

MULTISCALE TUMOR MODELING

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# ABSTRACT

## MULTISCALE TUMOR MODELING

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Cancer's complex behavior decreases success rates of the cancer therapies. The usual steps cancer therapy are, deciding phase of the cancer and planing the therapy according to medical guidelines and there is no room or chance for personalized medicine. Simulation systems that use patient specific data as input and up-to-date scientific evidence as business rules has chance to help clinicians for evidence based personalized medicine practice. In this study our aim is creating a basic model to guide researchers who are eager to start tumor modeling. Developed model tries to simulate adenocarcinoma which is a subtype of non-small cell lung carcinoma. Parameters of model gathered from literature is based on A549. In simulations effects of oxygen concentration and mutation rate are examined. Tumor cell number decreases and apoptosis frequency increases proportionally with oxygen concentration's decrease. When mutation rate decreases tumors become more vulnerable and apoptosis rate increases. All these results proves that model is consistent with tumor biology rules.

Keywords: cancer, tumor modeling, cellular automata, personalized medicine

# ÖZ

## ÇOK BOYUTLU TÜMÖR MODELLEME

Ünsal, Serbüent

Yüksek Lisans, Sağlık Bilişimi Bölümü

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Kanserin karmaşık yapısı tedavilerin başarı oranını düşürmektedir. Klasik tedavi kanserin tipinin ve hangi fazda olduğunun belirlenmesinin ardından tıbbi klavuzlara göre tedavi edilmesi ile devam eden bir süreçtir ve kişiselleştirilmiş tedaviler bu süreçte yer almamaktadır. Hastaya özel tıbbi veriyi ve güncel bilimsel kanıtları iş kuralları olarak kullanan simülasyon sistemleri hekimlere kişiselleştirilmiş, kanıta dayalı tedavi uygulama şansı tanımaktadır. Bu çalışmadaki hedefimiz, basit bir tümör modeli oluşturarak, tümör modelleme konusunda çalışmaya istekli araştırmacılar için bir başlangıç noktası oluşturmaktır. Geliştirilen model adenokarsinomanın benzetimini gerçekleştirmeyi amaçlamaktadır. Modelde kullanılan parametreler genellikle A549'a aittir. Simülasyonlar ile oksijen konsantrasyonunun ve mutasyon oranının etkileri incelenmiştir. Oksijen konsantrasyonu düşüşü ile orantılı olarak tümördeki hücre sayısının azaldığı ve apoptoz oranının arttığı gözlemlenmiştir. Mutasyon oranı düşürüldüğünde ise, tümör zayıflamakta ve apoptoz oranı artmaktadır. Bütün bu sonuçlar geliştirilen tümör modelinin tümör biyolojisinin kuralları ile uyumlu biçimde çalıştığını göstermektedir.

Anahtar Kelimeler: kanser, tümör modelleme, hücresel otomat, kişiselleştirilmiş tedavi

*to my beloved wife, who supports me at every critical decision in my life.*

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# CHAPTER 1

## INTRODUCTION AND RELATED WORK

### 1.1 Introduction

Despite all enhancements in medicine, cancer is still one of the most fatal word in our lives. Cancer's complex behavior decreases success rates of the cancer therapies. The usual steps of cancer therapy are, decision phase of the cancer upon gathering radiological, pathological and genetic information and planning the therapy according to medical guidelines. Afterwards, clinicians evaluate the patient's objective (based on evidence) and subjective (based patient's expression) responses to therapy and update the therapy plan. In this routine therapy plan, there is no room to calculate and predict patient's therapy response with scientific methods. The main cause for this situation is cancer's complexity. When the outcomes are to be predicted, one needs to process huge amount of data which is very cumbersome. Under these circumstances prediction of therapy outcomes becomes almost impossible for human but it might be possible for machines which have high computing power. Simulation systems that use patient specific data as input and up-to-date scientific evidence as business rules has chance to help clinicians for evidence based personalized medicine practice. By using these prediction softwares clinicians would be able to compare alternative therapy plans and predict results. Application areas of evidence based personalized models' abilities are not limited to clinics. These models have an important role in early drug development and development of therapy devices. It is possible to calculate and optimize dose and time parameters for clinical trials with simulation systems. When cost of cancer drug development is considered (approximately 1 billion for each drug), importance of these models could easily be understood. These simulation systems not only decrease cost of trials but also has potential to decrease their time time. In this review we aim to define a starting point for new researchers who would like to study tumor modeling.

### 1.2 Background of Tumor Modeling

Before creating a new model, a detailed literature review is essential. One can use different types of taxonomies to classify mathematical tumor models. We choose to classify them by modeling techniques. The first technique is the oldest one, "Continuous Modeling". Continuous models are based on differential equations. Differential are used for calculating amount of change. For example difference of speed versus time (acceleration) is a fundamental example for differential equations. Researchers try to find growth of tumors versus time by creating complex differential models, also known as continuous tumor models. On the other hand some researchers try to achieve same goal by "Discrete Models". Discrete models are mostly based on rules like cellular automata systems. The third way is creating a hybrid model that uses continuous and discrete modeling approaches together. Like most of the complex system models, tumor models also need submodels which are represents different parts of the tumor system. In this chapter, these sub-models are also reviewed. An overview of multiscale tumor modeling could be shown below:

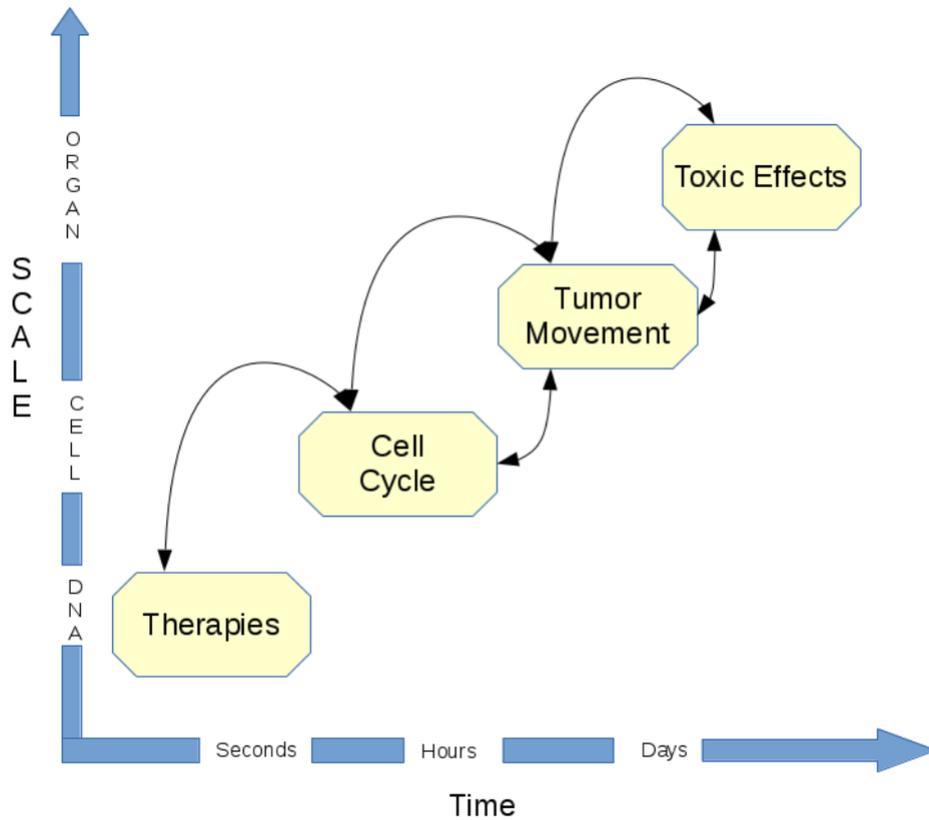


Figure 1.1: Multiscale Tumor Model

### 1.2.1 Cell Modeling

Mathematical modeling of cell is a complex work. Many scientists have tried to enlighten unknown features of the cell with different tools and methods. One of the methods is mathematical modeling. We can start the history of mathematical cell modeling with Rubinow. In 1969 Rubinow created a discrete model of hemopoiesis [1].

In another example Murray and Frenzen create a model to inspect cell populations with Gompertz equation [2]. In this model correlation of cell doubling time and growth patterns of cell are inspected [3]. Novak and Tyson's study is an important achievement in modeling of cell dynamics [4]. Their model explains autonomous cell division, controlled division of somatic cells and controlling mitosis with nucleocytoplasm rate. Mathematical modeling of cells is still an interesting research subject. For example, Chen et al. developed a continuous model and a simulation for chromosome replication and segregation cycle. Their model clarifies proteins regulatory effects in cell cycle [5].

### 1.2.2 Continuous Tumor Models

Continuous tumor models try to simulate entire tumor with one or more differential equations, hence it is a good option to model complex systems. This approach uses continuous mechanic's principles by means of partial differential equations to define model's variables. By comparison with discrete models, it is easier to obtain, inspect and control common continuous variables (i.e. substrate concentration, tumor density, cell volume fraction) [6]. These type of models could easily characterize global scale attributes of a tumor, however it is a non-trivial challenge to use them for simulating individual cell dynamics, or discrete events in cell or cell's microenvironment [7]. Because small scale changes may have big effects at cancer systems

which known as non-linear. This fact is an important factor at researches that investigates genetic and microenvironment parameters' effects on tumor behavior [8].

Greenspan's model is a frontier in continuous tumor modeling [9]. Most the continuous models includes one or more reaction-diffusion equation related differential equations [10].

The question which Greenspan focuses at his research is "What can we know about internal mechanics of a growing tumor ? ".Afterwards Ward and King developed Greenspan's model [11]. ]. In this study, dead and living cells are included to model. Controversially to Greenspan their model does not assume tumor is heterogeneous. According to model living cells divides and expand the tumor or dies and shrinks the tumor. This change creates a velocity field in a spheroid. We could say that model is successful example and has a good agreement with numerical data. One other example in this area belongs to Sherratt and Chaplain [12]. Their model simulates continuous density of proliferative, quiescence and necrotic cells based on nutrition and growth factor parameters. This study does mainly three contribution to literature. First study shows cell movement and proliferative edge, quiescence border and necrotic region structure's growing with partial differential equations. Secondly it shows effects of mobile living cells' and immobile necrotic cells' effects on tumors' shrinkage in a continuous model. Last one is drain of nutrients from tissue which tumor exits. This phenomenon could not observed in vitro bu has an important effect on tumor's growth and shape in vivo. Wise et al. developed more complex model which is a multiscale, 3D and takes angiogenesis into account. With this model, effects of malign cells, necrosis, tumor's response to therapy, triggering process of angiogenesis and tumor mutations could be simulated.

The model which was developed by Macklin et. al [13] is a multiscale model. In the study cell-cell adhesion, cell-ECM adhesion, ECM fractionation, tumor cell migration, proliferation and necrosis are considered. Tumor model also includes angiogenesis. In angiogenesis model, blood flow through vascular network, non-Newtonian effects, mechanical strain induced changes of vascular network model are considered. This study not only shows relationship between vascular network and tumor's progression but also effect of ECM fractionation over growth of vascular network and tumor's progression proved. Tumor growth is related very closely with residual stress. This phenomenon studied by Golneshan and Nematı [14]. They created a new continuous model to calculate residual stress. This model is important because there are only few studies which calculates tumor growth under continuous mechanics.

Applying cancer therapies under hyperthermia is an interesting technique. Zhu et al. studied tumor growth under hyperthermia [15]. This model also considers energy metabolism and Warburg effect among other parameters. Model assumes, tumor starts with one cell with a homogeneous growth pattern and tumor growth is associated with glucose concentration. Eventually, when results are inspected control group's results are consistent with simulation results. Therapy group's results are also consistent for first 15 days. After 15th day a variation occurred between model's assumption and therapy group results.

### **1.2.3 Discrete Tumor Models**

Most frequent methods for discrete tumor modeling are, cellular automata, agent based techniques and Potts model. Cellular automata developed by Stanislaw Ulam and John von Neumann at 1946 [16] [17]. Cellular automata (CA) is a discrete modeling technique which could also used to model different types of biological and physical phenomenon. CA consists of cells on a grid. These cells' states are effects each other. CAs used successfully for modeling tumors. In agent based models, each agent behaves independently but communicate with other agents and aware its environment. Potts model [18] ] is inherited from Ising model which uses electrons on a grid that has 2 states. In Potts model electrons states are more than 2 and they effect each other neighbor. Application of Potts model, benefited from Metropolis algorithm which based on Monte Carlo simulation [19]. Using Metropolis algorithm, state of each electron determined based on relevant parameter's probability distributions. When modeling biological systems, Potts uses 3 types of energy relationship. These are; cell-cell adhesion, cells' morphology changes and chemical effects. We

claim that CA, agent based approach and Potts model are fundamentally same discrete model. They consists from micro subunits which interacts with environment and each other. So there is no superiority on each other. In this chapter models which are developed using these three techniques inspected.

It is claimed that the study has been done by Kansal et. al. [20] is the first validated 3D realistic tumor model. Glioblastoma is modeled in the study with 4 parameters. These are, cell proliferation probability, necrotic tissue thickness in tumor, proliferative tissue thickness and tumor's maximum growth diameter. Study is also first example for use of voronoi mosaics with CA. Another remarkable feature of the model is including different types of tumor populations in same model. Results of the research are consistent with in vitro data. Effects of internal and external stress factors on mutations are postponed for future work by authors. The model which was developed by Mansury et. al. could be an another good example for agent based models [21]. Researchers inspected orientation mechanism of tumor to nutrients. In the model, virtual cells are migrating from areas that has high toxic waste concentration to areas that has high nutrient concentration. A feature specific for this model is, cell's nutrient search mechanism. Search mechanism works for both local and global scales. If global search results are dominant cells forms fewer number of clusters which are ephemeral, fast grown and bigger. If local search results are dominant, cells will form small but long lived clusters. These clusters have slower movement and growth. When local search is dominant on tumor, cells need fewer amount of nutrient. In the study model developed in 2D and aims to simulate aggressive behavior of tumor cells. Since smallest observable unit is cell, researchers prefer agent based simulation technique. After experiment results, each cell modeled as an individual who can decide independently. Model designed with discrete modeling to show sequential progression of brain tumors. Researchers avoided trivial deterministic approach and assumes cells acts in a probabilistic way. Simulation results are consistent with experiments. Turner and Sherratt preferred to use Potts model for their research which simulates cell and tissue adhesion and effects of proteolytic enzymes [22]. Study aims to find effects of cell adhesion on tumor growth. Results showed that cell-cell adhesion is less effective than cell-tissue adhesion on tumor invasion. Both adhesion effect are triggering proteolytic enzyme secretion and increases tumor invasion. In discussion, researches claimed high proliferation rate is not always means high invasion rate, controversy it leads to decrease of tumor invasion, according to in silico results.

Most of the tumor modeling studies ignores blood flow. Alarcon, Byrne and Maini aim to model this phenomenon in their research [23]. Study shows effect of blood flow and heterogeneity of red blood cells to tumor growth. Model developed in two stages. At first stage, distribution of oxygen in a natural vascular network was determined and included into the model. In stage two, vascular network assumed as static and independent from embracing tissue. By this way normal and tumor cell dynamics were studied. Study also emphasis heterogeneity of cell colonies as tumor and normal cells. Heterogeneity was emphasized because competition between cell colonies has important effects on tumor invasion. Discrete models are practical to integrate with other techniques. As an example Mansury, Diggory and Deisboeck integrated evolutionary game theory with an agent based model [24]. In study tells competence of two different cell genotypes in tumor which are migrative and proliferative types. Decision of cells for migration and proliferation adapted to game theory. Controversially to this study cells are continuously evaluating but this study could be a good base for modeling genetic effects on tumor's shape and movement. Nevertheless preliminary results of study is promising (i.e. effects of tumor environment on genotype could be observed in simulation). Also there are more detailed genetic pathway models in literature which simulates effects of genetic parameters on tumor movement [25].

Biomechanical forces should be considered to predict tumor movement with discrete models. As an example Macklin et. al. developed an agent based simulation for mechanics of ductal carcinoma in situ [26]. Cell motion simulated by using physical forces. In the model phenotype of each cell determined with stochastic mechanisms which uses genetic and microenvironment variables as input parameters. Tumor's shape, growth and death simulated with subsystems. Researchers also noted that their model is the first of its kind that considers clarifications but real breakthrough for this study is its calibration approach. Model could be calibrated by physicians according to pathological data after its first steps. By this way model can

be personalized and its validated. According to study personalized models are more accurate than regular models based on validation with clinical data. Except frequently used modeling methods which mentioned at introduction part, alternative methods also applied in literature. In their study Brown et. al. developed multi-agent model uses "Decentralized Markov Decision Process (DEC-MDP)" [27]. Model handles tumor cells and wild type cells as two teams which are competing for resources. DEC-MDP algorithm uses agents which has limited ability to sense environment and communicate each other. Behaviour of robots in a robot football tournament could be a good example for application of this algorithm. The difference between classic agent based simulations and DEC-MDP based agents lies on its decision process. In classical approach researchers defines rules to control agents, but agents uses DEC-MDP to define their own rules. This method could be a powerful approach in uncertain environments. According to us the study is a trial which is not based on clinical data but has some promising methods like modeling noise at signaling between cells.

#### 1.2.4 Hybrid Tumor Models

Continuous and discrete tumor models has different advantages and disadvantages. Hybrid tumor models used for make advantages both approach together. Study by Jiang et. al. is a good start point to demonstrate hybrid models [28]. This study inspects tumor at three different scales. First one is modeling cell scale dynamics like, proliferation, survival, adhesion. Potts model used at this scale. At another scale dynamics of tumor microenvironment like distribution of chemicals in cell microenvironment like nutrition, waste, growth/growth inhibitor hormone, modeled with diffusion equations. At last in molecular scale genetic networks modeled by using boolean networks. Data produced by simulation compared with mouse models of breast cancer (EMT6/Ro) and results are consistent with experimental data. Results supports hypothesis which suggests proliferation process is managed by a few number of proteins. These proteins act in G1 phase of the cell cycle. Simulation results suggested, the mainspring which stops cell growth is G1 regulatory proteins not physical factors arising from cell's diameter.

