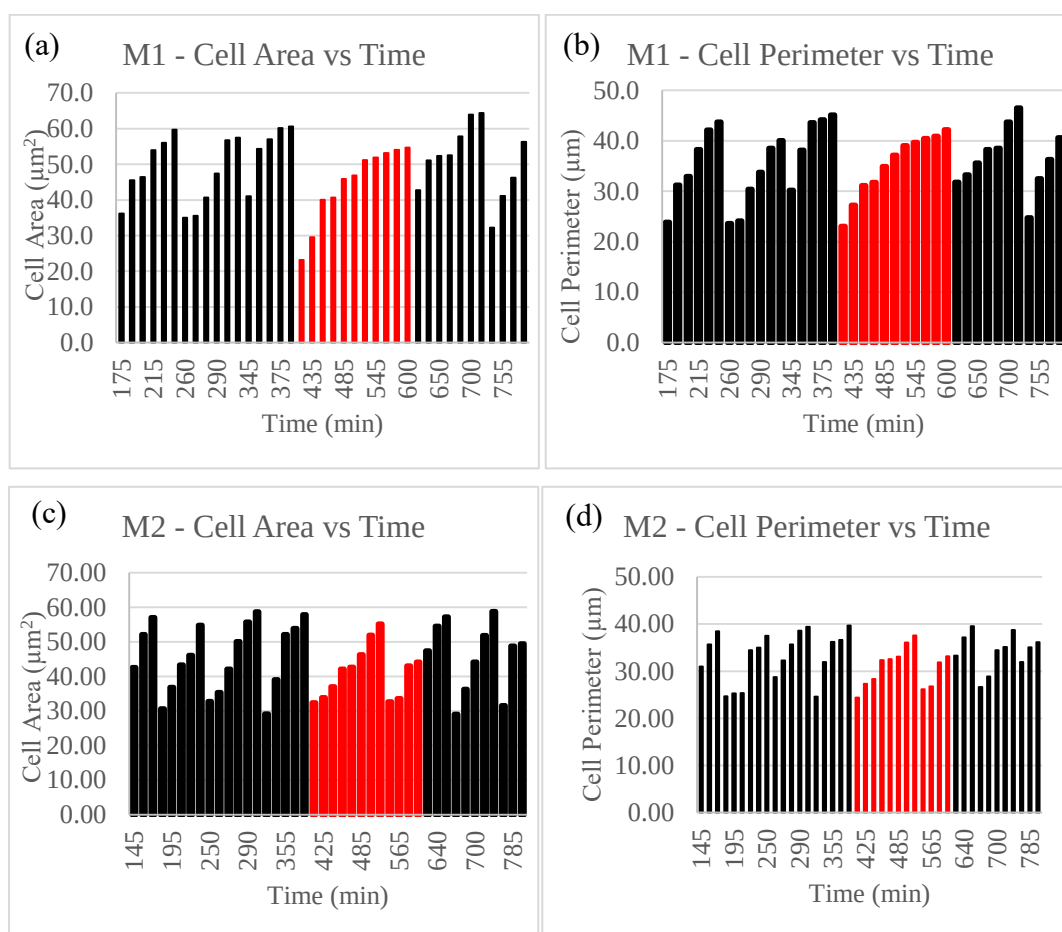


Figure 5.137. Graphical representation of processed data of chamber 1 (a) mother cell 1 area vs time graph (b) mother cell 1 perimeter vs time graph (c) mother cell 2 area vs time graph (d) mother cell 2 perimeter vs time graph – EXP 7

Table 5.43. Duration of cell cycle phases of the analyzed cells in chamber 1

Cell Name	Bud Number	Medium Type	G ₁ phase (min)	S/G ₂ /M phases (min)	Cell Cycle (min)	Area (μm ²)
M1	1	N1	40	65	105	35-60
M1	2	N1	30	65	95	35-57
M1	3	N1	25	60	80	40-60
M1	4	N2	50	185	225	35-55
M1	5	N1R	40	100	140	40-65

Table 5.43. Duration of cell cycle phases of the analyzed cells in chamber 1 (cont.).

Cell Name	Bud Number	Medium Type	G ₁ phase (min)	S/G ₂ /M phases (min)	Cell Cycle (min)	Area (μm ²)
M1	6	N1R	30	60	90	32-56
M2	1	N1	40	45	85	30-55
M2	2	N1	30	60	90	30-60
M2	3	N1-N2	25	40	65	30-60
M2	4	N2-N1R	60	110	170	30-55

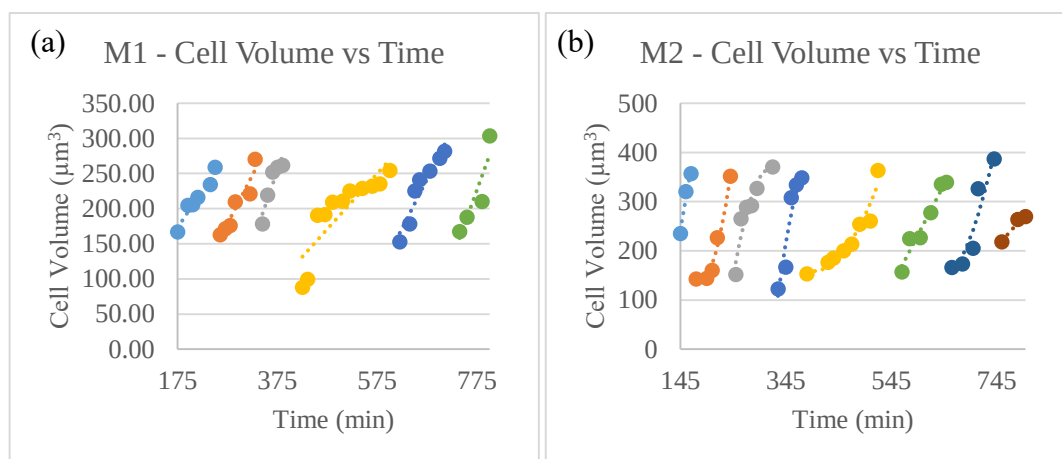


Figure 5.138. Graphical representation of processed data of chamber 1 (a) mother cell 1 volume vs time graph (b) mother cell 2 volume vs time graph – EXP7

Table 5.44. The fold change in area, perimeter and volume of the M1 and M2 cells in chamber 1

Time	M1-Cell Area Fold Change	M1-Cell Perimeter Fold Change	M1-Volume Fold Change	M2-Cell Area Fold Change	M2-Cell Perimeter Fold Change	M2-Volume Fold Change
145	-	-	-	1.00	1.00	1.00
155	-	-	-	1.22	1.15	1.36

Table 5.44. The fold change in area, perimeter and volume of the M1 and M2 cells in chamber 1 (cont.).

Time	M1-Cell Area Fold Change	M1-Cell Perimeter Fold Change	M1-Volume Fold Change	M2-Cell Area Fold Change	M2-Cell Perimeter Fold Change	M2- Volume Fold Change
165	-	-	-	1.34	1.24	1.52
175	1.00	1.00	1.00	1.00	1.00	1.00
195	1.26	1.31	1.23	1.20	1.02	1.01
205	1.28	1.38	1.23	1.41	1.03	1.12
215	1.49	1.60	1.30	1.50	1.40	1.59
240	1.55	1.76	1.40	1.79	1.42	2.46
250	1.65	1.83	1.55	1.00	1.00	1.00
260	1.00	1.00	1.00	1.08	1.04	1.74
270	1.01	1.02	1.05	1.29	1.17	1.90
280	1.16	1.29	1.08	1.53	1.30	1.92
290	1.35	1.43	1.29	1.70	1.40	2.15
320	1.62	1.63	1.36	1.79	1.43	2.44
330	1.64	1.70	1.66	1.00	1.00	1.00
345	1.00	1.00	1.00	1.34	1.30	1.36
355	1.32	1.26	1.23	1.78	1.47	2.52
365	1.39	1.44	1.42	1.85	1.49	2.73
375	1.46	1.46	1.45	1.98	1.61	2.85
385	1.48	1.49	1.47	1.00	1.00	1.00
425	1.00	1.00	1.00	1.05	1.12	1.15
435	1.27	1.18	1.13	1.14	1.16	1.21
455	1.73	1.35	2.16	1.30	1.33	1.30
470	1.75	1.38	2.17	1.32	1.33	1.39
485	1.98	1.51	2.37	1.43	1.36	1.65
505	2.02	1.61	2.39	1.60	1.48	1.70
520	2.21	1.69	2.55	1.70	1.54	2.37

Table 5.44. The fold change in area, perimeter and volume of the M1 and M2 cells in chamber 1 (cont.).

Time	M1-Cell Area Fold Change	M1-Cell Perimeter Fold Change	M1- Volume Fold Change	M2-Cell Area Fold Change	M2-Cell Perimeter Fold Change	M2- Volume Fold Change
620	1.00	1.00	1.00	1.45	1.27	2.29
640	1.20	1.05	1.17	1.67	1.42	2.77
650	1.23	1.12	1.48	1.75	1.51	2.80
660	1.23	1.20	1.58	1.00	1.00	1.00
680	1.35	1.21	1.66	1.25	1.08	1.04
700	1.50	1.37	1.78	1.52	1.29	1.24
710	1.51	1.46	1.85	1.78	1.32	1.96
740	1.00	1.00	1.00	2.02	1.45	2.33

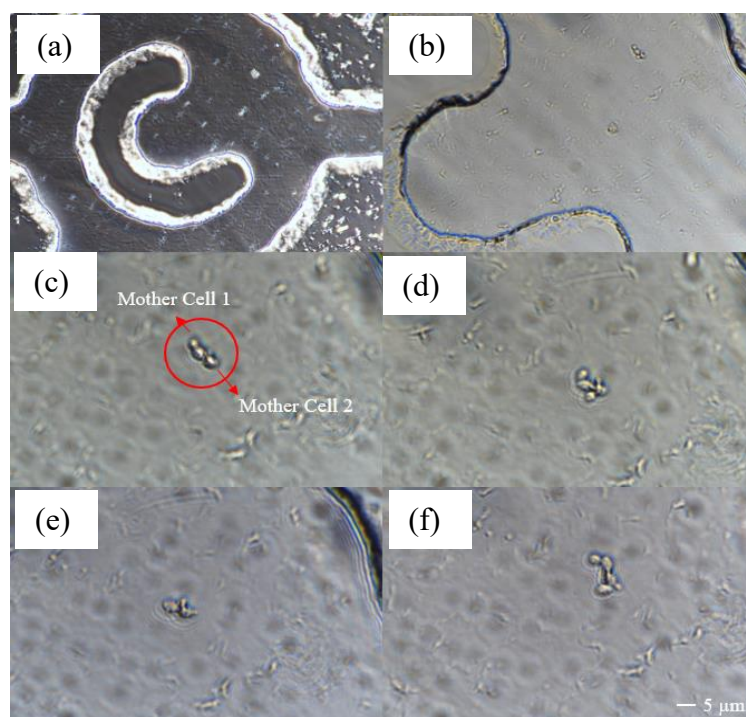


Figure 5.139. Image of chamber 4 (a) 20X (b) 40X (c) at 10th minute (d) at 35th minute (e) at 155th minute (f) at 205th minute under 90X

Table 5.45. Duration of cell cycle phases of the analyzed cells in chamber 2

Cell Name	Bud Number	Medium Type	G ₁ phase (min)	S/G ₂ /M phases (min)	Cell Cycle (min)	Area (μm ²)
M1	1	N1	-	85	>85	45-70
M1	2	N1	30	60	90	30-60
M1	3	N1	30	20	50	35-70
M2	1	N1	40	60	100	35-65
M2	2	N1	30	60	90	50-65

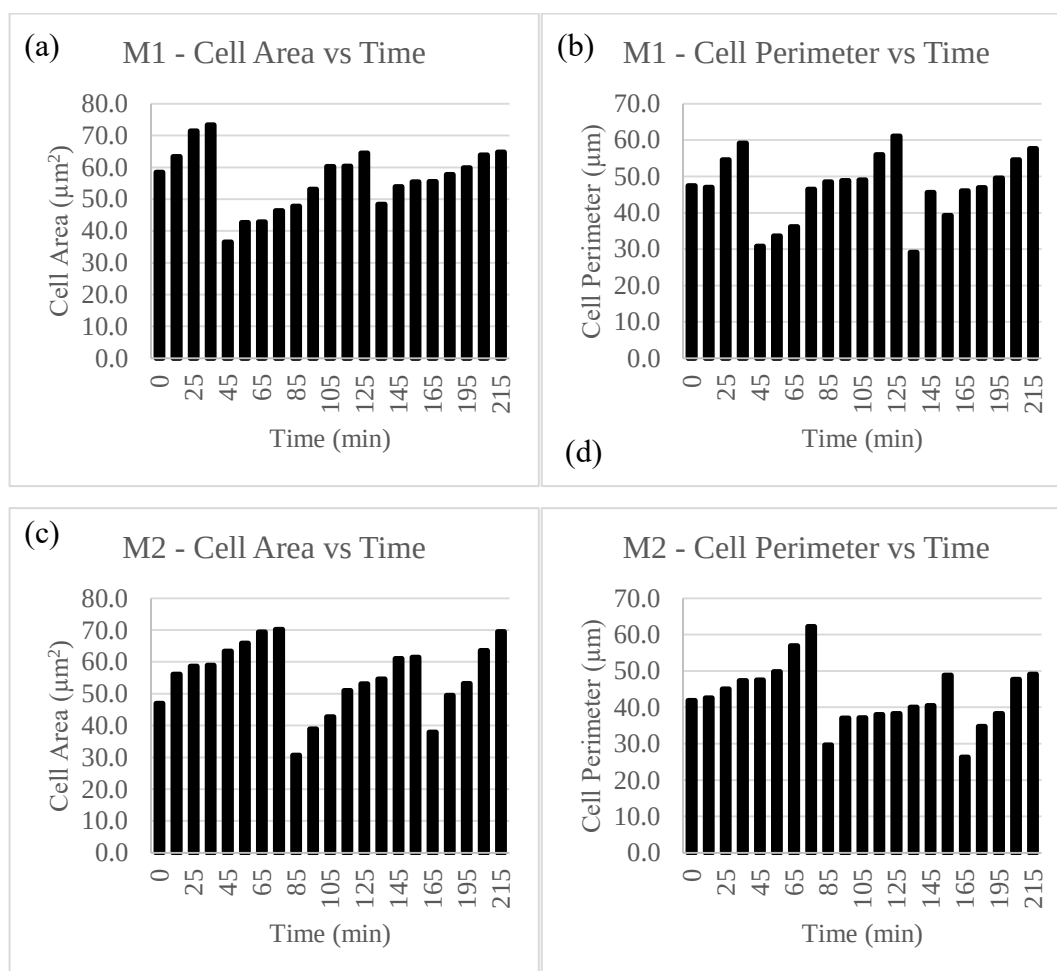


Figure 5.140. Graphical representation of processed data of chamber 2 (a) mother cell 1 area vs time graph (b) mother cell 1 perimeter vs time graph (c) mother cell 2 area vs time graph (d) mother cell 2 perimeter vs time graph – EXP 7

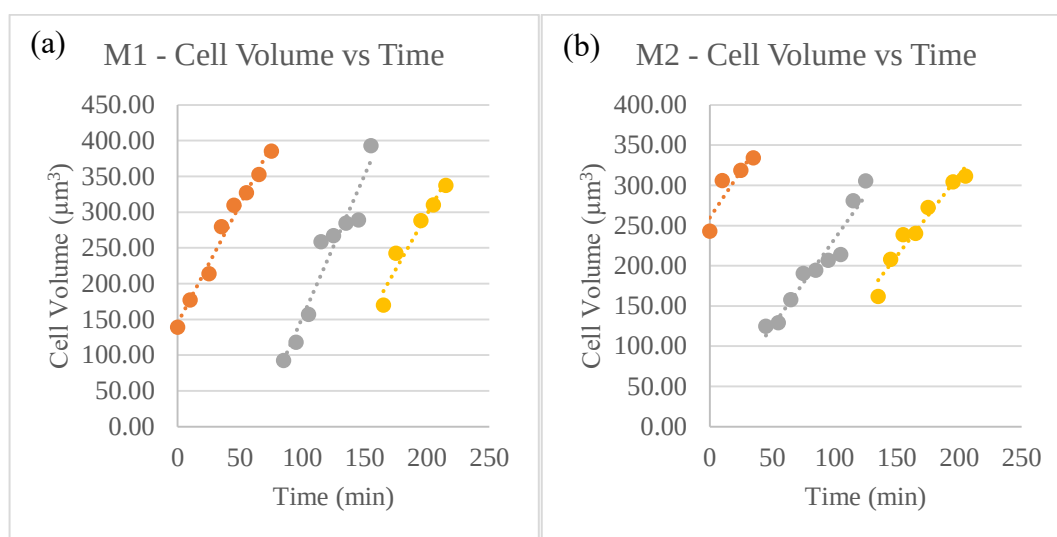


Figure 5.141. Graphical representation of processed data of chamber 2 (a) mother cell 1 volume vs time graph (b) mother cell 2 volume vs time graph – EXP7

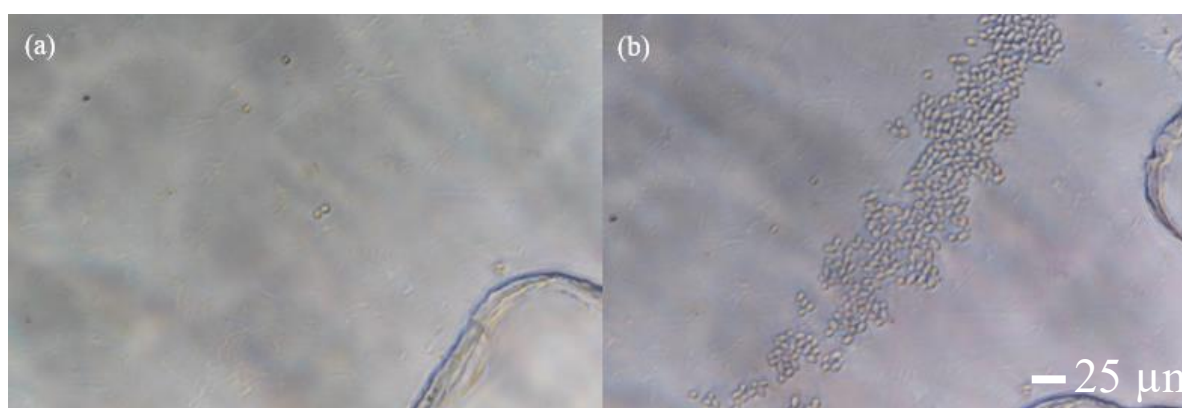


Figure 5.142. Images of the cells that were trapped in outlet (a) at 45th minute (b) 680th minute

Table 5.46. Duration of cell cycle phases of the analyzed cells (M1-D1) in outlet

Cell Name	Bud Number	Medium Type	G ₁ phase (min)	S/G ₂ /M phases (min)	Cell Cycle (min)	Area (μm^2)
M1	1	N1	50	60	110	30-55
M1	2	N1	50	-	>50	20-30
D1	1	N1	50	70	120	25-55

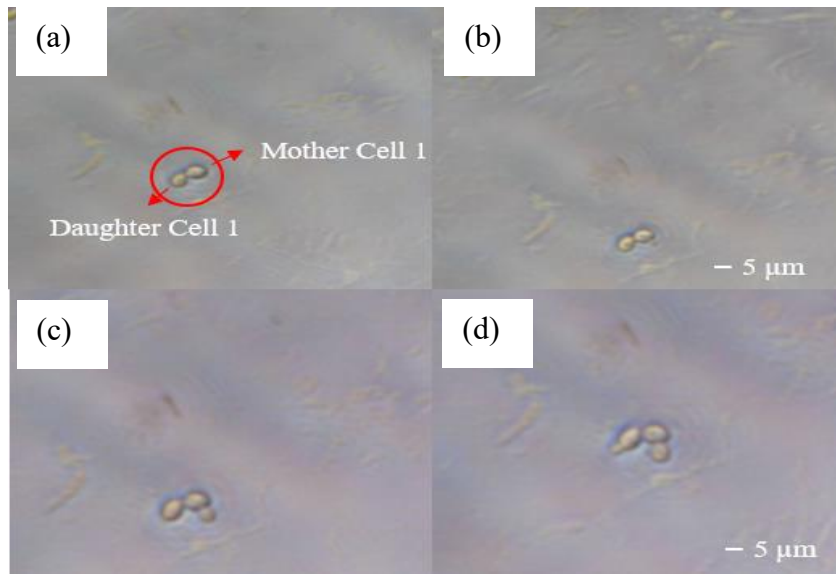


Figure 5.143. Images of the cells that were trapped in outlet at (a) 45th minute (b) 65th minute (c) 85th minute (e) 105th minute

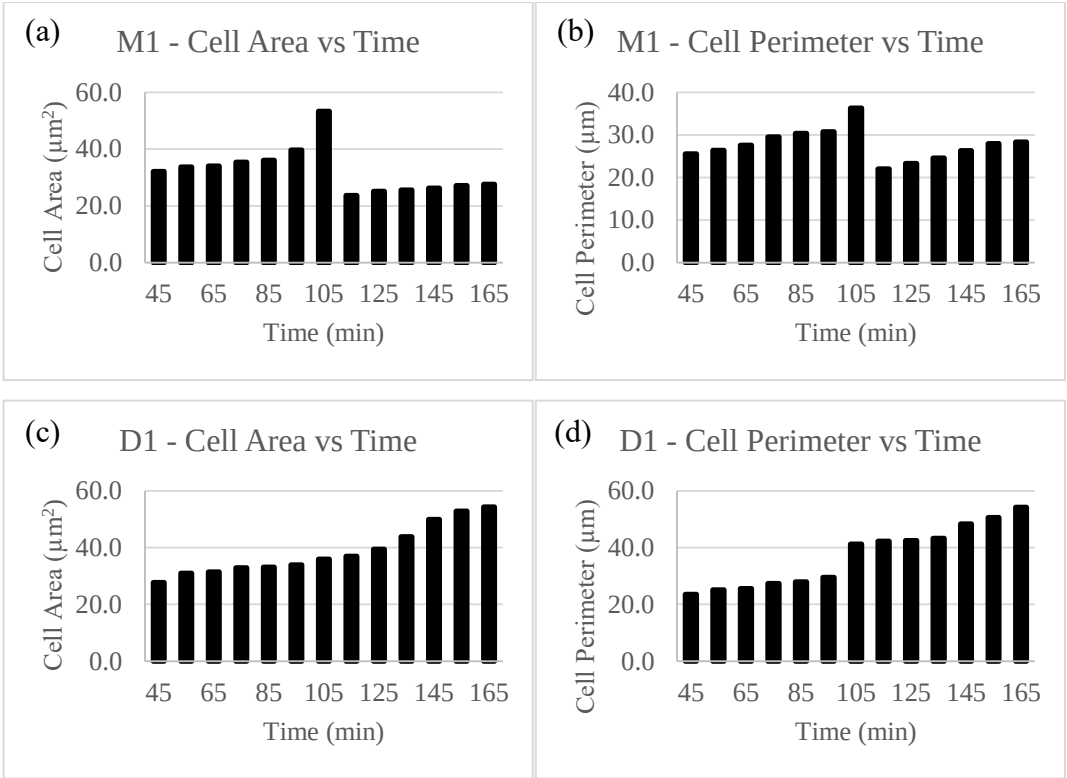


Figure 5.144. Graphical representation of processed data of outlet (a) mother cell 1 area vs time graph (b) mother cell 1 perimeter vs time graph (c) daughter cell 1 area vs time graph (d) daughter cell 1 perimeter vs time graph – EXP 7

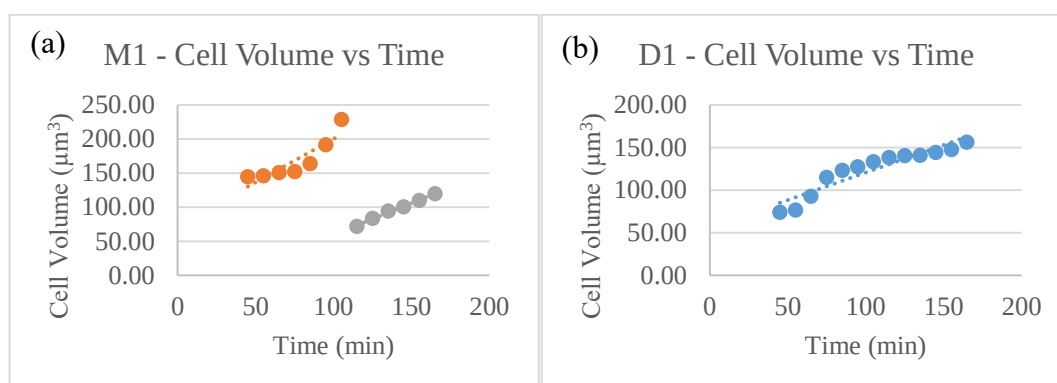


Figure 5.145. Graphical representation of processed data of outlet (a) mother cell 1 volume vs time graph (b) daughter cell 1 volume vs time graph – EXP7

Table 5.47. The fold change in area, perimeter and volume of the M1 and D1 cells in outlet

Time	M1-Cell Area Fold Change	M1-Cell Perimeter Fold Change	M1-Cell Volume Fold Change	D1-Cell Area Fold Change	D1-Cell Perimeter Fold Change	D1-Cell Volume Fold Change
45	1.00	1.00	1.00	1.00	1.00	1.00
55	1.05	1.03	1.01	1.12	1.07	1.04
65	1.06	1.08	1.04	1.13	1.08	1.26
75	1.10	1.16	1.05	1.19	1.16	1.55
85	1.12	1.19	1.13	1.19	1.19	1.66
95	1.24	1.20	1.32	1.22	1.25	1.72
105	1.66	1.42	1.58	1.30	1.75	1.80
115	1.00	1.00	1.00	1.33	1.79	1.87
125	1.06	1.06	1.17	1.42	1.80	1.90
135	1.08	1.12	1.31	1.58	1.83	1.90
145	1.11	1.20	1.40	1.80	2.05	1.95
155	1.15	1.27	1.53	1.91	2.15	1.99
165	1.17	1.29	1.67	1.96	2.30	2.11

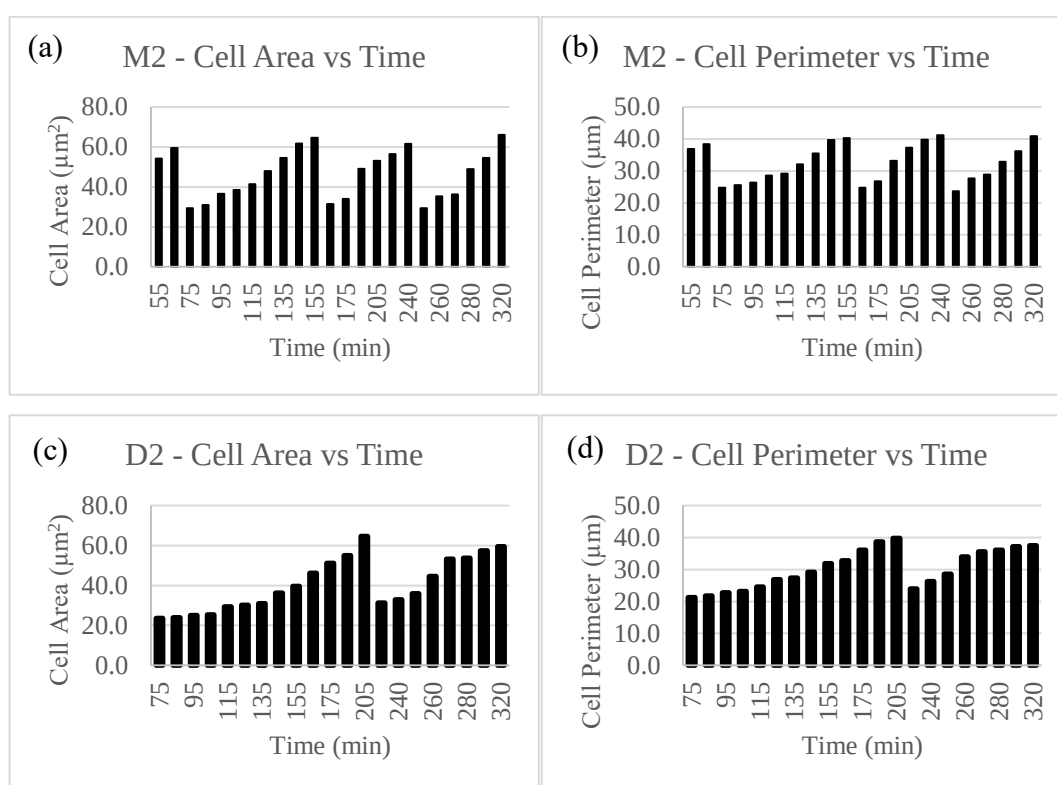


Figure 5.146. Graphical representation of processed data of outlet (a) mother cell 2 area vs time graph (b) mother cell 2 perimeter vs time graph (c) daughter cell 2 area vs time graph (d) daughter cell 2 perimeter vs time graph – EXP 7

Table 5.48. Duration of cell cycle phases of the analyzed cells (M2-D2) in outlet

Cell Name	Bud Number	Medium Type	G ₁ phase (min)	S/G ₂ /M phases (min)	Cell Cycle (min)	Area (μm ²)
M2	1	N1	-	>20	>20	55-60
M2	2	N1	40	60	100	30-65
M2	3	N1	40	55	95	30-60
M2	4	N1	30	50	80	30-65
D2	1	N1	60	70	130	20-65
D2	2	N1	65	50	115	30-60

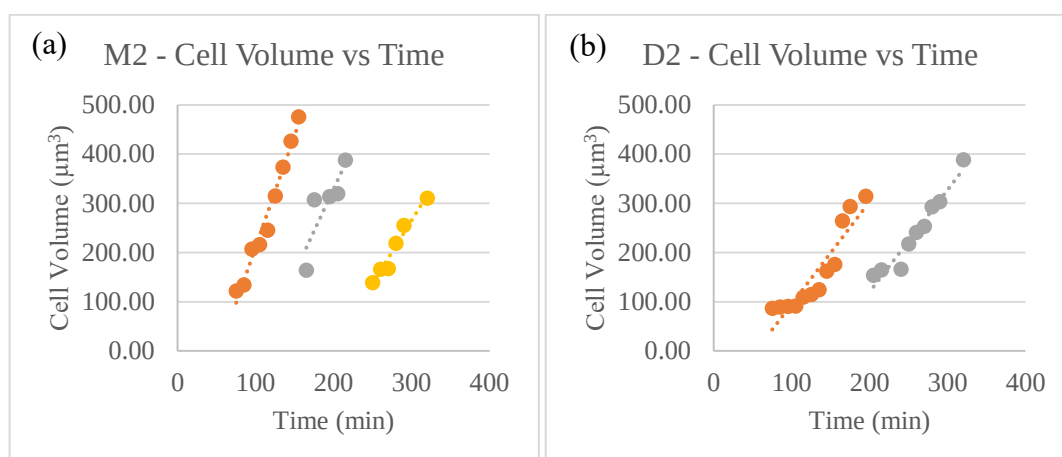


Figure 5.147. Graphical representation of processed data of outlet (a) mother cell 2 volume vs time graph (b) daughter cell 2 volume vs time graph – EXP7

Table 5.49. The fold change in area, perimeter and volume of the M2 and D2 cells in outlet

Time	M2-Cell Area Fold Change	M2-Cell Perimeter Fold Change	M2-Cell Volume Fold Change	D2-Cell Area Fold Change	D2-Cell Perimeter Fold Change	D2-Cell Volume Fold Change
75	1.00	1.00	1.00	1.00	1.00	1.00
85	1.06	1.03	1.10	1.01	1.03	1.03
95	1.25	1.06	1.70	1.06	1.07	1.05
105	1.31	1.15	1.78	1.08	1.09	1.05
115	1.41	1.18	2.02	1.24	1.16	1.26
125	1.63	1.29	2.59	1.28	1.26	1.32
135	1.86	1.43	3.07	1.31	1.29	1.43
145	2.10	1.60	3.50	1.53	1.38	1.87
155	2.20	1.62	3.91	1.68	1.50	2.02
165	1.00	1.00	1.00	1.95	1.55	3.05
175	1.09	1.08	1.07	2.16	1.70	3.38
195	1.56	1.34	2.01	2.33	1.82	3.62

Table 5.49. The fold change in area, perimeter and volume of the M2 and D2 cells in outlet
(cont.).