A parameter which is important as oxygen and nutrition, but ignored most of the time is,  $H^+$  concentration. This waste is result of cell activity and determines pH of cell microenvironment. Patel et. al. studied this phenomenon with a hybrid model based on cellular automata and partial differential equations [29]. The study is a pioneer to simulate effects of  $H^+$  ions on tumor growth. Aim of the study is measure effect of angiogenesis and pH on tumor's invasive character. According to simulation results are below; - Low pH is advantageous for tumor cells but disadvantageous for normal cells. - There is an optimal amount of vessel for tumors - If quantity of vessels are under this level both tumor cells and normal cells died because of low pH level. - If quantity of vessels are over this optimal threshold tumor cells lose their advantage over normal cells because of  $H^+$  drain. In addition to these results when  $H^+$  ions reaches to a specific threshold tumor cells switch from proliferative behavior to invasive character in a short time. But when  $H^+$  production stops, tumor growth became slower, and even stops. There is no experimental direct result presented in research but some indirect results are available in paper. Chemotaxis and adhesion is two other important factor in tumor growth phenomenon. Sander and Deisboeck examined this parameters on Glioblastoma Multiform (GBM) in their research [30]. Chemotaxis was grounded on for tumor's invasion dynamics. Homotypic adhesion assumed as main factor that triggers these dynamics. Researchers also validated their results with laboratory experiments. The volumetric growth model is Gompertzian and chemotaxis was modeled with Keller-Segel equation. Movement mechanism of cells uses snail-trail approach which basically means following of one cell by another. The hypothesis which assumes spreading of tumor with branching bases on chemotaxis and homotypic adhesion was validated by experimental results.

Another study which focuses on adhesion was conducted by Anderson [31]. Study also inspected effects of heterogeneity of tumor. Model based on 3 different cell types which are proliferative, migrative and destroyer (which destroy tissues). These phenotypes changes by epigenetic effects in model. Model bases on four parameters; tumor cells, host tissue (ECM), tissue degrading enzymes and oxygen content. When

movement modeled, effect of haptotaxis included but chemotaxis ignored. Mutations modeled in two different ways. First four different phenotypes modeled in a linear way (i.e first mutation1 occurs then mutation2 occurs etc.). Alternative method is choosing 100 different phenotypes randomly. One of the most interesting result was observed at this stage. Two different method gave almost same results for tumor growth. This phenomenon was explained with aggressive phenotypes' has becoming dominant in tumor, during evolution. In recent years many researches focuses on cancer stem cells to understand tumor growth dynamics. Sottoriva et. al. studied this subject with a hybrid virtual tumor model [32]. Most distinctive part of the study is, prediction type of cell after stem cell division. This approach also determines genetic variation of cells with simple probabilistic functions. The research is very important for literature because results are meticulously validated with in vivo experiments and observations. Model shows that any treatment that does not aims cancer stem cells will lead to an opposite result thus, tumor becomes more aggressive. There are lots of examples for tumor modeling in literature but only a few of them includes all dimensions of tumor growth process. Study conducted by Alarcon et. al. is one of these few examples [23]. Study includes vascular and avascular phases, angiogenesis, interactions between cells, adaptation of tumor to environment, cell cycle, VEGF production, important mutations at cellular and sub-cellular level. According to model normal cells' growth is consistent with Gompertz population dynamics but tumor cells' growth dynamics follows linear, exponential patterns and finally reaches saturation.

### **1.2.5 Modeling Tumor Movement**

Chemotaxis could be defined as movement reaction of cells to chemicals at their microenvironment. If movement is towards chemical named as positive chemotaxis, otherwise called as negative chemotaxis. The first model of chemotaxis developed by Patlak at 1953 [33]. Afterwards in 1970 Keller and Segel developed a similar model [34]. These models, pioneered chemotaxis modeling and have been transformed to Patlak-Keller-Segel model which known as a fundamental at this area. Lapidus and Schiller tried to predict chemotactic migration of a bacterial population with differential equations under certain boundary conditions at 1974 [35]. The diffusion theory of suspended particles was used to define model mathematically. Results are consistent with Segel-Jackson [36] and Nosell-Weiss [37] studies. In conclusion Keller-Segel's [38] and Dahlquist et. al.'s [39] models' results are discussed and compared with their results to validate their model. Spiro, Parkinson and Othmer researched cooperation between chemotactic and motor proteins in their model. The study based on both chemotaxis and cell signaling principles. During research substances that plays role at signaling for chemotaxis of E.Coli bacteria and pathways of these substances had modeled. Hillen, Painter and Schmeiser assumed that chemotaxis occurs in a certain field in their study [40]. The model claims that cells interacts with a certain field in their microenvironment. This study might be used to understand tissue invasion process for tumor cells.

### **1.2.6 Modeling Effects of Chemotherapies on Tumors**

Mathematical chemotherapy models provides us a chance to make assumptions about interactions between cells and chemotherapy outcomes, tumor prognosis or metastasis. As stated by Knolle [41] knowing dynamics of interactions between cells gives an advantage to us for predicting cells' responses to chemotherapy. Also Eisen denoted that, mathematic could help medicine to use it more efficiently [42]. At 1995 Panetta and Adam have put forth a model which intended to give chemotherapy according to specific cell cycle phase [43]. Model aims to find the most suitable dose and period for preventing tumor growth. Researchers have benefited from studies done by Agur et al. [44] and Cojocar-Agur [45]. The most convenient phase to give chemotherapeutic medicine indicated as S phase which is also weakest time interval of cell. Study enhances models of Birkhead [46], Webb [47] and Shiller [48]. Pulsed and continuous chemotherapies and their effects on healthy tissue added to model. Another method used in model was using growth factors to increase efficiency of chemotherapeutic agents that targets a certain cell cycle phase. Two different methods

used for modeling which are pulsed and piecewise. Piecewise model comes with more realistic results but from mathematical view of angle it is non-trivial to implement. On the other hand results of two methods are very close. Researchers advise pulsed method since it is easier to implement. For future work variability of parameters should be considered. For example chemotherapeutic agents effects on tumor decreases while effects on tissue increases during time. A year after Panetta developed a model basis on chemotherapy dose and period [49]. In study, tumor-normal cell interactions and therapy resistance were modeled more realistic than previous models. Especially therapy resistance elaborated since tumor become resistant to therapy during time. There are two types of therapy resistant groups, genetic and acquired. Model only handles acquired resistance. Growth factor is another important parameter handled by model. For example, before surgery normal cells can inhibit tumor by growth hormones which they secretes, but after surgery same hormones could stimulate tumor growth. Iliadis and Barbolosi defined pharmacokinetics of toxicity and anti-tumor efficiency of chemotherapeutics in 2000. Model also predicts plasma concentration, drug absorption and leukopenia parameters. Model optimizes drug dose for minimum tumor cell count under a constraint of white blood cell number threshold. Plasma and drug concentrations modeled separately. Study assumed tumor growth follows Gompertzian pattern. Model uses submodules as Pharmacokinetic Modeling, Pharmacodynamic-Efficacy Modeling, Pharmacodynamic-Toxicity Modeling, Maximum Drug Concentrations, and all effects can be observed independently. It is important that model's validation with clinical data for future studies. Jackson and Byrne compares their vascular tumor growth model with previous models in 2000 [50]. Heterogeneity ignored and predefined tumor growth rates used with logistic and Gompertzian equations. Tumor growth explained with spatially dependent. Transportation mechanisms of therapeutics were also inspected in study. Model parameters applied from experimental results which rats and doxorubicin used. Best response taken from tumors which have good vascularized peripheral and big avascular center. Povathill et. al. point out every cell should be handled independently at multiscale tumor and therapy models in their study [51]. Their study uses cellular automata to represent cells but uses PDEs to calculate intracellular dynamics. Researchers claims that cellular automata has higher prediction power but with no experimental supportive data. In study oxygen distribution inspected elaborately since hypoxic cells develops resistance to chemotherapy. According to us a remarkable feature of study is modeling of combined therapy which frequently used in clinic.

### 1.2.7 Modeling Effects of Immunotherapies on Tumors

Immunotherapies are very important for the future of cancer treatment. Virtual tumor modelers could not be indifferent to this phenomenon. Kuznetsov's (et. al.) study which defines nonlinear dynamics of immunogenic tumors is one of the first models in this area [52]. Kirschner and Panetta were also investigated this phenomenon and defined interactions between tumor cells, immune cells, IL-2 mathematically [53]. Their study explains both short and long terms oscillations of tumor growth. Another model from Kuznetsov and Knott also important since it was validated with experimental data [54]. In the study, a model which could predict tumor growth and tumor growth inhibition was presented. Actually aim of the study is understood "Tumor Dormancy" phenomenon. For many reasons tumors could grow up very slowly. During months or even years its existence may not be distinguished. This state of tumor is defined as "Tumor Dormancy" [55] [56]. Tumor dormancy can be caused by naturally or triggered after a therapy. There are two different ways for emergence of this phenomenon. First one is internal causes like inhibitor gene expressions, growth factors, receptors. Second way is, stabilizing of the competition between tumor and immune system. In both cases tumor seems stationary. This balance can be deteriorate for reasons like infections, stress, failures on immune system. The model can predict tumor regrowth after dormancy at following cases; - Impact of immune system decreases on tumor thus, balance can be broken in favor of tumor. - A mutation can occur in tumor that could not be effectively inhibited by immune system. Study uses experimental data to validate simulation results. Experiments done with rats and B Cell Lymphoma. Results of simulation are consistent with experimental data. Kiani et. al. developed a mathematical model with AVK method for optimal control of tumor cells with siRNA therapy [57]. Model also tries to predict effects of Interleukin-2 [58] therapy on

tumor growth. Results of simulation were evaluated as efficient and siRNA's inhibitive effect on TGF- $\beta$  observed as defined in literature. Study of Alberto d'Onofrio handles tumor-immune system interactions as a function of cancer cell count and investigate previous models [59]. Another model on interactions of TGF- $\beta$  was developed by Wilson and Levy [60]. In study interactions between tumor size, TGF- $\beta$  concentration, activated cytotoxic cells and T Cells was investigated. Numeric simulation and stability analysis were used to handle, natural tumor growth, anti-TGF- $\beta$  therapy, anti-TGF- $\beta$  and vaccine therapy cases. Study also validated with experimental results and results are consistent with experimental data.

### 1.2.8 Modeling Effects of Radiotherapies on Tumors

Radiotherapy response of cancers are investigated by many researchers thus lots of models developed for out-of-box personalized therapies. In this section these studies are tried to inspected.

Duechting et. al. develops models in 1992 and 1995 for response of in vitro tumor growth with control theory and cellular automata in 3D [61] [62]. In the study number of cells effected from radiation calculated with linear quadratic model, cell proliferation and cell-cell interactions defined. Total area of interest is 1 mm and a cubical mesh defined to locate cells. This method used by other studies to model internal cell dynamics. Guirado and Almodovar predicted therapeutic gain with a model to determine cells' sensitivity to radiation [63]. Model bases on damage analysis on DNA chain. Model can be calibrated for each patient to increase dose for radiation resistant regions. Monte Carlo simulation and analytical method was used to evaluate simulation results. Tumor control possibility increased on %10 of 40 patient in experiment. Researchers suggests more detailed radiosensitivity analysis are needed to develop personalized therapy protocols.

In 2004, Dionysiou et. al. developed a model for response of solid tumor growth to radiotherapy as 4D. Model algorithm developed based on fundamental biologic phenomenon. Model uses genetic variation's effect on radiosensitivity for "Glioblastoma Multiform". Model results were validated with patient data and consistency observed between results and data.

Zacharakia et. al. tried to optimize radiotherapy with in silico experiments [64] at 2004. Avascular growth of tumor and it's response to radiotherapy modeled by using Monte-Carlo simulation. Important parameters of model are oxygen and nutrient diffusion, survival rate of cells after radiotherapy, cell-cell interactions. 3D Visualization accomplished with AVS-Express software. Predicted histological structure for EMT6/Ro are consistent with published experimental data. Further more, morphology and radiation response of tumor seems satisfactory when compared with in-vitro experimental results.

In 2006 ,Nielsen et. al. accomplished a study which focuses on long term function loss of lung for Non-small cell lung cancer patients after radiotherapy. Researchers tried to predict function loss with forced expiatory volume values. In study, dose-function and dose-damaged volume relationships tried to explained. Since forced expiatory volume values are varying, it was emphasized that further studies needed. Dionysiou et. al. developed a model in 2006 to find effects of weekend radiotherapy and p53 gene for glioblastoma. During study, HART (Hyperfractionated Accelerated Radiotherapy) with 54 Gy and CHART (Continuous HART) with 54 Gy were compared. Model results showed that HART and CHART have approximately same long term effects on glioblastoma with same p53 state.

In 2007 Qi et. al. researched cell response to radiotherapy which also take p53 into account. Model also gives information about activation of ATM mutation. In study, to find optimal therapy plan, comparison of different simulations for different mutations (i.e. p53, MDM2) suggested. Kundrat's study at 2009 focuses on developing a probabilistic model of ionized radiation dose in cell inactivation. This model can predict survival curves which are also observed experimentally.

In 2010 Barazzuol et. al. researched response of brain tumor to combined radiotherapy and chemotherapy

(Temozolomide) treatment. Linear Quadratic Model used for radiotherapy response. Results were validated with clinical data supplied from European Organization for Research and Treatment of Cancer and The National Cancer Institute of Canada. Model shows addition of TMZ gives better results and a new therapy schema developed. Partridge et. al. study analyses and model literature of non-small cell lung carcinoma radiotherapy. Using 24 clinical experiment hyperfractionated and hypofractionated schemes compared. Phase II and phase III cancer cases analyzed. Best prognosis observed for hypofractionated schema.

### 1.2.9 Tumor Modeling Software

MASON is a fast discrete-event multiagent simulation library core in Java, designed to be the foundation for large custom-purpose Java simulations, and to provide more than enough functionality for many lightweight simulation needs. MASON contains both a model library and an optional suite of visualization tools in 2D and 3D [65].

CompuCell3D was originally written to model morphogenesis, the process in embryonic development where cells cluster into patterns which eventually differentiate into organs, muscle or bone. Through integration of multiple mathematical models into a software implementation with easy to use XML based syntax scientists were able to build models within few hours as opposed to weeks when writing source code from scratch. compuCell3D is based on Glazier-Garner-Hogeweg model (GGH) also known as the Cellular Potts Model (CPM). The model is capable of capturing key cellular behaviors: cell clustering as well as growth, division, death, intracellular adhesion, and volume and surface area constraints [66].

Dr Eye is a flexible and easy-to-use annotation platform (GUI) for quick and precise identification and delineation of tumors in medical images. The Platform design is clinically driven in order to ensure that the clinician can efficiently and intuitively annotate large number of 3D tomographic datasets. Both manual and well-known semi-automatic segmentation techniques are available in the platform allowing clinician to annotate multiple regions of interest at the same session. Additionally, it includes contour drawing, refinement and labeling tools that can effectively assist in the delineation of tumors. Furthermore, segmented tumor regions can be annotated, labeled, deleted, added and redefined. The platform has been tested over several MRI datasets to assess usability, extensibility and robustness with promising results [67].

The Multistage Weibull (MSW) time-to-tumor model and related documentations were developed principally (but not exclusively) for conducting time-to-tumor analyses to support risk assessments under the IRIS program. These programs and related documentation are made publicly available to ensure that the methods and calculations used for such analyses are transparent and reproducible [68].

Advancements in small-animal imaging technology over the past decade enable quantitative assessment of dynamic in vivo distribution of radiolabeled compounds as well as quantitative sub-organ analysis in preclinical studies. Drug distribution and targeting depend on a large number of factors including affinity, immunoreactive fraction, radiolabel, molecular weight, blood clearance, cellular internalization rate, antigen density, tumor vascularity, dose, and specific activity. inviCRO develops and employs biologically relevant mathematical models with physiologically meaningful parameters as tools for researchers in guiding the design, preclinical development, and translation of therapeutics and imaging biomarkers [69].

CellSys is a modular software tool for efficient off-lattice simulation of growth and organization processes in multicellular systems in two and three dimensions. It implements an agent-based model that approximates cells as isotropic, elastic and adhesive objects. Cell migration is modeled by an equation of motion for each cell. The software includes many modules specifically tailored to support the simulation and analysis of virtual tissues including real-time 3D visualization and VRML 2.0 support. All cell and environment parameters can be independently varied which facilitates species specific simulations and allows for detailed analyses of growth dynamics and links between cellular and multicellular phenotypes [70].



## CHAPTER 2

### METHOD

#### 2.1 Cellular Automata

##### 2.1.1 One Dimensional Cellular Automata

The brain behind the "Cellular Automata" idea is John Von Neuman. Actually he tried to make self replicating robots, but technology of 1950s does not allow such a futuristic idea. So, Stanislaw Ulam, (co-worker of Neuman from Los Alamos National Laboratory) came up with, idea of making self replicating robots by using abstract mathematic. The basic model was growth of cells on a lattice. Growth of cells was realized according to simple rules which are determined according to state of other cells.

Here are the fundamentals of cellular automata;

- Cellular Automata is based on Grids.
- Development of automata is presented with cells' states.
- New states are represented for each time step (i.e.  $t$ ,  $t+1$  etc.). thus system is discrete.
- State changes are based on rules determined according to state of neighbor cells.
- Size of the grid and cell number is finite

Chess is a good example to imagine cellular automata system. A chess board is a grid that consist of cells. Each cell has a state like, empty, queen, bishop, king... Of course rules aren't based on neighbor cells' states. A 1D cellular automata system could be defined with rules below;

- Check upper neighbors' state at each time step.
- Each cell should be empty or filled (as 0 or 1) .
- If all neighbors are empty then cell state is filled.
- If any of upper neighbors is filled then cell state will empty.

Figure below shows an example rule for a 1D cellular automata;

This rule structure is formulated for binary system by Stephen Wolfram [71] and could be shown as below;

This is called Rule1 as can be seen

$$2^8 = 256$$

and rules can be defined for 8 columns. For 90 iterations of Rule1, automata will be seen as below;

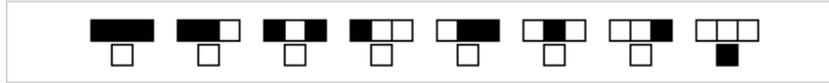


Figure 2.1: Example Rule

Table 2.1: Rule 1 of Wolfram

111	110	101	100	011	010	001	000
0	0	0	0	0	0	0	1

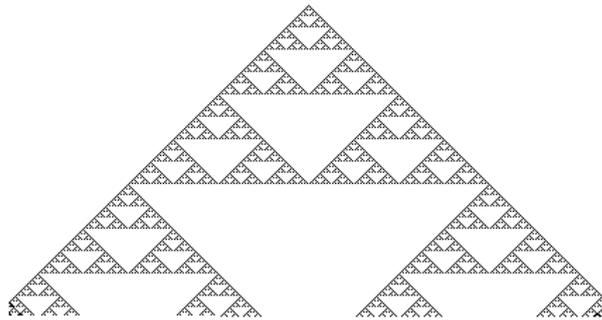


Figure 2.2: Rule1 on Cellular Automata after 90 Iterations

### 2.1.2 Two Dimensional Cellular Automata

For 2D cellular automata the most famous example is "Conway's Game of Life". The game is dependent upon a two-dimensional grid that each cell in one of two states, "living" or "dead".. The living squares at time  $t$  are controlled by cellular automata rules [72]:

1. If a live square has either two or three live neighbors, it will survive in the next generation; otherwise it will die.
2. If a dead square has exactly three live neighbors, there will be a "birth" in that square and it will be alive in the next generation.

Numerous diverse sorts of examples happen in the Game of Life, incorporating "Still Lifes", "Oscillators", and examples that deciper themselves in all cases ("spaceships"). Live units demonstrated in dark, and dead cells indicated in white.[73]

Still lifes		Oscillators		Spaceships	
Block		Blinker (period 2)		Glider	
Beehive		Toad (period 2)		Lightweight spaceship (LWSS)	
Loaf		Beacon (period 2)			
Boat		Pulsar (period 3)			

Figure 2.3: Game of Life Examples

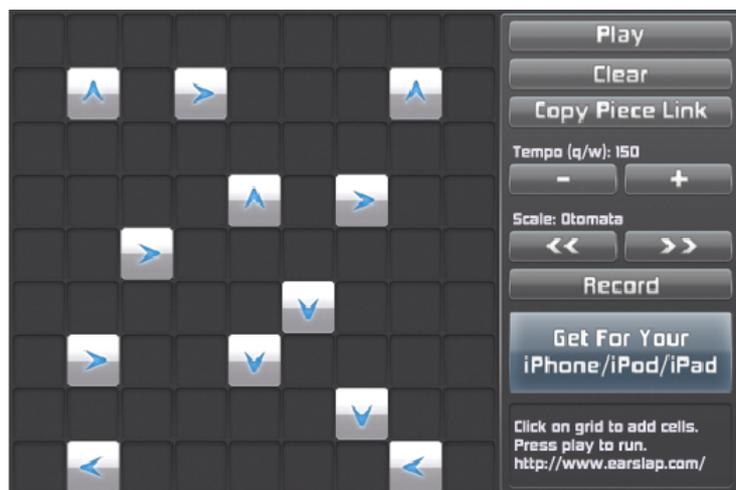


Figure 2.4: Otomata, cellular automata based musical instrument

Another important study is Greenberg-Hastings model which was developed to model diffusion equations. A phenomenon described by an universally stable balance state. This reaction is runs as a loop, and the framework soon comes back to its balance. [74]. Greenberg-Hastings model mostly used for stimuli-response type of problems like modeling behavior of heart muscle [75].

The world of cellular automata is unlimited. Literature includes many different application examples but accomplish a complete literature review is beyond this chapter. Thus, the last example of cellular automata be reviewed is on music. Batuhan Bozkurt a sound artist, computer programmer, performer created an application called Otomata [76].

Otomata is formed on 9x9 grid, which acts as a canvas that user could place cells on it. Each cell has 5 state (up, down, right, left, empty). Each cell moves in a direction according to its state. If any cell hits to wall, makes a sound, turns 180 degree and continues to move. If any cell hits to another cell, makes a sound, turns 90 degree at clockwise and continues to move. Each start condition creates a unique and endless melody until user stops this spectacular musical instrument. Figure shows the tool.

### 2.1.3 Using Cellular Automata for Tumor Modeling

Cellular Automata is one of the most preferred technique for tumor modeling. In this section two questions will seek to answer: "Why cellular automata preferred in this study for tumor modeling?" and "How can it be used for tumor modeling".

Let's start with the first question. Cellular automata is a potent technique which has lots of different application fields. In early days of tumor modeling, researchers were used PDEs, but cancer is a complex phenomenon and equations become harder to solve, at each time a parameter is added. When using cellular automata, for adding a biological parameter to model is trivial. Only a new property should be added to automata cell. Biologist also tries to find patterns and rules to explain tumor behavior. Each time a new pattern/rule discovered in biology, modeler just adds a new rule to automata without any effort. Also, as a discrete modeling technique, cellular automata can be easily parallelized.

Another advantage of cellular automata over continuous techniques is stated as its stability [77]. Expanding model with new parameters does not causes stability problems. Theoretical reviews can be found at literature, on this issue [78] [79].

Some well known problems of continuous techniques, makes use of cellular automata advantageous for tumor modeling. The first one is finding a solution for differential equations. A limited number of differential equations has analytical solution. Numerical methods like finite differences works well on with equations that has not closed-form solution, but has problems with irregular geometry. Irregular geometry is observed frequently in tumors [80].

As mentioned before second question that we tried to answer is: "How can cellular automata be used for tumor modeling ?" Most of the time cellular automata is used with continuous techniques. The logic behind this method is, solving easier parts of the problem with diffusion equations and modeling complexer modules with cellular automata.

Basics of tumor growth starts with consumption and production processes. Tumor needs oxygen and nutrition to grow, also produces waste. If tumor can't find enough oxygen it goes to hypoxia. If tumor can't find enough nutrition (glucosis), hypoglycemia starts. Producing waste ( $H^+$  ions) is another function of cell metabolism but also helps tumor to create an advantageous microenvironment for itself, because low Ph is favorable for most of tumors. Cell also decides how much oxygen and nutrition will he consume as a part of these process.

During his life, cell always has to get critical decisions. The first decision is about proliferation. Cell decides weather he proliferate or not based on genetic and microenvironment variables. Another critical decision is, triggering apoptosis. Cell also should manage his energy production. When cell detect oxygen level is below a specific threshold, he changes his energy metabolism from aerobic to anaerobic.

Migration is the key of tumor morphology. Tumor cells may decide to migrate if conditions are not suitable to live or proliferate. Deciding where to migrate is the next part of the migration decision process. Mechanisms like chemotaxis and haptotaxis is effective on this decision with genetic factors.

Tumor modeling is not only consists of cell decisions. Cell microenvironment has a deep impact on tumor growth. For example rules of physics are also valid for tumors as in the rest of the universe. Tumor could not grow if he can not degrade and infiltrate into extra cellular matrix (ECM) which is skeleton of soft tissues. Cells should separate from other cells to move. This means they should overcome adhesion.

Tumor cells also should compete with normal cells and should survive from immune system's attacks. These two mechanism also impacts tumor growth. Exterior forces are not limited to these. Actually therapies are the main exterior factors determines destiny of tumor. Cells goes to apoptosis or necrosis according to effects of chemotherapy and radiotherapy. Beyond all, DNA is the most influential factor in tumor growth

process. Cells' behavior is determined due to genetic inheritance and mutations.

After this brief overview, how we can transfer this knowledge to a tumor model, question should be answered. Distribution of nutrients and waste is suitable to model with partial differential equations as shown in figure.

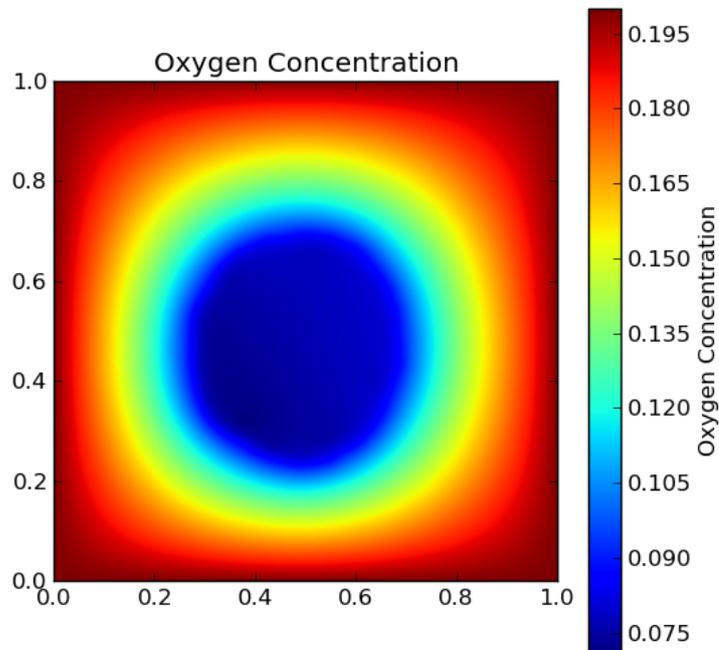


Figure 2.5: An example of oxygen distribution in cell microenvironment

When time comes for modeling decision making based on substance distribution, cellular automata comes to scene because its time to get complex decisions, based on many internal variables. Decision for consumption, proliferation, apoptosis are using cellular automata with stochastic decision making process. Migration mechanism, secretion of ECM degrading enzymes, control of immune system and normal cells could also be developed based on cellular automata.

At each time step submodules which uses continuous modeling calculates values for all grid cell coordinates. Each tumor cell scans his own grid and neighbor cells of mesh to read relevant parameter values. After obtaining all values each subsystem applies their rules with these values and makes decisions about cells destiny.

## 2.2 Solving PDEs for Modeling Tumor Microenvironment

Differential equations are an important part of applied mathematic and has lots of different application fields. Differential equations could be defined as derivative of one or more functions like

$$y = y(x)$$

but this is an very abstract definition. We can also define differential equations as functions that shows magnitude of change for a phenomenon.

Many scientific problems' definition includes changes of key variables according to other variables. If magnitude of change defined, many physical events could be formulated with mathematical equations. This means, results of physical events can be predicted on theoretical ground. Applications of differential equations are not limited with physics. Medicine, biology, statistics, chemistry, psychology are just a few samples from application fields of differential equations. Differential equations are obtained with application of rules and principals of events' minimal changes to a problem. Hence, to obtain a differential equation, problem should be defined in great detail, variables which effects problem should be determined and simplifications has to be made.

As a summary, equations which includes derivatives called as differential equations. Many phenomena like flow dynamics, electric current, heat transfer, seismic waves, population dynamics, could be defined, understood and predicted using differential equations. Differential equations express physical model and called as mathematical model. The aim of solving differential equation is learn about mathematical model that express physical model. The way of understanding a phenomenon is simplifying it. Hence, we first need to know about simplified versions to make sense of phenomena which are represented by models and equations [81].

Sometimes a differential equation includes conditions that should be satisfied by solution.

$$y'' = 3x^2 + x \quad ; \quad y(1) = 0$$

Equation above should be solved but when independent variable's value is 1 and value of y is 0. Problems that supplies a solution for equation but also satisfies conditions determined at the beginning are called as initial-value problems. Initial value problems and boundary value problems are important parts of differential equations.

A Boundary value problem is a system of differential equations with solution and derivative values specified at more than one point. Most commonly, the solution and derivatives are specified at just two points (the boundaries) defining a two-point boundary value problem [82].

### 2.2.1 PDEs

Differential equations are divided into two main types. Ordinary differential equations and Partial differential equations.

A partial differential equation (or briefly a PDE) is a mathematical equation that involves two or more independent variables, an unknown function (dependent on those variables), and partial derivatives of the unknown function with respect to the independent variables. The order of a partial differential equation is the order of the highest derivative involved. A solution (or a particular solution) to a partial differential equation is a function that solves the equation or, in other words, turns it into an identity when substituted into the equation. A solution is called general if it contains all particular solutions of the equation concerned.

The term exact solution is often used for second- and higher-order nonlinear PDEs to denote a particular solution.

Partial differential equations are used to mathematically formulate, and thus aid the solution of, physical and other problems involving functions of several variables, such as the propagation of heat or sound, fluid flow, elasticity, electrostatics, electrodynamics, etc.

The preceding discussion pertains to the exact or analytical solution of PDEs. For example, in the case of a heat equation or a wave equation, an exact solution would be a function  $w=f(x,t)$  which, when substituted

into the respective equation would satisfy it identically along with all of the associated initial and boundary conditions.

Although analytical solutions are exact, they also may not be available, simply because we do not know how to derive such solutions. This could be because the PDE system has too many PDEs, or they are too complicated. In this case, we may have to resort to an approximate solution. That is, we seek an analytical or numerical approximation to the exact solution.

Perturbation methods are an important subset of approximate analytical methods. They may be applied if the problem involves small (or large) parameters, which are used for constructing solutions in the form of asymptotic expansions. Unlike exact and approximate analytical methods, methods to compute numerical PDE solutions are in principle not limited by the number or complexity of the PDEs. This generality combined with the availability of high performance computers makes the calculation of numerical solutions feasible for a broad spectrum of PDEs (such as the Navier–Stokes equations) that are beyond analysis by analytical methods [83].

### 2.2.2 Diffusion Equations

Diffusion is spread of molecules without external forces. Diffusion moves from gradient which means that molecules moves from high concentration region to low concentration region. Diffusion rate is a function of concentration gradient. How greater the concentration difference is, molecules spreads (diffuse) faster from high to low concentration. Forces like electrical and magnetic fields may effect movement of molecules [84]. Diffusion rate is also influenced by heat. When heat increases molecules moves faster.

Partial differential equation

$$\frac{\partial c}{\partial t} = D \frac{\partial^2 y}{\partial x^2}$$

is convenient to represent ordinary diffusion. In this equation  $c$  represents concentration of diffusive substance,  $t$  is time,  $x$  is coordinate vector and  $D$  is diffusion coefficient.

Since diffusion is an stochastic process where a single particle can move in each direction with the same probability, diffusion coefficient could be defined as, the rate at which a diffusing substance is transported between opposite faces of a unit cube of a system when there is unit concentration difference between them [85]. Another description of the diffusion coefficient is the equation:

$$D = \frac{x^2}{2t}$$

where  $t$  is the time and  $x^2$  is the mean squared displacement of the particles at this time.

### 2.2.3 Solving PDEs with Computational Methods

In ordinary differential equations (ODE) , solution functions which satisfies ODE or ODE system, forms general solution family. When constants assigned to general solution which also forms a solution to problem, this family of solutions named as particular solution. For PDEs solutions are not trivial as in ODEs. Finding a general solution is almost same as in ODEs. Since a set of initial/boundary conditions which is valid for any PDEs is not known, finding a particular solution (if available) to PDEs is a non-trivial job. The

known theory could only explain these conditions for a limited set of PDEs. Hence we can not talk about a general solution method for PDEs. An alternative to solve PDEs is using numerical methods.

Second degree PDEs are commonly encountered when a model for a physical phenomenon modeled but analytical solutions for PDEs are still unsatisfying. Trying to solve PDEs with numerical methods be a good alternative. It should be noted that, neither analytical nor numerical solutions are not precise.

PDEs includes multi-variable functions. Even if we already know exact solution of a problem, whether analytical or numerical, creating tables from numeric data is a difficult task. Instead, using computers for this task offers more convenient solution to us. Nowadays, based on these two property of PDEs, numerical analysis to solve PDEs are using more frequently.

Naturally solution field of PDEs is a 3D space. To transform PDEs from an abstract mathematical expression to a physical model solution field and boundary conditions (behavior of system at boundaries) should be defined.

Most common numerical methods using to solve PDEs are, Finite Difference Method, Method of Lines, Finite Element Method, Finite Volume Method, Spectral Method, Meshfree Methods, Domain Decomposition Methods, Multigrid Methods. From these methods, Finite Difference Method, Method of Lines, Finite Element Method, Finite Volume Method are most popular ones. When Finite Element Method is mostly used for structural models, Finite Differences Method is mostly used for fluid flows problems. If problem includes conservation of energy Finite Volume Method would be a good choice [86]. Detailed explanation could be found in literature. [87].

# CHAPTER 3

## INTERNALS OF TUMOR MODEL

### 3.1 Big Picture

In this study a basic tumor model was developed. The aim of study is creating a pathway to make a tumor model from scratch for new researchers who are eager to start tumor modeling. Before begin to explain details of the model, it should be said that model of Gerlee and Anderson [88] was very helpful to create a base for this model. If an ideal tumor model is described, figure will show components should have and methods may use in this context.

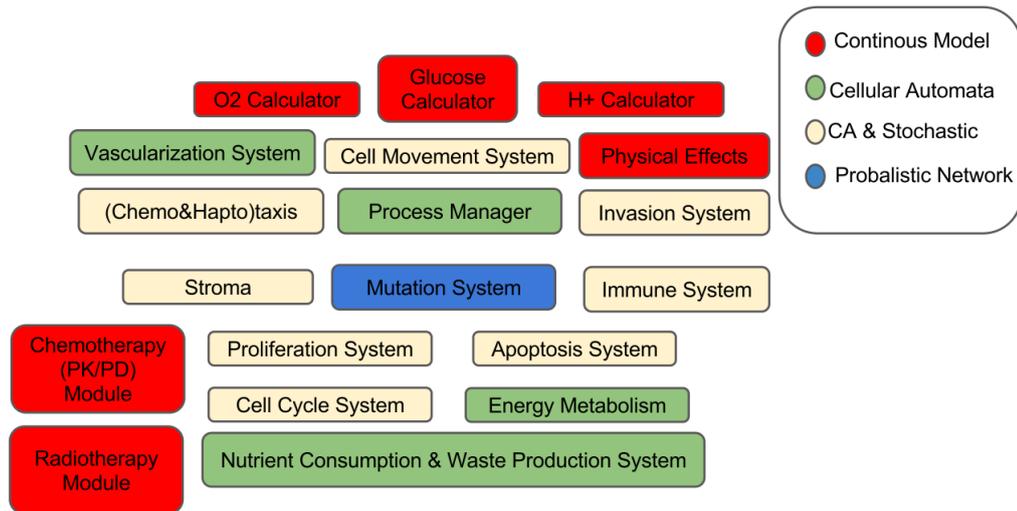


Figure 3.1: Example for a General Tumor Model

As mentioned before, only essentials of a tumor model is covered in this study. Model is specified for adenocarcinoma of non-small cell lung cancer. Most of the parameters taken from in vitro and in vivo experiments found in literature. Implemented modules are shown in the figure.

In this chapter each module will be discussed in detail. Since details of technology used for simulation, is a technical issue (not scientific), is excluded from chapter. Briefly, Python [89] is used as main platform for implementation of model. FiPy [90] is used to solve PDEs and Cython [91] was very helpful to increase efficiency of simulation. Finally PyGame [92] is used for creating graphic user interface.

Before simulation starts a grid with 400x400 cell established. This is the main structure which cellular automata works on. Then "Process Manager" (PM) module created. PM initializes and manages all other modules also refreshes graphic interface when needed. Initialization of tumor cells are performed by PM.