Time	M2-Cell Area Fold Change	M2-Cell Perimeter Fold Change	M2-Cell Volume Fold Change	D2-Cell Area Fold Change	D2-Cell Perimeter Fold Change	D2-Cell Volume Fold Change
205	1.69	1.51	2.05	2.73	1.88	3.77
215	1.80	1.61	2.09	1.00	1.00	1.00
240	1.96	1.66	2.54	1.05	1.09	1.01
250	1.00	1.00	1.00	1.15	1.19	1.32
260	1.20	1.17	1.19	1.42	1.42	1.46
270	1.23	1.22	1.20	1.70	1.48	1.54

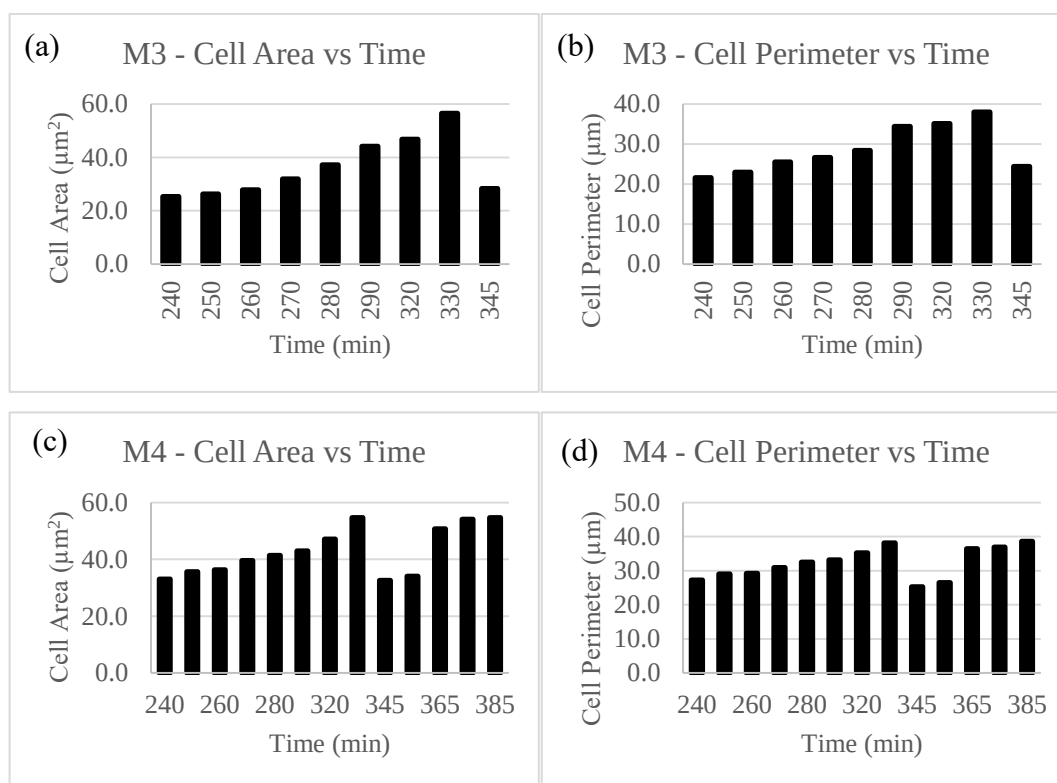


Figure 5.148. Graphical representation of processed data of outlet (a) mother cell 3 area vs time graph (b) mother cell 3 perimeter vs time graph (c) mother cell 4 area vs time graph (d) mother cell 4 perimeter vs time graph – EXP 7

Table 5.50. Duration of cell cycle phases of the analyzed cells (M3-M4) in outlet

Cell Name	Bud Number	Medium Type	G ₁ phase (min)	S/G ₂ /M phases (min)	Cell Cycle (min)	Area (μm ²)
M3	1	N1	30	70	100	25-60
M4	1	N1	-	80	>80	30-55
M4	2	N1	35	40	75	30-55

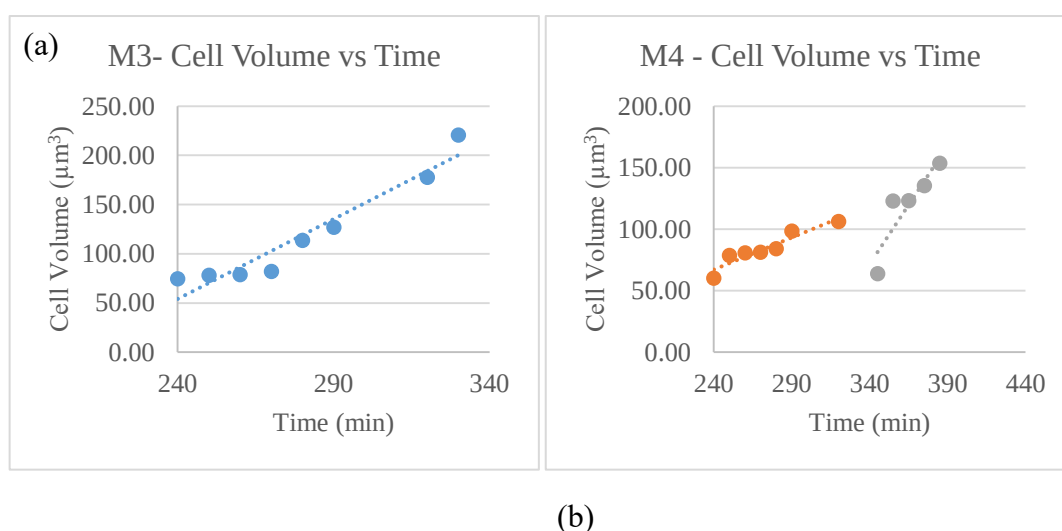


Figure 5.149. Graphical representation of processed data of outlet (a) mother cell 3 volume vs time graph (b) mother cell 4 volume vs time graph – EXP7

Table 5.51. The fold change in area, perimeter and volume of the M3 and M4 cells in outlet

Time	M3-Cell Area Fold Change	M3-Cell Perimeter Fold Change	M3-Cell Volume Fold Change	M4-Cell Area Fold Change	M4-Cell Perimeter Fold Change	M4-Cell Volume Fold Change
240	1.00	1.00	1.00	1.00	1.00	1.00
250	1.04	1.07	1.05	1.08	1.06	1.31
260	1.10	1.18	1.06	1.10	1.07	1.34

Table 5.51. The fold change in area, perimeter and volume of the M3 and M4 cells in outlet (cont.).

Time	M3-Cell Area Fold Change	M3-Cell Perimeter Fold Change	M3-Cell Volume Fold Change	M4-Cell Area Fold Change	M4-Cell Perimeter Fold Change	M4-Cell Volume Fold Change
270	1.26	1.24	1.10	1.20	1.13	1.35
280	1.47	1.32	1.52	1.25	1.19	1.40
290	1.75	1.59	1.70	1.30	1.22	1.64
320	1.85	1.63	2.38	1.43	1.29	1.77
330	2.24	1.76	2.96	1.66	1.40	2.62

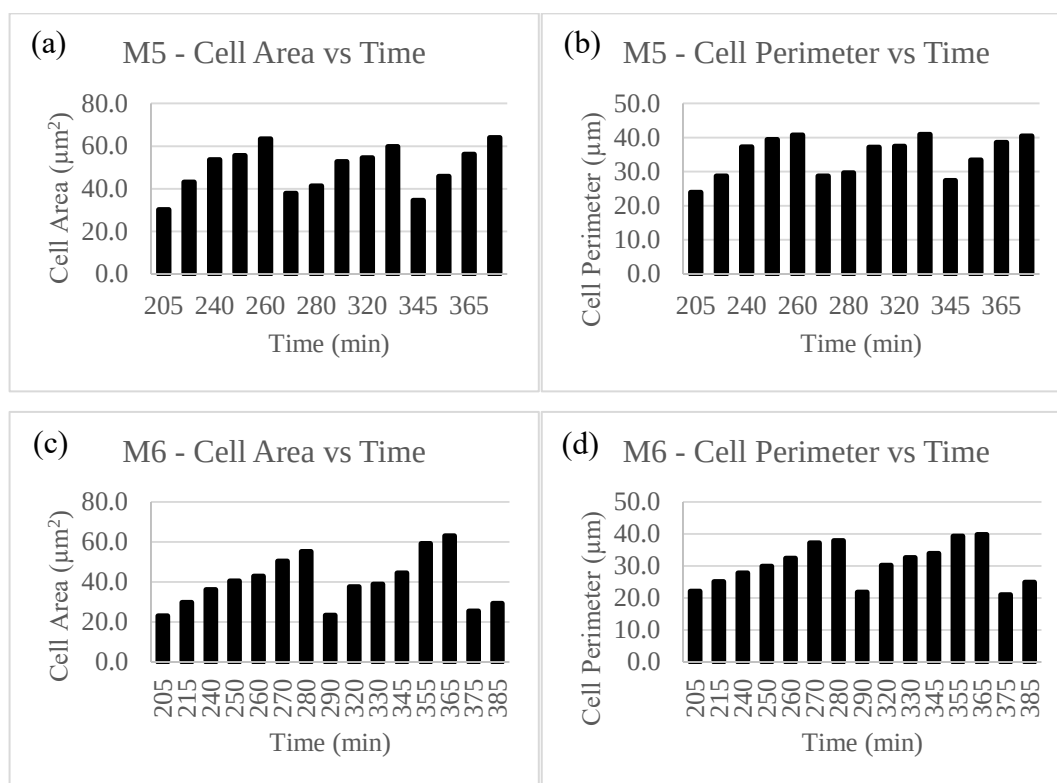


Figure 5.150. Graphical representation of processed data of outlet (a) mother cell 5 area vs time graph (b) mother cell 5 perimeter vs time graph (c) mother cell 6 area vs time graph (d) mother cell 6 perimeter vs time graph – EXP 7

Table 5.52. Duration of cell cycle phases of the analyzed cells (M5-M6) in outlet

Cell Name	Bud Number	Medium Type	G ₁ phase (min)	S/G ₂ /M phases (min)	Cell Cycle (min)	Area (μm ²)
M5	1	N1	-	55	>55	30-65
M5	2	N1	30	55	85	35-60
M5	3	N1	25	35	60	35-65
M6	1	N1	35	50	85	20-55
M6	2	N1	50	45	95	20-65

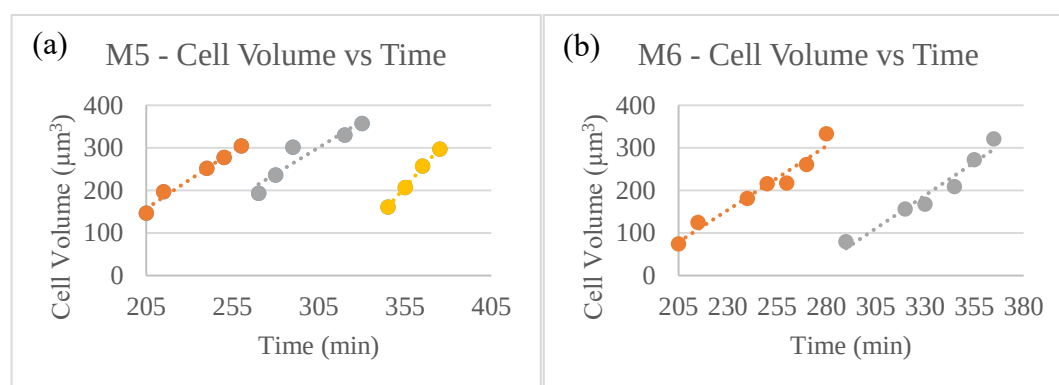


Figure 5.151. Graphical representation of processed data of outlet (a) mother cell 5 volume vs time graph (b) mother cell 6 volume vs time graph – EXP7

Table 5.53. The fold change in area, perimeter and volume of the M5 and M6 cells in outlet

Time	M5-Cell Area Fold Change	M5-Cell Perimeter Fold Change	M5-Cell Volume Fold Change	M6-Cell Area Fold Change	M6-Cell Perimeter Fold Change	M6-Cell Volume Fold Change
205	1.00	1.00	1.00	1.00	1.00	1.00
215	1.43	1.20	1.34	1.29	1.13	1.68

Table 5.53. The fold change in area, perimeter and volume of the M5 and M6 cells in outlet (cont.).

Time	M5-Cell Area Fold Change	M5-Cell Perimeter Fold Change	M5-Cell Volume Fold Change	M6-Cell Area Fold Change	M6-Cell Perimeter Fold Change	M6-Cell Volume Fold Change
240	1.78	1.56	1.71	1.57	1.26	2.42
250	1.84	1.65	1.89	1.75	1.35	2.89
260	2.10	1.70	2.07	1.85	1.46	2.91
270	1.00	1.00	1.00	2.18	1.68	3.50
280	1.09	1.03	1.22	2.39	1.71	4.47
290	1.39	1.29	1.56	1.00	1.00	1.00
320	1.44	1.30	1.71	1.61	1.38	1.96
330	1.58	1.43	1.85	1.67	1.50	2.10
345	1.00	1.00	1.00	1.91	1.56	2.62

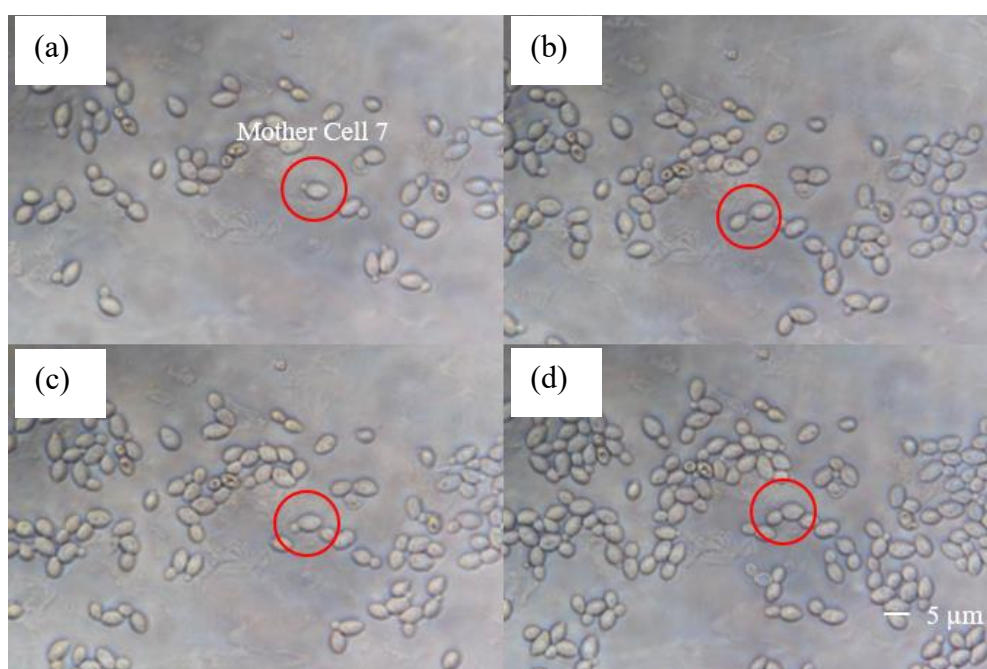


Figure 5.152. The trapped cells in outlet under 90X magnification (a) 425th minute (b) 505th minute (c) 520th minute (d) 600th minute

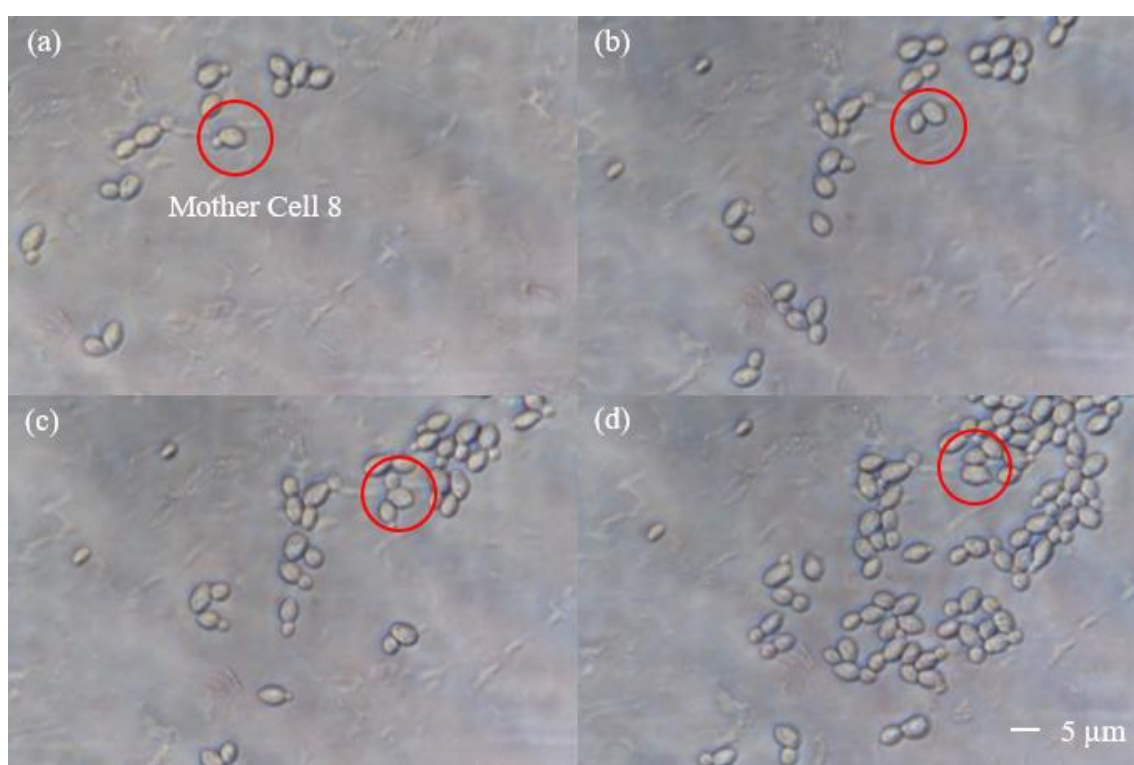


Figure 5.153. The trapped cells in outlet under 90X magnification (a) 425th minute (b) 485th minute (c) 520th minute (d) 565th minute

Table 5.54. Duration of cell cycle phases of the analyzed cells (M7-M8) in outlet

Cell Name	Bud Number	Medium Type	G ₁ phase (min)	S/G ₂ /M phases (min)	Cell Cycle (min)	Area (μm ²)
M7	1	N2	30	65	95	35-55
M7	2	N2	40	75	115	25-50
M7	3	N1R	40	60	100	25-60
M7	4	N1R	30	70	105	35-55
M8	1	N2	45	110	155	25-60
M8	2	N2-N1R	35	100	135	35-55
M8	3	N1R	30	75	105	30-60

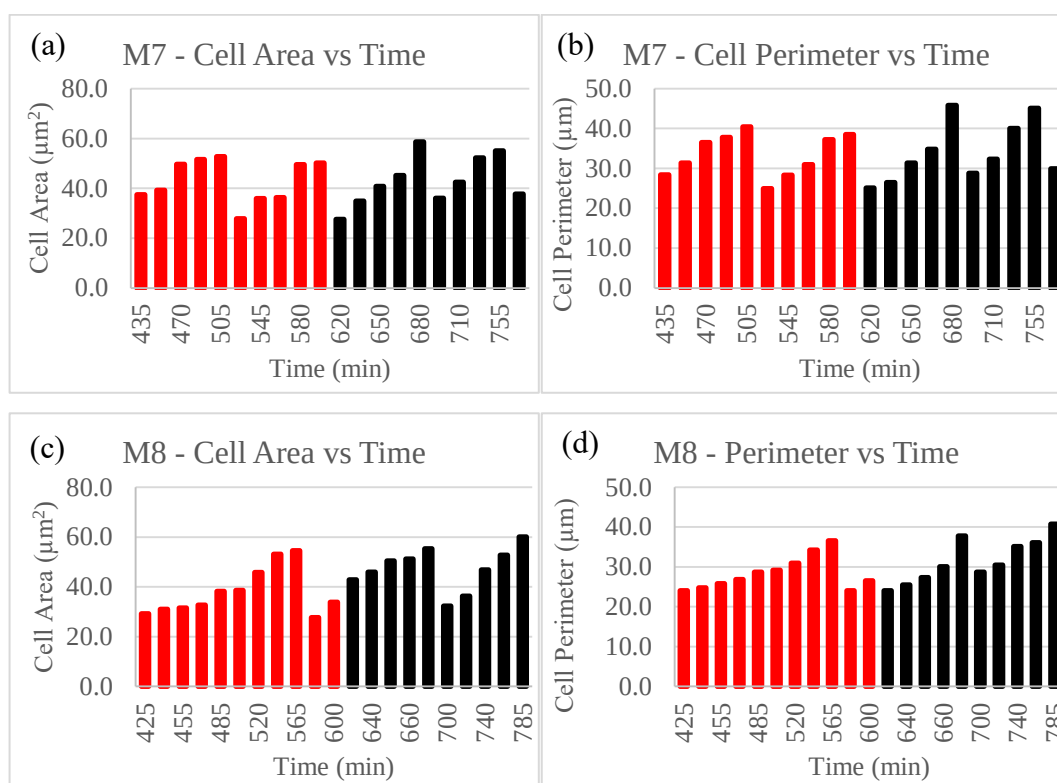


Figure 5.154. Graphical representation of processed data of outlet (a) mother cell 7 area vs time graph (b) mother cell 7 perimeter vs time graph (c) mother cell 8 area vs time graph (d) mother cell 8 perimeter vs time graph – EXP 7

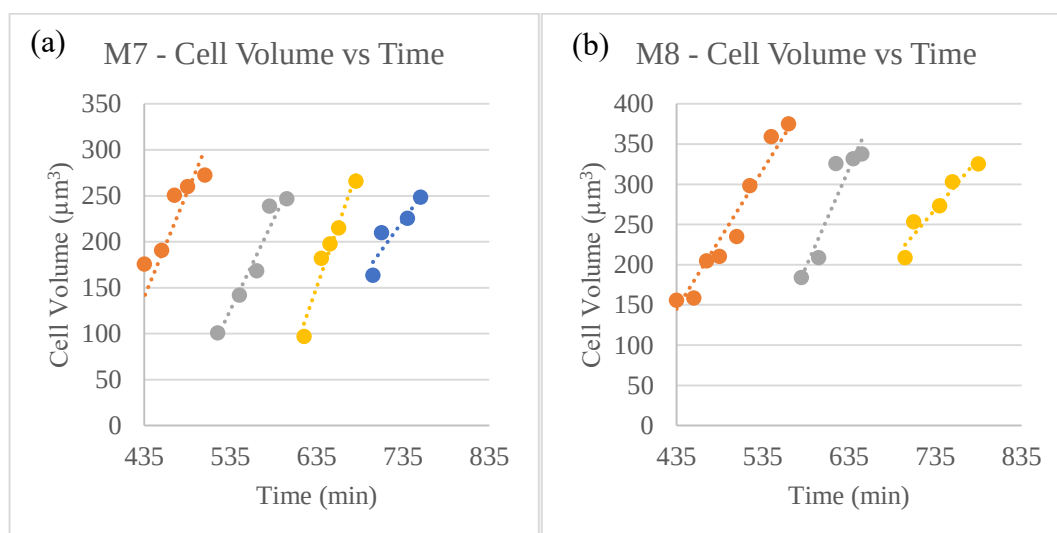


Figure 5.155. Graphical representation of processed data of outlet (a) mother cell 7 volume vs time graph (b) mother cell 8 volume vs time graph – EXP7

Table 5.55. The fold change in area, perimeter and volume of the M7 and M8 cells in outlet

Time	M7-Cell Area Fold Change	M7-Cell Perimeter Fold Change	M7-Cell Volume Fold Change	M8-Cell Area Fold Change	M8-Cell Perimeter Fold Change	M8-Cell Volume Fold Change
425	1.00	1.00	1.00	1.00	1.00	1.00
435	1.61	1.23	2.57	1.06	1.03	1.01
455	1.69	1.36	2.79	1.08	1.07	1.03
470	2.14	1.58	3.67	1.12	1.12	1.33
485	2.23	1.64	3.81	1.30	1.20	1.36
505	2.28	1.76	3.99	1.32	1.22	1.53
520	1.00	1.00	1.00	1.57	1.29	1.94
545	1.29	1.14	1.41	1.82	1.43	2.33
565	1.31	1.24	1.67	1.87	1.52	2.44
580	1.78	1.49	2.37	1.00	1.00	1.00
600	1.81	1.55	2.45	1.23	1.10	1.47
620	1.00	1.00	1.00	1.55	1.00	1.63
640	1.26	1.15	1.88	1.66	1.06	1.85
650	1.48	1.36	2.04	1.83	1.14	2.88
660	1.63	1.51	2.21	1.85	1.25	2.93
680	2.12	1.99	2.74	2.00	1.57	2.99
700	1.00	1.00	1.00	1.00	1.00	1.00
710	1.18	1.12	1.28	1.12	1.06	1.21
740	1.45	1.39	1.38	1.45	1.23	1.31
755	1.53	1.57	1.52	1.64	1.26	1.45
785	1.00	1.00	1.00	1.86	1.42	1.56

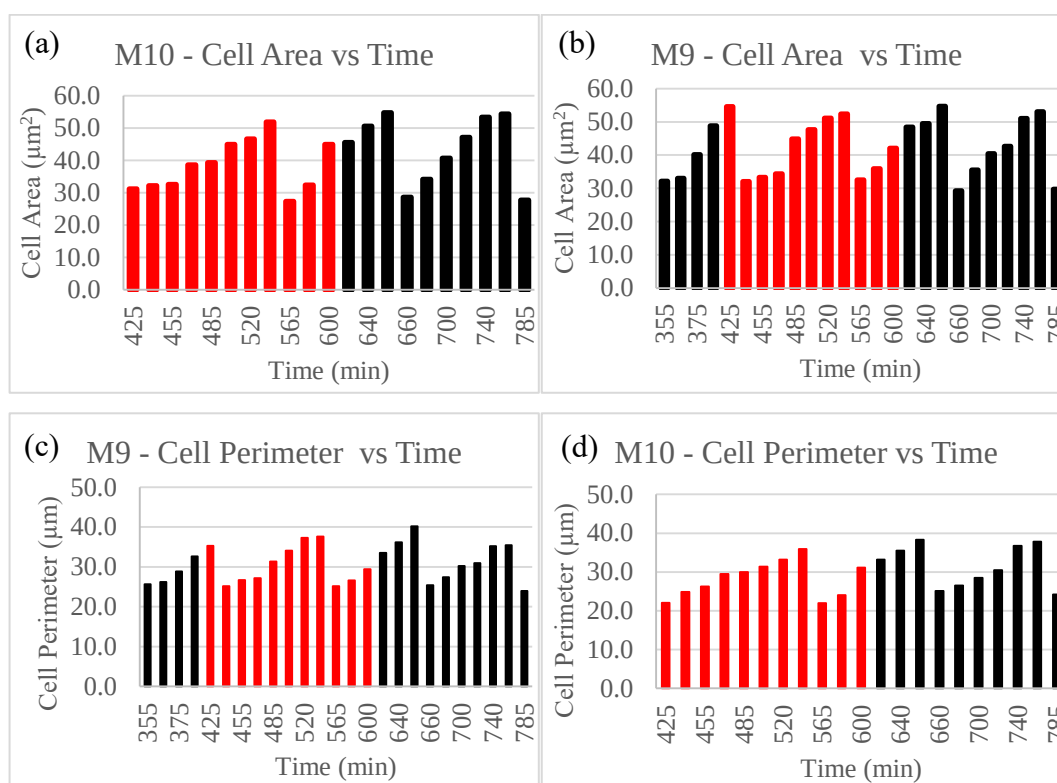


Figure 5.156. Graphical representation of processed data of outlet (a) mother cell 9 area vs time graph (b) mother cell 9 perimeter vs time graph (c) mother cell 10 area vs time graph (d) mother cell 10 perimeter vs time graph – EXP 7

Table 5.56. Duration of cell cycle phases of the analyzed cells (M9-M10) in outlet

Cell Name	Bud Number	Medium Type	G ₁ phase (min)	S/G ₂ /M phases (min)	Cell Cycle (min)	Area (μm ²)
M9	1	N1 - N2	30	60	90	30-55
M9	2	N2	45	95	150	30-55
M9	3	N2 - N1R	55	60	115	30-55
M9	4	N1R	30	105	135	30-55
M10	1	N2	45	95	150	30-55
M10	2	N2 – N1R	55	60	115	25-55
M10	3	N1R	30	105	135	25-55

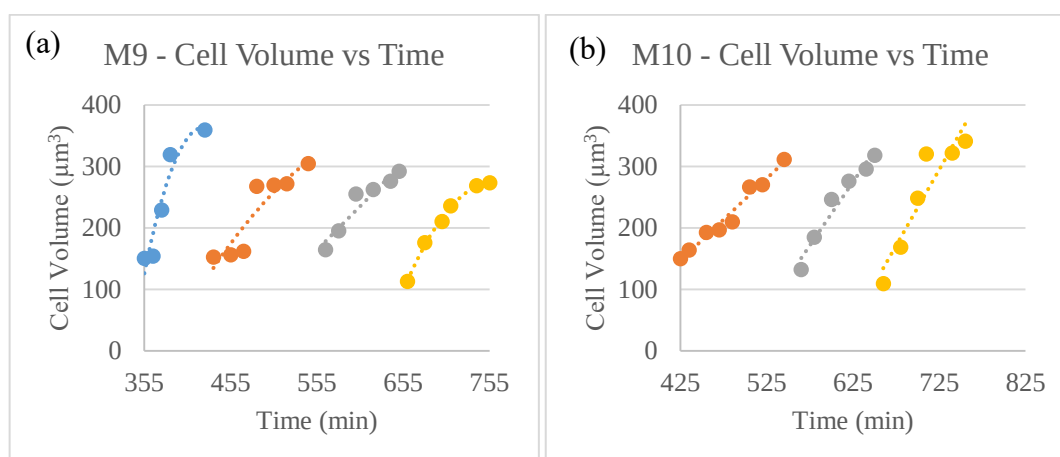


Figure 5.157. Graphical representation of processed data of outlet (a) mother cell 9 volume vs time graph (b) mother cell 10 volume vs time graph – EXP7

Table 5.57. The fold change in area, perimeter and volume of the M9 and M10 cells in outlet

Time	M9-Cell Area Fold Change	M9-Cell Perimeter Fold Change	M9-Volume Fold Change	M10-Cell Area Fold Change	M10-Cell Perimeter Fold Change	M10-Volume Fold Change
355	1.00	1.00	1.00	-	-	-
365	1.03	1.02	1.03	-	-	-
375	1.25	1.12	1.53	-	-	-
385	1.52	1.27	2.12	-	-	-
425	1.70	1.38	2.39	1.00	1.00	1.00
435	1.00	1.00	1.00	1.03	1.13	1.09
455	1.04	1.06	1.03	1.05	1.19	1.29
470	1.07	1.08	1.06	1.24	1.34	1.31
485	1.40	1.24	1.75	1.26	1.36	1.40
505	1.49	1.35	1.77	1.44	1.43	1.78
520	1.60	1.48	1.78	1.50	1.51	1.80
545	1.63	1.50	2.00	1.67	1.63	2.08

Table 5.57. The fold change in area, perimeter and volume of the M9 and M10 cells in outlet (cont.).

Time	M9-Cell Area Fold Change	M9-Cell Perimeter Fold Change	M9- Volume Fold Change	M10-Cell Area Fold Change	M10-Cell Perimeter Fold Change	M10- Volume Fold Change
565	1.00	1.00	1.00	1.00	1.00	1.00
580	1.10	1.06	1.19	1.19	1.09	1.40
600	1.29	1.17	1.55	1.64	1.42	1.86
620	1.49	1.33	1.60	1.67	1.51	2.08
640	1.52	1.44	1.68	1.85	1.61	2.23
650	1.68	1.60	1.77	2.00	1.74	2.40

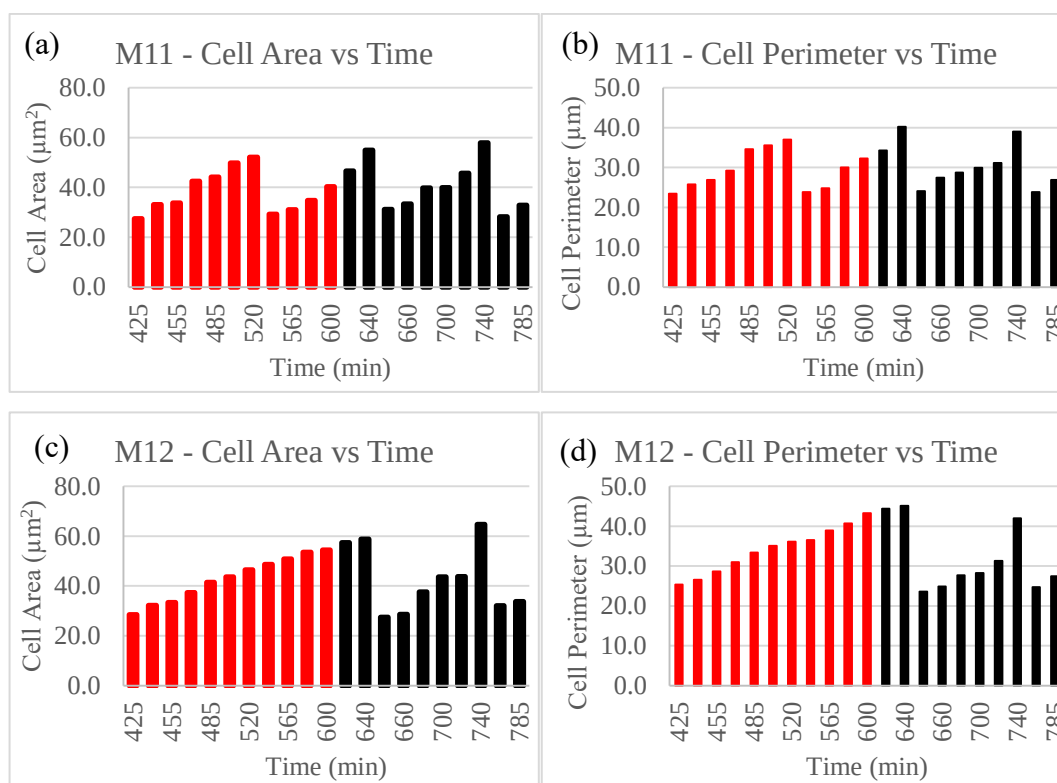


Figure 5.158. Graphical representation of processed data of outlet (a) mother cell 11 area vs time graph (b) mother cell 11 perimeter vs time graph (c) mother cell 12 area vs time graph (d) mother cell 12 perimeter vs time graph – EXP 7

Table 5.58. Duration of cell cycle phases of the analyzed cells (M11-M12) in outlet

Cell Name	Bud Number	Medium Type	G ₁ phase (min)	S/G ₂ /M phases (min)	Cell Cycle (min)	Area (μm ²)
M11	1	N2	30	90	120	25-55
M11	2	N2 - N1R	45	85	130	25-55
M11	3	N1R	40	75	115	30-60
M12	1	N2	60	165	225	25-60
M12	2	N1R	40	75	115	25-60

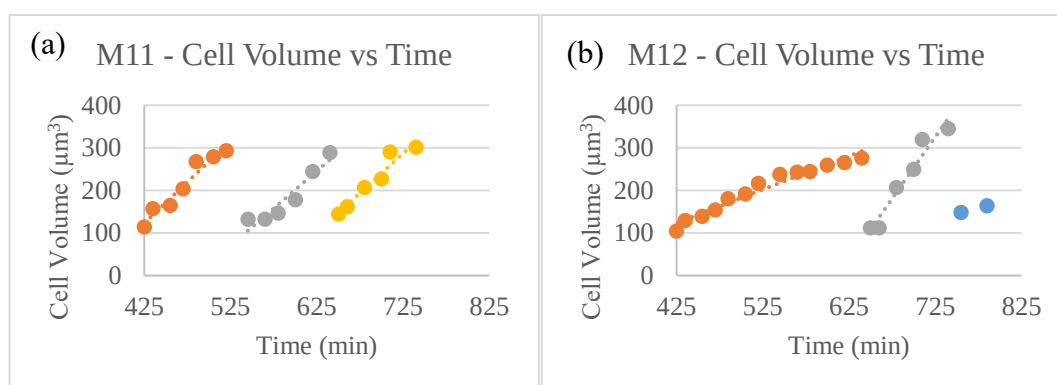


Figure 5.159. Graphical representation of processed data of outlet (a) mother cell 11 volume vs time graph (b) mother cell 12 volume vs time graph – EXP7

Table 5.59. The fold change in area, perimeter and volume of the M11 and M12 cells in outlet

Time	M11-Cell Area Fold Change	M11-Cell Perimeter Fold Change	M11-Cell Volume Fold Change	M12-Cell Area Fold Change	M12-Cell Perimeter Fold Change	M12-Cell Volume Fold Change
425	1.00	1.00	1.00	1.00	1.00	1.00
435	1.20	1.10	1.38	1.13	1.05	1.24

Table 5.59. The fold change in area, perimeter and volume of the M11 and M12 cells in outlet (cont.).

Time	M11-Cell Area Fold Change	M11-Cell Perimeter Fold Change	M11-Cell Volume Fold Change	M12-Cell Area Fold Change	M12-Cell Perimeter Fold Change	M12-Cell Volume Fold Change
455	1.23	1.15	1.44	1.17	1.13	1.33
470	1.54	1.25	1.78	1.31	1.22	1.47
485	1.61	1.48	2.34	1.46	1.32	1.73
505	1.81	1.52	2.44	1.54	1.38	1.83
520	1.89	1.58	2.57	1.63	1.43	2.08
545	1.00	1.00	1.00	1.00	1.00	1.00
565	1.06	1.04	1.00	1.04	1.07	1.02
580	1.19	1.26	1.11	1.10	1.11	1.03
600	1.38	1.35	1.35	1.12	1.19	1.09
620	1.59	1.44	1.85	1.18	1.22	1.12
640	1.88	1.69	2.18	1.21	1.24	1.16
650	1.00	1.00	1.00	1.00	1.00	1.00
660	1.07	1.14	1.12	1.04	1.06	1.00
680	1.27	1.19	1.43	1.37	1.18	1.84

Table 5.60. Duration of cell cycle phases of the analyzed cells in different environments

Medium	G₁ phase (min)	Ave. G₁ Phase (min)	S/G₂/M phases (min)	Ave. S/G₂/M phases (min)	Cell Cycle (min)	Ave. Cell Cycle (min)
N1	25-65	40	40-75	60	60-130	100
N1-N2	25-70	45	50-110	75	80-170	115
N2	30-50	45	65-110	90	95-150	130
N2-N1R	35-60	50	60-185	95	115-235	145
N1R	30-50	40	55-105	80	90-140	115

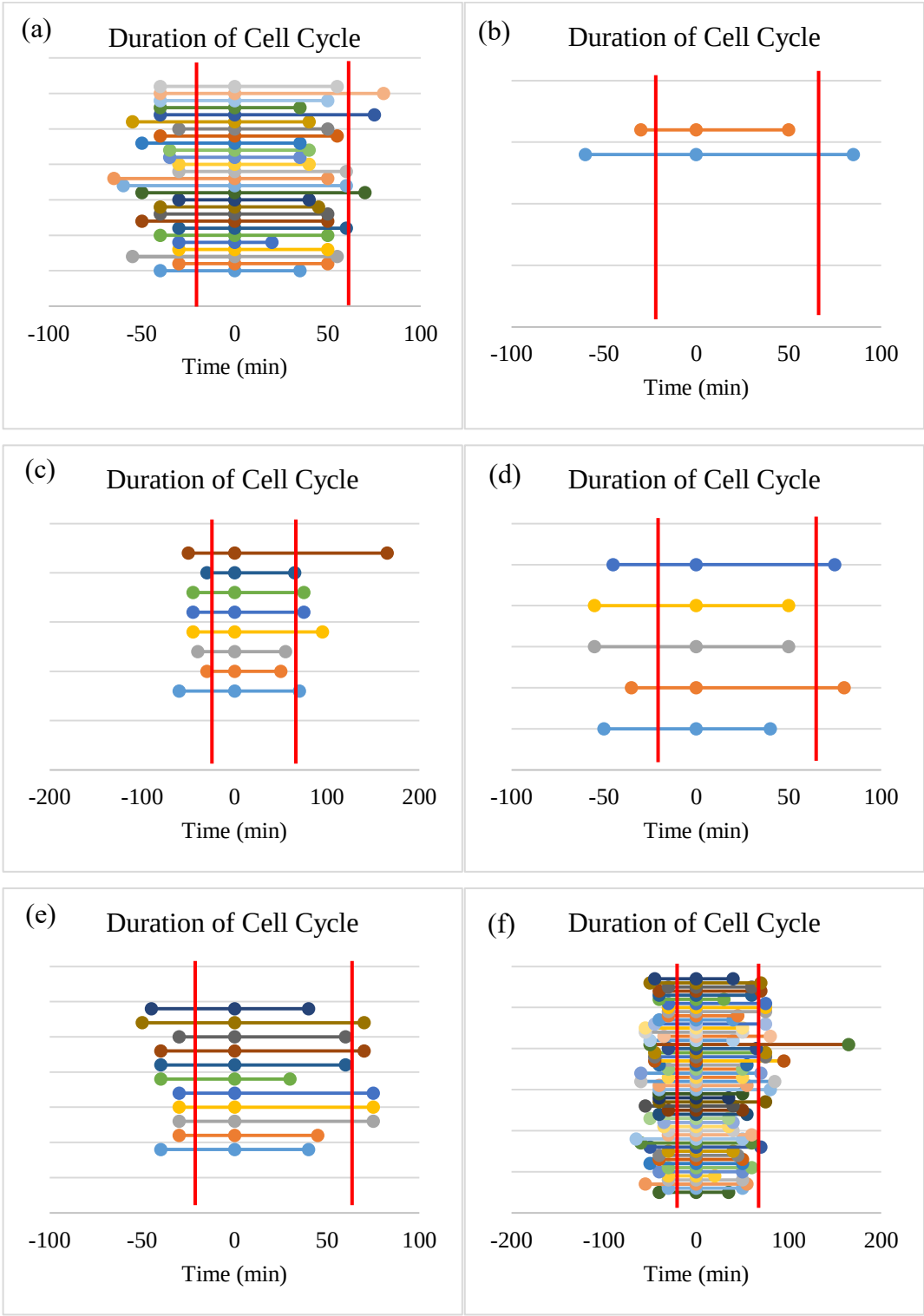


Figure 5.160. The duration of cell cycle (a) in N1 medium (b) in N1-N2 medium (c) in N2 medium (d) in N2-N1R medium (e) N1R medium (f) in all media

Table 5.61. The duration of the cell cycle phases in different environments

Medium Type	2 % Glucose		0.2 % Glucose	
	G ₁	S/G ₂ /M	G ₁	S/G ₂ /M
N1	35	65	40	60
N1-N2	45	95	45	75
N2	55	95	45	90
N2-N1R	55	125	50	95
N1R	35	85	40	80

Table 5.62. The comparison of the duration of cell cycle phases between daughter and mother cells

Cell Name	Medium Type	Ave. G ₁ (min)	Ave. S/G ₂ /M (min)	Ave. Cell Cycle (min)
Mother	N1	38	47	85
Daughter	N1	59	63	122

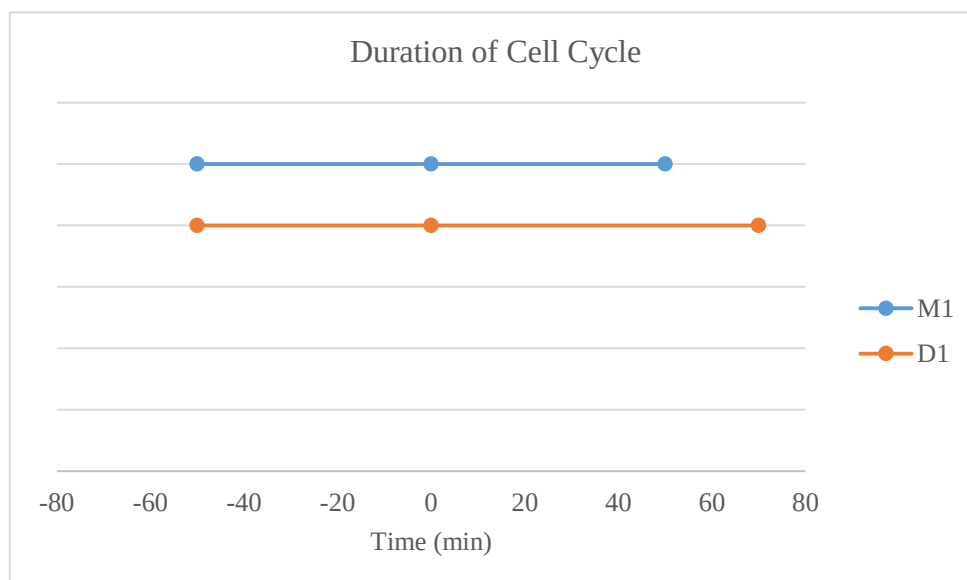


Figure 5.161. The comparison of the duration of cell cycle between mother and its daughter cells in calorie restricted YPD media

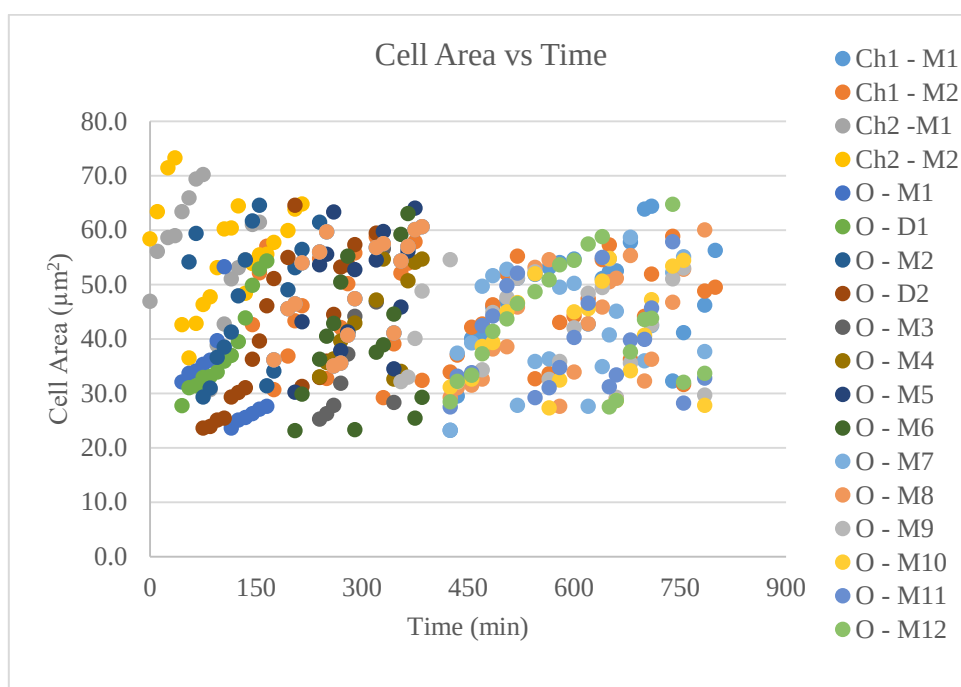


Figure 5.162. The comparison of cell area

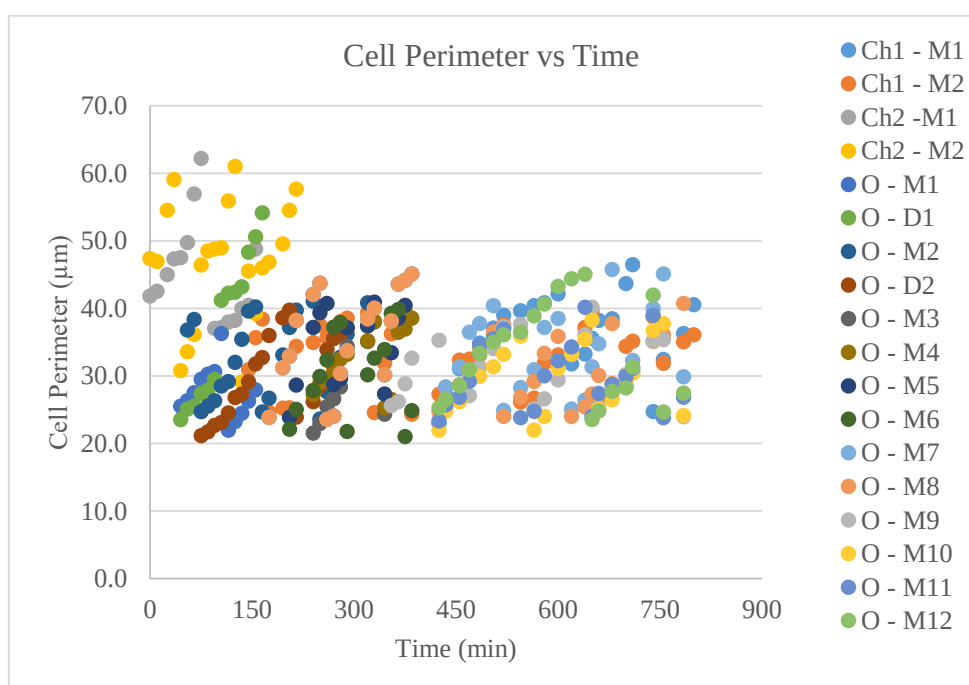


Figure 5.163. The comparison of cell perimeter

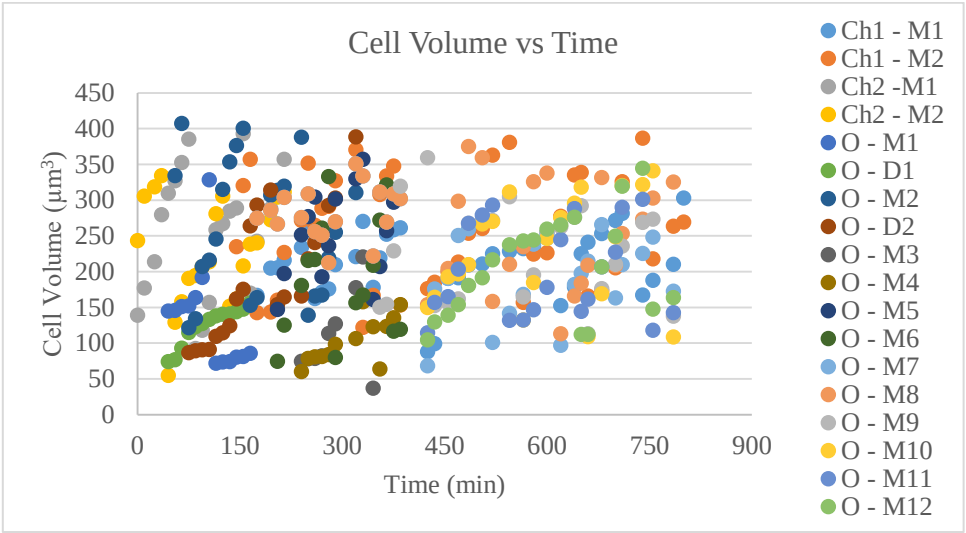


Figure 5.164. The comparison of cell volume

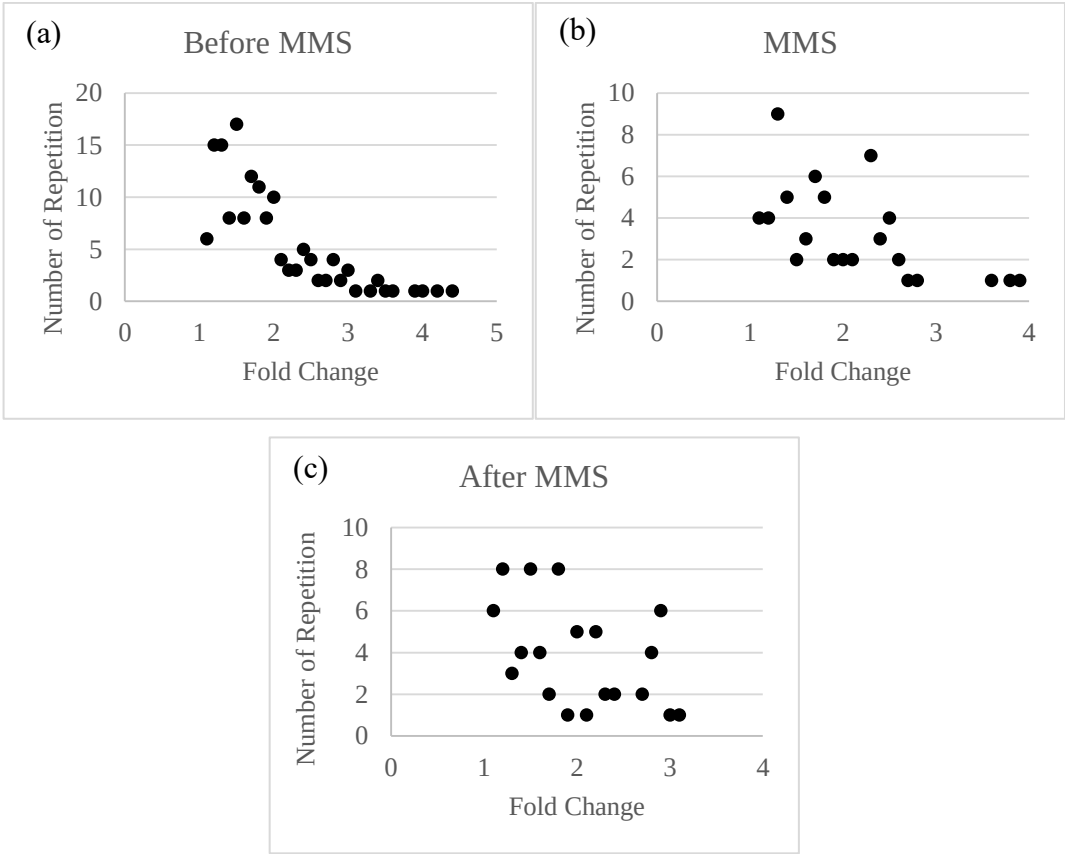


Figure 5.165. The volumetric fold change of the cells (a) in glucose limited YPD media (b) in glucose limited YPD media containing 0.01 MMS (c) in fresh glucose limited YPD medium

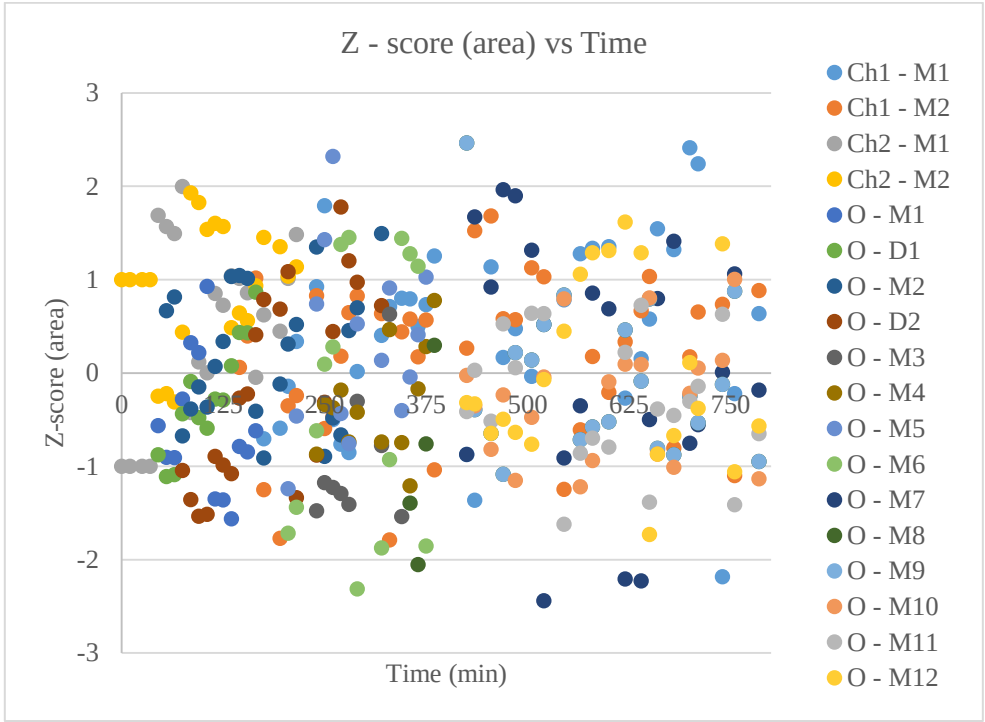


Figure 5.166. Graphical demonstration of Z-score for cell area

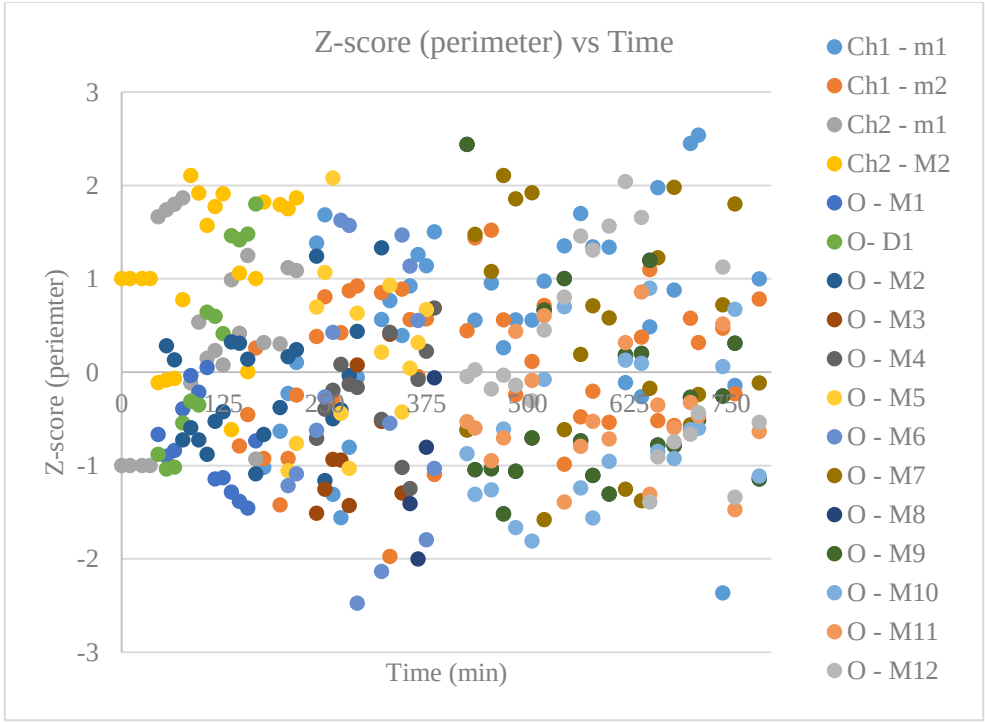


Figure 5.167. Graphical demonstration of Z-score for cell perimeter

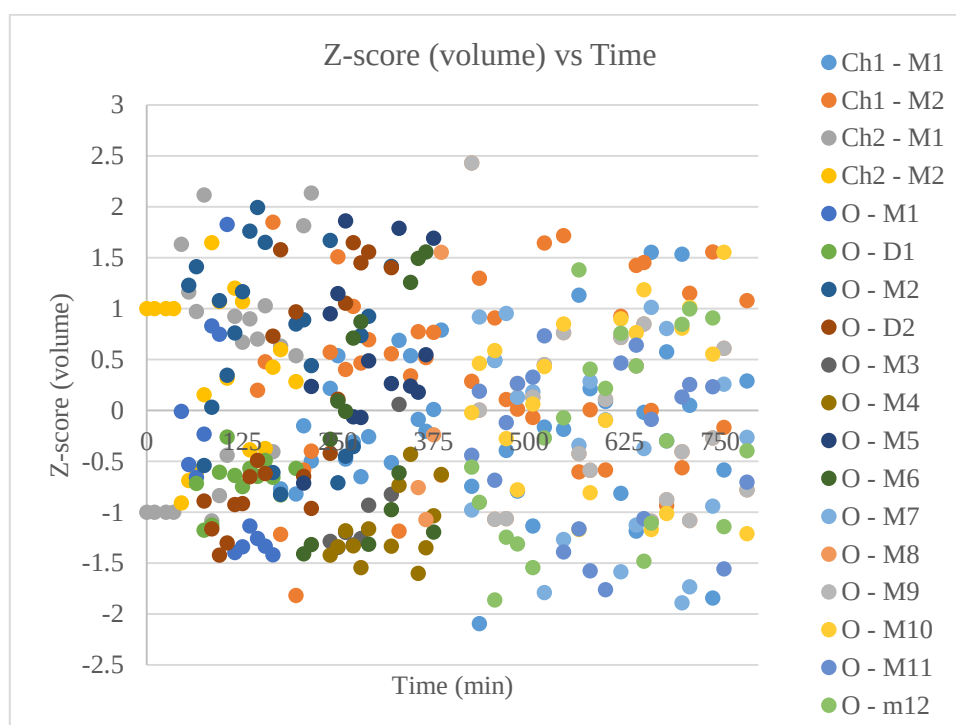


Figure 5.168. Graphical demonstration of Z-score for cell volume

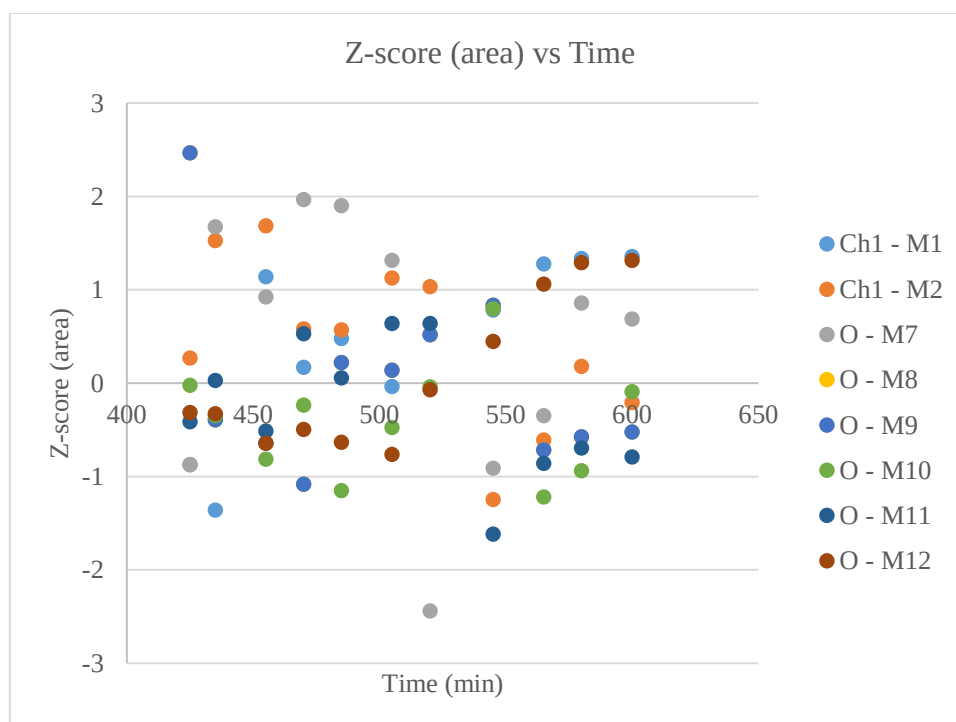


Figure 5.169. Graphical demonstration of Z-score for cell area for the cells under effect of inhibitor

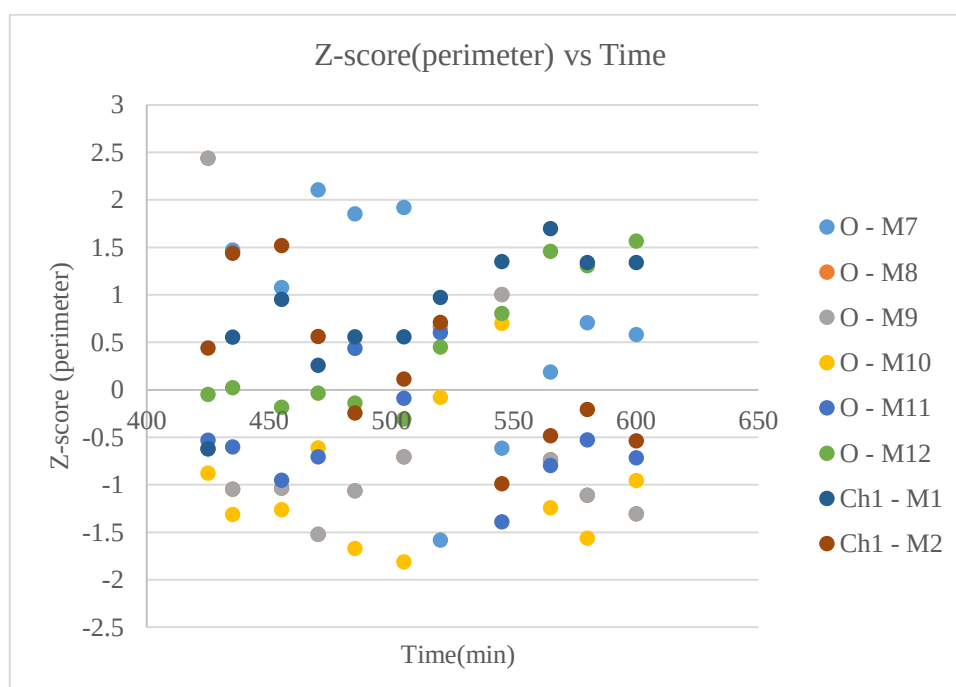


Figure 5.170. Graphical demonstration of Z-score for cell perimeter for the cells under effect of inhibitor