Tumors needs to reach a critical threshold to continue their existence. Consequently, simulation starts with

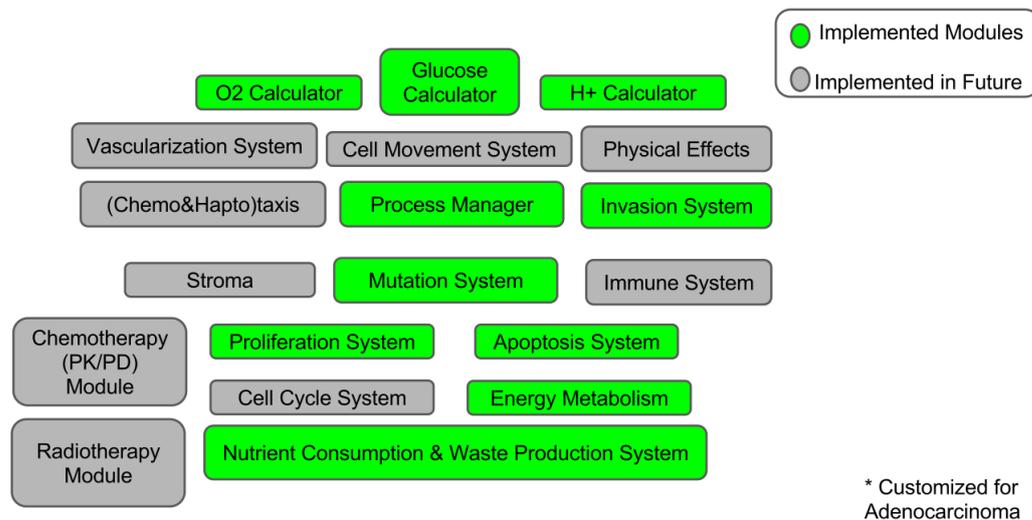


Figure 3.2: Implemented Modules in Study

an initial tumor which has 20 cell radius. Actually, for each grid cell in this radius from center has %60 chance to be a tumor cell. Creation of initial tumor is responsibility of PM.

Proliferation system, apoptosis system, energy metabolism and mutation system are part of tumor cell and managed by cell, but spread and diffusion of substances like oxygen, glicosis are independent from cell. These concentrations are calculated by independent modules and information of concentration at each grid cell stored in matrices. Creation of these matrices are also done by PM.

Cellular automata is an discrete modeling technique which means at each time step, time stops, calculations are performed, values are stored and user interface is refreshed. In this study each time step assumed as 22 hours which is doubling time of adenocarcinoma (A549) [93].

Concentration of oxygen, glicosis and H<sup>+</sup> ions are calculated at the beginning of each time step for each grid cell coordinate and stored to use them when needed in this time step. After, all grid cells scanned and following steps are performed:

- Oxygen consumption calculated according to cell metabolism (which can be aerobic or anaerobic), cell's state (proliferative or quiescence) and oxygen consumption rate ( oxygen uptake rate constant as mol/(cell\*second) )
- Glucose consumption calculated with cell metabolism, cell's state and glucose consumption rate.
- H<sup>+</sup> ion production is result of cell metabolism and calculated based on cell metabolism, cell's state and H<sup>+</sup> production rate.

Finally cell will decide his next state also with the help of these calculations. A simplified flow chart 3.3 for the model is presented below:

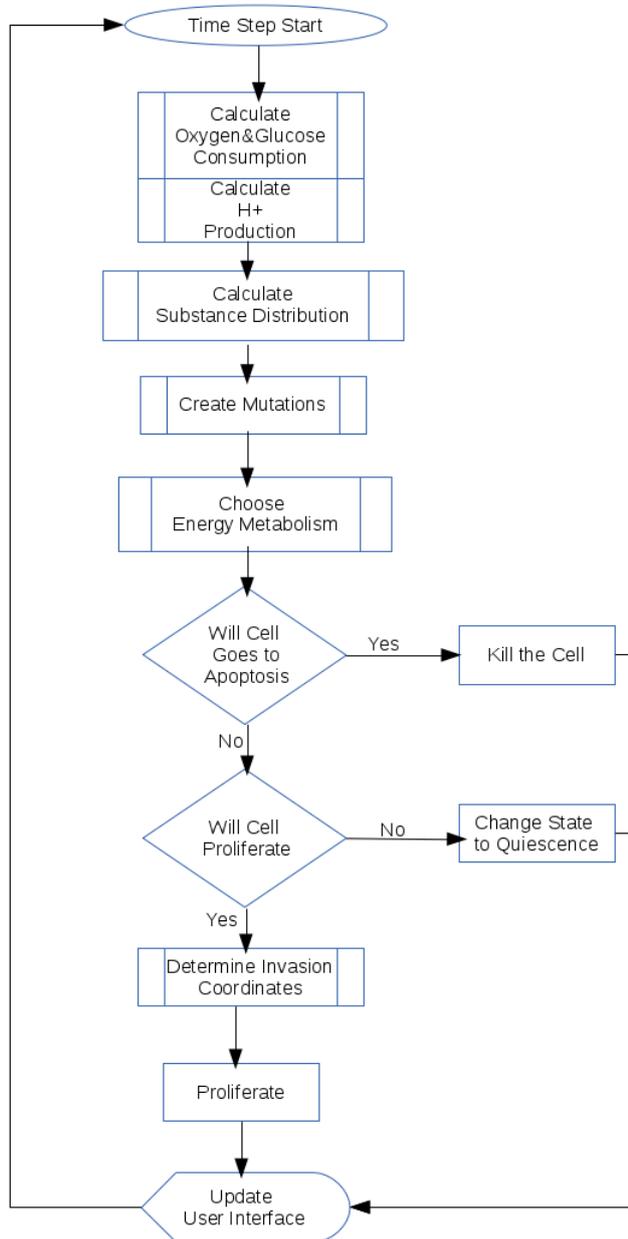


Figure 3.3: Overview of Flow

### 3.1.1 What is New

Every year more researchers are studying on tumor modeling. There are lots of different approaches and models available in literature. According to our best knowledge, our model is the first one that is specific to adenocarcinoma of non-small cell lung carcinoma. Tumor microenvironment parameters are mostly specific to A549, a widely known adenocarcinoma cell line. Also bayesian genetic network of tumor model is created from data belongs to A549. Finally probability functions introduced in following sections are developed by us based on literature. Details of calculations and decision process will be discussed but an important part of tumor modeling, "Non-dimensionalization" will be explained at first.

### 3.2 Parameters of Model

Cancer is a complex phenomenon, thus, many parameters is needed to determine when a tumor model is developed (even a simple one as in this study). These parameters which will be non-dimensionalize, is gathered from various sources from literature. Most of the parameters belongs to adenocarcinoma of non-small cell lung cancer, others are general parameters for tumors or tumor microenvironments. A549 cell line of adenocarcinoma is selected to gather data, since it is very common tumor example which is used in experiments. Table shows parameters, symbols, values with references.

Table 3.1: Parameters Used In Model

Parameter	Symbol	Value	Specific to A549
Tumor Cell Doubling Time	$\delta_t$	22 h [93]	Yes
Oxygen Background Concentration	$c_o$	$4.375 \times 10^{-1} mM$ (calculated from [94])	Yes
Oxygen Diffusion Constant	$d_o$	$8.0 \times 10^{-9} m^2 sec^{-1}$ [95]	No
Oxygen Uptake Rate	$u_o$	$0.91 \times 10^{-15} mol \ min^{-1}$ [94]	Yes
Hypoxia Induced Apoptosis Threshold	$ho_a$	$0.015 \times c_o$ [96]	Yes
Hypoxia Induced Glycolysis Threshold (Upper Limit)	$ho_{gu}$	$0.05 \times c_o$ [96]	Yes
Hypoxia Induced Glycolysis Threshold (Lower Limit)	$ho_{gl}$	$0.01 \times c_o$ [96]	Yes
Hypoxia Induced Apoptosis Rate	$ho_{ar}$	%55 [96]	Yes
Oxygen Threshold Proliferative to Quiescence	$o_{pq}$	1 mmHg [97]	No
Glucose Background Concentration	$c_g$	17 mM [95]	No
Glucose Diffusion Constant	$d_g$	$1.350.13 \times 10^{-5} cm^2 sec^{-1}$ [98]	Yes
Glucose Aerobic Uptake Rate	$u_{ga}$	$4.1 \times 10^{-17} molcell^{-1} sec^{-1}$ [99]	Yes
Glucose Anaerobic Uptake Rate	$u_{gan}$	$6 \times 4.1 \times 10^{-17} molcell^{-1} sec^{-1}$ ( Calculated from [99] )	Yes
Hypoglycemia Induced Apoptosis Threshold	$hg_a$	8 mM [100]	No
Glucose Threshold Proliferative To Quiescence	$g_{pq}$	12 mM [100]	No
Hydrogen Ion Diffusion Constant	$d_h$	$1.4 \times 10^{-6} cm^2 sec^{-1}$ [101]	No
Hydrogen Ion Production Rate	$p_h$	$1.5 \times 10^{-18} molcell^{-1} sec^{-1}$ [88]	No
Background pH level	$c_{ph}$	7.35 [102]	Measured in Lung Tissue
H+ Background Concentration	$c_p$	$1.11 \times 10^{-13} molcm^2$ (Calculated from [102])	Measured in Lung Tissue
pH Induced Apoptosis Threshold	$ph_a$	5.5 [103]	No
Optimal pH for Tumor	$ph_o$	6.8 [104]	No

Gathering all these different parameters and using them in a single model was a non-trivial job. So, some details on parameter calculations and conversation are discussed in this section to lead the way for reader.

The first parameter is "Oxygen Background Concentration" which is calculated from Molter et. al. [94]. Total oxygen in background is 7 ppm in study. 7 ppm means 7 mg/liter. Since 1 mol oxygen atom is 16 g (monoatomic weight) there is 0.4375 mol oxygen in 1 liter which equals  $4.375 \times 10^{-1} mM$  .

"Glucose Aerobic Uptake Rate" is calculated from Shankland et. al. [99]. We assume that glucose uptake should be 16 times more in glicosis. Because, aerobic metabolism produces 32 ATP when anaerobic metabolism only produce 2 ATP. But, in simulation 16 times more consumption collapses the system at early phases of tumor growth. Thus, decreasing coefficient step by step 6 times more consumption gives

optimal results for simulation.

"H+ Background Concentration" is converted from pH. As a beginning,  $\text{pH} = -\log[\text{H}^+]$  and unit of H+ is M which means mol/L and our total volume is  $1 \times 1 \times 0.0025 \text{ cm}^3$  and 1 liter is 1000 cc we can convert background pH as  $\frac{10^{-7.35} \times 0.0025}{1000}$ .

Finally some conversions between units are needed. Many resources uses miliMolar (mM) others uses  $\text{mol}/\text{cm}^2$  in literature. Since our volume is  $0.0025 \text{ cm}^3$ , 1 mol is 1000 milimol and 1 liter is  $1000 \text{ cm}^3$ , to convert miliMolar to  $\text{mol}/\text{cm}^2$ , value of parameter multiplied by a coefficient for miliMolar to  $\text{mol}/\text{cm}^2$  which is represented as  $c f_M$ :

$$c f_M = 0.0025 \times 0.0001 \times 0.001 \quad (3.1)$$

Also conversion from mmHg to  $\text{mol}/\text{cm}^2$  is frequently used for oxygen concentration. To convert mmHg to miliMolar we use  $a = 1.39 \times 10^{-3}$  as solubility coefficient of oxygen in plasma and find coefficient for  $pO_2$  mmHg to  $\text{mol}/\text{cm}^2$  which is represented as  $c f_{\text{mmHg}}$ :

$$c f_{\text{mmHg}} = 1.39 \times 10^{-3} \times 0.0025 \times 0.0001 \times 0.001 \quad (3.2)$$

### 3.3 Non-dimensionalization

Dimension is measure of physical size like length, mass, time. There is seven main dimensions, mass (kg), length (m), time (sec), heat (K), electrical current (A), light amount (cd) and substance amount (mol). All other dimensions could be produced from these dimensions like: Speed = length/time. In an equation dimensions should be homogeneous, which means units should be same in an equation. Instead of converting lots of units to each other non-dimensionalization is an efficient way of forming equations and making calculations in modeling. Also normalizing different values in same scale is a must in calculations non-dimensionalization is used for this propose. Non-dimensionalization can be done with dividing each term of an homogeneous equation to parameters which have same units [105].

Diffusion constants and consumption/production rates were non-dimensionalized with following equations:

$$d^* = \frac{3600 \times \delta_t \times d}{a} \quad (3.3)$$

$$r^* = \frac{3600 \times \delta_t \times r \times n}{c_b} \quad (3.4)$$

These are generalized form of non-dimensionalization equations. In equation (3.3),  $d^*$  is non-dimensional diffusion constant. Tumor cell doubling time ( $\delta_t$ ) multiplied by 3600 to convert hour to second. Original diffusion constant represented with  $d$ . Area ( $a$ ) is total area (as  $\text{cm}^2$ ) of the grid which tumor model grows.

When non-dimensionalizing consumption/production rates with equation (3.4),  $r^*$  represents non-dimensional consumption/production rate. Original rate is represented by  $r$ . Maximum number of tumor cells (which equals to number of grid's cells) is represented by  $n$ . Finally background concentrations are represented by  $c_b$ .

Using these equations (3.3) - (3.4),  $u_o, u_{ga}, u_{gan}, p_h, d_o, d_g, d_h$  were non-dimensionalized successfully.

### 3.4 Diffusion of Substances in Tumor Microenvironment

When a diffusion equation is created, actually a partial differential equation was formed. General form of diffusion could be written as [106]:

$$\frac{\partial m(\vec{c}, t)}{\partial t} = D \nabla^2 m(\vec{c}, t) \quad (3.5)$$

The left part of the equation (3.5) represents change in concentration of substance at coordinates  $\vec{c}$  according to time.  $m(\vec{c}, t)$  means concentration of substance at coordinates  $\vec{c}$  at time  $t$ .  $D$  is diffusion constant of substance. Since our model is 2D,

$$\vec{c} = (x, y) \quad (3.6)$$

The tricky part of the equation (3.5) is  $\nabla^2$  which is a Laplace operator. Laplace operator means divergence the gradient of the function. Divergence stands for magnitude of a flow at a specified coordinate. Gradient is derivative of an multi-variable function which says us change according to variables. In our example it stands for amount of change for a substance at specified coordinate. Thus, laplacian gives us amount of change in magnitude for a flow in a vector field. In other words we could know concentration of substance at specified coordinates at time  $t$ . To solve equation it could also be written as below:

$$\frac{\partial m(\vec{c}, t)}{\partial t} = D \left( \frac{\partial^2 m}{\partial x^2} + \frac{\partial^2 m}{\partial y^2} \right) \quad (3.7)$$

A detailed explanation for this equation could be found in literature [106]. Physical representation of diffusion phenomenon which is respresented as equation (3.7) could be seen in figure:

After the basic equation of diffusion is formed, only a source term is needed which represents consumption or production of the substance for a coordinate at a specific time [106]:

$$s(\vec{c}, t) = s(x, y, t)$$

Oxygen and glucose which are consumed by cells are subtracted from equation (3.7):

$$\frac{\partial m(\vec{c}, t)}{\partial t} = D \left( \frac{\partial^2 m}{\partial x^2} + \frac{\partial^2 m}{\partial y^2} \right) - s(\vec{c}, t) \quad (3.8)$$

and source term for hydrogen ions (H<sup>+</sup>) which are produced at the end of glycolysis is added:

$$\frac{\partial m(\vec{c}, t)}{\partial t} = D \left( \frac{\partial^2 m}{\partial x^2} + \frac{\partial^2 m}{\partial y^2} \right) + s(\vec{c}, t) \quad (3.9)$$

Finally, initial and boundary conditions for equations (3.8) - (3.9) will be determined [107]. We assume that boundaries of grid with  $L \times L$  size, consists of veins and concentration of substances equals to background concentration values. Initial conditions could be defined as

$$\begin{aligned} m(x, y, 0) &= f(x, y), \quad \forall (x, y) \in R, \\ R &= [0, L] \times [0, L] \end{aligned} \quad (3.10)$$

with Dirichlet boundary conditions

$$\begin{aligned} m(x, 0, t) &= m(x, L, t) = 1, \quad 0 \leq x \leq L, \quad \forall t \geq 0 \\ m(0, y, t) &= m(L, y, t) = 1, \quad 0 \leq y \leq L, \quad \forall t \geq 0 \end{aligned} \quad (3.11)$$

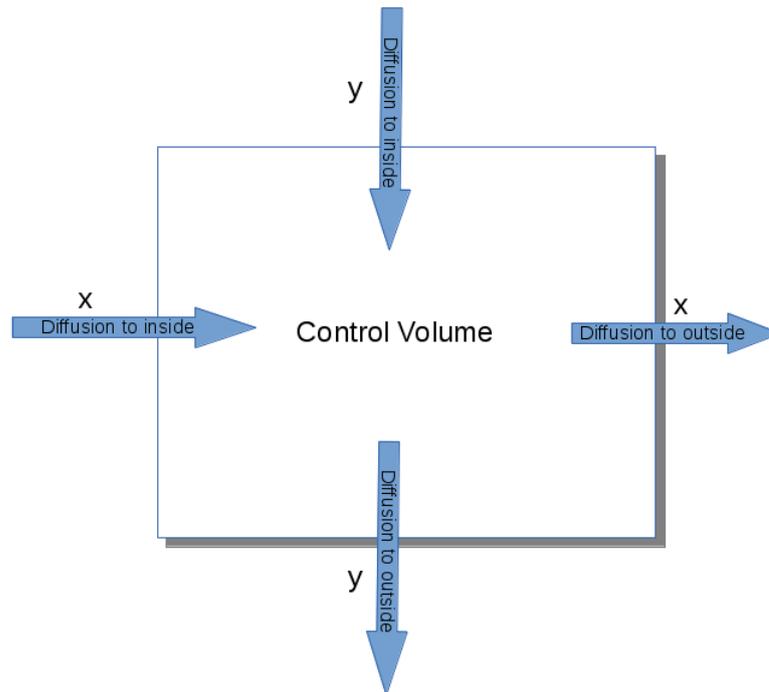


Figure 3.4: Diffusion as a Physical Phenomenon

After conditions of tumor's microenvironment determined, internal dynamics of tumor cells' could be explored.

### 3.5 Intracellular Model

The key question in tumor modeling is "How will tumor cell behave in the future ?" In this section this question is discussed. In the model at each time step, each cell should decide what he does until next time step. Cell should decide on many subjects, like will he die or not, how he will produce energy, how much substance he consume etc.

When decision making process begins, genetic subsystem is triggered to decide if any mutations will occurs. This should be completed before other decisions are taken, since nearly all other subsystems are effected by genetic variations.

After genetic changes occurred, cell starts to explore its environment to find is there any other cells and detect amount of vital substances to use them in decision making process. Then cell decides, how he will produce energy, by using aerobic or anaerobic energy metabolisms. Finally cell decides for his next state as apoptosis, quiescence or proliferative. Decision process is based on probabilisty functions formed by us. Constants used in functions are our assumptions since there is no data found in literature. Details of how this decisions produced in subsystems were explained in subsections.

### 3.5.1 Proliferation

Each cell's proliferation subsystem decides whether will cell proliferate or not. Both quiescence and proliferate cells could be tested for proliferation. If cell's state is quiescence cell's age and free space around cell is tested. If all these parameters are satisfied then cell's state is changed to proliferative.

Even cell's state is proliferative, the cell is still a candidate for proliferation and there are still dices that should be played. It is assumed that the maximum proliferation rate, will take place at optimal conditions. When cell's microenvironment conditions become closer to optimal conditions proliferation probability increases otherwise probability decreases. Parameters used for proliferation decision are oxygen concentration, glucose concentration and pH. Genetic effects are also used in probability function.

To clarify see the following function which is used for deciding proliferation.  $P_r$  represents, weighted proliferation probability:

$$P_r(P_g, P_h, P_d) = \begin{cases} 0.2 \times p_o + 0.5 \times p_g + 0.3 \times p_h + p_d, & \text{if Cell's Metabolism is Aerobic} \\ 0.7 \times p_g + 0.3 \times p_h + p_d, & \text{if Cell's Metabolism is Anaerobic} \end{cases} \quad (3.12)$$

In equation (3.12),  $P_o$  is probability coefficient for oxygen.  $P_o$  is based on oxygen concentration at coordinates of cell and represented with  $m_o$ . Calculation of  $P_o$  is shown in equation below,

$$P_o = \frac{m_o - ho_a}{c_o - ho_a} \quad (3.13)$$

In equation (3.13),  $ho_a$  is hypoxia induced apoptosis threshold which is minimum oxygen concentration for proliferation and  $c_o$  is background oxygen concentration which is maximum oxygen concentration for cell's microenvironment as mentioned in table 3.1. Probability coefficient for glucose  $P_g$ , also calculated in a similar way by using  $m_g$  which represents glucose concentration at coordinates of cell.

$$P_g = \frac{m_g - hg_a}{c_g - hg_a} \quad (3.14)$$

Calculation of probability coefficient for pH which is represented by  $P_h$  is a little complexer than  $P_o$  and  $P_g$ , because for oxygen and glucose higher concentration means higher probability for proliferation, but for pH cell needs optimal pH level to have highest proliferation chance. Thus,  $P_h$  is calculated as a piecewise function:

$$P_h = \begin{cases} \frac{m_{ph} - min_{ph}}{ph_o - min_{ph}}, & \text{if } m_{ph} < ph_o \\ \frac{|m_g - max_{ph}|}{|ph_o - max_{ph}|}, & \text{if } m_{ph} > ph_o \\ 1, & \text{if } m_g = ph_o \end{cases} \quad (3.15)$$

In equation (3.15)  $min_{ph}$ ,  $max_{ph}$  are minimum and maximum values of pH that a tumor cell can live,  $ph_o$  is optimal pH level for proliferation as mentioned in table 3.1 and  $m_{ph}$  is pH level at coordinates of cell.

Finally,  $P_d$  represents effects of cell's DNA on proliferation probability and will be explained in subsection 3.5.6.

### 3.5.2 Invasion

After a cell decides for proliferation, next question is about finding most convenient place around cell. Invasion system answers this question based on microenvironment conditions. Invasion system uses oxygen, glucose and H<sup>+</sup> concentration as input parameters. When scanning neighbor cells with traditional methods

for invasion, a strange effect is occurred as mentioned by Gerlee and Anderson [88]. Tumor grows like a tree with branches. Although we couldn't explain this effect, solution is scanning cells orthogonal and diagonal at different time steps. For example choose orthogonal cells in even time steps and diagonal ones in odd time steps.

Finding optimal cells with oxygen and glucose is easy, maximum concentration is better. For H+ concentration cell should look for optimal pH level or nearest level to optimal which is already defined as  $ph_o$  in table 3.1. Invasion convenience score  $s_{inv}$  could be calculated as:

$$s_{inv} = \begin{cases} 0.2 \times m_o + 0.5 \times m_g + 0.3 \times m_h, & \text{if Cell's Metabolism is Aerobic} \\ 0.7 \times m_g + 0.3 \times m_h, & \text{if Cell's Metabolism is Anaerobic} \end{cases} \quad (3.16)$$

Choosing cell with highest score does not always give most realistic result in simulation because tumor grows to a single direction continuously in this case. To overcome this problem an error margin ( $e_{inv}$ ) should be determined which simulates insensitivity of cells. When maximum  $s_{inv}$  is determined each cell's  $s_{inv}$  is tested against maximum  $s_{inv}$ . If difference between them is not more than  $e_{inv}$  then this cell is a candidate for invasion. After all candidates are determined, one candidate is selected randomly from candidate list and a new tumor cell is born at coordinates of chosen cell.

### 3.5.3 Apoptosis

Beyond natural apoptosis, three cases are considered for apoptosis in model. Hypoxia, hypoglycemia and extremely low pH level could cause apoptosis.

When oxygen level decreases below %1.5 hypoxia started until oxygen runs out (%0). Researches show that %55 of NSCLC Adenocarcinoma (A549) cells were died when oxygen level reaches to %0. Also it is known that natural apoptosis rate is %10 for A549 cells [96].

Since  $\frac{55-10}{1.5-0} = 3$  for each %0.1, change at oxygen level, apoptosis survival probability will increase %3. For example if oxygen level is %1, probability of apoptosis could be calculated as,  $((1.5 - 1)/0.1) * 3 + 10 = 25$ . Hence, hypoxia induced apoptosis probability ( $P_{ha}$ ) for this cell will be %25. If this method is generalized:

$$P_{ha}(M_o, P_{da}) = \frac{\frac{(1.5-m_{po})}{0.1} \times 3 + 10}{100} - P_{da} \quad (3.17)$$

In equation (3.17), oxygen concentration at coordinates of cell as percentage represented with  $m_{po}$  and probability of genetic effects decreases apoptosis chance is shown as  $p_{da}$ .

Cells can live without oxygen but not without glucose, so cell will definitely die when glucose runs out. In other words, cells die when glucose level reaches to %0. By using this knowledge hypoglycemia induced apoptosis probability ( $P_{ha}$ ) will be calculated when  $m_g < hg_a$  as below:

$$P_{ha}(M_g, P_{da}) = \frac{m_g}{hg_a} - P_{da} \quad (3.18)$$

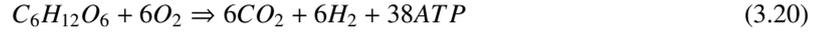
The last factor which causes apoptosis is pH level. Acidic microenvironment is favorable for tumor because cancerous cells are more resistant to acidic environment than parenchyma. But, when pH decreases to lower levels all kind of cells start to die. When this effect is modeled, pH at coordinates of cell represented with  $m_h$  and  $min_{ph}$  stands for minimum pH level of cell microenvironment. Thus, we can calculate  $P_{ha}$  (apoptosis probability based on pH) as:

$$P_{ha}(M_h, P_{da}) = \frac{m_h - min_{ph}}{ph_a - min_{ph}} - P_{da} \quad (3.19)$$

Apoptosis probability calculated using oxygen, glucose and pH but even cell decides to start apoptosis, it will wait for a delay because in tumor cell biology events are occurred as after a while cell waive to his apoptosis decision. This delay also protect cell from instant oscillations of microenvironment signals. There are more complicated methods could be found in literature to model delayed systems [108].

### 3.5.4 Energy Metabolism

Tumor cells could produce energy by using two different metabolisms, aerobic and anaerobic. In aerobic metabolism oxygen and glucose are used to produce energy as:



In anaerobic metabolism only glucose is used to produce energy as can be seen in the simplified reaction below:



In the model, shift in energy metabolism only based on oxygen concentration, genetic effects are ignored implicfor the sake of simplicity. Metabolism shift starts at %5 oxygen concentration with %20 possibility and reaches %100 possibility at %1 oxygen concentration [96]. If concentration is over %5 then cell chooses aerobic metabolism. Oxygen concentration at coordinates of cell as percentage ( $m_{po}$ ) is obtained by:

$$m_{po} = m_o/C_0 \quad (3.22)$$

and probability of cell's metabolism change from aerobic to anaerobic is calculated by:

$$P_e = \begin{cases} \frac{m_{po}^{-1}}{100}, & \text{if } 0.01 \leq m_{po} \leq 0.05 \\ 1, & \text{if } m_{po} < 0.01 \end{cases} \quad (3.23)$$

For example if concentration is 0.05 then probability of metabolism change will  $\frac{(5/100)^{-1}}{100}$  which is %20 and if concentration is 0.01 then probability will %100. For concentrations below 0.01 probability will also %100. There is also a delay between decision and metabolism shift. A cell should detect conditions which are needed to shift metabolism from aerobic to anaerobic seven times successively for making a change in his metabolism. Using this way noise and oscillations could be eliminated.

### 3.5.5 Oxygen - Glucose Consumption and Acid Production

Tumor cells have oxygen consumption rate, represented by  $u_o$  as seen at table 3.1 but this rate could be changed based on conditions. For example cell will not use oxygen in anaerobic metabolism. So, the consumption will be 0 in this condition. Also cell's state will effect oxygen consumption. We assume that cell will consume %50 more oxygen in proliferative state than quiescence state.

Glucose consumption ( $u_g$ ) is also calculated in a similar way. Glucose consumption in aerobic and anaerobic rates are calculated based on literature and observations of our model's simulation results. The assumption of proliferative cells' %50 more consumption, is also used to calculate glucose.

Acid production with hydrogen ions is observed in anaerobic metabolism when glycolysis occurred. Acid production rate ( $p_h$ ) is determined by using literature [88]. At proliferative state of cell production increases %50, since glucose consumption increases with same ratio.

### 3.5.6 Genetic Effects on Tumor

Genes are the key of life. They are also the key of cancer therapies. In the near future, personalized therapies will basis on genetic variations of people. Genetic data is also huge. Every day a new part of this mystery is solved but there is still a long way to go. When modeling cancer, simulating genetic events with all variations is a non-trivial job. Also aim of this study is only creating a simple proof of concept model. Thus, just a few important mutations of A549 are included into this research based on literature [109].

Model uses inheritance mechanism. When a cell proliferate, he copies DNA of his ancestor, so mutations are transferred between generations.

In the beginning, relationship between mutations should be determined and important questions about relationships should be answered. For example: "Which mutation will be occurred first ?", "What is the shape of dependency relationship ?"

After this step, probabilities of mutations should be determined. At last, effects of mutations should be added to model. In the model, only two types of mutation effects are considered, mutations' effects on increasing proliferation chance and decreasing apoptosis probability. For both, if any mutation occurs in a cell, it is assumed that proliferation probability increases %5 or apoptosis probability decreases %5 based on mutation type. These relationships and effects are showed in table below:

Table 3.2: Mutations of A549 Used In Model

<b>Mutation</b>	<b>Minimum Probability</b>	<b>Maximum Probability</b>	<b>Ancestors</b>	<b>Effect Type</b>
EGFR [110]	10	35	None	Proliferation
KRAS [111]	33	-	None	Proliferation
NTRK3 [112]	3	-	None	Proliferation
TP53 [112]	30	50	KRAS	Apoptosis
ATM [112]	10	20	KRAS	Proliferation
STK11 [112]	18	-	TP53, NTRK3	Apoptosis
CDK2NA [112]	5	-	TP53	Apoptosis

Modeling for mutations is based on a naive Bayesian network approach. Using this information model works as follows. At each time step cell triggers his own mutation system. For each mutation, preconditions are checked. For example for A549 EGFR mutation almost never occurred in tumors with KRAS mutation.

After ancestors of the mutation is validated for their existence a mutation probability determined between mutation's minimum and maximum probability for occurrence of mutation. Finally, when all mutations occurred, effects of mutations, whether for proliferation or apoptosis, applied to cell to use it in his decisions.



## CHAPTER 4

### RESULTS

#### 4.1 Results

Results of all experiments and simulations were presented in Appendix A. In this section only notable results will be discussed.

Using limited number of subsystem and data it is hard to create a model which has same properties with real tumor. So, in this study consistency of model was success indicator for us.

For example tumor cell numbers decreases proportionally with oxygen concentration as seen on table 4.2. Also size of the apoptotic zone (blue) is another result of hypoxia shown in table. In graphics red cells are proliferative at outer zone of tumor and green cells are quiescence.

Table 4.1: Tumor at  $t=300$  for  $O_2=0.2$ ,  $O_2=0.5$ ,  $O_2=1$

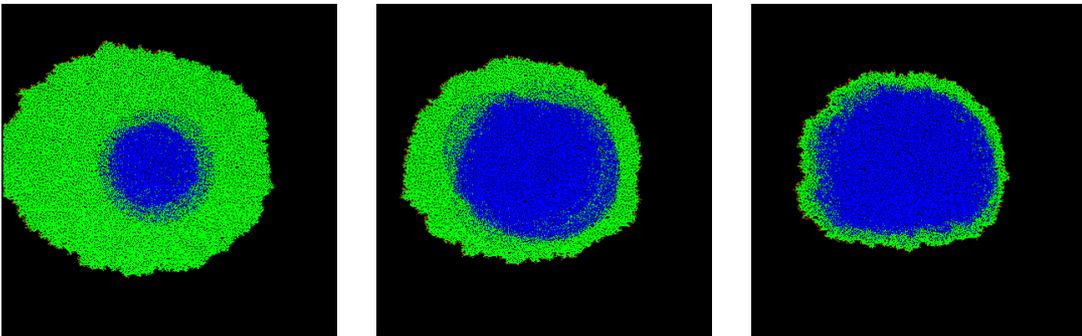
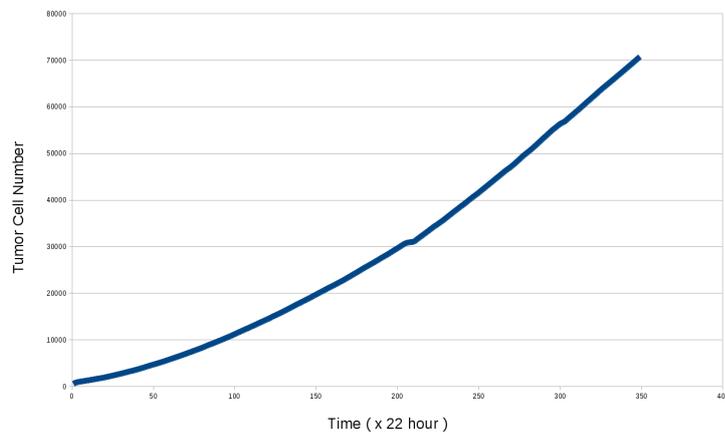
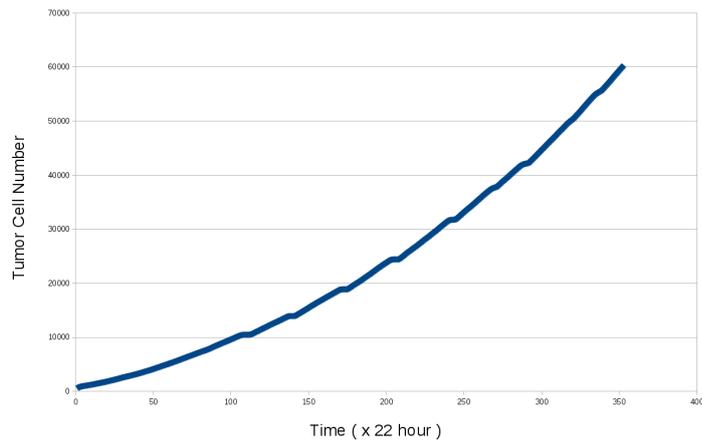
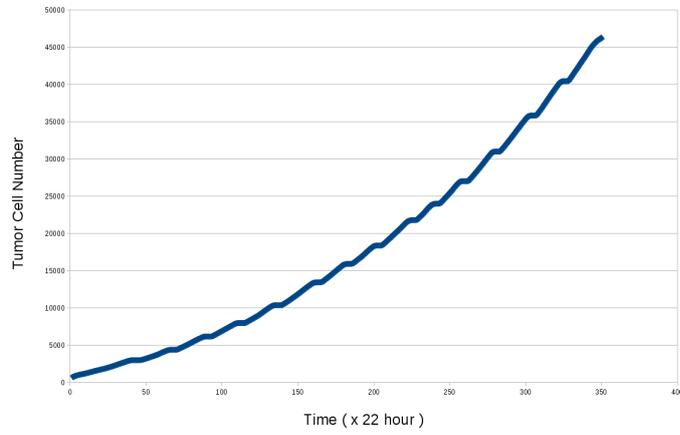
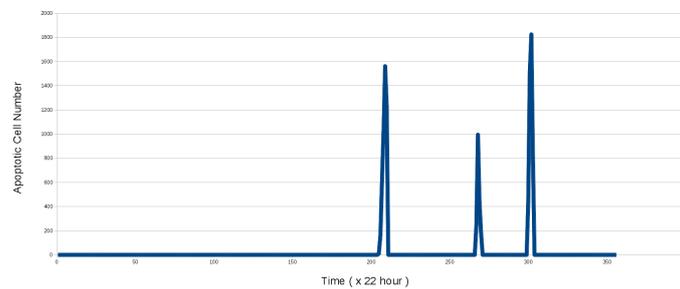
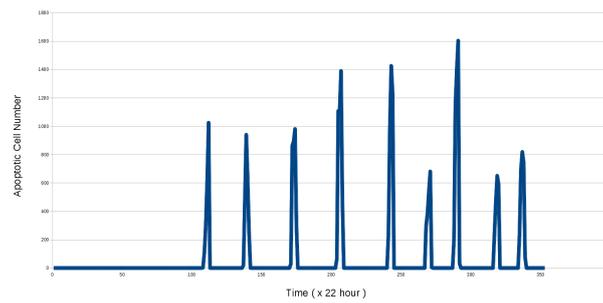
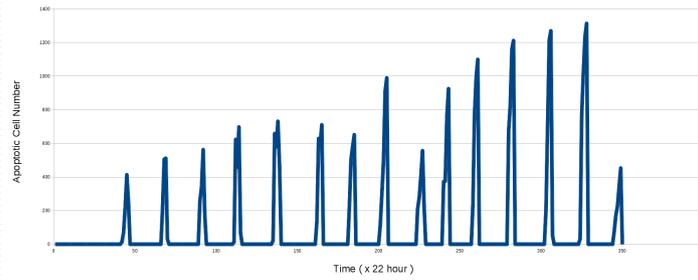


Table 4.2: Tumor Cell Numbers for  $O_2=0.2$ ,  $O_2=0.5$ ,  $O_2=1$



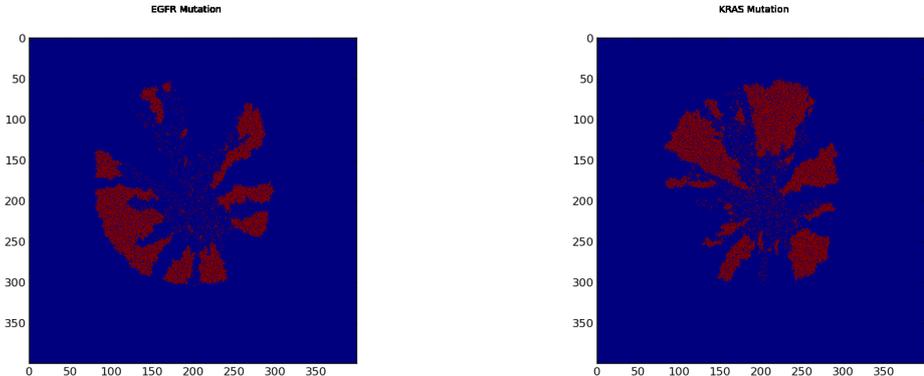
With decreasing oxygen concentration apoptosis occurs more frequently. This effect could be seen on graphs 4.3. At each peak in graphs means death of a group of cells in a time frame mostly because of nutrition shortage. After each death process, nutrition shortage ends since total cell number decreases. Thus, apoptosis ends and tumor starts to grow again until next starvation time.