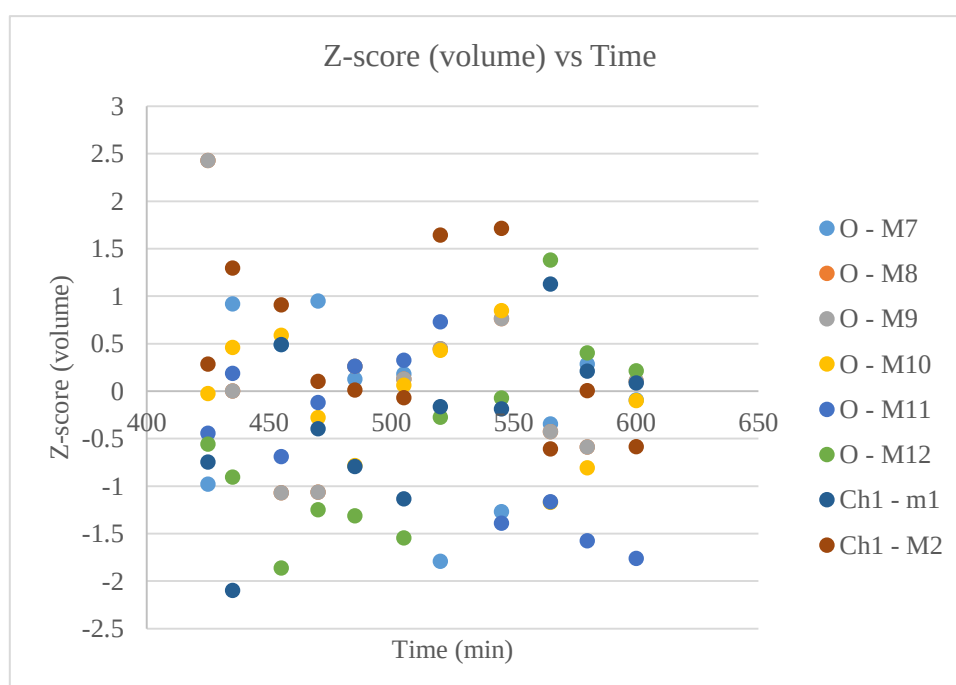


Figure 5.171. Graphical demonstration of Z-score for cell volume for the cells under effect of inhibitor

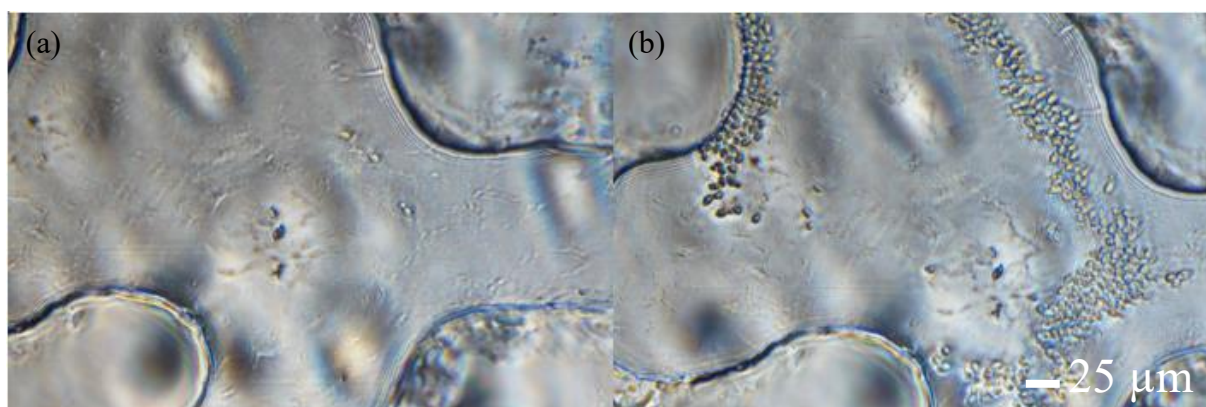


Figure 5.172. Images of the cells that were trapped in chamber 1 (a) at 0th minute (b) 728th minute

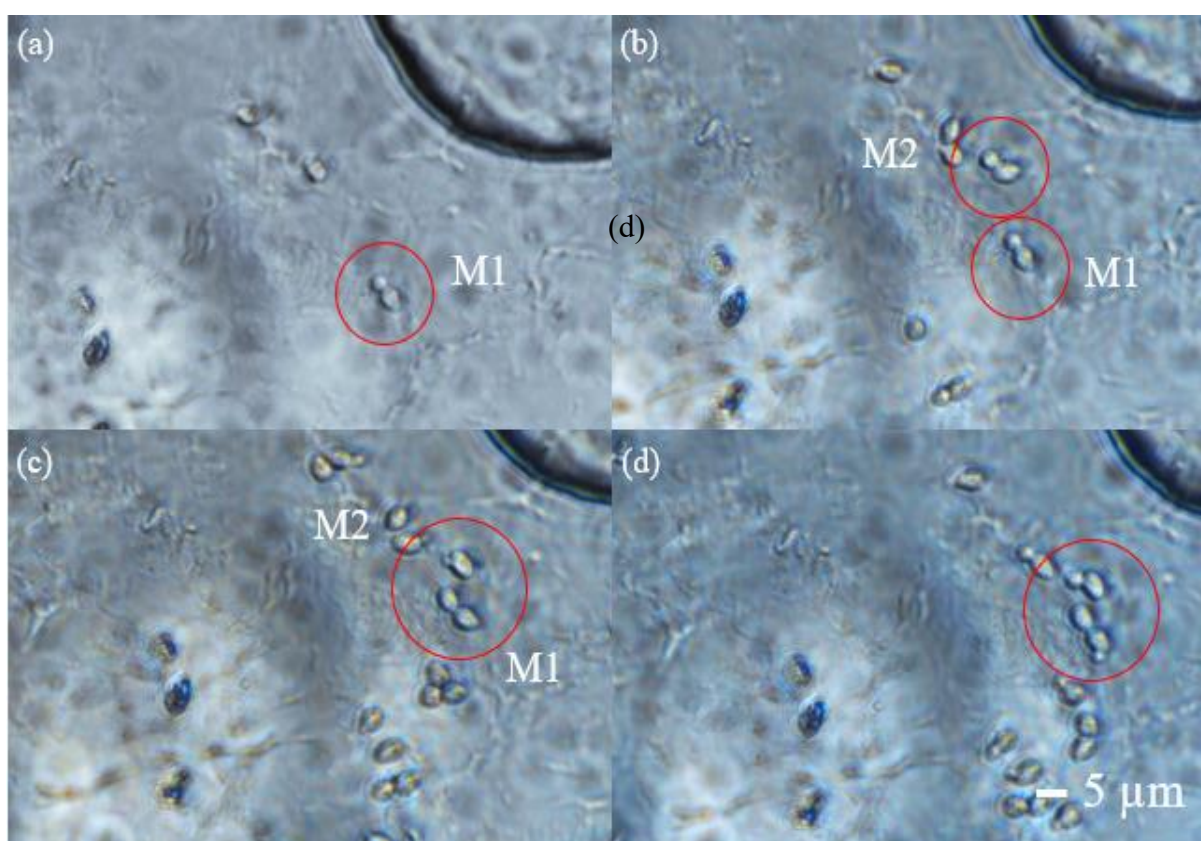


Figure 5.173. The image of the mother cells at (a) 0th minute (b) 3th minute (c) 45th minute (d) 68th minute under 90X magnification

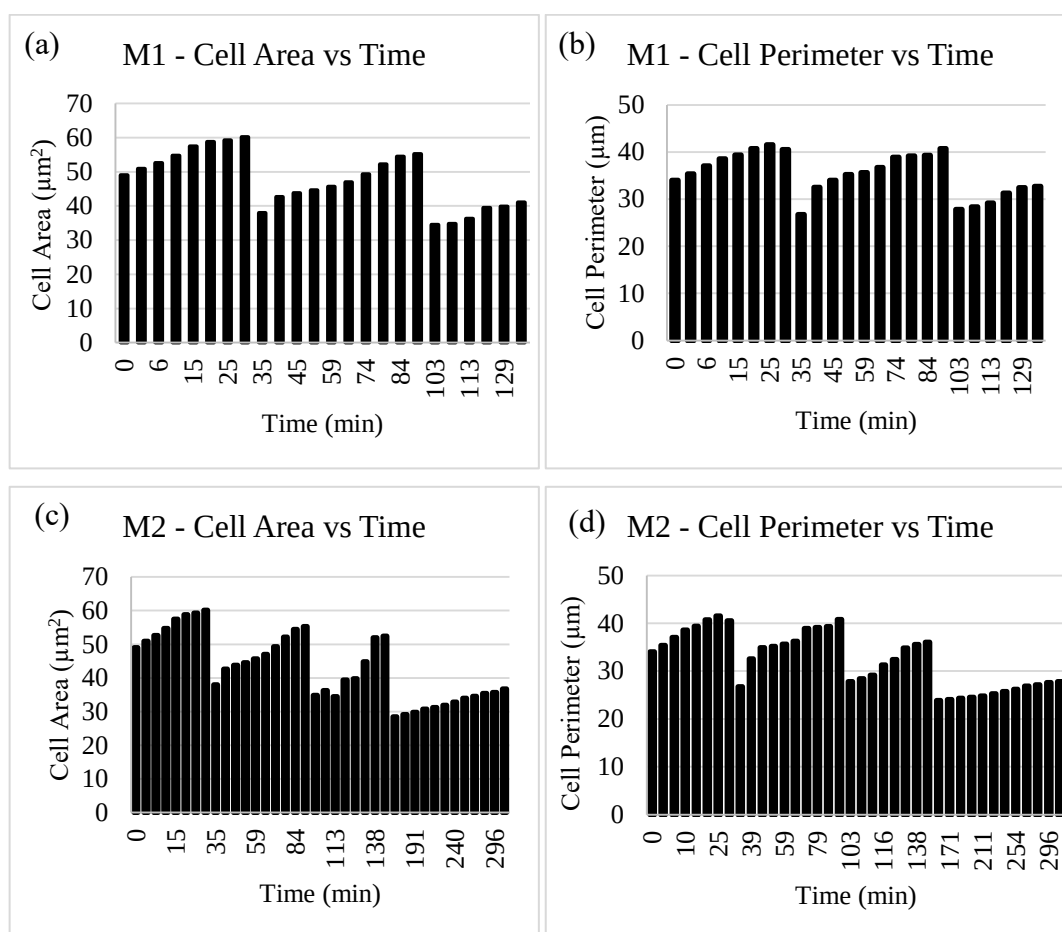


Figure 5.174. Graphical representation of processed data of chamber 1 (a) mother cell 1 area vs time graph (b) mother cell 1 perimeter vs time graph (c) mother cell 2 area vs time graph (d) mother cell 2 perimeter vs time graph – EXP 8

Table 5.63. Duration of cell cycle phases of the analyzed cells in chamber 1

Cell Name	Bud Number	Medium Type	G ₁ phase (min)	S/G ₂ /M phases (min)	Cell Cycle (min)	Area (μm^2)
M1	1	N1	-	35	>35	50-60
M1	2	N1	24	44	68	40-60
M2	1	N1	-	31	>31	50-60
M2	2	N1	24	48	68	35-55
M2	3	N1	16	51	67	35-55

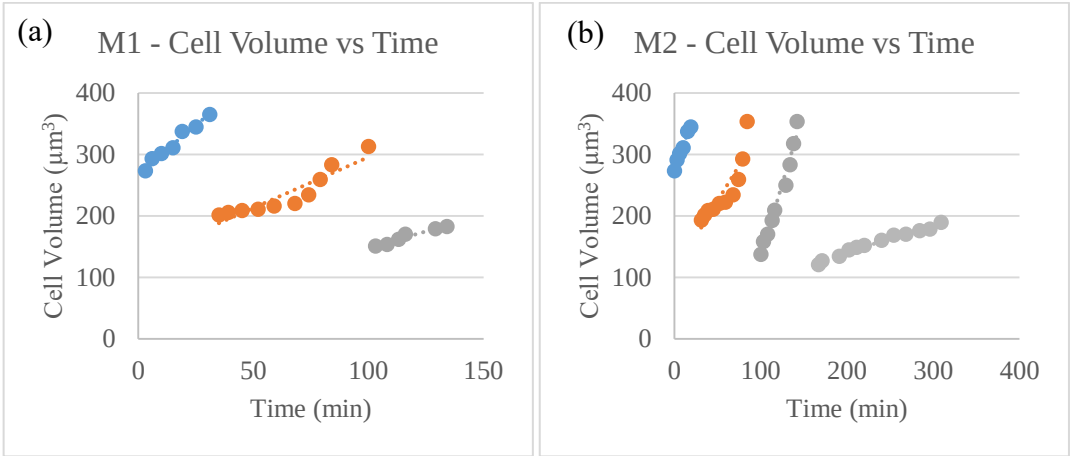


Figure 5.175. Graphical representation of processed data of chamber 1 (a) mother cell 1 volume vs time graph (b) mother cell 2 volume vs time graph – EXP8

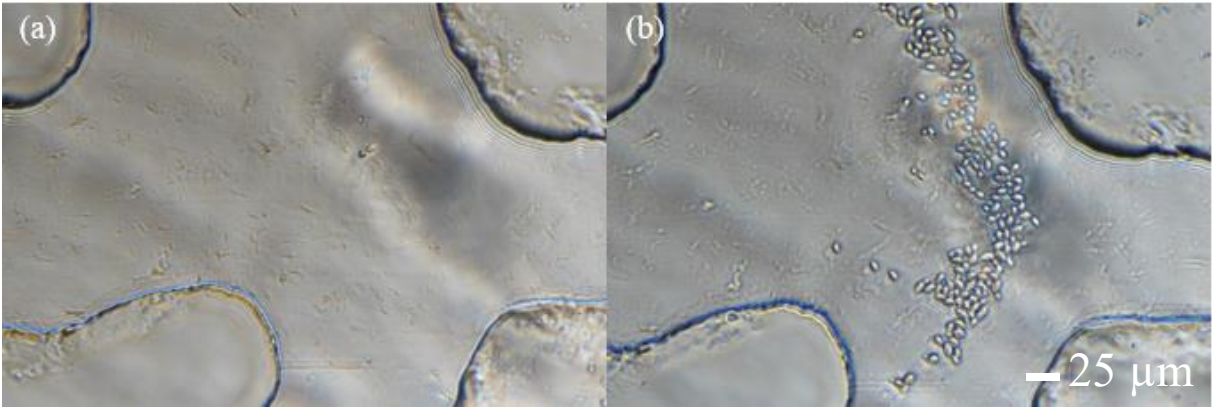


Figure 5.176. Images of the cells that were trapped in chamber 2 (a) at 240th minute (b) 728th minute

Table 5.64. The fold change in area, perimeter and volume of the M1 and M2 cells in chamber 1

Time	M1-Cell Area Fold Change	M1-Cell Perimeter Fold Change	M1- Volume Fold Change	M2-Cell Area Fold Change	M2-Cell Perimeter Fold Change	M2- Volume Fold Change
0	1.00	1.00	1.00	1.00	1.00	1.00
3	1.04	1.04	1.07	1.04	1.04	1.07

Table 5.64. The fold change in area, perimeter and volume of the M1 and M2 cells in chamber 1 (cont.).

Time	M1-Cell Area Fold Change	M1-Cell Perimeter Fold Change	M1- Volume Fold Change	M2-Cell Area Fold Change	M2-Cell Perimeter Fold Change	M2- Volume Fold Change
6	1.07	1.09	1.10	1.07	1.09	1.10
10	1.12	1.13	1.14	1.12	1.13	1.14
15	1.17	1.16	1.23	1.17	1.16	1.23
19	1.20	1.20	1.26	1.20	1.20	1.26
25	1.21	1.22	1.34	1.21	1.22	1.34
31	1.23	1.19	1.37	1.23	1.19	1.44
35	1.00	1.00	1.00	1.00	1.00	1.00
39	1.12	1.22	1.02	1.12	1.22	1.04
45	1.15	1.27	1.04	1.15	1.31	1.05
52	1.17	1.32	1.05	1.17	1.32	1.10
59	1.20	1.33	1.07	1.20	1.33	1.10
68	1.24	1.37	1.10	1.24	1.36	1.16
74	1.30	1.46	1.16	1.30	1.46	1.29
79	1.37	1.47	1.29	1.37	1.47	1.45
84	1.43	1.47	1.41	1.43	1.47	1.76
100	1.46	1.53	1.55	1.46	1.53	1.84
103	1.00	1.00	1.00	1.00	1.00	1.00
108	1.01	1.02	1.02	1.04	1.02	1.07
113	1.05	1.05	1.07	1.11	1.05	1.22
116	1.14	1.12	1.13	1.13	1.12	1.32
129	1.16	1.16	1.19	1.14	1.16	1.58
134	1.19	1.18	1.21	1.29	1.25	1.79
138	-	-	-	1.49	1.28	2.00
142	-	-	-	1.51	1.29	2.23
167	-	-	-	1.00	1.00	1.00

Table 5.64. The fold change in area, perimeter and volume of the M1 and M2 cells in chamber 1 (cont.).

Time	M1-Cell Area Fold Change	M1-Cell Perimeter Fold Change	M1- Volume Fold Change	M2-Cell Area Fold Change	M2-Cell Perimeter Fold Change	M2- Volume Fold Change
220	-	-	-	1.12	1.06	1.26
240	-	-	-	1.16	1.08	1.33
254	-	-	-	1.19	1.10	1.40
268	-	-	-	1.22	1.13	1.41
284	-	-	-	1.24	1.14	1.46
296	-	-	-	1.26	1.16	1.47
309	-	-	-	1.29	1.17	1.56

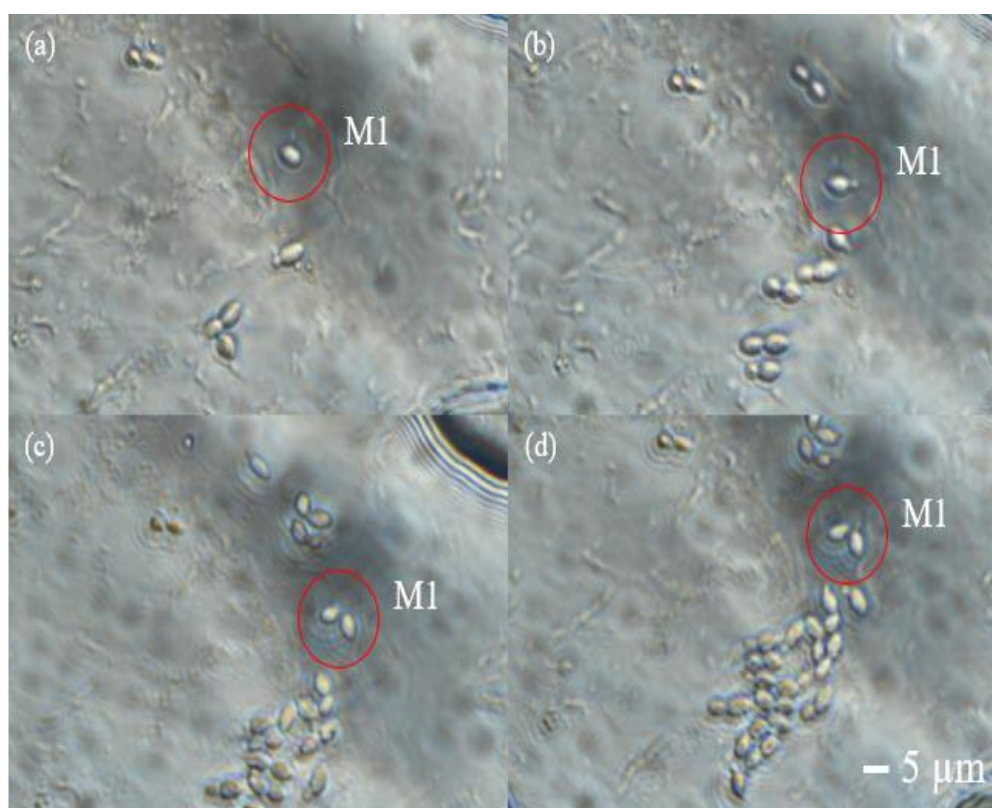


Figure 5.177. The image of the mother cell at (a) 254th minute (b) 284th minute (c) 344th minute (d) 359th minute under 90X magnification

Table 5.65. Duration of cell cycle phases of the analyzed cells in chamber 2

Cell Name	Bud Number	Medium Type	G ₁ phase (min)	S/G ₂ /M phases (min)	Cell Cycle (min)	Area (μm^2)
M1	1	N1	30	40	70	35-55
M1	2	N1-N2	35	52	87	30-55
M1	3	N2	43	42	85	30-50
M1	4	N2-N1R	35	52	87	30-55

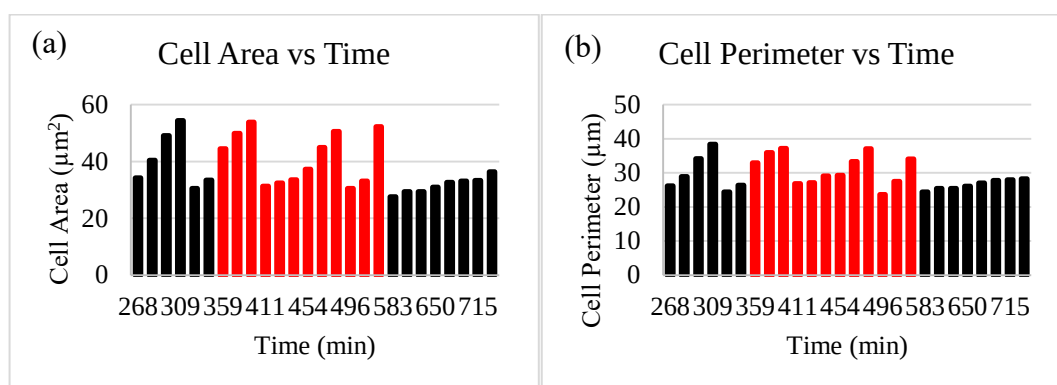


Figure 5.178. Graphical representation of processed data of chamber 2 (a) mother cell area vs time graph (b) mother cell perimeter vs time graph– EXP 8

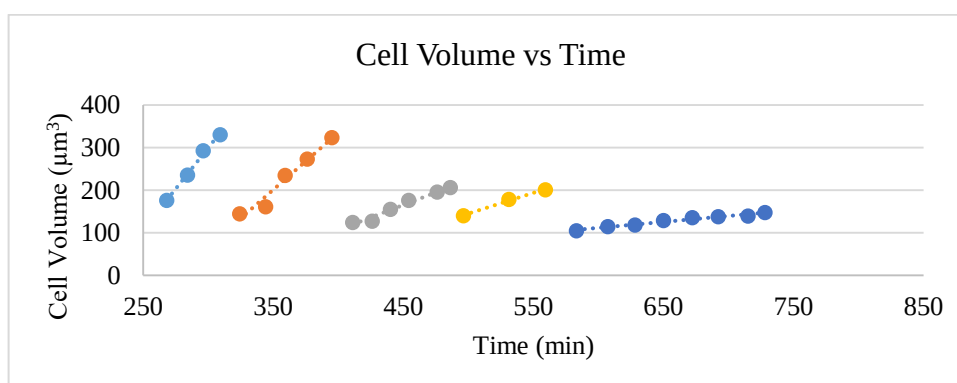


Figure 5.179. Graphical representation of processed data of chamber 2, mother cell volume vs time graph – EXP8

Table 5.66. The fold change in area, perimeter and volume of the cell in chamber 2

Time	Cell Area Fold Change	Cell Perimeter Fold Change	Volume Fold Change
268	1.00	1.00	1.00
284	1.18	1.11	1.34
296	1.44	1.31	1.66
309	1.59	1.47	1.88
324	1.00	1.00	1.00
344	1.10	1.08	1.11
359	1.46	1.35	1.62
376	1.63	1.47	1.89
395	1.77	1.52	2.24
411	1.00	1.00	1.00
426	1.03	1.01	1.03
440	1.07	1.08	1.25
454	1.19	1.09	1.42
476	1.43	1.24	1.57
486	1.61	1.38	1.66
496	1.00	1.00	1.00
531	1.08	1.16	1.27
559	1.71	1.44	1.44
583	1.00	1.00	1.00
607	1.06	1.04	1.09
628	1.06	1.04	1.13
650	1.12	1.07	1.23
672	1.18	1.11	1.30
692	1.20	1.14	1.32
715	1.21	1.15	1.33
728	1.32	1.16	1.41

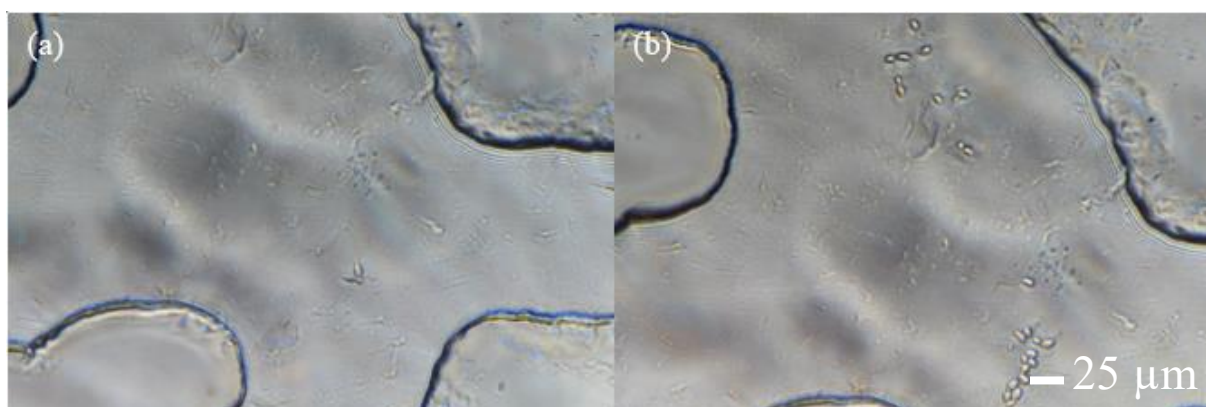


Figure 5.180. Images of the cells that were trapped in chamber 3 (a) at 454th minute (b) 728th minute

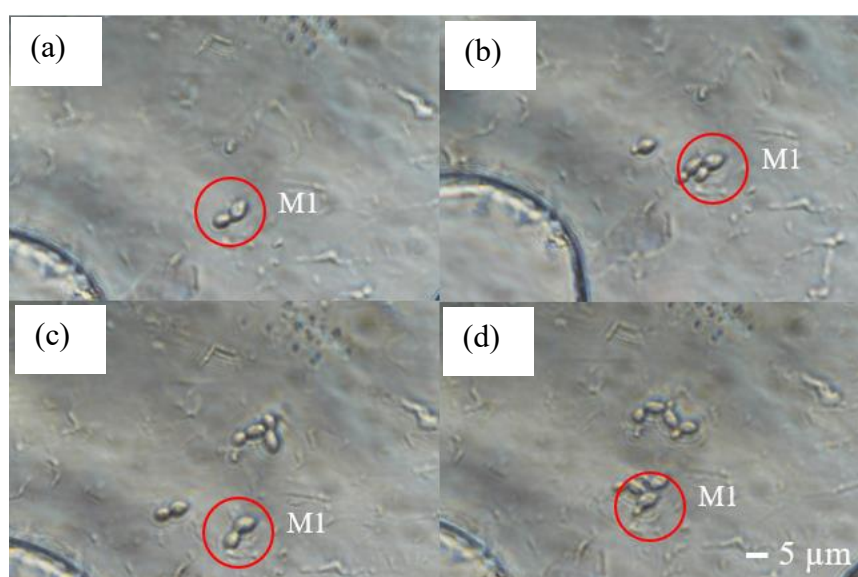


Figure 5.181. The image of the mother cell at (a) 454th minute (b) 496th minute (c) 559th minute (d) 583th minute under 90X magnification

Table 5.67. The fold change in area, perimeter and volume of the cell in chamber 3

Time	Cell Area Fold Change	Cell Perimeter Fold Change	Volume Fold Change
454	1.00	1.00	1.00
476	1.08	1.02	1.20

Table 5.67. The fold change in area, perimeter and volume of the cell in chamber 3 (cont.).

Time	Cell Area Fold Change	Cell Perimeter Fold Change	Volume Fold Change
486	1.25	1.12	1.53
496	1.30	1.16	1.62
531	1.57	1.25	1.75
559	1.85	1.48	1.82
583	1.00	1.00	1.00
607	1.07	1.04	1.15
628	1.12	1.07	1.22
650	1.15	1.08	1.27
672	1.16	1.09	1.28
692	1.18	1.11	1.30
715	1.19	1.14	1.31
728	1.20	1.15	1.31

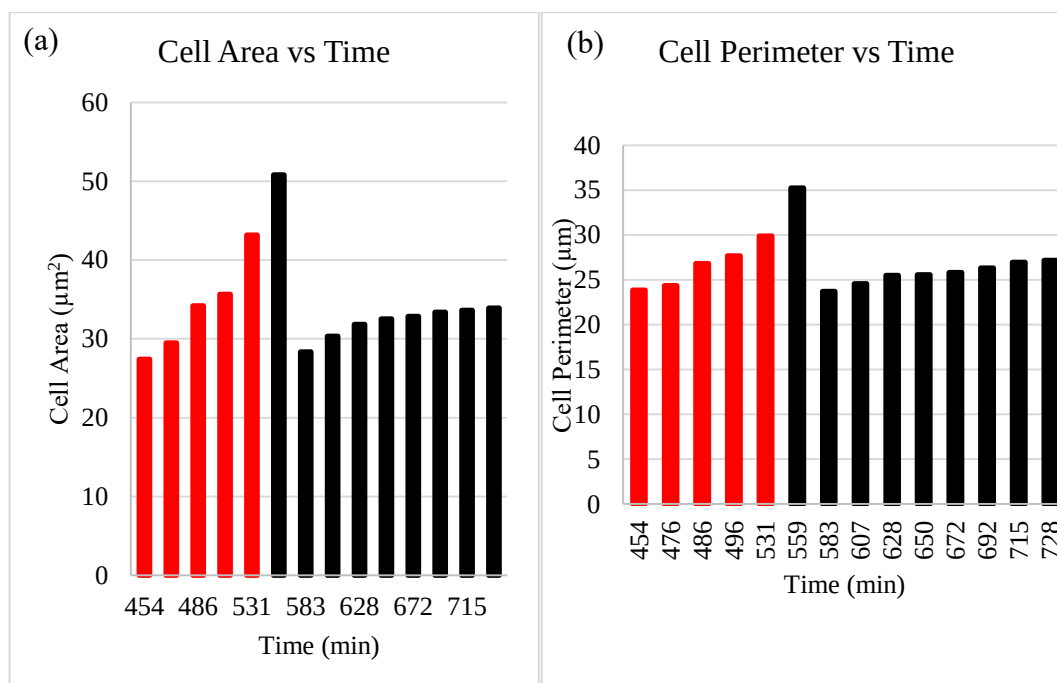


Figure 5.182. Graphical representation of processed data of chamber 3 (a) mother cell area vs time graph (b) mother cell perimeter vs time graph – EXP 8

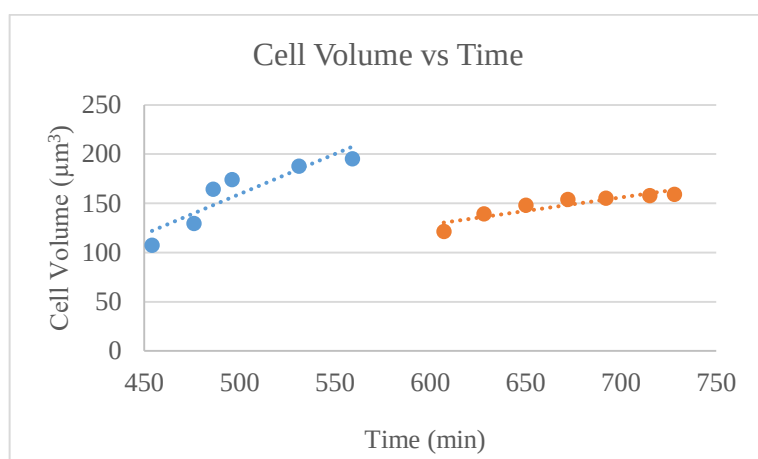


Figure 5.183. Graphical representation of processed data of chamber 3, mother cell volume vs time graph – EXP8

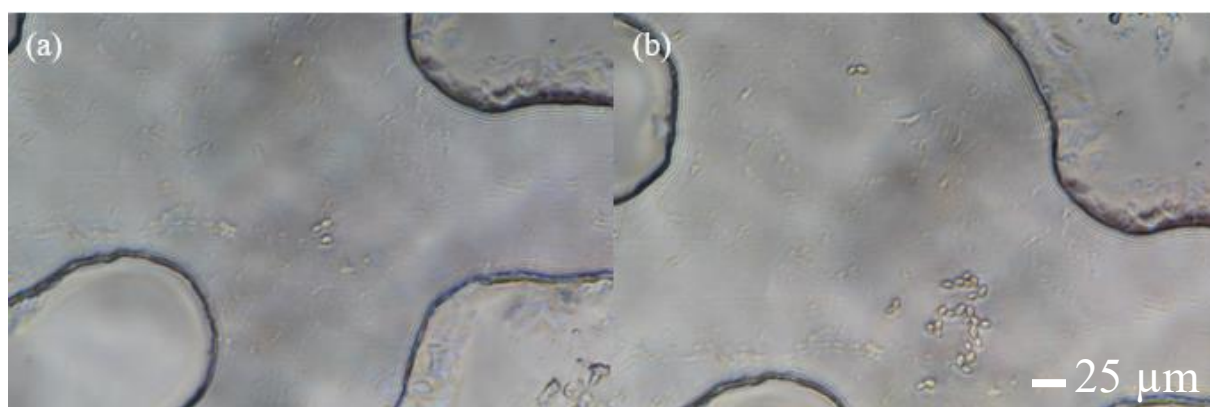


Figure 5.184. Images of the cells that were trapped in chamber 4 (a) at 344th minute (b) 728th minute

Table 5.68. Duration of cell cycle phases of the analyzed cells in chamber 4

Cell Name	Bud Number	Medium Type	G ₁ phase (min)	S/G ₂ /M phases (min)	Cell Cycle (min)	Area (μm^2)
M1	1	N1-N2	15	36	51	30-50
M1	2	N2	31	50	81	25-50
M1	3	N2	20	63	83	25-50

Table 5.68. Duration of cell cycle phases of the analyzed cells in chamber 4 (cont.).

Cell Name	Bud Number	Medium Type	G ₁ phase (min)	S/G ₂ /M phases (min)	Cell Cycle (min)	Area (μm ²)
M1	4	N2-N1R	25	>145	>170	30-40
M2	1	N1-N2	36	45	81	25-50
M2	2	N2	36	55	91	25-50
M2	3	N2-N1R	28	48	76	25-50

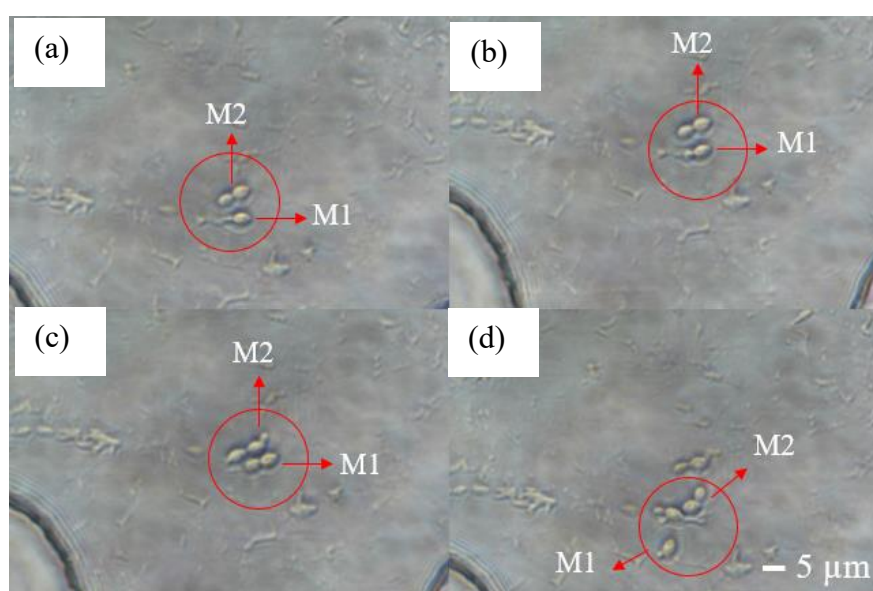


Figure 5.185. The image of the mother cell at (a) 344th minute (b) 359th minute (c) 395th minute (d) 426th minute under 90X magnification

Table 5.69. The fold change in area, perimeter and volume of the M1 and M2 cells in chamber 4

Time	M1-Cell Area Fold Change	M1-Cell Perimeter Fold Change	M1-Cell Volume Fold Change	M2-Cell Area Fold Change	M2-Cell Perimeter Fold Change	M2-Cell Volume Fold Change
344	1.00	1.00	1.00	-	-	-

Table 5.69. The fold change in area, perimeter and volume of the M1 and M2 cells in chamber 4 (cont.).

Time	M1-Cell Area Fold Change	M1-Cell Perimeter Fold Change	M1- Volume Fold Change	M2-Cell Area Fold Change	M2-Cell Perimeter Fold Change	M2- Volume Fold Change
359	1.27	1.09	1.19	1.00	1.00	1.00
376	1.47	1.39	1.52	1.33	1.17	1.18
395	1.00	1.00	1.00	1.46	1.24	1.28
411	1.33	1.17	1.22	1.63	1.41	1.45
426	1.46	1.24	1.55	1.80	1.54	1.54
440	1.63	1.41	1.63	1.00	1.00	1.00
454	1.80	1.54	1.75	1.17	1.12	1.25

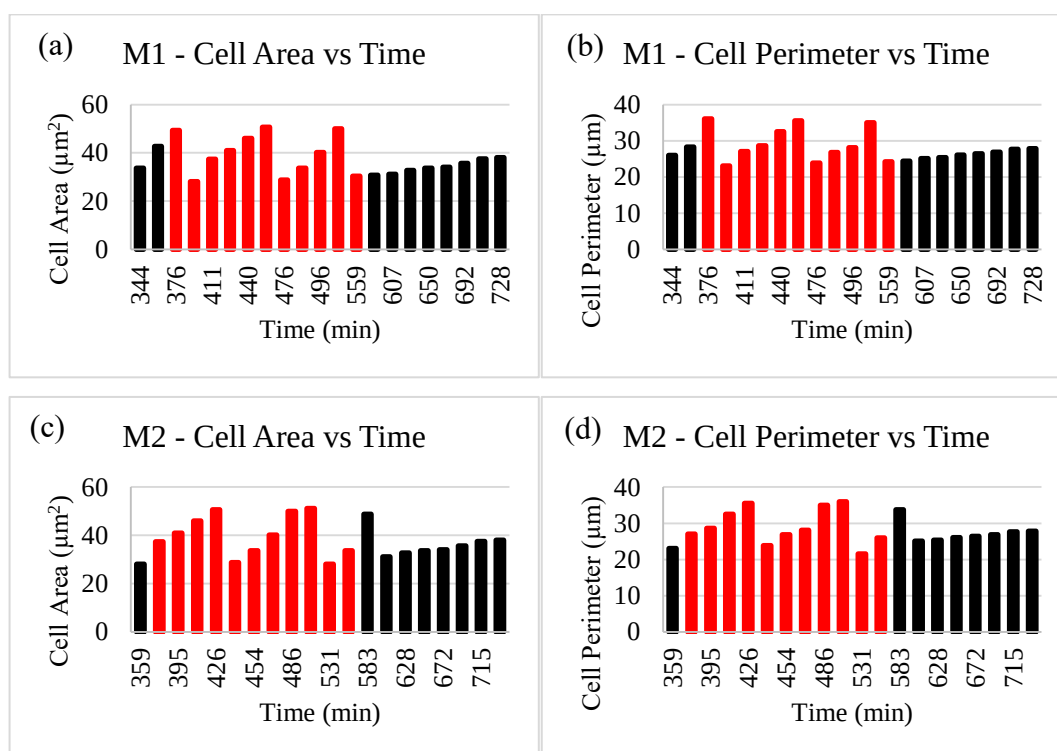


Figure 5.186. Graphical representation of processed data of chamber 4 (a) M1 area vs time graph (b) M1 perimeter vs time graph (c) M2 area vs time graph (d) M2 perimeter vs time graph – EXP 8

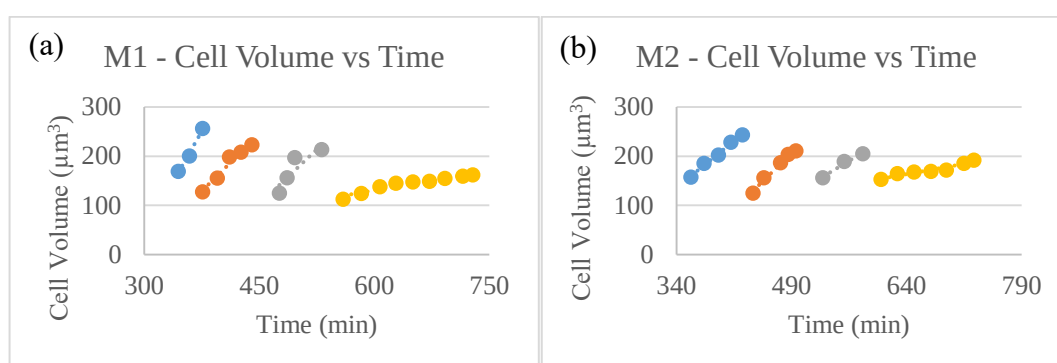


Figure 5.187. Graphical representation of processed data of chamber 4 (a) mother cell 1 volume vs time graph (b) mother cell 2 volume vs time graph – EXP8

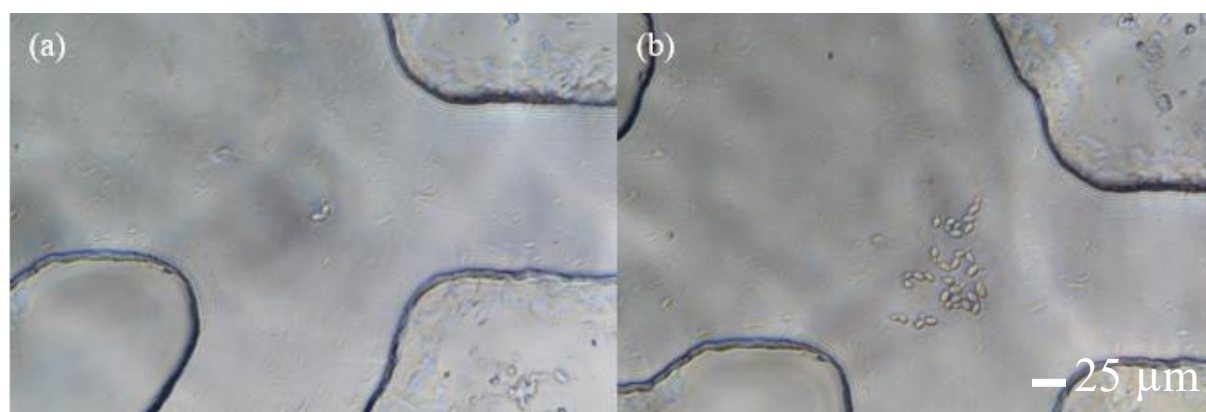


Figure 5.188. Images of the cells that were trapped in chamber 5 (a) at 309th minute (b) 728th minute

Table 5.70. Duration of cell cycle phases of the analyzed cells in chamber 5

Cell Name	Bud Number	Medium Type	G ₁ phase (min)	S/G ₂ /M phases (min)	Cell Cycle (min)	Area (μm ²)
M1	1	N1	-	35	>35	50-60
M1	2	N1-N2	32	50	82	30-55
M1	3	N2	28	42	70	30-50
M1	4	N2	35	52	87	30-50
D1	1	N1-N2	36	45	81	30-55

Table 5.70. Duration of cell cycle phases of the analyzed cells in chamber 5 (cont.).

Cell Name	Bud Number	Medium Type	G ₁ phase (min)	S/G ₂ /M phases (min)	Cell Cycle (min)	Area (μm ²)
D1	2	N2	36	55	91	30-50
D1	3	N2-N1R	28	91	119	25-50
M2	1	N1-N2	17	50	67	30-55
M2	2	N2	28	42	70	30-50

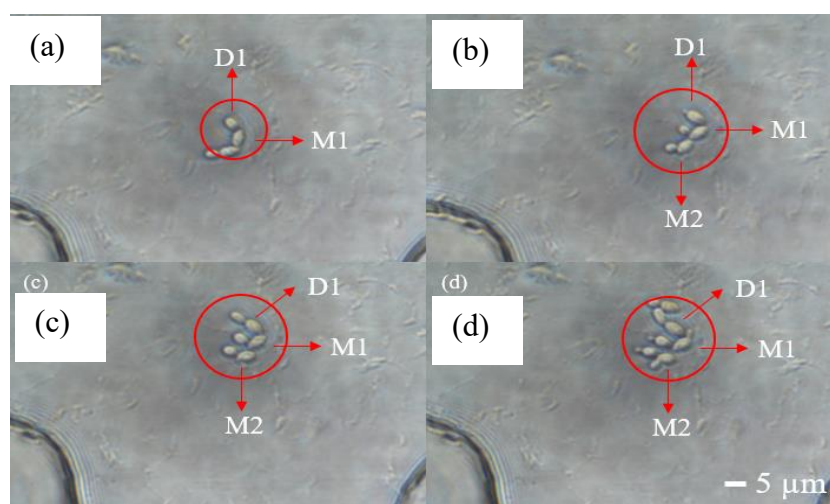


Figure 5.189. The image of the mother cells at (a) 259th minute (b) 395th minute (c) 426th minute (d) 454th minute under 90X magnification

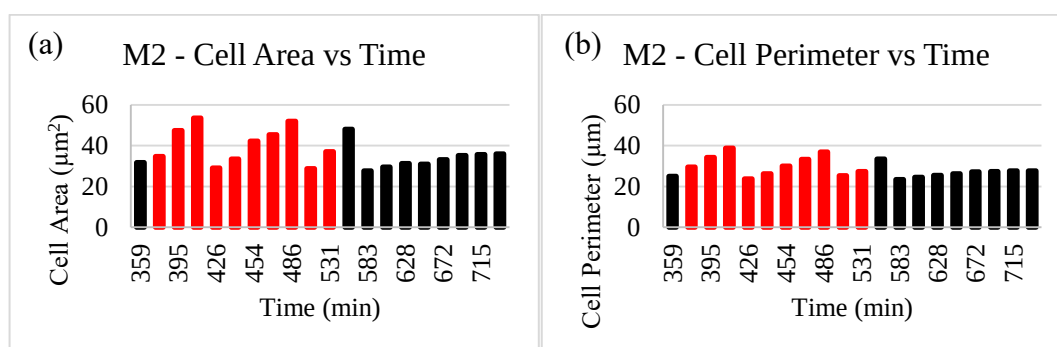


Figure 5.190. Graphical representation of processed data of chamber 5 (a) mother cell 2 area vs time graph (b) mother cell 2 perimeter vs time graph

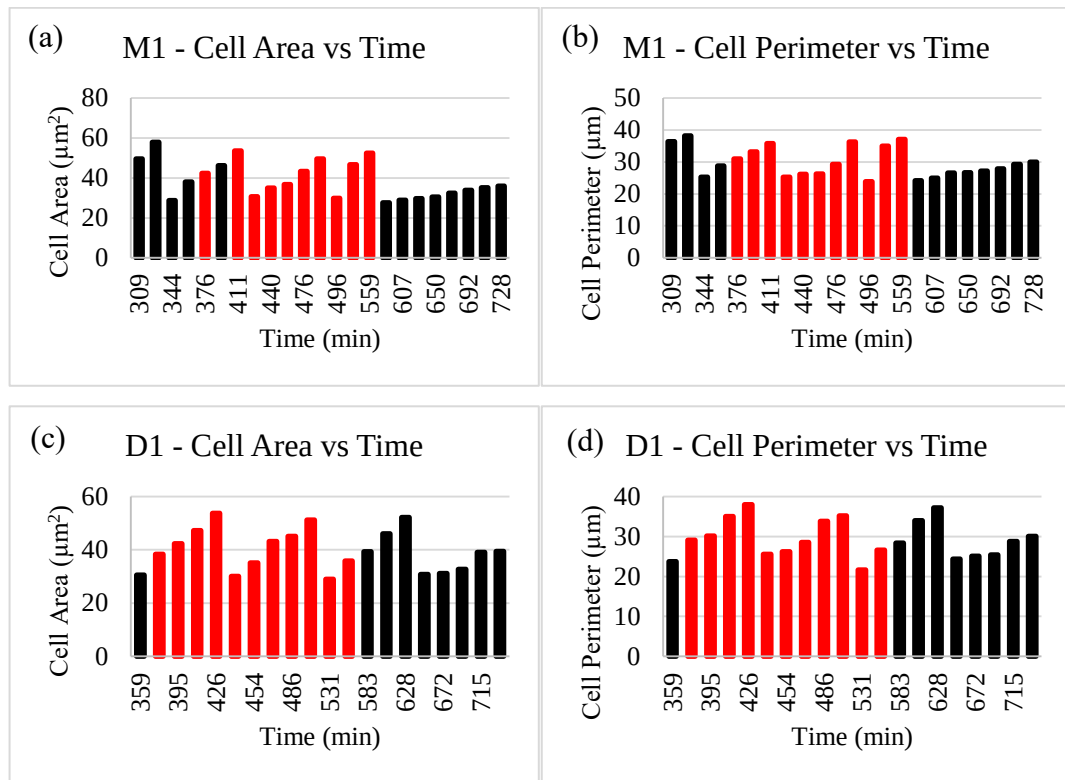


Figure 5.191. Graphical representation of processed data of chamber 5 (a) mother cell 1 area vs time graph (b) mother cell 1 perimeter vs time graph (c) daughter cell 1 area vs time graph (d) daughter cell 1 perimeter vs time graph – EXP 8

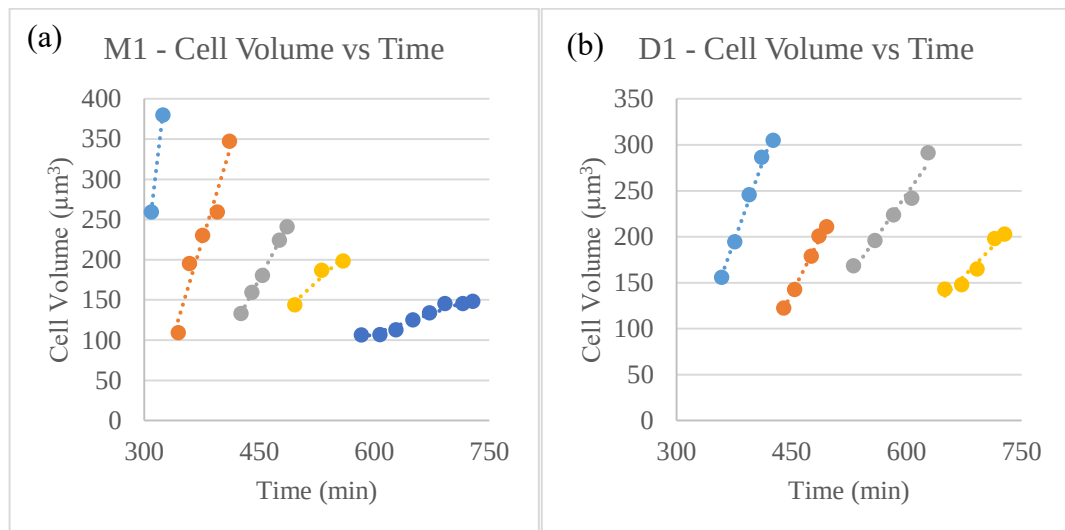


Figure 5.192. Graphical representation of processed data of chamber 5 (a) mother cell 1 volume vs time graph (b) daughter 1 volume vs time graph – EXP8

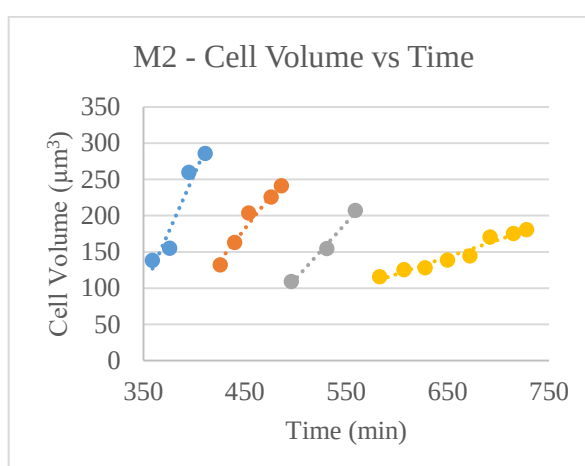


Figure 5.193. Graphical representation of processed data of chamber 5, mother cell 2 volume vs time graph – EXP8

Table 5.71. The fold change in area, perimeter and volume of the M1 and D1 cells in chamber 5

Time	M1-Cell Area Fold Change	M1-Cell Perimeter Fold Change	M1-Volume Fold Change	D1-Cell Area Fold Change	D1-Cell Perimeter Fold Change	D1-Volume Fold Change
309	1.00	1.00	1.00	-	-	-
324	1.17	1.05	1.47	-	-	-
344	1.00	1.00	1.00	-	-	-
359	1.32	1.14	1.79	1.00	1.00	1.00
376	1.47	1.22	2.11	1.25	1.23	1.25
395	1.61	1.31	2.37	1.39	1.27	1.57
411	1.86	1.42	3.18	1.55	1.48	1.84
426	1.00	1.00	1.00	1.76	1.60	1.95
440	1.14	1.03	1.20	1.00	1.00	1.00
454	1.20	1.04	1.36	1.17	1.02	1.16
476	1.41	1.16	1.68	1.43	1.12	1.46
486	1.62	1.43	1.81	1.50	1.32	1.64

Table 5.71 The fold change in area, perimeter and volume of the M1 and D1 cells in chamber 5 (cont.).

496	1.00	1.00	1.00	1.70	1.38	1.72
531	1.56	1.47	1.30	1.00	1.00	1.00
559	1.75	1.56	1.38	1.19	1.23	1.16
583	1.00	1.00	1.00	1.31	1.31	1.33
607	1.05	1.04	1.00	1.53	1.56	1.44
628	1.08	1.10	1.06	1.73	1.72	1.73
650	1.11	1.11	1.17	1.02	1.00	1.00
672	1.17	1.13	1.26	1.03	1.03	1.03
692	1.23	1.15	1.37	1.09	1.04	1.15
715	1.27	1.21	1.37	1.30	1.18	1.39

Table 5.72. The fold change in area, perimeter and volume of the M2 cell in chamber 5

Time	M2-Cell Area Fold Change	M2-Cell Perimeter Fold Change	M2-Volume Fold Change
359	1.00	1.00	1.00
376	1.10	1.18	1.12
395	1.49	1.37	1.88
411	1.69	1.56	2.07
426	1.00	1.00	1.00
440	1.15	1.10	1.23
454	1.45	1.26	1.54
476	1.56	1.40	1.71
486	1.79	1.56	1.83
496	1.00	1.00	1.00
531	1.29	1.08	1.42
559	1.67	1.32	1.90
583	1.00	1.00	1.00
607	1.07	1.05	1.09

Table 5.72. The fold change in area, perimeter and volume of the M2 cell in chamber 5
(cont.).

Time	M2-Cell Area Fold Change	M2-Cell Perimeter Fold Change	M2-Volume Fold Change
628	1.13	1.09	1.11
650	1.12	1.12	1.20
672	1.20	1.16	1.25
692	1.27	1.17	1.47

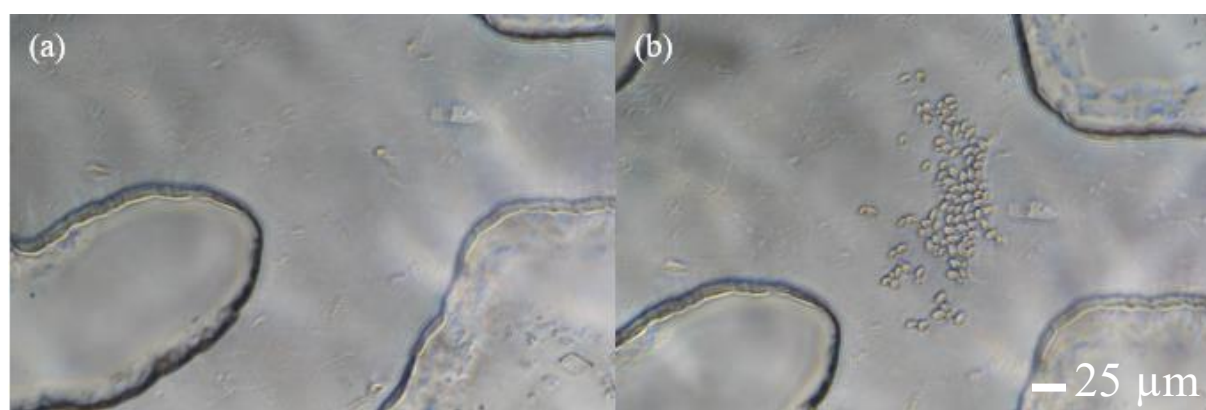


Figure 5.194. Images of the cells that were trapped in chamber 6 (a) at 240th minute (b) 728th minute

Table 5.73. Duration of cell cycle phases of the analyzed cells in chamber 6

Cell Name	Bud Number	Medium Type	G₁ phase (min)	S/G₂/M phases (min)	Cell Cycle (min)	Area (μm²)
M1	1	N1	28	28	56	25-55
M1	2	N1	28	35	63	30-60
M1	3	N1-N2	17	50	67	35-50
M1	4	N2	28	42	70	30-50
M1	5	N2-N1R	35	52	87	25-50
M2	1	N2	35	43	78	30-45

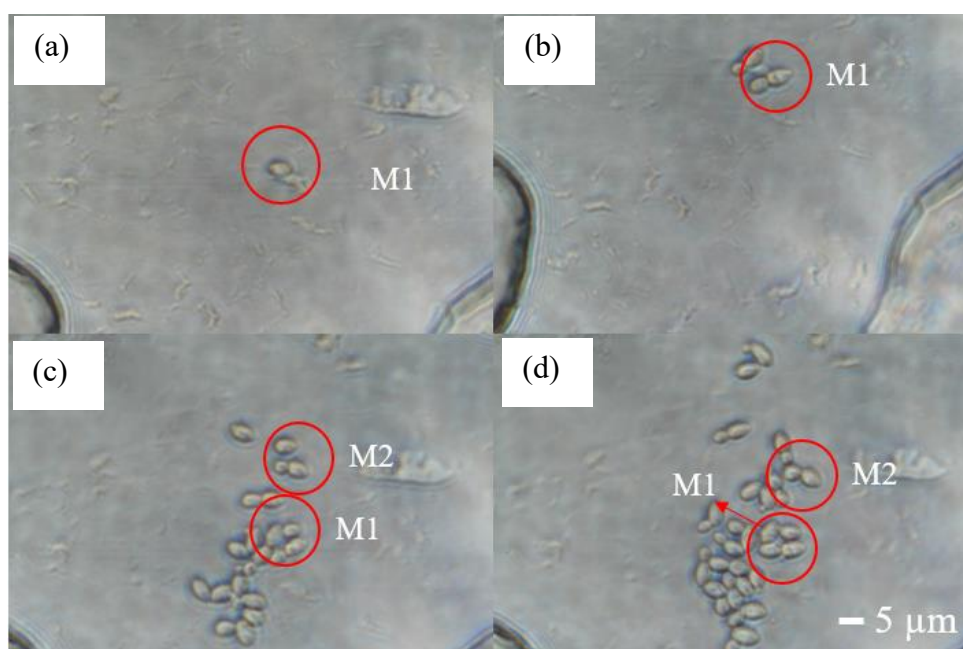


Figure 5.195. The image of the mother cells at (a) 240th minute (b) 284th minute (c) 376th minute (d) 411th minute under 90X magnification

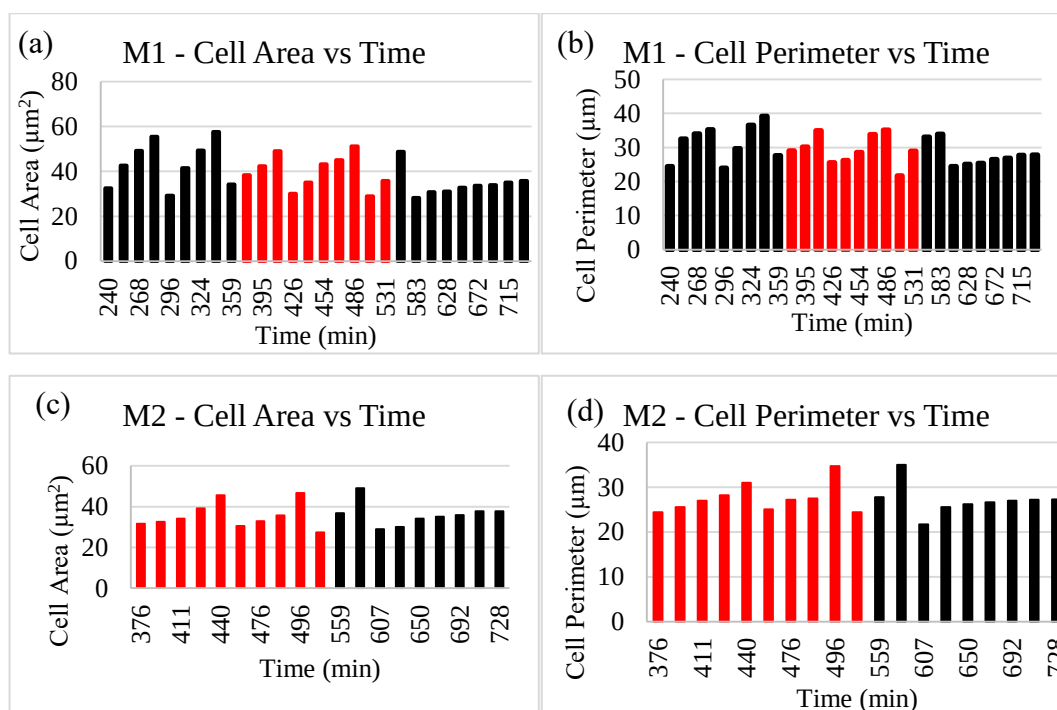


Figure 5.196. Graphical representation of processed data of chamber 6 (a) M1 area vs time graph (b) M1 perimeter vs time graph (c) M2 area vs time graph (d) M2 perimeter vs time graph – EXP 8

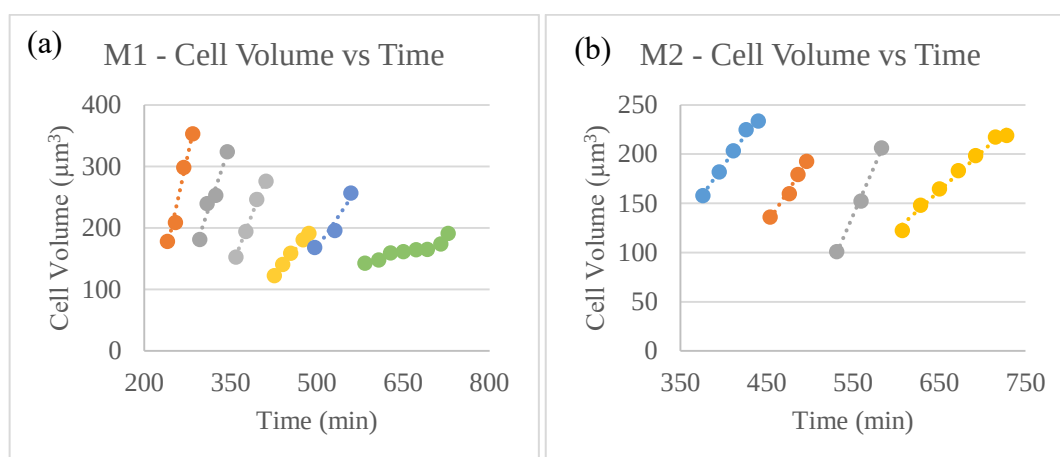


Figure 5.197. Graphical representation of processed data of chamber 6 (a) mother cell 1 volume vs time graph (b) mother cell 2 volume vs time graph – EXP8

Table 5.74. The fold change in area, perimeter and volume of the M1 and M2 cells in chamber 6

Time	M1-Cell Area Fold Change	M1-Cell Perimeter Fold Change	M1- Volume Fold Change	M2-Cell Area Fold Change	M2-Cell Perimeter Fold Change	M2- Volume Fold Change
240	1.00	1.00	1.00	-	-	-
254	1.31	1.33	1.17	-	-	-
268	1.52	1.39	1.67	-	-	-
284	1.71	1.44	1.98	-	-	-
296	1.00	1.00	1.00	-	-	-
309	1.42	1.24	1.32	-	-	-
324	1.68	1.53	1.40	-	-	-
344	1.97	1.63	1.79	-	-	-
359	1.00	1.00	1.00	-	-	-
376	1.12	1.05	1.27	1.00	1.00	1.00
395	1.24	1.09	1.61	1.03	1.05	1.15
411	1.44	1.27	1.81	1.08	1.11	1.29
426	1.00	1.00	1.00	1.23	1.16	1.42

Table 5.74. The fold change in area, perimeter and volume of the M1 and M2 cells in chamber 6 (cont.).