Table 4.3: Apoptotic Cell Numbers for  $O_2=0.2$ ,  $O_2=0.5$ ,  $O_2=1$



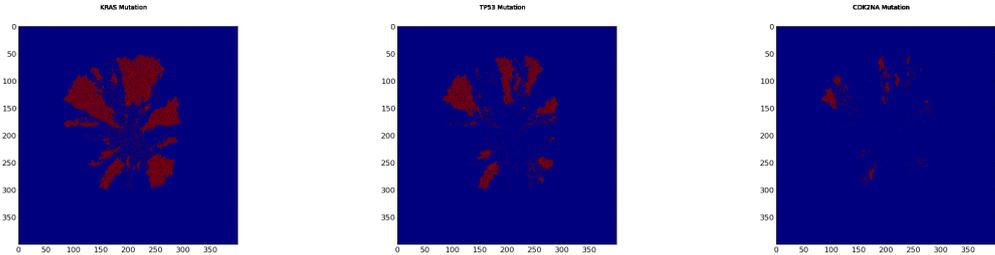
Rules of genetic subsystem was mentioned before. For example for A549 EGFR mutation almost never occurred in tumor cells which has KRAS mutation already. This effect could be seen on figures below. Coordinates of EGFR and KRAS mutations are entirely different.

Table 4.4: EGFR & KRAS Mutations Compared at 300th time step



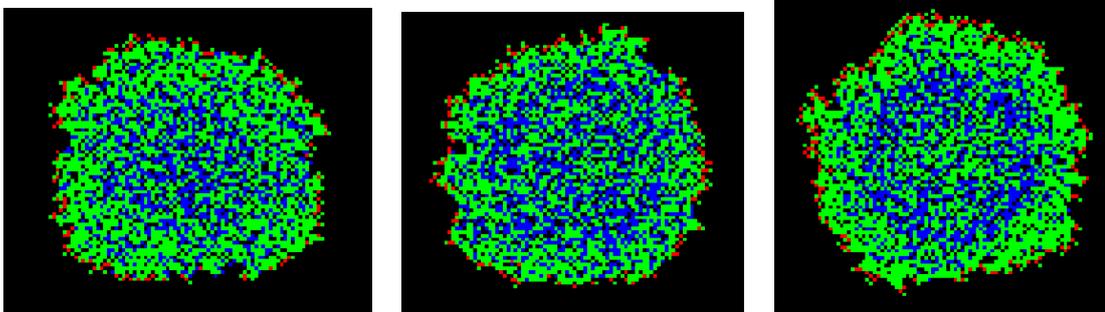
Genetic subsystem also works with dependency mechanism between mutations like TP53 is dependent to KRAS and CDK2NA is dependent to TP53. This dependency relationship could easily be seen in figures. CDK2NA could only found at the coordinates of cell that have TP53 mutated genes and TP53 mutation could be seen only at coordinates where KRAS mutation occurred.

Table 4.5: Dependency Between CDK2NA, TP53 and KRAS



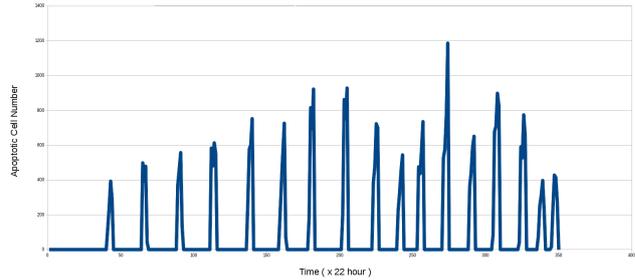
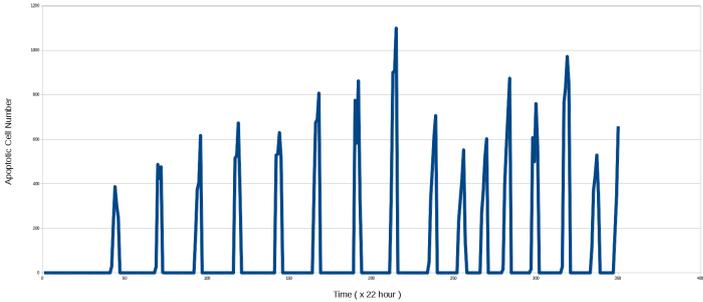
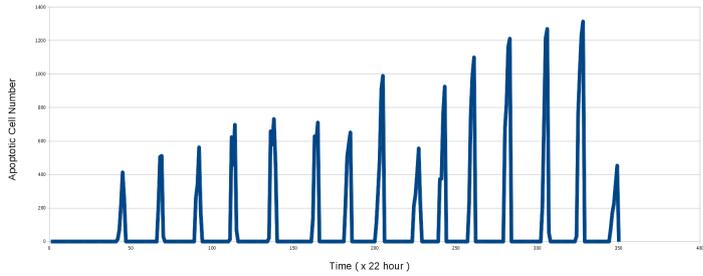
Effects of mutation rate could be seen on tumor vulnerability. Figures shows tumor at time step 50 with different mutation rates at  $O_2 = 0.2$  and Glucose = 1. First one is with with normal mutation rate which is 1. The second image is from simulation with mutation rate 1/10 and for the last tumor mutation system shuts down.

Table 4.6: Tumor Growth for  $m_r = 1$ ,  $m_r = 1/10$ ,  $m_r = 0$



Effect of low rate mutations and tumor without a mutation system compared with a normal tumor could be seen at apoptosis frequency. This effect is shown below:

Table 4.7: Apoptotic Cell Numbers for  $m_r = 1, m_r = 1/10, m_r = 0$



## CHAPTER 5

### CONCLUSION AND FUTURE WORK

#### 5.1 Conclusion

In this study our aim is creating a basic model to guide researchers who are eager to start tumor modeling. The model consists from modules, Oxygen Diffusion Calculator, Glucose Diffusion Calculator, Acid Diffusion Calculator, Invasion System, Mutation System, Proliferation System, Apoptosis System, Energy Metabolism, Nutrient Consumption Waste Production System and a Process Manager which controls and organize all these systems.

Oxygen Diffusion Calculator, Glucose Diffusion Calculator, Acid Diffusion Calculator systems are created by using continuous modeling with PDEs. Invasion System, Proliferation System, Apoptosis System, Energy Metabolism, Nutrient Consumption and Waste Production System uses cellular automata with stochastic decision making. Mutation System forms with a probabilistic network. Process Manager also uses cellular automata.

Model tries to simulate adenocarcinoma which is a subtype of non-small cell lung carcinoma. Parameters of model gathered from literature mostly for A549. A549 cells are human alveolar basal epithelial cells commonly used for adenocarcinoma experiments.

In simulations effects of oxygen concentration and mutation rate inspected. Tumor cell number decreased and apoptosis frequency increases proportionally with oxygen concentration. When mutation rate decreases tumors become more vulnerable and apoptosis rate increases. All these results proves that model is consistent with tumor biology rules.

#### 5.2 Future Work

The aim of tumor modeling should be applying personalized medicine for cancer. In the current workflow, physicians use standard medical guidelines to form a therapy plan. Therapy plans and guidelines are varying according to institutions and countries but all of them uses results of clinical experiments which are optimized for average mass. After application of therapy, physicians evaluate results and changes therapy plan. This is primitive trial and error approach. Decisions for determining delay time between chemotherapy cures, using radiotherapy and chemotherapy together or consequently, choosing radiotherapy type as hypofractionated or hyperfractionated are based on clinicians own choice. However for a personalized therapy these decisions should be based on scientific evidence which is customized for each individual.

A tumor model which is developed and updated bases on latest scientific evidence could help physicians to predict results of alternative therapy plans. Model could also uses patient specific variables (demographic, genetic etc.) as the part of input parameters to create a personalized therapy plan.

Tumor models will also be a part of drug development process. A new drug costs about a billion dollar. Costs of drug developed could be decreased by using pharmacodynamics and pharmacokinetics on tumor model with determining optimal dosage and trial plans.

Tumor models are also important for medical education process. Education of oncology specialists is a long and hard process. Using tumor models, candidates could easily try different therapy plans and get the results of their decisions during education.

In this study model was not validated with real data, because the aim was developing a consistent model with rules of tumor biology. But, eventually more realistic models will be developed. For a model that could be used in daily routine, development process should be planned with laboratory experiments.

## CHAPTER 6

### APPENDIX A

#### 6.1 Experiments

Results of simulation with different parameters sets based on oxygen, glucose and mutation rate could be seen in this section.

In ideal conditions (Oxygen = 1 , Glucose = 1 , Mutation Rate ( $m_r$ ) = 1) which means oxygen and glucose level equals background concentration and at each time step cell will try to mutate. Tables 6.1 - 6.2 - 6.3 shows results. Graphics shows number of tumor cells, cells choose apoptosis and mutated cells versus time. Red cells are proliferative at outer zone of tumor. Green cells are quiescence and blue cells are dead cells (apoptotic).

Table 6.1: Tumor Growth for  $O_2=1$ , Glucose=1,  $m_r = 1$

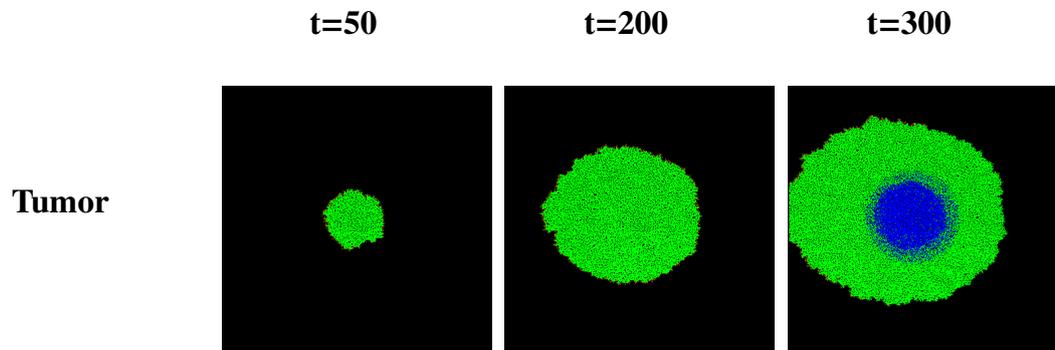


Table 6.2: Diffusion in Microenvironment for  $O_2=1$ , Glucose=1,  $m_r = 1$

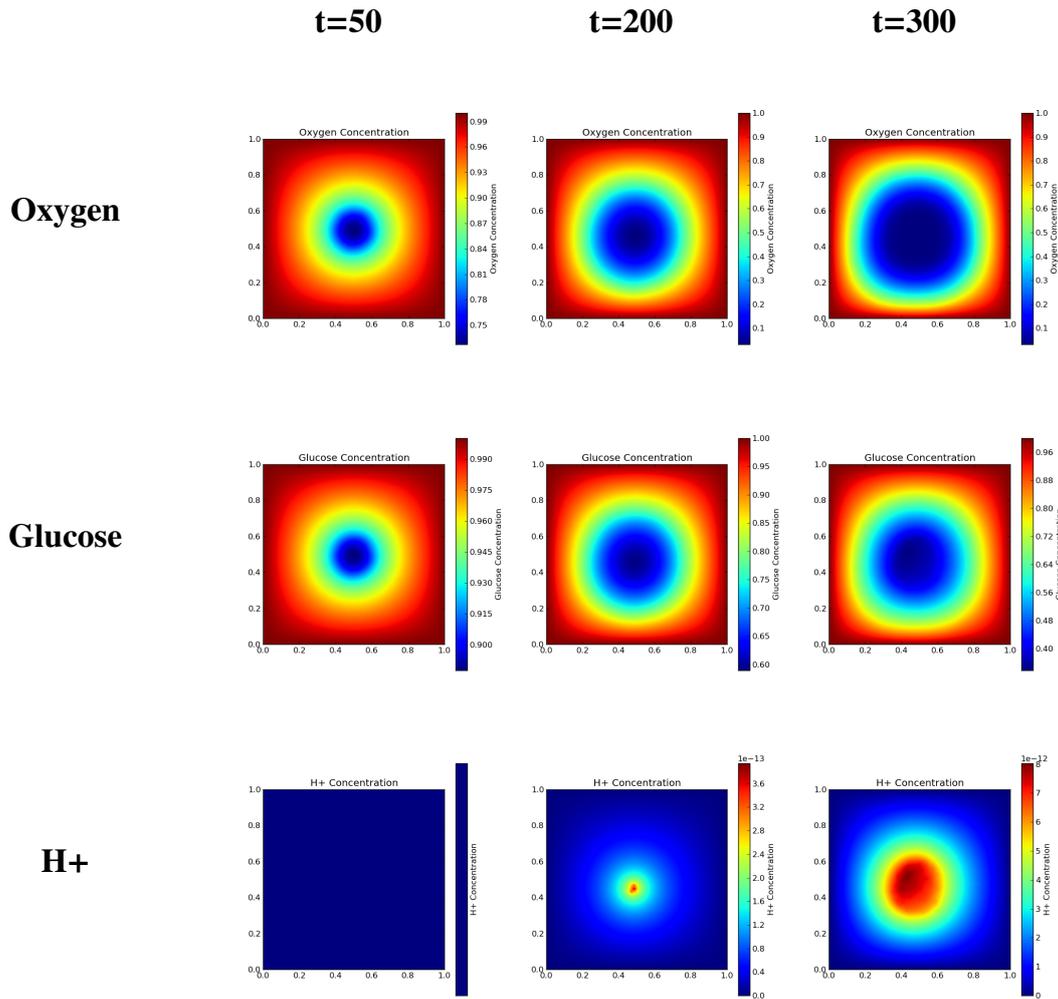
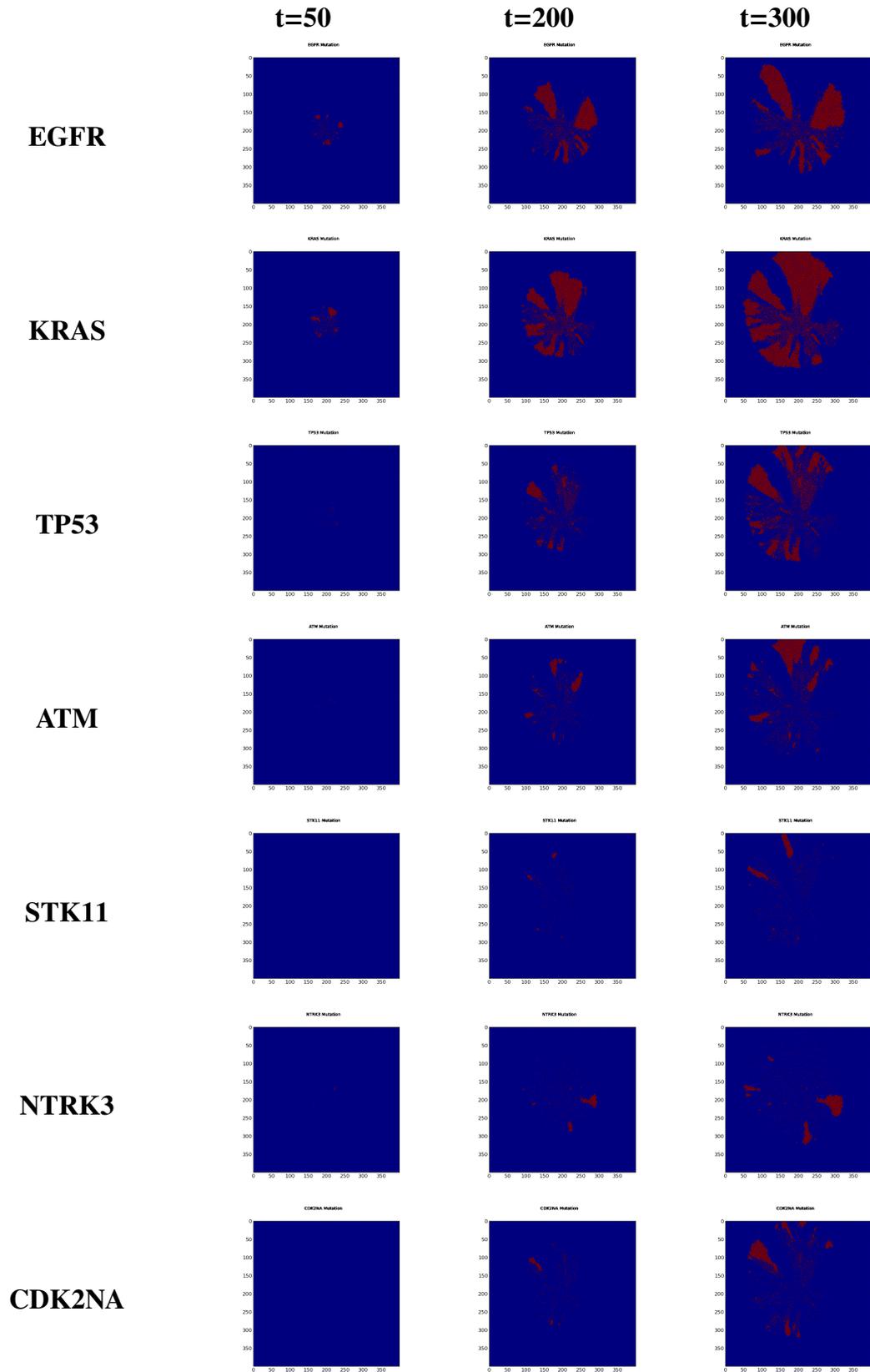