Time	M1-Cell Area Fold Change	M1-Cell Perimeter Fold Change	M1- Volume Fold Change	M2-Cell Area Fold Change	M2-Cell Perimeter Fold Change	M2- Volume Fold Change
440	1.17	1.02	1.15	1.44	1.27	1.48
454	1.43	1.12	1.30	1.00	1.00	1.00
476	1.50	1.32	1.48	1.08	1.09	1.18
486	1.70	1.38	1.56	1.17	1.10	1.32
496	1.00	1.00	1.00	1.53	1.39	1.42
531	1.24	1.33	1.16	1.00	1.00	1.00
559	1.69	1.53	1.53	1.35	1.14	1.51
583	1.00	1.00	1.00	1.79	1.43	2.04
607	1.09	1.10	1.03	1.00	1.00	1.00
628	1.10	1.13	1.12	1.04	1.18	1.21
650	1.16	1.14	1.13	1.18	1.21	1.34
672	1.19	1.19	1.15	1.21	1.23	1.50

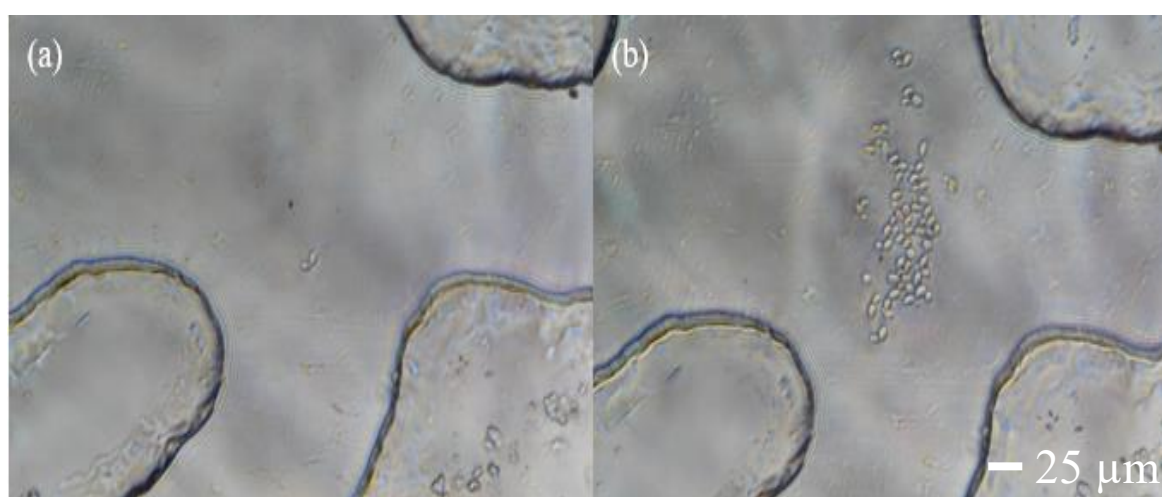


Figure 5.198. Images of the cells that were trapped in chamber 7 (a) at 268th minute (b) 728th minute

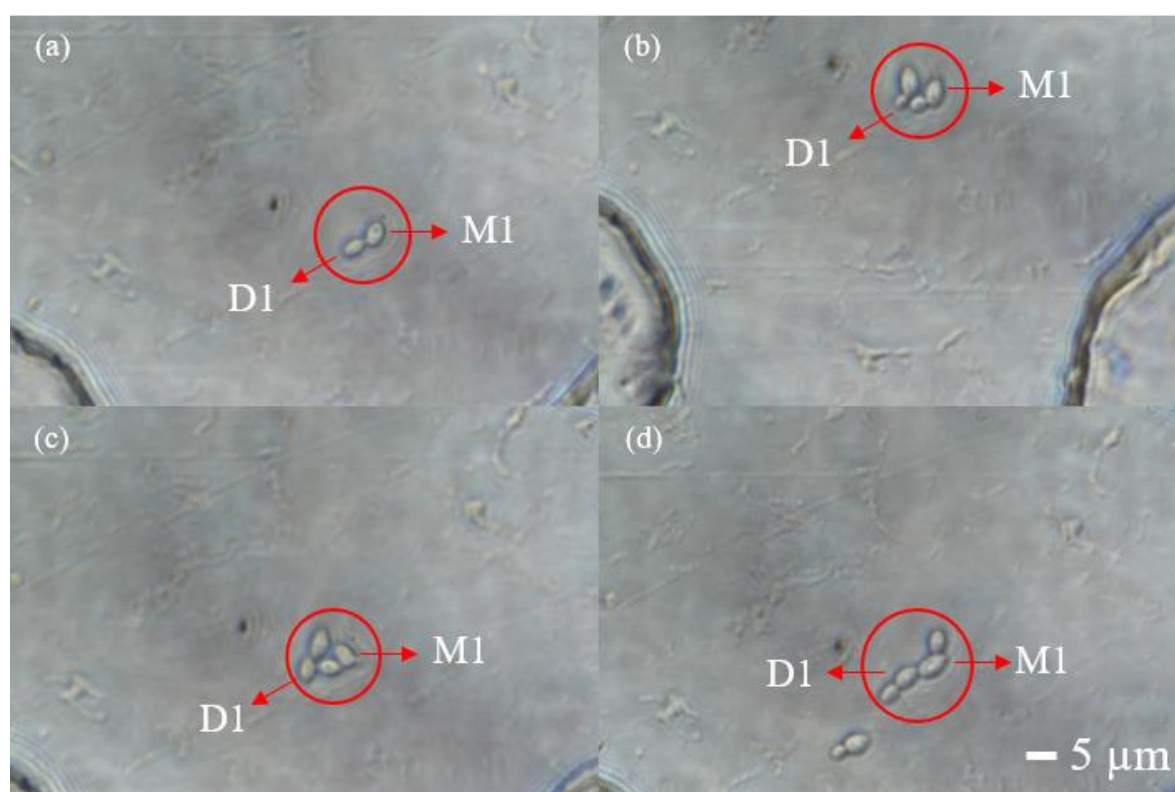


Figure 5.199. The image of the mother cells at (a) 268th minute (b) 309th minute (c) 359th minute (d) 411th minute under 90X magnification

Table 5.75. Duration of cell cycle phases of the analyzed cells in chamber 7

Cell Name	Bud Number	Medium Type	G ₁ phase (min)	S/G ₂ /M phases (min)	Cell Cycle (min)	Area (μm ²)
M1	1	N1	25	35	60	40-55
M1	2	N1-N2	32	35	67	30-55
M1	3	N2	29	36	65	35-55
M1	4	N2-N1R	20	87	107	35-55
M2	1	N1	25	50	75	30-55
M2	2	N1-N2	36	45	81	30-50
M2	3	N2	46	73	119	35-50
M2	4	N2	76	>121	>197	30-40

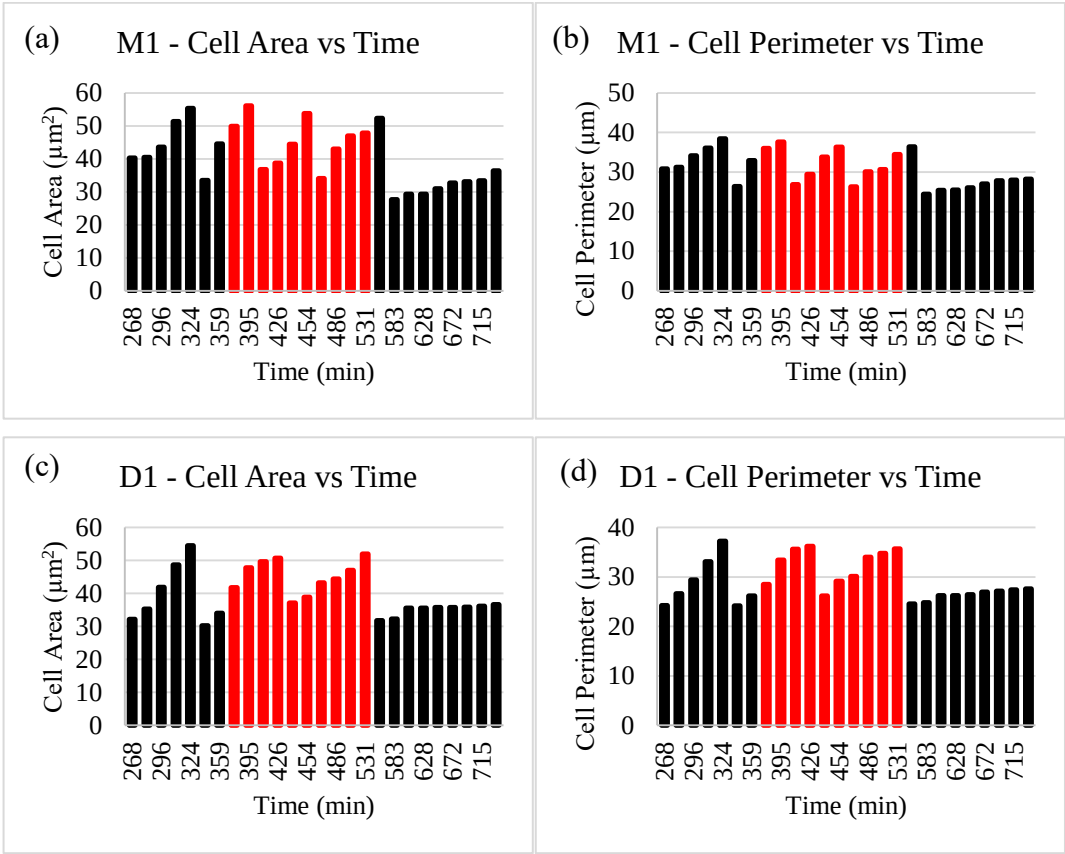


Figure 5.200. Graphical representation of processed data of chamber 7 (a) mother cell 1 area vs time graph (b) mother cell 1 perimeter vs time graph (c) daughter cell 1 area vs time graph (d) daughter cell 1 perimeter vs time graph – EXP 8

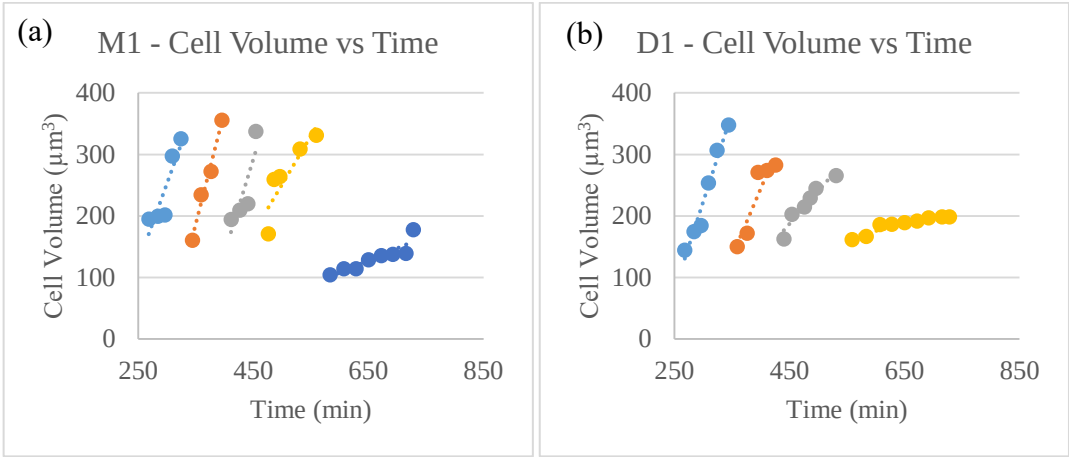


Figure 5.201. Graphical representation of processed data of chamber 7 (a) mother cell 1 volume vs time graph (b) daughter cell 1 volume vs time graph – EXP8

Table 5.76. The fold change in area, perimeter and volume of the M1 and D1 cells in chamber 7

Time	M1-Cell Area Fold Change	M1-Cell Perimeter Fold Change	M1- Volume Fold Change	D1-Cell Area Fold Change	D1-Cell Perimeter Fold Change	D1- Volume Fold Change
268	1.00	1.00	1.00	1.00	1.00	1.00
284	1.00	1.01	1.03	1.10	1.10	1.21
296	1.08	1.11	1.04	1.30	1.21	1.28
309	1.27	1.17	1.53	1.51	1.36	1.76
324	1.37	1.25	1.67	1.70	1.54	2.13
344	1.00	1.00	1.00	1.00	1.00	1.00
359	1.33	1.25	1.46	1.12	1.08	1.14
376	1.49	1.36	1.70	1.38	1.18	1.80
395	1.67	1.43	2.22	1.58	1.38	1.83
411	1.00	1.00	1.00	1.64	1.48	2.32
426	1.05	1.10	1.08	1.68	1.50	1.88
440	1.21	1.26	1.13	1.00	1.00	1.00
454	1.46	1.35	1.74	1.05	1.11	1.25
476	1.00	1.00	1.00	1.17	1.15	1.32
486	1.26	1.14	1.52	1.20	1.30	1.41
496	1.38	1.17	1.55	1.27	1.33	1.51
531	1.41	1.31	1.81	1.40	1.36	1.63
559	1.54	1.38	1.94	1.00	1.00	1.00
583	1.00	1.00	1.00	1.02	1.01	1.03
607	1.06	1.04	1.09	1.12	1.07	1.15
628	1.06	1.04	1.10	1.12	1.07	1.16
650	1.12	1.07	1.23	1.13	1.08	1.17
672	1.18	1.11	1.30	1.13	1.10	1.19
692	1.20	1.14	1.32	1.13	1.10	1.22

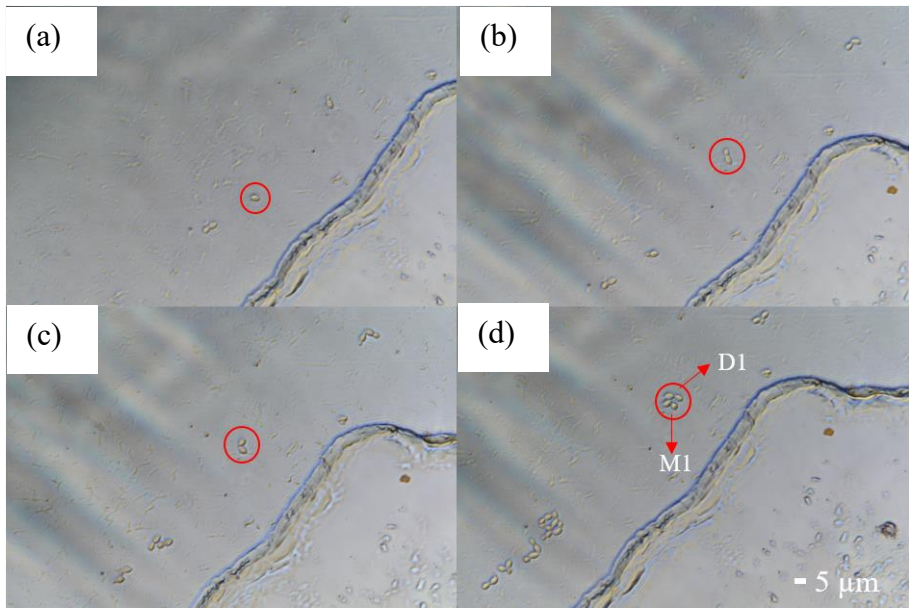


Figure 5.202. The image of the mother cells at (a) 376th minute (b) 395th minute (c) 440th minute (d) 531th minute under 40X magnification

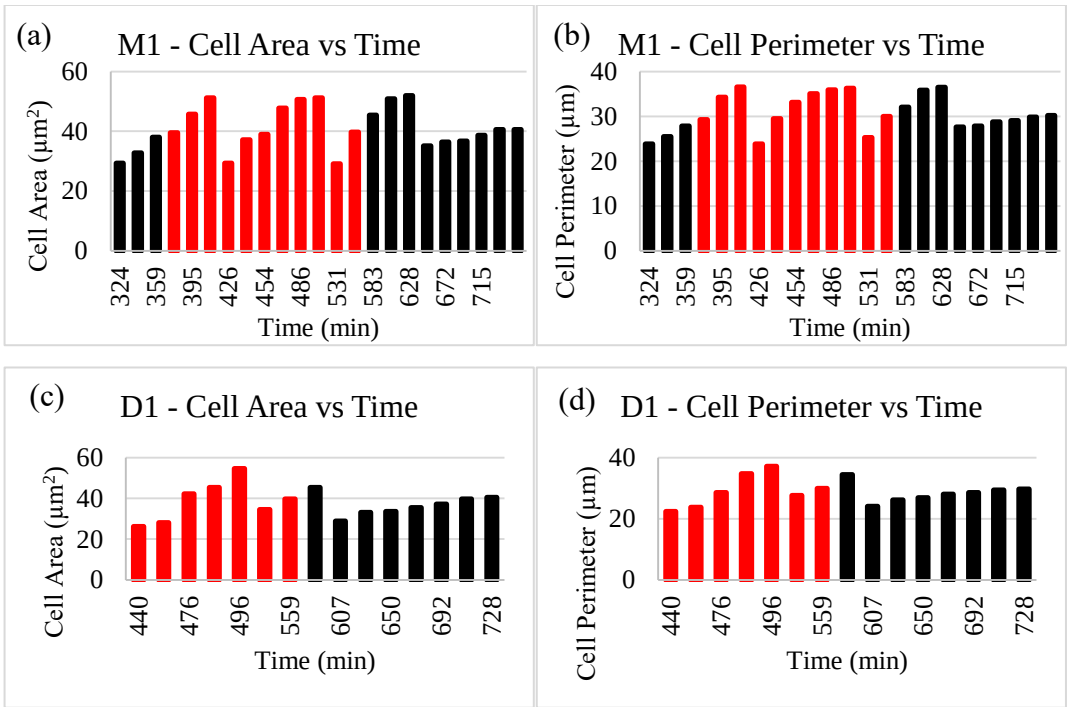


Figure 5.203. Graphical representation of processed data of outlet (a) mother cell 1 area vs time graph (b) mother cell 1 perimeter vs time graph (c) daughter cell 1 area vs time graph (d) daughter cell 1 perimeter vs time graph – EXP 8

Table 5.77. Duration of cell cycle phases of the analyzed cells in outlet

Cell Name	Bud Number	Medium Type	G₁ phase (min)	S/G₂/M phases (min)	Cell Cycle (min)	Area (μm²)
M1	1	N1-N2	35	67	102	30-50
M1	2	N2	50	55	105	30-55
M1	3	N2-N1R	52	67	119	30-50
D1	1	N2	50	55	105	25-55
D1	2	N2-N1R	28	48	76	35-45

Table 5.78. The fold change in area, perimeter and volume of the M1 and D1 cells in outlet

Time	M1-Cell Area Fold Change	M1-Cell Perimeter Fold Change	M1-Volume Fold Change	D1-Cell Area Fold Change	D1-Cell Perimeter Fold Change	D1-Volume Fold Change
324	1.00	1.00	1.00	-	-	-
344	1.12	1.07	1.21	-	-	-
359	1.30	1.17	1.58	-	-	-
376	1.35	1.23	1.59	-	-	-
395	1.56	1.44	1.73	-	-	-
411	1.75	1.53	2.13	-	-	-
426	1.00	1.00	1.00	-	-	-
440	1.27	1.23	1.14	1.00	1.00	1.00
454	1.33	1.39	1.28	1.07	1.06	1.16
476	1.63	1.47	1.50	1.61	1.27	1.47
486	1.73	1.50	1.66	1.73	1.55	1.65
496	1.74	1.52	1.77	2.08	1.66	1.89
531	1.00	1.00	1.00	1.00	1.00	1.00

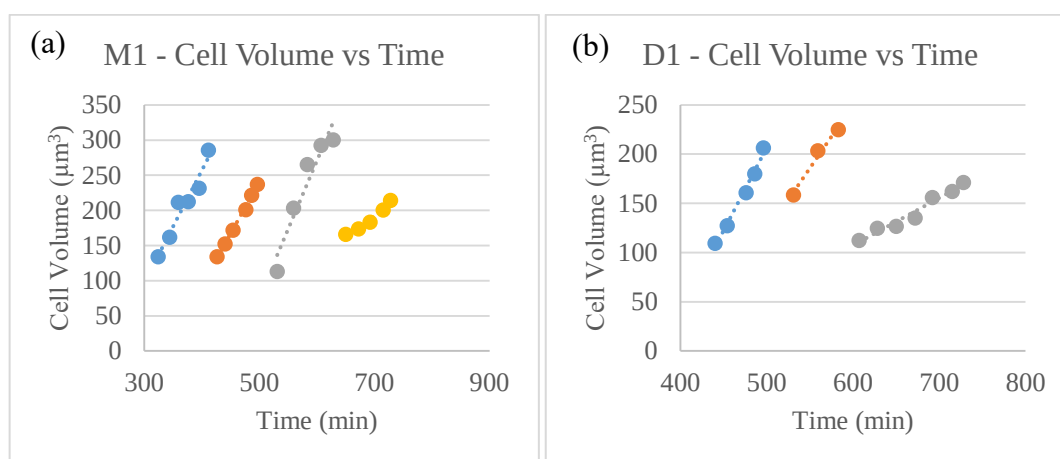


Figure 5.204. Graphical representation of processed data of outlet (a) mother cell 1 volume vs time graph (b) daughter cell 1 volume vs time graph – EXP8

The last view of the chambers were given in Figure 5.205 and Figure 5.206.

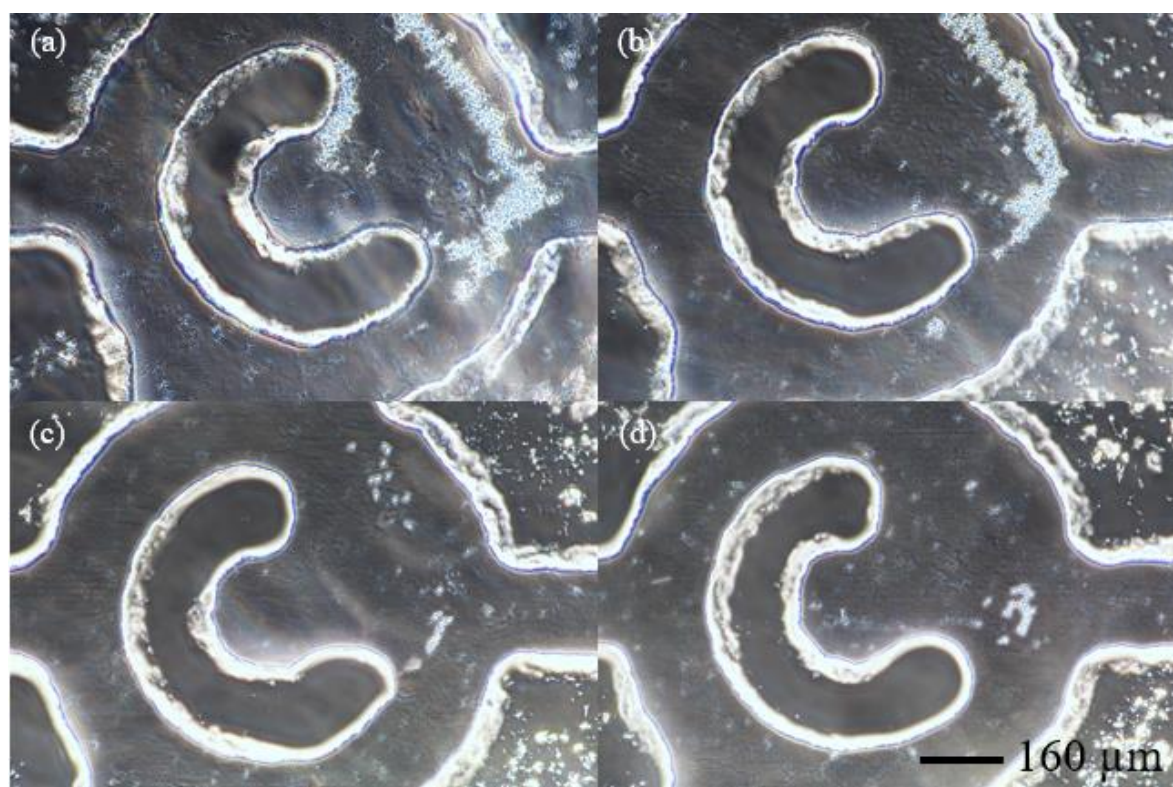


Figure 5.205. Images of (a) chamber 1 (b) chamber 2 (c) chamber 3 (d) chamber 4 at 728th minute under magnification of 20X



Figure 5.206. Images of (a) chamber 5 (b) chamber 6 (c) chamber 7 (d) outlet at 728th minute under magnification of 20X

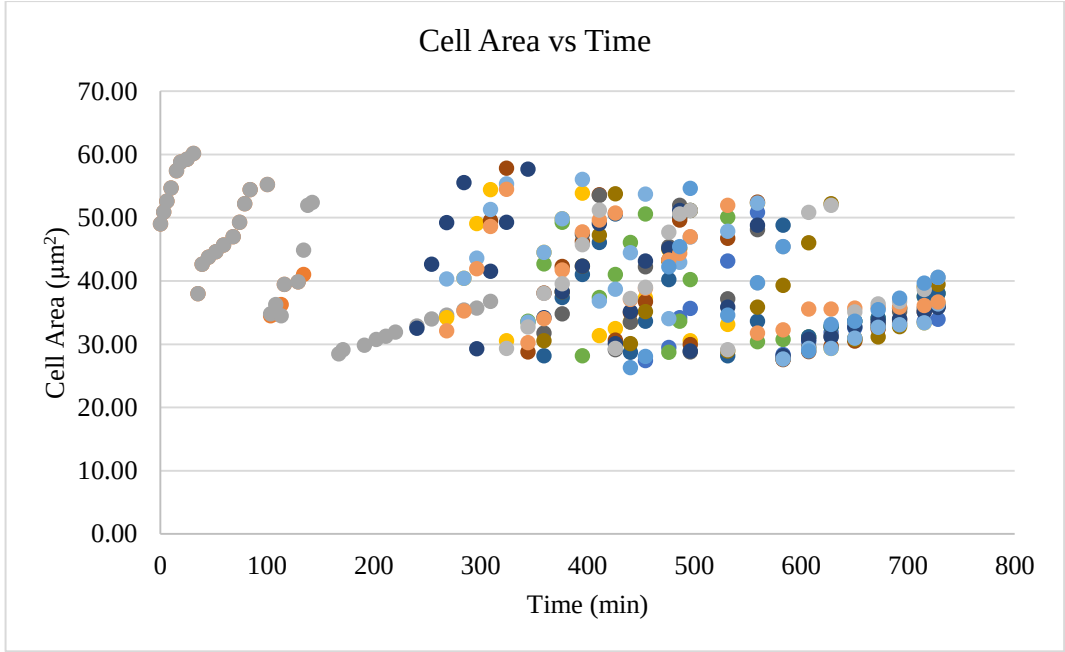


Figure 5.207. The comparison of cell area

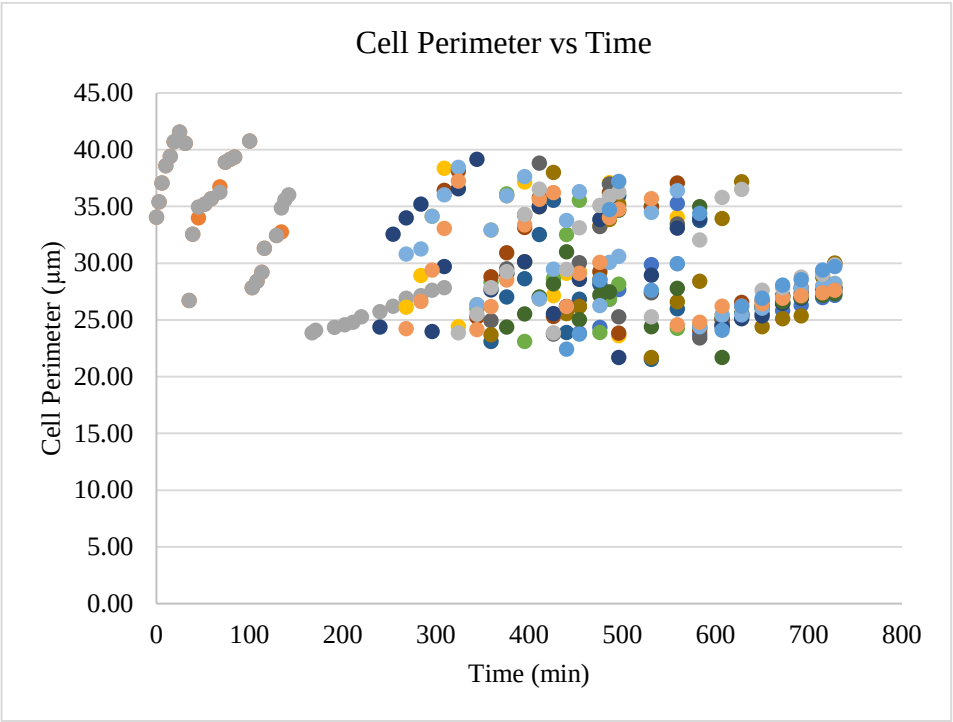


Figure 5.208. The comparison of cell perimeter

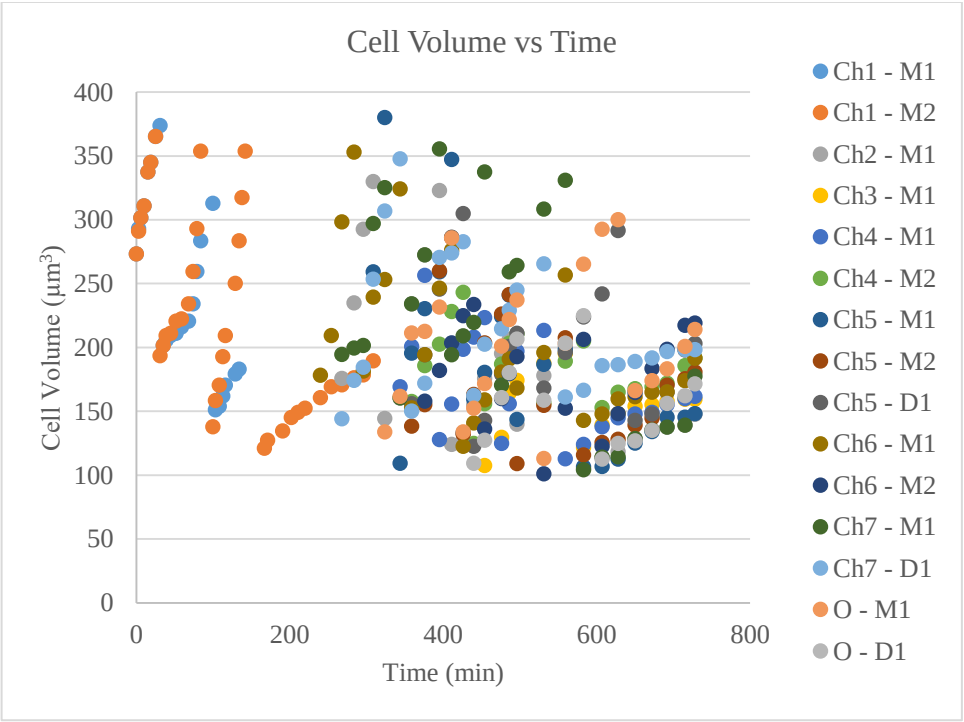


Figure 5.209. The comparison of cell volume

Table 5.79. Duration of cell cycle phases of the analyzed cells in different environments

Medium	G₁ phase (min)	Ave. G₁ Phase (min)	S/G₂/M phases (min)	Ave. S/G₂/M phases (min)	Cell Cycle (min)	Ave. Cell Cycle (min)
N1	16-30	25	28-50	40	35-75	60
N1-N2	17-36	30	35-67	50	67-102	80
N2	28-50	36	36-73	50	70-119	85
N2-N1R	20-35	30	48-91	60	76-119	90
N1R	52-76	64	67-121	94	119-197	158

The volumetric fold change of the cells were compared in each media (Figure 5.210). The size of the cells increased as almost 1.5 of their initial size (average=1.40) in glucose rich YPD media and YPD media containing MMS (average=1.52), whereas the the change in the cell sizes after feeding fresh medium were decreased to 1.32. In this case, the volume change of the cells was not significant.

Table 5.80. The comparison of the duration of cell cycle phases between daughter and mother cells

Cell Name	Medium Type	Ave. G₁ (min)	Ave. S/G₂/M (min)	Ave. Cell Cycle (min)
Mother	N1-N2	27	51	78
	N2	36	48	84
	N2-N1R	30	58	88
	N1R	64	94	158
Daughter	N1-N2	36	45	81
	N2	36	55	91
	N2-N1R	28	91	119
	N1R	65	-	>65

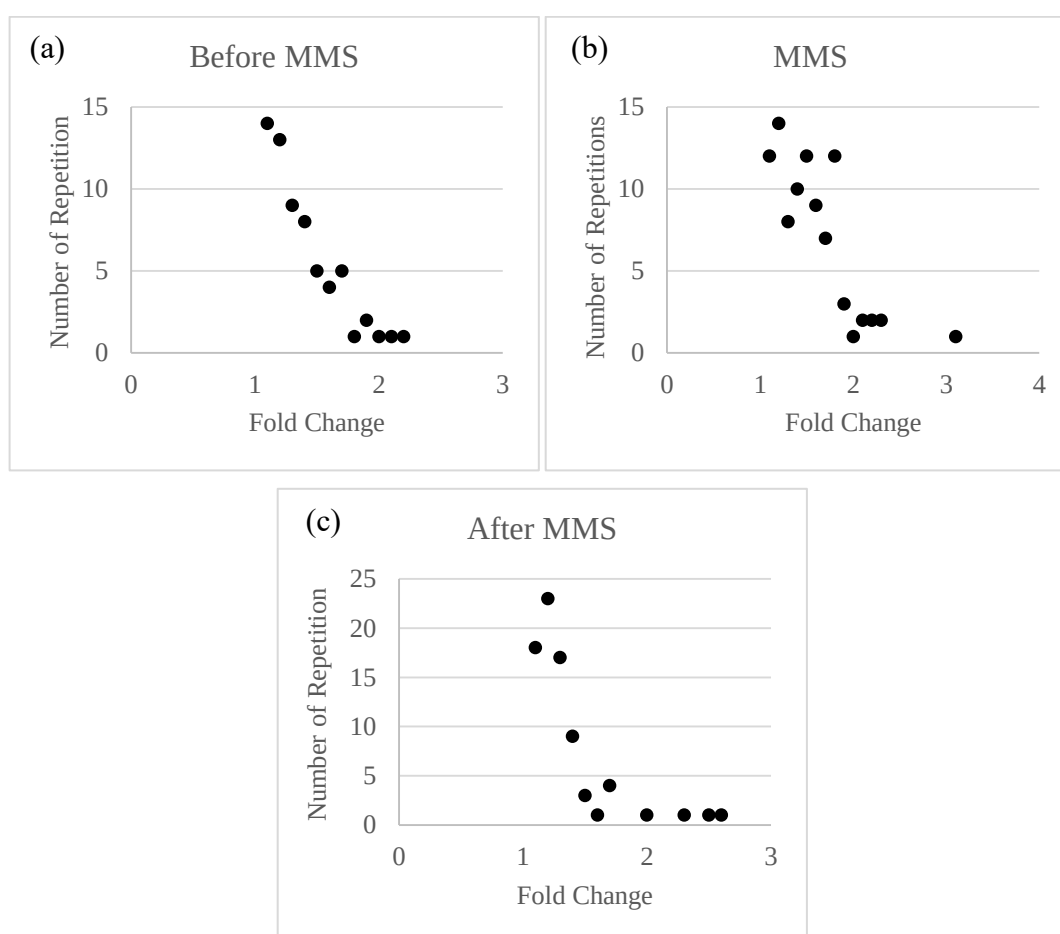


Figure 5.210. The volumetric fold change of the cells (a) in glucose rich YPD media (b) in glucose rich YPD media containing 0.01 MMS (c) in fresh glucose rich YPD medium

Table 5.81. The comparison of the duration of the cell cycle phases of *sld7Δ* mutant and wild type cells in glucose rich YPD medium

Medium Type	2 % Glucose – W303 – <i>sld7Δ</i>		2 % Glucose – BY4743 - WT	
	G ₁	S/G ₂ /M	G ₁	S/G ₂ /M
N1	35	65	25	40
N1-N2	45	95	30	50
N2	55	95	36	50
N2-N1R	55	125	30	60
N1R	35	85	64	94

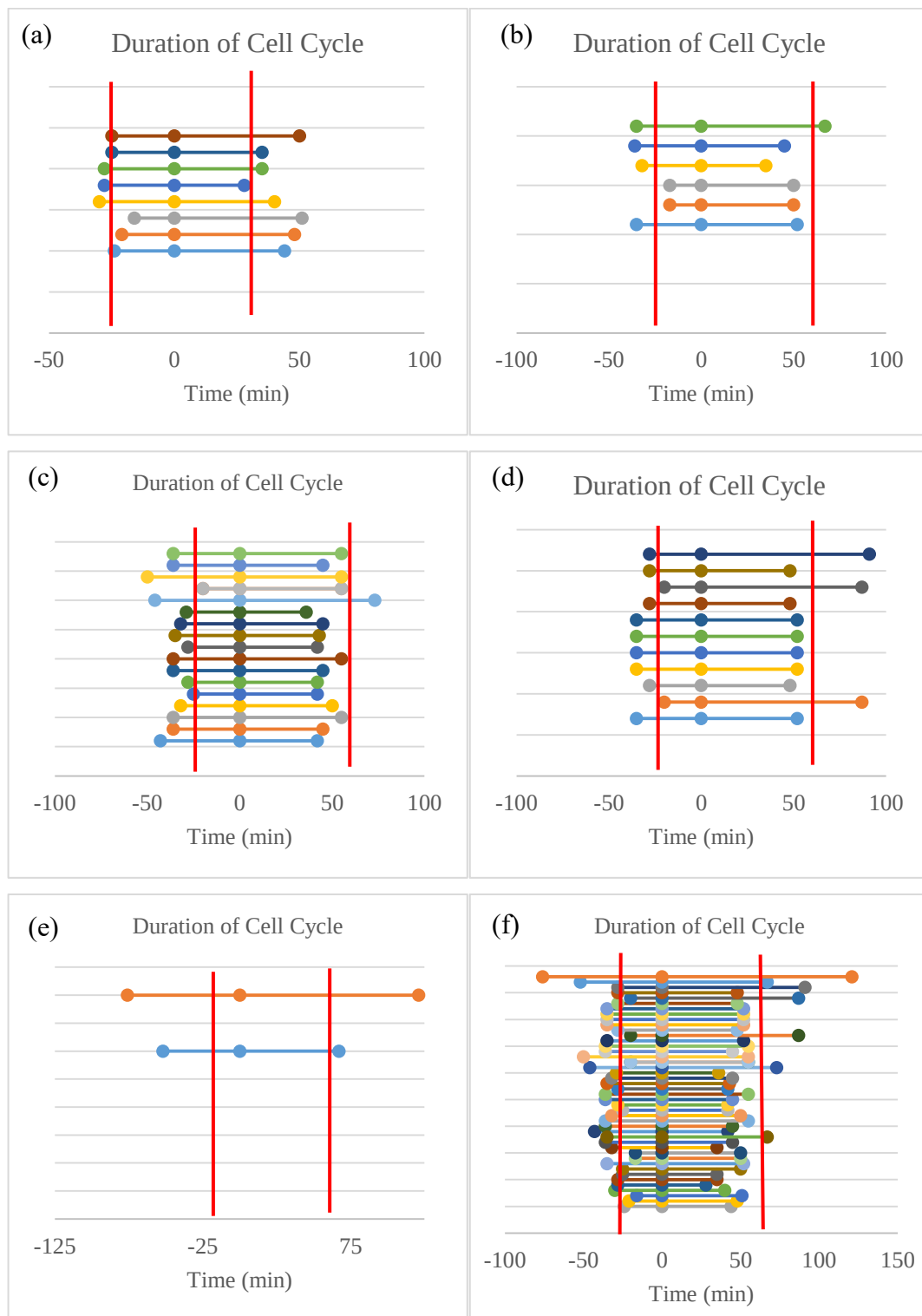


Figure 5.211. The duration of cell cycle (a) in N1 medium (b) in N1-N2 medium (c) in N2 medium (d) in N2-N1R medium (e) N1R medium (f) in all mediums

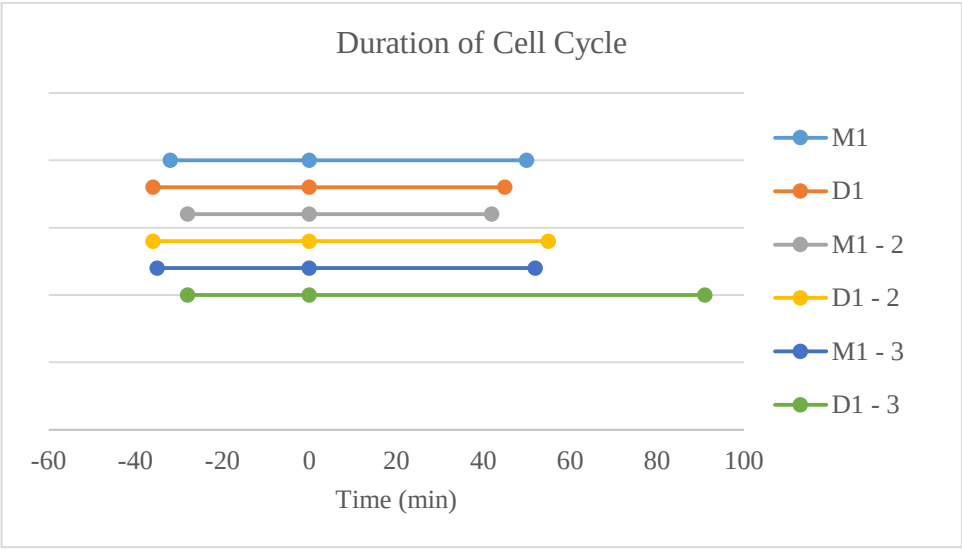


Figure 5.212. Comparson of the cell cycle phases of mother and thier daughter cells

6. DISCUSSION

6.1. Cell Cycle and Chronological Aging Mechanism of Sld7p

The putative molecular functions of Sld7 were studied by computational methods using an integrated approach of transcriptomics and interactomics. In the published literature, Sld7 was hitherto understood as one of the non-essential proteins with a role in the initiation of DNA replication in budding yeast (Tanaka *et al.*, 2011a; Tanaka *et al.*, 2011b). However, in our previous experimental work on the effects of medium acidity on chronological aging using BY4742 strain of *S. cerevisiae*, the transcriptomics analysis revealed that the expression level of Sld7 was 0.5 (log₂-fold change) under pH controlled conditions, being in the top 10 among 882 genes (Yucel *et al.*, 2014). This raises the question on a putative and critically important role of the Sld7 protein in aging.

The cell is prepared for the initiation of DNA replication in G₁ phase, and then proceeds to S phase, in which DNA replication occurs. The readiness of the cell is controlled through checkpoints in G₂ phase before M phase, in which cell divides (Cooper, 2000). The initiation of DNA replication consists of two steps, which are origin licensing and activation. Origin recognition complex (ORCs) legitimizes the replication origin at double stranded DNA (dsDNA). Cdc6 and Cdt1 bind to ORC in order to load inactive minichromosome maintenance (MCM) proteins onto replication origin (Aparicio, 2013), and then leave dsDNA. The loaded complex and loading steps were called as pre-replication complex (pre-RC) and licensing, respectively. Pre-loading complex (pre-LC), which involves Dpb11, Sld2, GINS (partner of Sld5, Sld1, Sld2 and Sld3) and Pol ϵ , bind to CDK-phosphorylated Sld3 (Tanaka *et al.*, 2007; Zegerman and Diffley, 2007). Cdc45 moves along the replication fork during replication of DNA, whereas pre-LC and Sld3 do not (Tanaka and Araki, 2013). Sld7 decreases the affinity of Sld3 to Cdc45 in early firing origin (Tanaka *et al.*, 2011b). The GINS binds to Mcm2-7 to form Cdc45-Mcm-GINS (CMG) complex in late origin (Bruck and Kaplan, 2015), which is called replication fork helicase or preinitiation complex (pre-IC). The CMG complex is activated by DDK, and then unwinds dsDNA in S-phase (Figure 6.1).

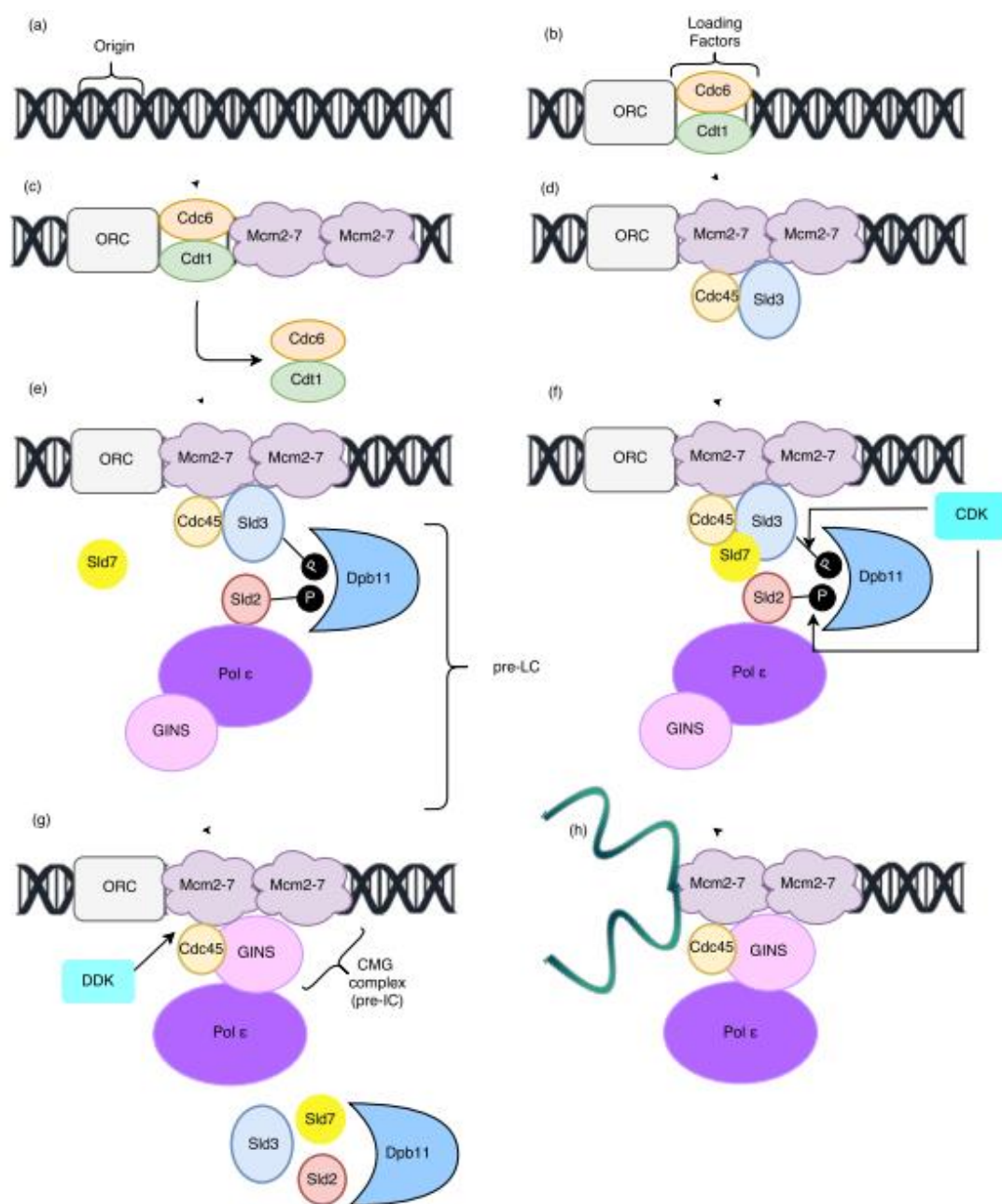


Figure 6.1 (a) DNA replication origin (b) ORC and loading factors binding to the origin (c) Formation of pre-RC (d) Cdc45-Sld3 bind into pre-RC (e) Pre-LC binding to Sld3 (f) Sld7 binding to Sld3 and decreasing the affinity of Sld3 towards Cdc45 (g) Formation and activation of pre-IC (h) Initiation of DNA replication.

We analyzed the physical interactions of Sld7 with other proteins in the reconstructed cell cycle and chronological aging networks of budding yeast. Several possible scenarios presented in the literature on the function of Sld7 were tested via analyzing the information

flow patterns in the reconstructed networks. In addition, the co-expression patterns were analyzed via combining transcriptome data from several experiments with the interactome networks presented here to propose possible roles of Sld7 protein in aging of budding yeast. In these networks the highly interacting protein clusters and significantly co-expressed gene clusters around Sld7 were further examined by functional enrichment analyses. The computational methods used here to annotate a function to Sld7 point out a dual role both in chronological and replicative (cell cycle) aging processes.

The comparison of the results of the enriched clusters revealed that among 107 different terms the most encountered function annotation term was “macromolecular complex binding (GO: 44877)”, which means a protein interacting selectively and non-covalently with any macromolecular complex. Sld7 recruits GINS to Mcm2-7 by binding onto pre-LC, a macromolecular complex (Takayama *et al.*, 2003), and decreases the affinity of Sld3 to Cdc45 in early firing origin in late G₁ phase. This binding allows the formation of CMG complex (active helicase) that initiates the DNA replication by unwinding dsDNA. The examined information flows from Mcm2-7 to Sld2 and Dpb11 supports the theory about the role of Sld7 as ‘macromolecular complex binding’ by its complex formation with Sld3 and Cdc45 and disassociating their interactions. In addition, Sld7 was encountered in paths from ORC to Cdc7, which is responsible for activation of DNA initiation by phosphorylating Mcm2-7 for early and late origins (Labib, 2010). The upstream and downstream proteins, that were encountered in analyzed paths, include Mis1, Hta2, Ssb1, Hmo1, Clb5, Cdc28, Ssa1, Sld3, Srp40, Hek2, Mth1, Psf1 and Pex13, some of which have a role in DNA binding, localize generally in nucleus, cytoplasm and DNA-initiation complex, and they are here suggested to function as assist proteins in DNA replication. The molecular functions of some of these proteins, such as Srp40, Mth1 and Psf1, are also unknown. These proteins might have role(s) in the formation of complexes in G₁ phase of cell cycle in budding yeast.

Other most encountered function annotation terms were “transcriptional factor activity, RNA polymerase II transcription factor binding (GO: 1076)”, “RNA polymerase II C-terminal domain phosphoserine binding (GO: 1990269)” and “mRNA binding (GO: 3729)”. The regulator protein of Sld7, Med2 (mediator complex), is a subunit of RNA polymerase II and it is an essential protein for transcriptional regulation in yeast (Venters *et al.*, 2011). The genetic interactor of Sld7, Cdc7 has molecular functions of “transcriptional

factor activity, RNA polymerase II transcription factor binding (GO: 1076)” and “RNA polymerase II C-terminal domain phosphoserine binding (GO: 1990269)”. The molecular functions of Hek2 and Nab2, the physical interactors of Sld7, are mRNA binding and rRNA binding, respectively (Irie *et al.*, 2002; Mitchell *et al.*, 2013). The transcription process and rRNA binding occur in nucleus and ribosome, respectively. Similarly, the localization of Sld7 was reported as nucleus (Huh *et al.*, 2003; Hazbun *et al.*, 2003) and nuclear envelope-ER (Hazbun *et al.*, 2003). Sld7 also co-localizes with spindle pole body (Huh *et al.* 2003). The results of GO enrichment analyses indicate that one of the putative functions of Sld7 might be related to ‘transcriptional repressor activity binding’ to RNA polymerase directly.

If Sld7 is not expressed during the cell cycle, the CMG complex, which is an active DNA helicase at replication fork (Tanaka *et al.*, 2011b), cannot be formed and DNA replication cannot be initiated. During nutrient depletion the cells’ arrest in G₁ phase causes chronological senescence (Burhans and Weinberger, 2007). We did experiments in 1 nL microbioreactors, where the *sld7Δ* mutant cells were grown in both glucose rich (2% glucose) and caloric restricted (0.2% glucose) environment. The duration of G₁ phase is observed to increase in caloric restricted medium. In our computational analysis, the enriched molecular functions of the clusters, obtained using aging co-expression dataset (GDS362, Fry *et al.*, 2003) and CAN (Yucel *et al.*, 2014), are “heme-copper terminal oxidase activity (GO: 15002)”, “oxidoreductase activity, acting on a heme group of donors (GO: 16675)” and “cytochrome-c oxidase activity (GO: 4129)”. The damaged DNA induces the genes involved in cellular redox homeostasis in yeast (Tkach *et al.*, 2012), and leads to increased reactive oxygen species (ROS) concentration, and thus prevents cell proliferation, and causes cell death (Martindale *et al.*, 2012).

The oxidative stress forces cells to autophagic cell death, where cells begin to shrink, bleb and die (Zakeri *et al.*, 1995). It was predicted, though still unclear, that the main source of oxidative stress was mitochondria (Burhans and Weinberger, 2007). The cellular component of the proteins, Cox4, Cox5, Cox6, Cox8, Cox9, Cox12 and Cox13, responsible for these oxidation related functions and present in highly correlated clusters of co-expressed genes, are mitochondria. Altmann and Westermann (2005) did a study about the essential cellular genes/proteins in mitochondrial morphogenesis in yeast, and suggested that the proteins, *erg29*, *fcf1*, *pga2* and *sld7*, with unknown molecular function might be contributing

to mitochondrial inheritance directly or indirectly. Hence, in addition to its role in cell cycle, Sld7 might have a role in keeping the level of ROS low and enhancing life span of the cell by reducing oxidative damage in mitochondrial DNA. In literature, the other protein with a dual role in both chronological and replicative life span is Tor1, and similar to Sld7 it is also responsible for macromolecular complex binding in yeast (Wullschleger *et al.*, 2006).

The number of mitochondria transmitted to daughter cells at mitosis presents broad fluctuations (das Neves *et al.*, 2010; Johnston *et al.*, 2012) and identified heterogeneity in mitochondrial content as a source of variability in transcription rate (das Neves *et al.*, 2010). RNA Pol II transcription elongation was variable from cell to cell, mainly due to the high sensitivity of this activity to the concentration of ATP, which is directly linked to mitochondrial levels in individual cells (das Neves *et al.*, 2010; Guantes *et al.*, 2016). Guantes *et al.* (2016) explored how mitochondrial content affects cellular protein content and whether the correlation observed between mitochondria and protein is due to covariation with cell cycle stage. They found that the cell cycle contributes minimally to protein variability.

The transcription process (gene expression) involves different steps subjected to control: one is the regulation of the transcription machinery. RNA Pol II and the general transcription initiation complex TFIID both co-varied with mitochondria in amounts comparable to protein variability. The transcriptional activity was monitored as a function of mitochondrial content using both RNA Pol II transcription elongation and mRNA accumulation, and it showed ~50% contribution. The changes in mitochondrial content can account for ~50% of the variability observed in protein levels. The cells with high mitochondrial content were more active in protein synthesis, supported by the high dependency of translation elongation factors and the synthetic ribosome machinery on mitochondria. Once RNA Pol II elongating molecules complete the transcription cycle, they become free and diffuse throughout the nucleoplasm. The cells with more mitochondria contained more copies of RNA Pol II active molecules on average and a lower number of elongating RNA Pol II molecules per gene than cells with low mitochondrial content. The cells with high mitochondrial content is supposed to transcribe a higher number of genes than cells with low mitochondrial mass.

In conclusion, natural variability in mitochondrial content may account for roughly half the variability in protein levels, and no two cells in a clonal population are identical. Mitochondria influence both the content of RNA Pol II and its function. Mitochondrial levels affect both the content of translation machinery and its activity, particularly ribosome biosynthesis. This is confirmed by the strong covariation in individual cells of mitochondria and ribosome metabolism proteins. Mitochondrial DNA depletion causes increased reactive oxygen species (ROS) production. ROS contributes to RNA Pol II transcription and ATP production. Mitochondrial DNA copy number reduction has been associated with breast cancer (Tseng *et al.* 2006).

In higher eukaryotes, the proteins that have a role in the initiation of DNA replication are replication-stimulating protein (Treslin) (homolog of Sld3) and MDM two binding protein (MTBP) (putative homolog of Sld7) with topoisomerase-II beta-binding protein 1 (TopBP1) (homolog of Dpb11) (Boos *et al.*, 2013). The expression level of MTBP has been found to be elevated in several cancer types (Alam *et al.*, 2012; Boos *et al.*, 2013, Grieb *et al.*, 2014; Bassi *et al.*, 2016; Chen and Butte, 2016; Han *et al.*, 2016). In human cells, the regulation of tumor suppressor protein p53 is controlled via MDM2, which inhibits the activity of p53. The regulation of this protein depends on a variety of cellular stresses that cause DNA damage, which in turn may cause abnormality or cancer (Leri *et al.*, 1999; Smith *et al.*, 2000). Once p53 is activated under stress conditions, the cell cycle is arrested in G1 phase and the system tries to repair the damaged DNA or leads to apoptosis to eliminate damaged cell (Ioannou and Chen, 1996; Haupt *et al.*, 1996). However, if MDM2 is overexpressed, it blocks p53 function and leads to tumor development. Thus, it can be suggested that MTBP has a role in the regulation of p53 indirectly via the MDM2 pathway (Alam *et al.*, 2012).

6.2. Choice of Material for Microfluidic Device Fabrication

Polystyrene (PS), which has high transparency and good material rigidity, is one of the most widely used biocompatible materials in microfluidic applications. It has high resistivity against alcohols, polar solvents and alkaline (Becker and Gartner, 2000). The chemical compatibility of polystyrene against different solvents are given in Table 6.1. The surface wettability can be manipulated by using plasma treatment, irradiation and corona

discharge (Chen *et al.*, 2008). The surface of PS is available for long-term cell studies. Despite all these appealing characteristics, the thermopress bonding between PS pieces has rarely been utilized, and not enough attention has been put to push the research further into device applications (Cao *et al.*, 2015). The list of the articles that include polystyrene made microfluidic devices and cell-based studies, were given in Table 6.2. Yet, yeast based studies were not encountered in literature.

Table 6.1. The chemical compatibility of polystyrene, “O” denotes good, “—” denotes moderate, “X” denotes poor

Solvent Type	Resistivity
Acetone	-
Ethanol	~
Ethylene Glycol	+
Glycerol	+
Isopropyl Alcohol	~
Methanol	~
Phenol	-
Water	+

Table 6.2. The articles that include polystyrene made microfluidic devices and cell-based studies.

No	Organism/ Protein	Polymers	Reference
1	Bovine immunoglobulin Mushroom tyrosinase Horseradish peroxidase	PS-block-PVP micellar	Kumar <i>et al.</i> , 2007
2	-	Surface Treatment	Bhattacharyya <i>et al.</i> , 2007
3	-	3D chips fabrication	Chen <i>et al.</i> , 2008
4	BSA	PS fabrication	Toft <i>et al.</i> , 2008

Table 6.2. The articles that include polystyrene made microfluidic devices and cell-based studies (cont.).

No	Organism/ Protein	Polymers	Reference
5	Mammalian cell	Chip Fabrication PS, PETG or COC combination with PDMS or PU membrane	Mehta <i>et al.</i> , 2009
6	RBL or MV1 Lu cells	Fabrication, Surface treatment	Ktari <i>et al.</i> , 2010
7	-	PDMS – PS Fabrication	Goral <i>et al.</i> , 2011
8	Oligonucleotides IgG antibody	PC, PS, COC, COP fabrication – not detailed	Rendl <i>et al.</i> , 2011
9	Human umbilical vein endothelial cells (HUVECs)	PS fabrication	Young <i>et al.</i> , 2011
10	HepG2 cells	PS, PMMA, PC and COC	van Midwoud <i>et al.</i> , 2012
11	Escherichia coli and Staphylococcus epidermidis	PS coating to the fabricated microchip	Sun <i>et al.</i> , 2012
12	IgG molecules	PS-block-PMMA	Hahm <i>et al.</i> , 2014,
13	Escherichia coli	PS sheet microchip fabrication	Dong and Zhao, 2015
14	-	PS-film chip fabrication	Cao <i>et al.</i> , 2015
15	Cytochrome-c protein	PS chip fabrication	Hu <i>et al.</i> , 2015

The comparison of the polymers that are used in microfluidic device fabrication was given in Table 6.3. According to given information, the properties of COP and PS are remarkable. One disadvantageous property of PS material is its high fluorescent background.

To understand the role and localization of Sld7p during cell cycle, the fluorescence images of the Sld7: GFP tagged cells were taken. However, the luminescence of the fluorescence signal of the tagged protein could not be distinguished from the noise on the surface because of the high fluorescence background of the PS (Figure 5.31).

Table 6.3. Property summary of the materials used in fluidic chip fabrication (Nevitt, 2013)

Property	PS	COP	PMMA
Optical transparency	O	O	O
Low fluorescence	--	O	--
Moisture barrier	--	O	X
Low binding	O	O	X
Chemical resistance	O	O	X
Dimensional stability	O	O	--
Processing (micro/nano scale)	--	O	--
Functionalizable	O	--	O
Break resistance	--	O	O
Incinerable	O	O	O

Polystyrene is also widely used in molecular and cell biology due to its biocompatibility. It is being used for petri dishes, test tubes, microplates, and other laboratory containers (Kodzius *et al.* 2011). In our studies, the cells' adhesion on the PS surface was very low. To change the loaded medium, the tubing of the medium inlet had to be taken out of the pipette tips. Taking out the tubing causes high pressure difference in the system and causes backflow, and then the already trapped cells were swept away. Therefore, it was difficult to observe the response of the same cells under different environmental conditions. To prevent this problem, y-junctions can be used so that it will not be necessary to take out the inlet tubings, and the sweeping problem of cells can be partially prevented.

In addition to, the trapping areas such as C-shapes might be coated with BSA or any other material that might increase the adhesion of the cells to the surface.

6.3. Fabrication steps

The hot embossing and thermal bonding of polymer pieces were performed by using Carver Hydraulic Press Machine. The thickness of the polystyrene pieces was 1.5 mm. The surface areas of the PS pieces were 3 cm x 3 cm. The optimization of the parameters of the hot embossing and thermal bonding methods were determined by considering the properties of the polystyrene, design of the mold, and height of the channels. The design formation via hot embossing can be performed about 20-30°C above the glass transition temperature of the polymer piece (Kricka *et al.*, 2002).

The fabrication steps of PS made microbioreactor is as follows; first the polymer piece was cleaned by distilled water and dried by pressurized nitrogen. Then the microchannels were formed at 98°C ($T_g = 85^\circ\text{C}$) under 35 bar pressure for 10 minutes. The polymer was demolded after 10 minutes. After replication, the microchannel featured 1.5 mm thick PS piece was drilled at the inlet and outlet positions of each channel. The 1.5 mm thick cover piece and featured piece were again washed by 2-propanol, distilled water and 2-propanol, and dried by pressurized nitrogen. For the bonding process, these two pieces were aligned between glass pieces of 5 cm x 5 cm. The optimized bonding temperature and pressure for PS pieces were 93 °C and 8 bar for an hour, respectively. After an hour, the microbioreactor was maintained under same pressure for half an hour to prevent the decollement of the bonded pieces because of the high temperature difference between clean room and the microbioreactor/carver. The microbioreactor was tested with ethanol to investigate whether the channels were open or clogged.

The c-shapes of each chamber were more collapse than any part of the microbioreactor because of the high aspect ratio. To prevent collapsing, the bonding pressure was decreased till 8 bar. Yet, the bonding pressure less than 8 bar causes leakage inside the microbioreactor. These collapses caused clogging at chamber 1, which prevented medium flow thorough the reactor and diffusion of nutrients to the cells. Young *et al.* (2011) mentioned about the potential for channel collapse when width-to-height aspect ratios are

too high. In this case, the aspect ratio of the trapping areas ($180\ \mu\text{m} \times 10\ \mu\text{m}$) might be decreased or the height of the reactor might be increased.

6.4. Growth in microbioreactor

It was reported that the Sld7p have a role in the initiation of DNA replication in G_1 phase. The reconstructed networks and other analysis that were done to estimate the role of Sld7 also supported the role of Sld7 in G_1 phase. To understand the role of Sld7p by experimental methods, the response of the Sld7-GFP tagged BY4741, *sld7 Δ* mutant (W303) and wild type BY4743 cells were observed under different environmental conditions in the PS made microbioreactor (Table 6.4). The fluorescence images of the GFP tagged cells were taken, yet the auto-fluorescence background of the PS is not appropriate to observe the fluorescence signal of any protein (Figure 5.31).

Table 6.4. The summary of the experiments

Strain Type	Haploid/diploid	Cell Type	Medium Type	Glucose Ratio (w/v)	Inhibitor (MMS)
BY4741	Haploid	Wild type Sld7 – GFP	YNB	2 %	-
BY4741	Haploid	Wild type Sld7 – GFP	YNB	2 %	+
W303	Diploid	<i>sld7Δ</i> mutant	YPD	2 %	-
W303	Diploid	<i>sld7Δ</i> mutant	YPD	2 %	+
W303	Diploid	<i>sld7Δ</i> mutant	YPD	0.2 %	+
BY4743	Diploid	Wild type	YPD	2 %	+

The loading velocities of the medium and yeast pre-culture were given with their duration of loading in Table 6.5 for all experiments. Increasing the flowrate of the medium while filling the microbioreactor, decreases the duration of loading. However, loading the yeast preculture at high volumetric flowrate prevented the trapping of the yeasts. The duration of the loading of yeast preculture should not exceed 20 minutes. If it passes 20

minutes, the yeast cells may cause clogging in the first chamber after a while and the experiment must end. The loading flowrate of the medium after yeast preculture should not exceed 0.2 $\mu\text{L}/\text{min}$. Feeding the medium higher than the 0.2 $\mu\text{L}/\text{min}$ flowrate causes sweeping of the trapped cells away.