Table 6.3: Mutations for  $O_2=1$ , Glucose=1,  $m_r = 1$



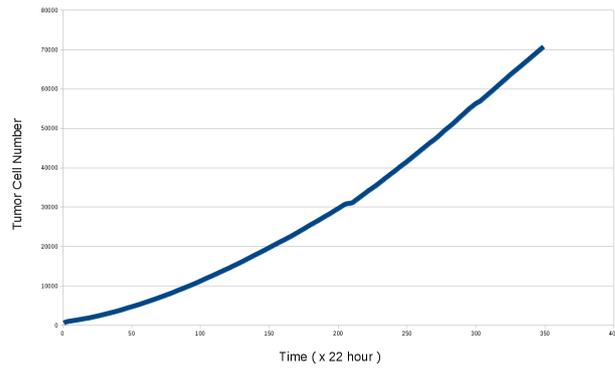


Figure 6.1: Tumor Cell Number for  $O_2=1$ , Glucose=1,  $m_r = 1$

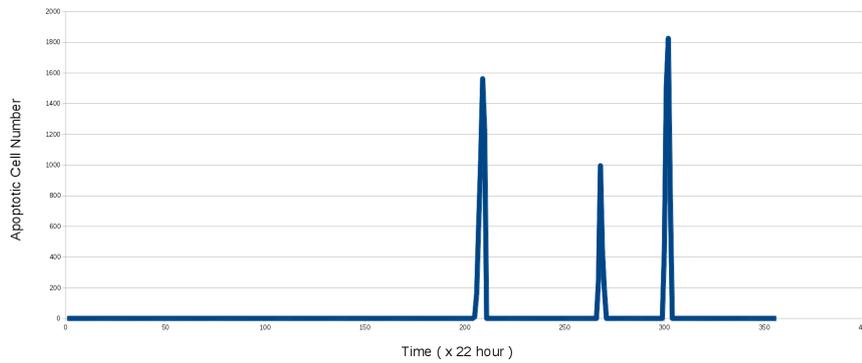


Figure 6.2: Apoptotic Cell Number for  $O_2=1$ , Glucose=1,  $m_r = 1$

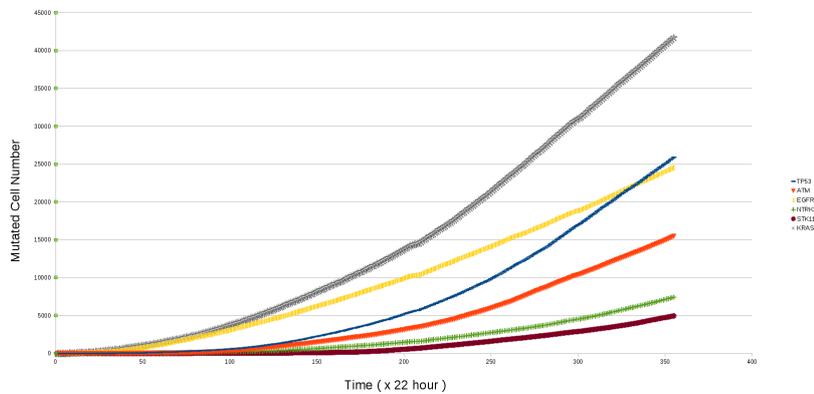


Figure 6.3: Mutated Cell Number for  $O_2=1$ , Glucose=1,  $m_r = 1$

In the second case oxygen concentration decreased to half of original, glucose concentration and mutation rate is same.

Table 6.4: Tumor Growth for  $O_2=0.5$ , Glucose=1,  $m_r = 1$

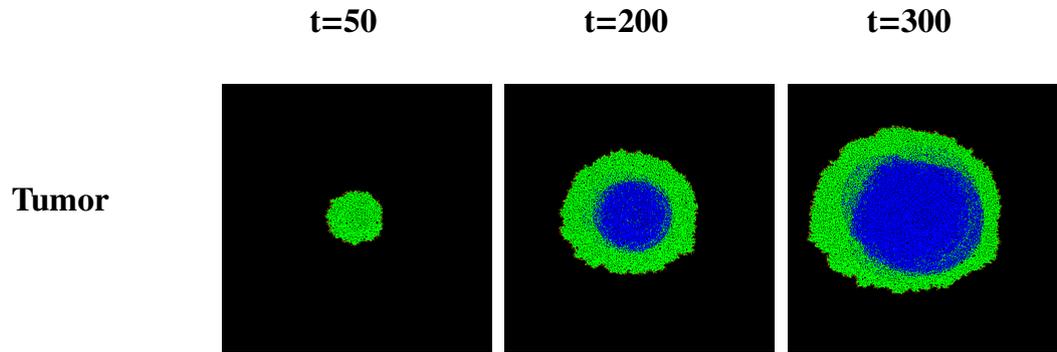


Table 6.5: Diffusion in Microenvironment for  $O_2=0.5$ , Glucose=1,  $m_r = 1$

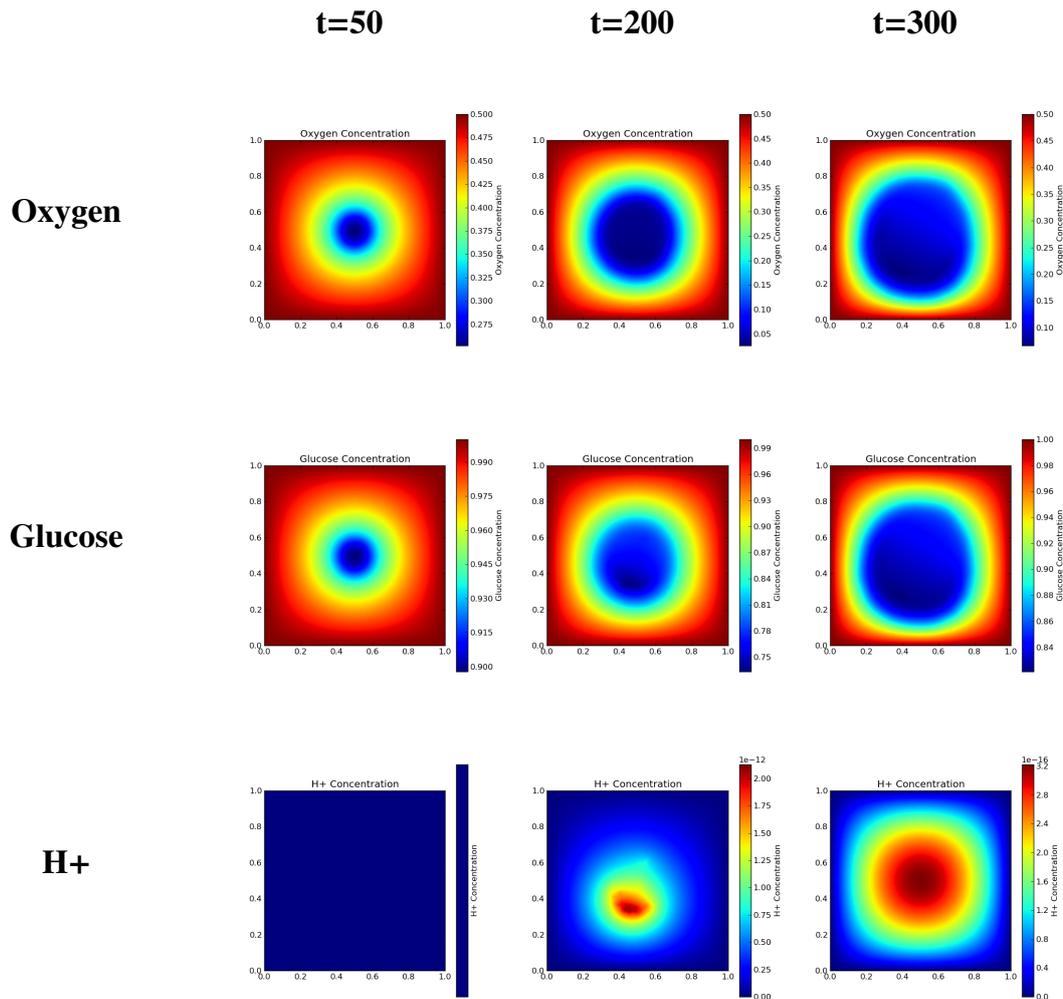
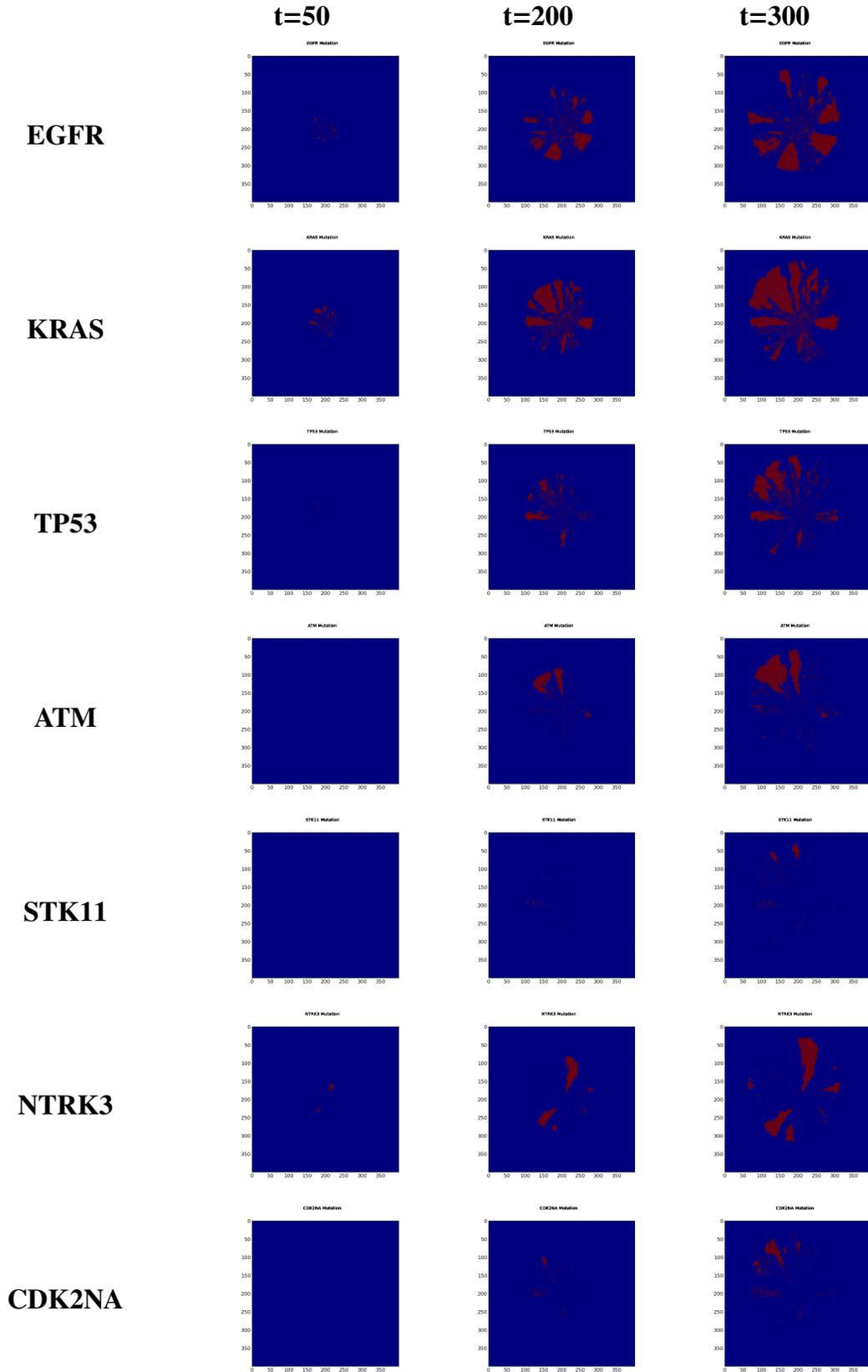


Table 6.6: Mutations for  $O_2=0.5$ , Glucose=1,  $m_r = 1$



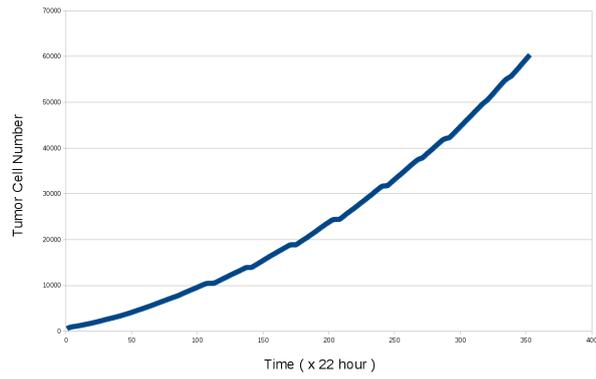


Figure 6.4: Tumor Cell Number for  $O_2=0.5$ , Glucose=1,  $m_r = 1$

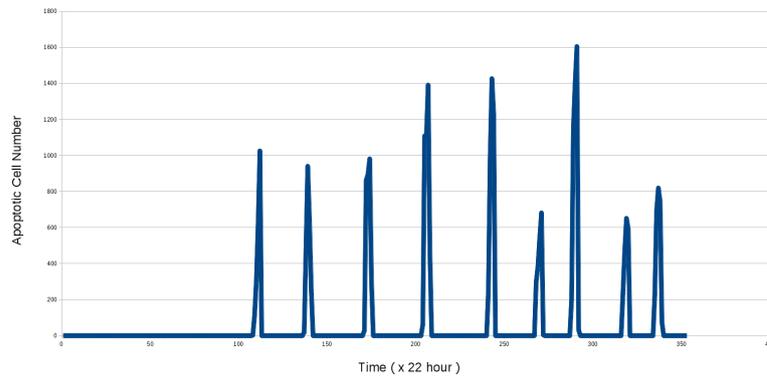


Figure 6.5: Apoptotic Cell Number for  $O_2=0.5$ , Glucose=1,  $m_r = 1$

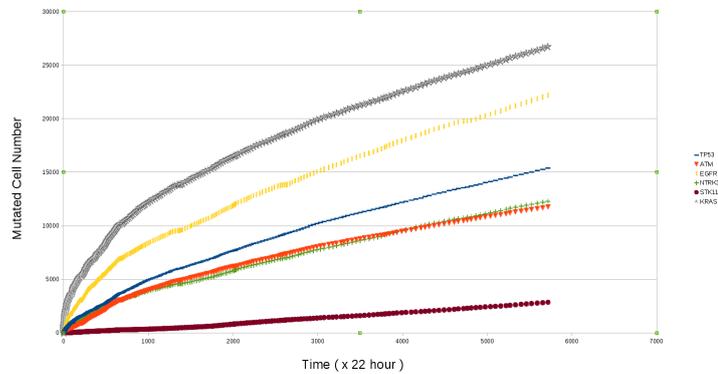


Figure 6.6: Mutated Cell Number for  $O_2=0.5$ , Glucose=1,  $m_r = 1$

For the third simulation oxygen concentration is decreased to 1/5 of original background concentration, glucose concentration and mutation rate is same.