Table 6.5. Inlet velocities in the experiments

Exp #	Cell Type	Media Type	Glucose Ratio (w/v)	MMS	Loading Velocity ($\mu\text{L}/\text{min}$)				Loading Time (h)				
					M	C	N1	N2	M	C	N1	N2	N1R
1&2	Wild type BY4741	YNB	2 %	-	3&5	0.5	-	-	4	15	-	-	-
3	Wild type BY4741	YNB	2 %	+	1	0.1	0.1	0.1	10	2	8.5	10	-
4&5	sld7 Δ mutant	YPD	2 %	-	5	3	0.1	0.1	1	0.3	8	-	-
6	sld7 Δ mutant	YPD	2 %	+	5	0.5	0.1	0.1	1	0.25	5	2.5	5
7	sld7 Δ mutant	YPD	0.2 %	+	5	0.5	0.1	0.1	1	0.3	7	3	3
8	Wild type BY4743	YPD	2 %	+	5	0.5	0.1	0.1	2	0.3	6	3	3

6.4.1. Comparison of PS and COP made microbioreactors for yeast cultivation

The total sizes of the trapped cells (BY4741) were compared in COP and PS made microbioreactors (Table 5.1). Even though the number of trapped cells were different, the cell area per cell count and cell perimeter per cell count were similar. In this case, it can be said that both polymeric microbioreactors were appropriate to observe the growth of yeast cells. However, the auto fluorescent background of these two thermoplastics are indeed different (Table 6.3). The fluorescence signal of PS is 7.24 times higher than the fluorescence signal of COP bringing a disadvantage for the observation of the response of green fluorescently tagged protein of the yeast cells under different environmental conditions.

6.4.2. Comparison of growth behavior of haploid BY4741 wild type cells and diploid *Sld7* deletion mutants

The growth of haploid BY4741 cells was observed in YNB media, whereas that of *sld7Δ* mutant cells was followed in YPD media. The duration of the cell cycle phases of haploid cells could not be analyzed since they were trapped as colony. Even though they were trapped as single cell in experiment 3, the duration of cell cycle could not be determined because some of these cells were swept away or exposed to MMS before completing their cell cycle. The durations of cell cycle phases of the diploid *sld7Δ* mutant cells were measured under different environmental conditions (Table 6.8) and discussed in section 6.4.3. It was reported that replication of haploid cells (A364a background) in the presence of 0.015% MMS took approximately 3-4 h, whereas replication in the absence of MMS takes fewer than 90 min (Paulovich and Hartwell, 1995). Talia *et al.* (2007) reported that the duration of cell cycle and its phases of the haploid cells were barely longer than that of diploid cells. The duration of the haploid cell cycle was affected by medium type, and it is 140 minutes in YNB media, whereas 90 min in YPD media (Sherman, 2002).

In the present study, the area, perimeter and volume of the different cell types were compared under different environmental conditions in Tables 6.6-6.8. The volume of our haploid and diploid wild type cells changed between 15-210 μm^3 and 121-324 μm^3 . Milo and Phillips (2015) measured the cell sizes of the haploid cells (BY4741) as approximately half of the diploid cells (BY4743) (Milo and Phillips, 2015). The sizes of the haploid and diploid cells (SC288c background) were reported as 42 and 82 μm^3 (Jorgensen *et al.*, 2002). The haploid and diploid cells were reported to change two-fold in size before division (Brewer *et al.*, 1984).

The *sld7Δ* mutant cells increased their cell sizes between 1.5 – 5.5 fold, whereas wild type cells increased their sizes between 1.5 and 2.5 fold before division (Tables 6.6-6.8). There was significant difference between the initial size of the diploid *sld7Δ* mutant and diploid wild type cells. The volumes of all the cell types decreased after they were exposed to inhibitor. The volume of the *sld7Δ* mutant cells increased after they were fed with fresh medium but not as much as their initial sizes.

Table 6.6. Comparison of the area of the cells in different environmental medium

Exp #	Cell Type	Haploid/Diploid	Medium Type	Glucose Ratio (w/v)	Area (μm^2)	Area (μm^2) - inhibitor	Area (μm^2) – After inhibitor
1&2	WT BY4741 Sld7:GFP	Haploid	YNB	2 %	-	-	-
3	WT BY4741 Sld7:GFP	Haploid	YNB	2 %	4-30	4-24	-
4&5	sld7 Δ mutant	Diploid	YPD	2 %	23-84	-	-
6	sld7 Δ mutant	Diploid	YPD	2 %	25-65	26-65	30-63
7	sld7 Δ mutant	Diploid	YPD	0.2 %	23-73	23-55	28-65
8	Wild type BY4743	Diploid	YPD	2 %	28-60	26-52	27-52

Table 6.7. Comparison of the perimeter of the cells in different environmental medium

Exp #	Cell Type	Haploid/Diploid	Medium Type	Glucose Ratio (w/v)	Perimeter (μm)	Perimeter (μm) - inhibitor	Perimeter (μm) – After inhibitor
1&2	WT BY4741 Sld7:GFP	Haploid	YNB	2 %	-	-	-
3	WT BY4741 Sld7:GFP	Haploid	YNB	2 %	7-19	6-17	-
4&5	sld7 Δ mutant	Diploid	YPD	2 %	18-60	-	-
6	sld7 Δ mutant	Diploid	YPD	2 %	25-60	25-62	25-55
7	sld7 Δ mutant	Diploid	YPD	0.2 %	21-65	23-45	24-46
8	Wild type BY4743	Diploid	YPD	2 %	25-42	22-37	21-36

Table 6.8. Comparison of the volume of the cells in different environmental medium

Exp #	Cell Type	Haploid/Diploid	Medium Type	Glucose Ratio (w/v)	Volume (μm^3)	Volume (μm^3) – under inhibitor	Volume (μm^3) – After inhibitor
1&2	WT BY4741 Sld7:GFP	Haploid	YNB	2 %	-	-	-
3	WT BY4741 Sld7:GFP	Haploid	YNB	2 %	15-210	210-5	-
4&5	sld7 Δ mutant	Diploid	YPD	2 %	55-400	-	-
6	sld7 Δ mutant	Diploid	YPD	2 %	80-315	51-300	65-325
7	sld7 Δ mutant	Diploid	YPD	0.2 %	36-410	70-380	110-390
8	Wild type BY4743	Diploid	YPD	2 %	121-324	150-330	104-300

After being exposed to fresh medium (N1R), the diploid *sld7* mutant cells recovered their DNA and continued to replicate. However, the haploid cells BY4741 did not form any bud after they were exposed to MMS (Table 6.9).

Table 6.9. Comparison of rates of volume changes in mother and daughter cells

Strain	Medium	Mother cell volume change rate	Daughter Cell volume change rate	Max Number of buds	Time (hour)
WT BY4741 (SLD7:GFP)	N1	0.20	0.20	1	8.5
	N2	-0.13	-0.05	-	8
	N1R	-	-	-	-

Godin *et al.* (2016) did a study about the effect of MMS at different temperature and medium conditions, which are also effective in DNA damage sensitivity. They observed that the cell can proliferate in YPD medium containing MMS at 30 °C. Yet, the rate of proliferation was lower at 23 °C but not effective as expected. The medium was changed into synthetic complete (SC) medium and the proliferation of cells was observed at the same temperatures. The DNA-damaging agents were more effective in this medium even at 30 °C. The most effective condition to see the effect of DNA-damaging agents was determined as SC medium at 23 °C (Figure 6.2).

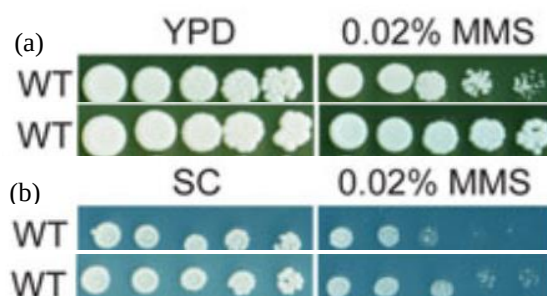


Figure 6.2. DNA damage in different environments (a) incubated for 4 days at 23 C or 2 days at 30 C onto YPD medium with or without 0.02 % MMS (b) incubated for 4 days at 23 C or for 2 days at 30 C onto SC medium medium with or without 0.02 % MMS (Godin *et al.*, 2016).

Table 6.10. Comparison of rates of volume changes in mother and daughter cells (cont.).

Strain	Medium	Mother cell volume change rate	Daughter Cell volume change rate	Max Number of buds	Time (hour)
WT BY4743	N1	3.04	2.46	3	6
	N2	1.44	1.37	2	3
	N1R	0.40*	0.51*	1	3
SLD7Δ of W303 background	N1	1.50	1.14	3	5
	N2	0.65	0.55	1	2.5
	N1R	1.18	0.26	2	5
SLD7Δ of W303 background**	N1	2.70	1.6	3	7
	N2	1.82	-	2	3
	N1R	1.84	-	2	3

**0.2% glucose -CR+ alkylating agent-inhibitor

The DNA response genes such as Mec1, Dun1, Rad53 or Rad9 have a role in apoptosis (Burhans *et al.*, 2003). The effect of MMS on the expression of the genes that are dependent on Mec1 or Dun1 (responsible for protein kinase activity), was observed by Gasch *et al.* (2001). When the wild type and mutant cells were grown in YPD medium with 0.02% MMS, the alterations in gene expression began within 15 min of MMS exposure and >45% of the wild type cells survived, whereas only 1% of the *mec1Δ* cells survived. Deletion of *mec1* or *dun1* affected the expression of more than thousand genes in a medium involving MMS (Gasch *et al.*, 2001). It had already been demonstrated that Rad53 is necessary for DNA damage-induced cell cycle regulation at the G1-S phase transition (Allen *et al.*, 1994) and that both Mec1 and Rad53 are necessary for regulation at the S-M phase (Allen *et al.*, 1994; Weinert *et al.*, 1994) and G2-M phase (Weinert *et al.*, 1994) transitions. Rad53 is an essential protein for mitochondrial inheritance and it is one of the genetic interactor of Sld7 in yeast (Stirling *et al.*, 2012).

It was observed that the replication of the cells delayed if they were exposed to MMS before S phase, otherwise the life span was not effected (Gasch *et al.*, 2001). MMS could repress the activity of COX-genes, which are cytochrome-c oxidase subunit (Gasch *et al.*, 2001). The repression of these genes alter the oxidation state of cells and change the level of reactive oxygen species (ROS) (Gasch *et al.*, 2001; Madeo *et al.*, 1999). Salmon *et al.* (2004) showed that the intracellular level of ROS increased in the cells that were exposed to MMS.

Fbp1 protein is required for glucose metabolism. The transcription level of this protein was reported to decrease due to either proteasome-mediated or autophagy-mediated degradation depending on growth conditions (Kitanovic and Wöfl, 2006). The Fbp1 was overexpressed in chronologically aged cells or after exposed to MMS and caused ROS production (Kitanovic and Wöfl, 2006). MMS induce the expression of Fbp1, which regulates the production of ROS. However, the level of ROS was lower in the mutant cells than the WT cells when exposed to MMS. It was observed that the onset of ROS production delayed in aged *fbp1Δ* cells. The *fbp1Δ* cells survived and reproduced even after long-term MMS treatment because of the low level of ROS production. (Kitanovic and Wöfl, 2005). Herker *et al.* (2004) found that high accumulation of ROS with chronological aging leads to an apoptotic phenotype.

6.4.3. Comparison of diploid wild type BY4743 cells with diploid *Sld7* deletion mutants

To understand the role of *Sld7p*, the response of the *sld7Δ* mutant cells were observed under different environmental conditions. The duration of cell cycle phases, number of formed buds and the volume of the cells were compared (Tables 6.9-6.12).

The duration of the G₁ and S/G₂/M phases of *sld7Δ* mutant (W303) and wild type (BY4743) cells were observed under same environmental conditions (Table 6.10). The cell cycle phases of the *sld7Δ* mutant cells were longer than those of the wild type cells as expected. The average number of the buds, that the *Sld7* deletion mutants formed, was 3 (8 hours in Exp5) and 2 (5 hours in Exp6) in glucose rich YPD medium.

It was reported that the depletion of *Sld7* slows down DNA replication and cell growth in budding yeast (Tanaka *et al.*, 2011b). Tanaka *et al.* (2011b) found the doubling time of

sld7 Δ cells and wild type cells (W303) in liquid YPD media at 30°C as 86.2 min and 77.5 min, respectively.

Table 6.11. The comparison of the duration of cell cycle phases of different strains under different conditions

Medium Type	2 % Glucose – W303 – sld7 Δ		2 % Glucose – BY4743 - WT		0.2 % Glucose – W303 – sld7 Δ	
	G ₁	S/G ₂ /M	G ₁	S/G ₂ /M	G ₁	S/G ₂ /M
N1	35	65	25	40	40	60
N1-N2	45	95	30	50	45	75
N2	55	95	36	50	45	90
N2-N1R	55	125	30	60	50	95
N1R	35	85	64	94	40	80

In the present study, the duration of the cell cycle phases of both cell types (BY4741 and Sld7 deletion mutant) increased after they were exposed to MMS (Figure 6.3). It was observed that sld7 Δ mutant cells continued to reproduce after they were refreshed with N1R medium, whereas wild type cells (BY4743) did not (Table 6.11). The loading time of the N2 media was 3 and 5 hours for BY4743 wild type cells and sld7 Δ mutant cells, respectively. It was observed that the duration of the cell cycle of mutant cells were decreased after they were exposed to fresh media (Table 6.12). In this case, it can be said that the loading time of the fresh medium was not enough for diffusing to the cells and repairing their DNA. Since we want to see the role of Sld7p experimentally, it is more appropriate to repeat this experiment.

In literature reports, the sld7 Δ mutant yeast cells were transiently exposed to 0.01 % MMS in YPD media (Tanaka *et al.*, 2011b). It was observed that MMS reduced the viability of mutant cells compared to wild-type cells (Figure 6.4). Sld7 is a non-essential protein that has a function in cooperation with the Dpb11 and Sld- proteins to provide initiation of DNA replication efficiently (Tanaka *et al.* 2011b). Based on the information provided in the literature, it was expected that the sld7 Δ mutant cells exposed to MMS will be affected dramatically due to DNA damage and increased ROS production, but instead it was observed

that the cells continued to regenerate themselves after feeding fresh medium. This may be because the loading time of MMS was not sufficient for diffusing into the cells.

Table 6.12. Comparison of cell cycles of the mother and daughter cells in the present study

Strain	Medium 2% glucose	Mother cell G1 phase	Daughter Cell G1 phase	Mother cell cycle (min)	Daughter Cell Cycle (min)	Time (hour)
WT BY4741 (SLD7:GFP)	N1	ND	ND	ND	ND	8.5
	N2	ND	ND	ND	ND	8
	N1R	-	-	-	-	-
WT BY4743	N1	28	36	65	81	6
	N2	35	50	85	91	3
	N1R	64*	65*	158*	>65*	3
SLD7 Δ of W303 background	N1	35	-	100	-	5
	N2	55	50	145	125	2.5
	N1R	35	-	120	-	5
SLD7 Δ of W303 background**	N1	38	59	98	85	7
	N2	45	-	135	-	3
	N1R	40	-	120	-	3

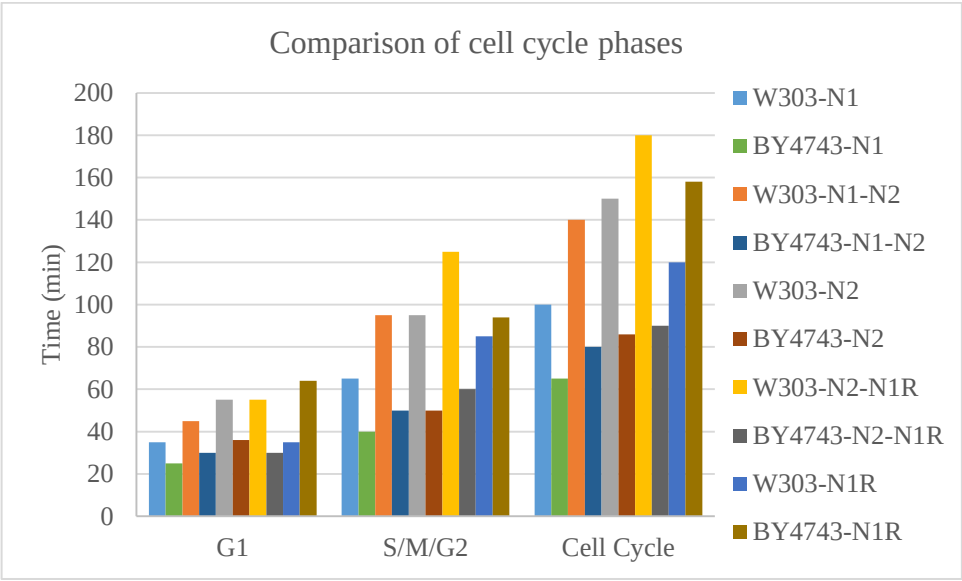


Figure 6.3. The comparison of the duration of the cell cycle and its phases of the *sld7Δ* mutant and wild type BY4743 cells in glucose rich YPD media

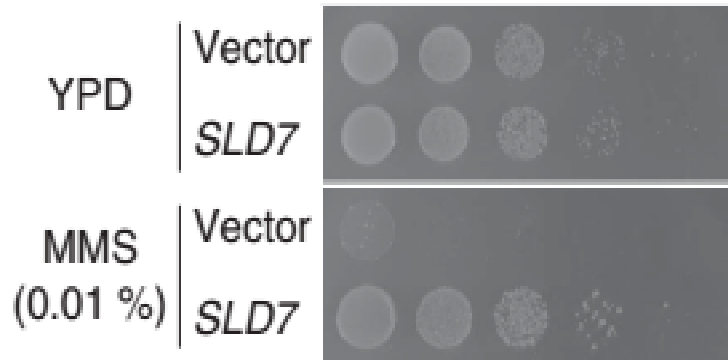


Figure 6.4. Sensitivity of *sld7Δ* cells to MMS. Plasmids with or without the *SLD7* gene were introduced into *sld7Δ* cells. Tenfold serial dilutions of the cells were spotted onto YPD plates containing 0.01% MMS (Tanaka *et al.*, 2011b).

The cell division times of wild type cells of different backgrounds reported in literature were compared with those of our cells (Table 6.12). The duration of the cell cycle is dependent on the background. It was observed that the duration of the cell cycle was similar in haploid and diploid cells (Jorgensen *et al.*, 2002). Tanaka *et al.* (2011b) reported that the duration of cell cycle of the *sld7* mutant cells (W303) were 10 minutes longer than

the wild type (W303) cells. In the present study, it was observed that the duration of the cell cycle of *sld7* mutant (W303) cell were 35 minutes longer than the wild type (BY4743) cells.

Table 6.13. Comparison of cell cycles of *Saccharomyces cerevisiae* strains

Strain	Type	Mother cell cycle (min)	Daughter Cell Cycle (min)	Growth medium	Reference
Wild type	diploid	90 min	ND	ND	Kraikivski <i>et al.</i> , 2015
WT A364A D5	diploid	99 min	93-142 min	ND	Brewer <i>et al.</i> , 1984
WT W303	diploid	77.5 min	ND	YPD+2% glucose	Tanaka <i>et al.</i> , 2011
SLD7 Δ of W303 background	diploid	86.2 min	ND	YPD+2% glucose	Tanaka <i>et al.</i> , 2011
WT	haploid	87 $\pm$ 6 min	ND	ND	Jorgensen <i>et al.</i> , 2002
WT	diploid	87 $\pm$ 6 min	ND	ND	Jorgensen <i>et al.</i> , 2002
WT BY4741 (SLD7:GFP)	haploid	ND	ND	YNB +2%glucose	Present study
WT BY4743	diploid	65 min	80 min	YPD +2%glucose	Present study
SLD7 Δ of W303 background	diploid	100 min	120 min	YPD +2%glucose	Present study

**0.2% glucose -CR+ alkylating agent-inhibitor

The volumes of the wild type BY4743 cells were found to be higher than those of *sld7Δ* (W303) mutant cells (Table 6.8). In fact, the areas of the both wild type and deletion mutant cells were similar in the beginning ($26\text{--}28\ \mu\text{m}^3$) but they decreased barely after the cells were exposed to MMS ($25\text{--}28\ \mu\text{m}^3$). After the fresh medium was fed, the cells returned to their normal sizes (Table 6.8).

The rates of volume changes of the mother and daughter cells reveal that mother cells have higher rates of volume changes than their daughters (Table 6.9). In general the volume change of the daughter cells were 75% less than that of the mother cells. Under the effect of inhibitor, the rate of volume change almost halved from 3.04 to 1.44 for wild type cells, 1.50 to 0.65 for deletion mutants. Under caloric restriction and inhibitor addition simultaneously, the rate of volume change decreased from 2.70 to 1.82 for deletion mutant cells (Table 6.9).

Budding yeast cells divide asymmetrically, with a certain fraction f ($f=0.4586$) of division mass going to daughter cell at division (Kraikivski *et al.*, 2015). The concentration of the critical regulatory proteins, which regulates the volume of the cells, is a key factor to cell cycle control. The cell cycle continues till the cells reach their critical cell sizes which is 25 fL ($25\ \mu\text{m}^3$) (Cross *et al.*, 2002). These observations revealed that there is a correlation between size regulation and the expression of critical genes. The differences in background of the strains also affect the cell size (Table 6.13).

Recent measurements have indicated that the single gene deletions affected the yeast cell size significantly. In some of these deletion mutants, the median volume was only 40% of the wild type size, whereas in others it was larger than wild type by $>70\%$ (Jorgensen *et al.*, 2002). It remains unclear how genetic and environmental changes shift the median cell size in yeast. There is a significant difference between the size of the *sld7Δ* mutant cells and wild type (BY4743) cells. Based on these results, it can be said that the lack of *sld7Δ* affects not only genotype of the cells but also their phenotype. The lack of *sld7* not only delays the S/G2/M phase, but also change the cell size .

Table 6.14. Comparison of volumes of *Saccharomyces cerevisiae* strains

Strain	Type	Volume	Mass doubling time (min)	Growth medium	Reference
WT	diploid	150 fL V=2.37 fold change at division	90 min	ND	Kraikivski <i>et al.</i> , 2015
WT BY4741 (WHI5:GFP)	haploid	30→75 μm^3 M+D 0→30 μm^3 D	ND	SC + 2%glucose	Ferrezuelo <i>et al.</i> , 2012
A2587 of W303 background	Diploid	35→60 μm^3	ND	YEPD	Bryan <i>et al.</i> , 2010
A11311 of W303	diploid	20→40 μm^3	ND	YEPD	Bryan <i>et al.</i> , 2010
WT	haploid	41 μm^3	87±6 min	ND	Jorgensen <i>et al.</i> , 2002
WT	diploid	82 μm^3	87±6 min	ND	Jorgensen <i>et al.</i> , 2002
WT BY4741 (SLD7:GFP)	haploid	M:15-210 μm^3 D:6-60 μm^3	ND	YNB +2%glucose	Present study
WT BY4743	diploid	M:121-324 μm^3 D:100-200 μm^3	65 min	YPD +2%glucose	Present study
Sld7 Δ of W303 background	diploid	M: 55-400 μm^3 D:50-360 μm^3	100 min	YPD +2%glucose	Present study

SC=synthetic complete medium+2%glucose

6.5. Simultaneous application of inhibitor and caloric restriction on mutant yeast cells

Yucel *et al.* (2014) completed an experimental work on the effects of medium acidity on chronological aging using BY4742 strain of *S. cerevisiae*. The transcriptomics analysis revealed that the expression level of Sld7 was 0.5 (log₂-fold change) under pH controlled conditions, being in the top 10 among 882 genes (Yucel *et al.*, 2014). This raises the question on a putative and critically important role of the Sld7 protein in aging.

Caloric restriction (CR) is one of the tools to study chronological aging and life span. Dietary restriction or drug (caloric restriction mimetics-CRM) treatment for health preservation and life span extension lead to reorganization of cellular metabolism by activating stress responses which eventually help to establish mitochondrial hormesis and chronological longevity. In our study, the responses of the *sld7Δ* mutant cells in YPD medium containing 2% (nutrient rich) or 0.2 % glucose (nutrient limited-caloric restriction) were observed. Then, these cells were exposed to YPD medium containing 0.01% MMS for a while, afterwards these cells were fed with fresh medium. When the medium type was N1, the G₁ phase of these cells was 5 minutes longer in 0.2 % glucose containing media than that in 2% glucose containing media, whereas the S/G₂/M phases were 5 minutes shorter (Tables 6.10 and 6.11). The response of the cells that were exposed to MMS were similar in both volumetric fold change and number of formed buds. The average number of the formed buds was same (3) in both cases. The durations of the cell cycles of the mutant cells were almost same in both experiments (Figure 6.5).

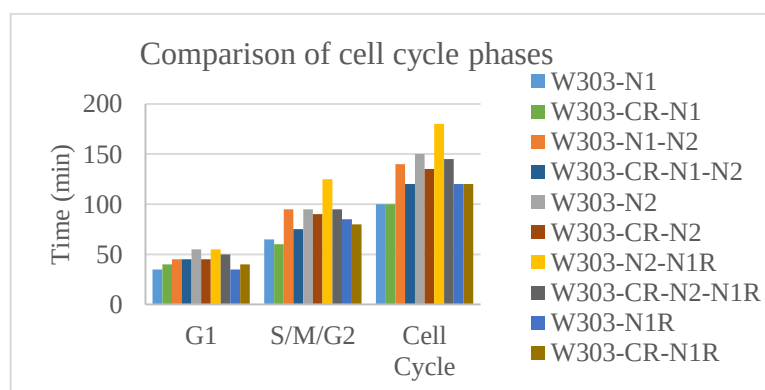


Figure 6.5. The comparison of the duration of the cell cycle and its phases of the *sld7Δ* mutant cells in glucose rich and calorie restricted (CR) YPD media

It was reported that the CR did not prolong the life span of yeast cells of W303R background at 0.5% glucose (Kaeberlein and Powers, 2007) and further reduction in the level of glucose concentration shortens life span of the cells dramatically.

Goldberg *et al.* (2009) investigated the effect of caloric restriction on wild type BY4742 strain in nutrient rich YPD medium (1% yeast extract and 2% peptone) containing 0.2%, 0.5%, 1% or 2% glucose as the carbon source. The reason of choosing YPD medium instead of synthetic medium was that to understand the effect of glucose rather than amino acids or nutrients in both chronological and replicative aging. It was observed that the growth rate and the glucose consumption in logarithmic phase were similar for cells cultured in any of the four media, whereas the maximum cell density was obtained in stationary phase in YPD medium containing 0.2% glucose. It was observed that the cells that were grown on 0.2% or 0.5% glucose lived longer than the cells that were grown under normal conditions (YPD medium containing 1% or 2% glucose). The ATP levels and growth dynamics of CR and no-CR cells were similar. It was hypothesized that CR cells remodel their metabolism to have same level of ATP produced in non-CR yeast and this remodeling extends their life span (Goldberg *et al.*, 2007). On the other hand, the cells cultured in YPD medium with 0.05% glucose lived shorter than the cells cultured in YPD medium containing 0.5% glucose.

Growing a cell in a calorically restricted environment involving DNA-damage agent such as MMS, is an effective method in treatment of cancer by forcing the cancerous cells to either apoptosis or autophagy. The cells that were grown in a calorically restricted environment involving MMS are forced to either apoptosis or autophagy. This method may have a high potential for anti-cancer therapy strategies (Kopeina *et al.*, 2016).

7. CONCLUSIONS

7.1. Conclusions

This thesis described both computational and experimental works on cell cycle network of yeast using systems biology approaches. The following achievements were obtained from this study:

- The visualization and analysis of reconstructed cell cycle networks were done via Cytoscape software. The molecular functions of the favorable clusters were examined via GO enrichment analysis. The enriched function terms point out a dual role for Sld7 in both cell cycle and chronological aging processes in yeast.
- The putative molecular functions of Sld7 were determined as “macromolecular complex binding (GO: 44877)” and “oxidoreductase activity, acting on a heme group of donors (GO: 16675)”.
- The designed microfluidic device was successfully produced via hot embossing and thermal bonding techniques from polystyrene.
- Yeast cells were trapped and cultured in determined c-shaped regions within the microbioreactor.
- The yeast cell experiments were performed in glucose rich YNB medium, glucose rich and glucose-limited YPD media in the presence and absence of an inhibitor.
- The behavior of GFP tagged (Sld7:GFP) BY4741 cells, *sld7* Δ mutant (W303) cells and wild-type BY4743 cells was followed in the fabricated Lab-on-a-Chip platform under various environmental stress conditions. The genotypic and phenotypic responses of the cells were compared in different environments such as glucose rich YNB medium, glucose rich and glucose-limited YPD media in the presence and absence of an inhibitor.
- The brightfield microscopy images of the cells were captured by inverted microscope (Nikon Eclipse-T) at regular time intervals during the experiments. The images were processed via ImageJ software by using the macros that were written for brightfield images. The changes on cell count, cell area, cell perimeter and cell volume were analyzed in each experiment.

- The cell area of the *sld7* Δ mutant cells changed between 25 and 65 μm^2 , and the cell area of BY4743 wild type cells changed between 28 and 60 μm^2 . The cell areas of the mutant cells were barely smaller than those of the wild type BY4743 cells. The volume of the *sld7* Δ mutant cells (50-60 μm^3) were half of the wild type BY4743 cells (120-140 μm^3). In literature, the volumes of the haploid and diploid wild type cells were given as 41 and 82 μm^3 , respectively. Deletion of some regulatory genes in the cell cycle causes changes in the cell size (either increase or decrease). In the present case, lack of Sld7 might have a role in changing not only the genotype of the cells but also the phenotype.
- The durations of G₁ phase and S/G₂/M phases in cell cycle were determined for *sld7* Δ mutant of W303 background and wild type cells of BY4743 background. The duration of G₁ phase was found as 35 minutes and 25 minutes in *sld7* Δ mutant and BY4743 wild type cells. With the application of caloric restriction, the duration of the G₁ phase of *sld7* Δ mutant cells increased from 35 to 40 minutes, while the duration of S/G₂/M phases decreased from 85 to 80 minutes.
- After the cells were exposed to MMS, the duration of G₁ phase and S/G₂/M phases of diploid cells (BY4743) increased between 5-10 minutes and 10-20 minutes, respectively. After a period of fresh medium feeding, the DNA of the mutant cells repaired itself, and then the duration of the cell cycle phases decreased, but not as much as it increased. On the other hand, the wild type cells (BY4743) could not repair their DNA, and they did not reproduce even they were fed with fresh medium, N1R. The loading time of the fresh medium was 3 and 5 hours for wild type (BY4743) and *sld7* Δ mutant cells. The loading time of N1R fresh medium might not be enough for diffusion of fresh medium to the wild type cells, and then it may not be possible to observe any reproduction from the cells, that were arrested in G₁ phase.
- The accumulating evidence suggests that the combination of DNA damage and caloric restriction has a synergistic effect on cancer cell death. Therefore, the response of the *sld7* deletion mutant cells in calorically restricted environment involving 0.01 % MMS were followed to elucidate the role of Sld7 in yeast and consequently to understand the role of its putative human homolog MTBP better. In caloric restricted medium involving DNA alkylating agent, the duration of the G₁ phase increased from 35 to 40 minutes, whereas the duration of S phase decreased

from 65 to 60 minutes. In that case the duration of cell cycle did not change.

7.2. Recommendations

The ultimate aim of this study was to determine putative molecular function of Sld7 protein in yeast by systems biology approaches and validate the predicted results by experiments in a microbioreactor made of polystyrene. The response of the *S. cerevisiae* yeast cells of different genetic backgrounds was monitored after applying environmental stress via changing the concentration of nutrient media or applying replication inhibitor such as MMS. The results were used to get an insight on the behavior of the cells in the case of presence and lack of Sld7p, which has a role in the initiation of the DNA replication of the cell cycle process. Nevertheless, some additional studies should be done to annotate a function to uncharacterized Sld7p protein by using the fabricated microbioreactor. The elucidation of the role of Sld7 in yeast can help to understand the role of its putative human homolog MTBP better, and will shed light on the development of novel strategies for the treatment of cancers and aging related diseases.

- To get an insight about the response of the *sld7Δ* mutant cells in stressed environment more precisely, the glucose level might be less than 0.2 % (w/v) in YNB medium.
- To understand the molecular function of Sld7p, the duration of the loading time of MMS and fresh medium for recovery need to be changed in order to compare the response of the wild-type cells with that of *sld7Δ* mutant cells.
- In order to be able to observe the same cells during the experiment, the y-junction can be used in the middle inlet on PMMA inlet apparatus. The different media can be loaded into the microbioreactor without changing the tubing, and that is expected to prevent the sweeping of the cells.
- To increase the efficiency of the trapping, the design of the microbioreactor might be modified by arranging the aspect ratio of the trapping areas, i.e. increasing the height and decreasing the width of these areas.
- Since the polystyrene has high auto fluorescence background, the visualization of the fluorescent signal of Sld7p is unattainable. In order to visualize the effect of MMS on Sld7p in haploid cells, these cells can be grown in COP made microbioreactor.

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