Table 6.7: Tumor Growth for  $O_2=0.2$ , Glucose=1,  $m_r = 1$

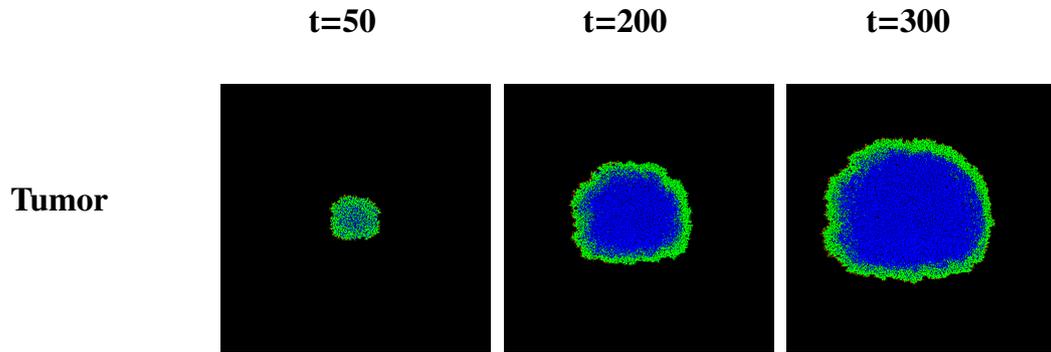


Table 6.8: Diffusion in Microenvironment for  $O_2=0.2$ , Glucose=1,  $m_r = 1$

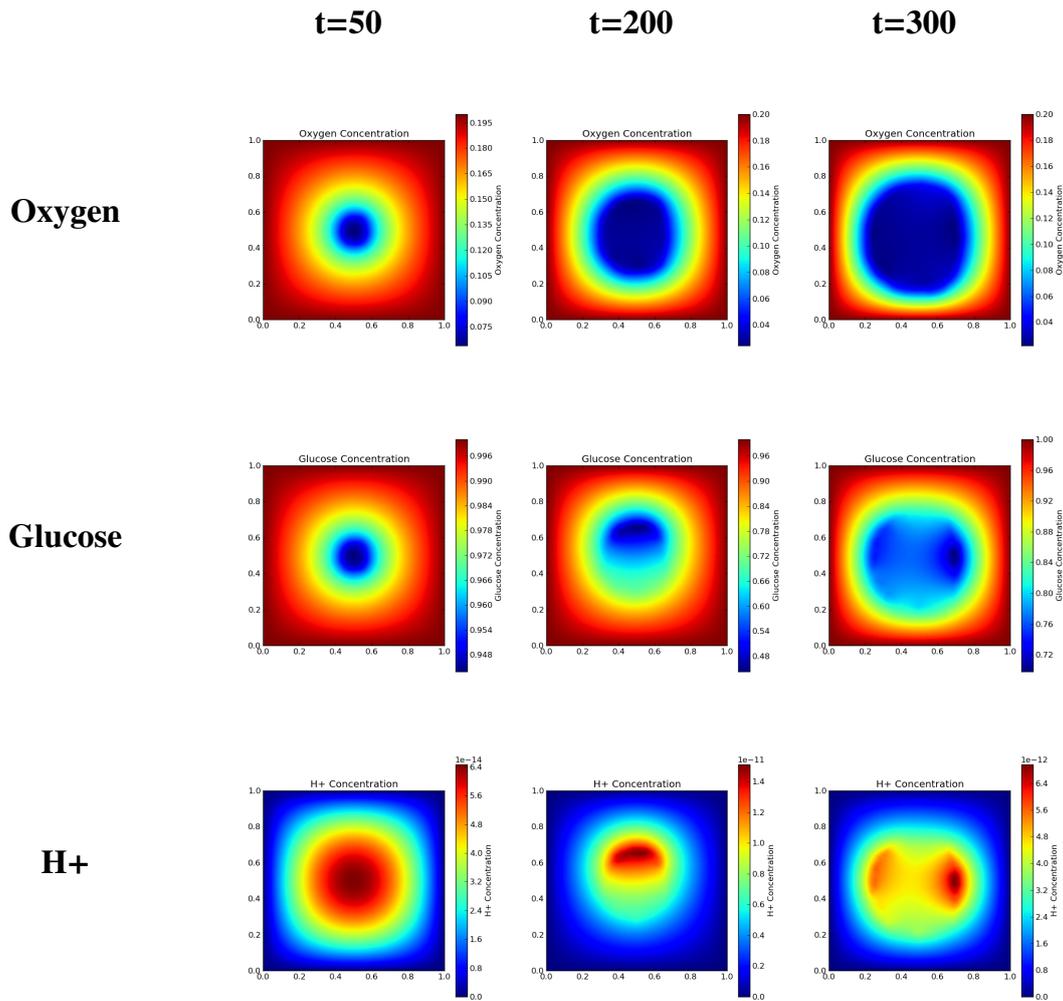
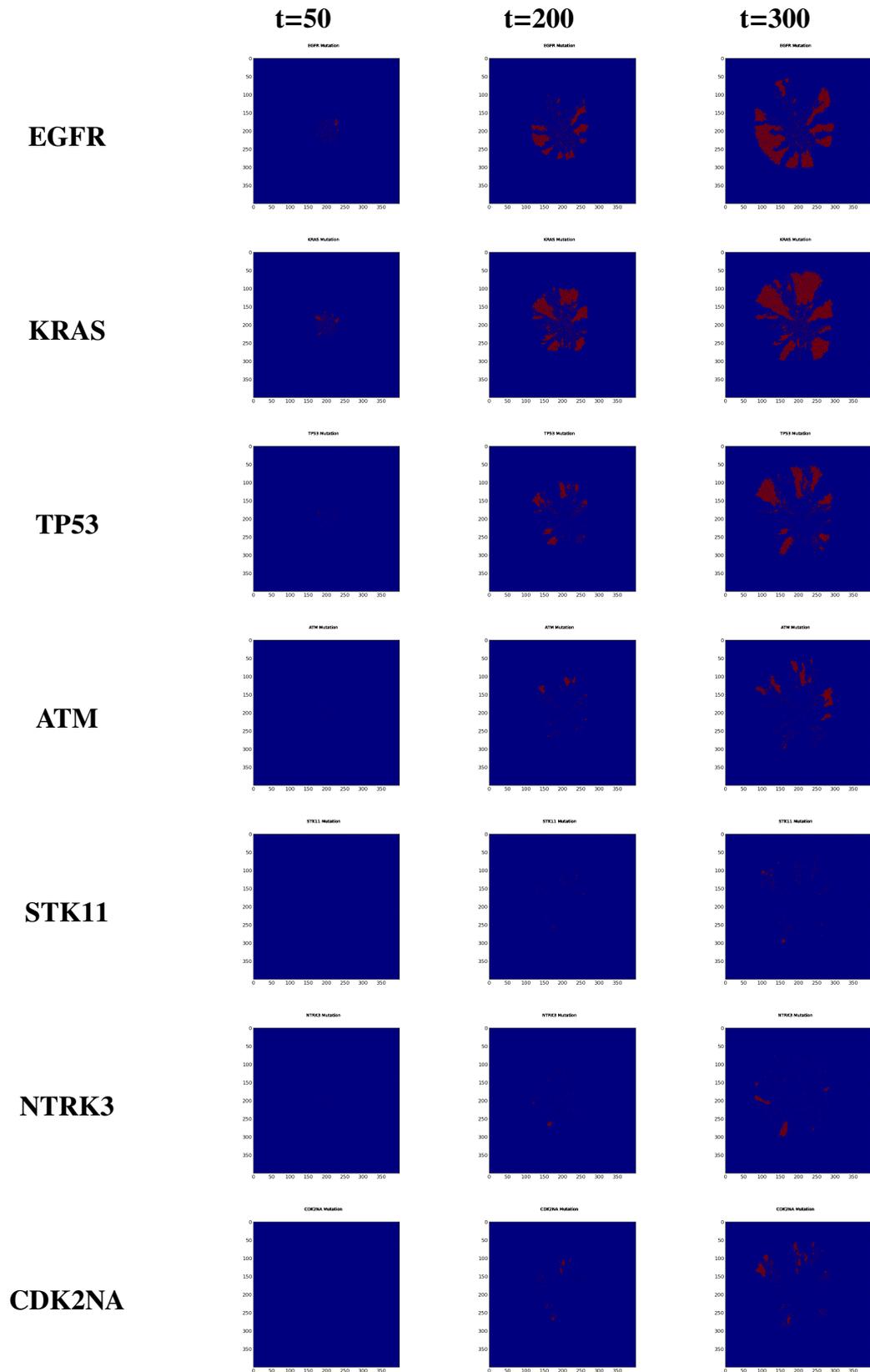


Table 6.9: Mutations for  $O_2=0.2$ , Glucose=1,  $m_r = 1$



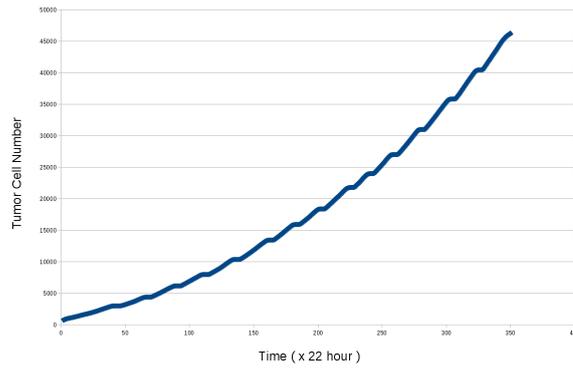


Figure 6.7: Tumor Cell Number for  $O_2=0.2$ , Glucose=1,  $m_r = 1$

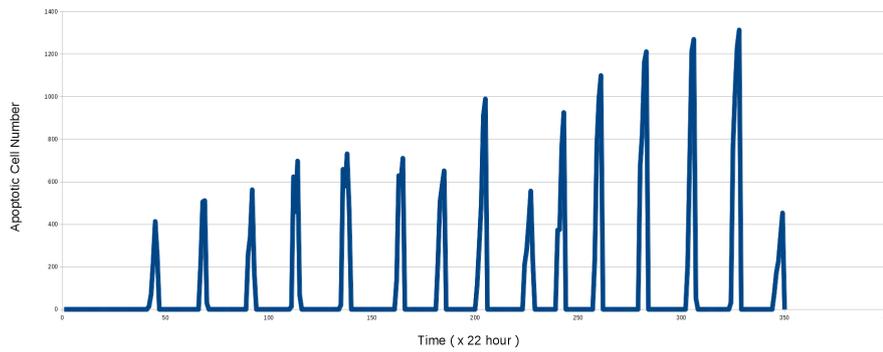


Figure 6.8: Apoptotic Cell Number for  $O_2=0.2$ , Glucose=1,  $m_r = 1$

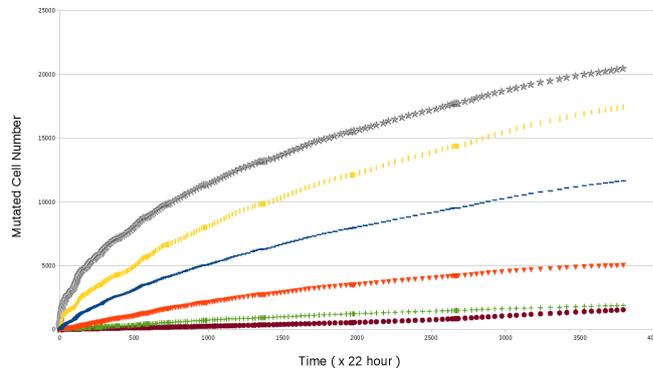


Figure 6.9: Mutated Cell Number for  $O_2=0.2$ , Glucose=1,  $m_r = 1$

Now mutation rate decreased 1/10 which means cell tries to mutate each 10th time step. Oxygen concentration is 1/5 of original background concentration and glucose level is same as original. Results shown below.

Table 6.10: Tumor Growth for  $O_2=0.2$ , Glucose=1,  $m_r = 0.1$

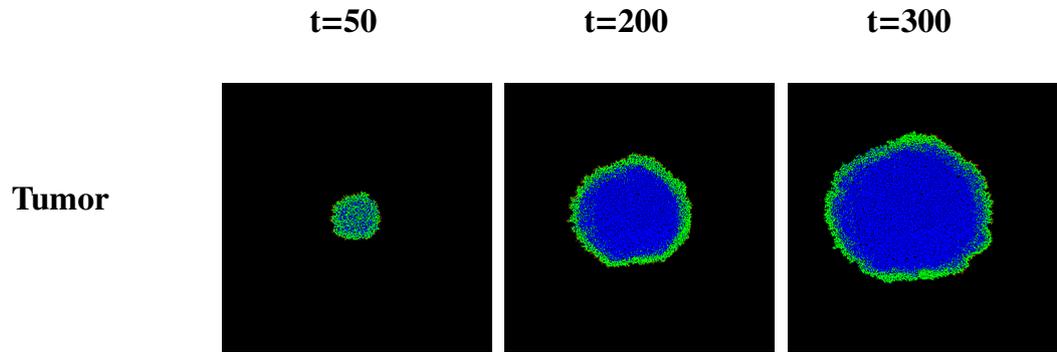


Table 6.11: Diffusion in Microenvironment for  $O_2=0.2$ , Glucose=1,  $m_r = 0.1$

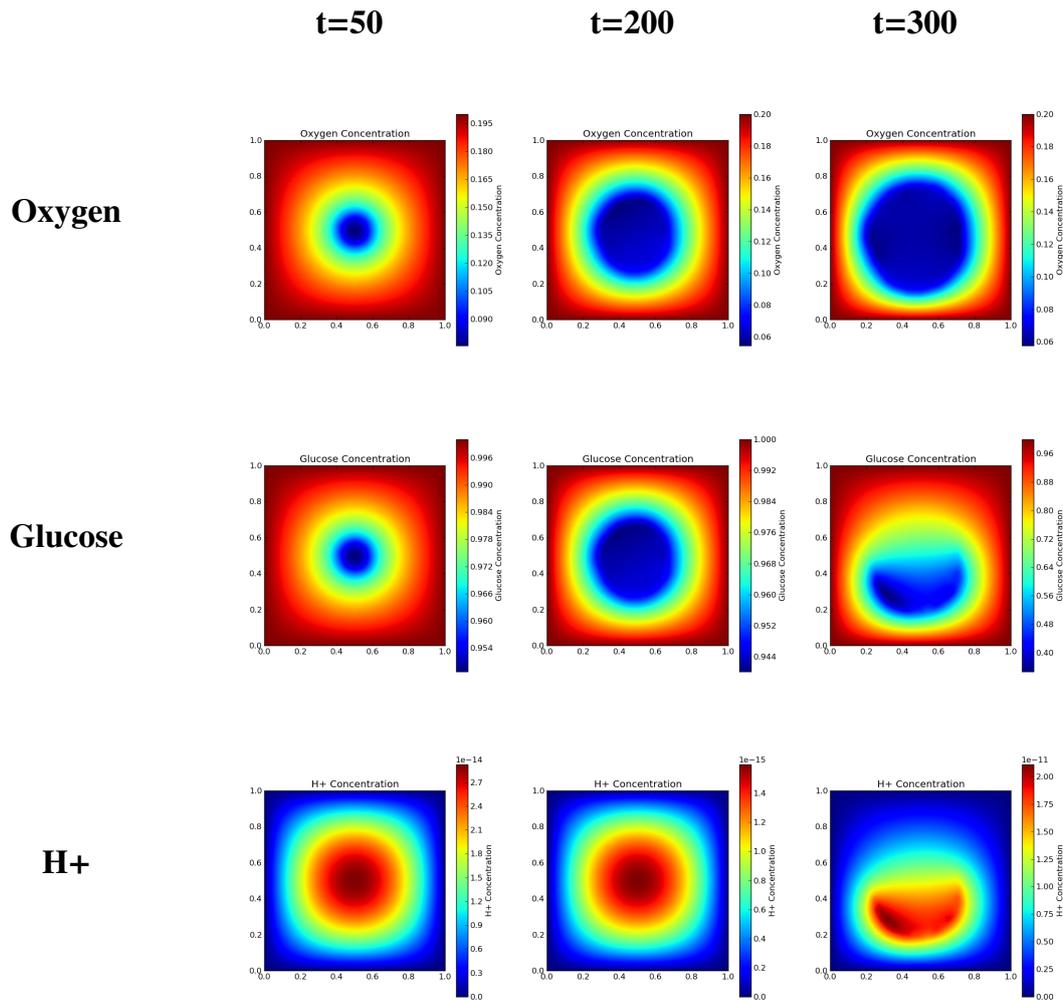
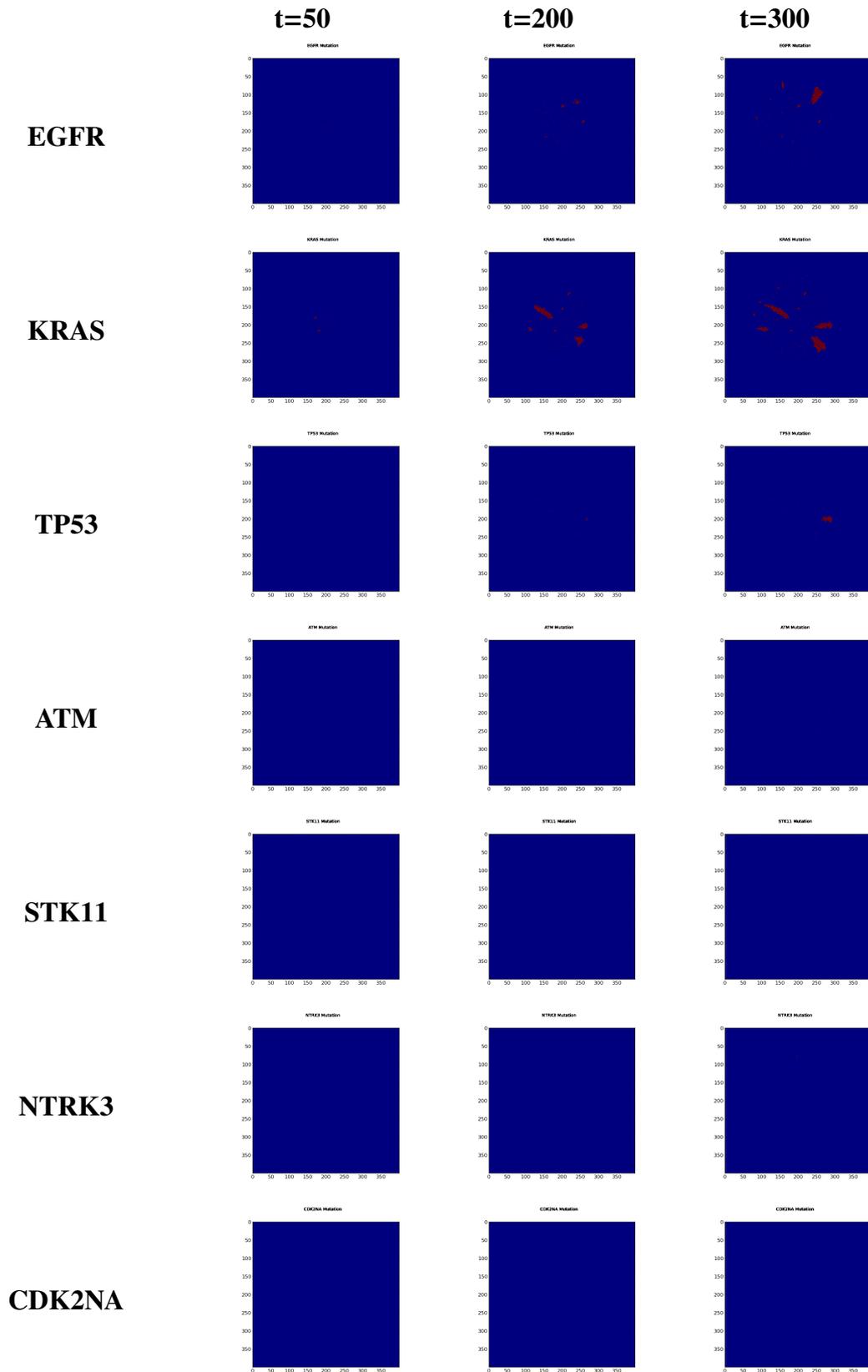


Table 6.12: Mutations for  $O_2=0.2$ , Glucose=1,  $m_r = 0.1$



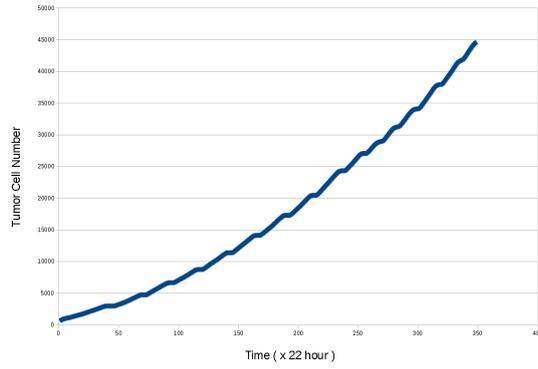


Figure 6.10: Tumor Cell Number for  $O_2=0.2$ , Glucose=1,  $m_r = 0.1$

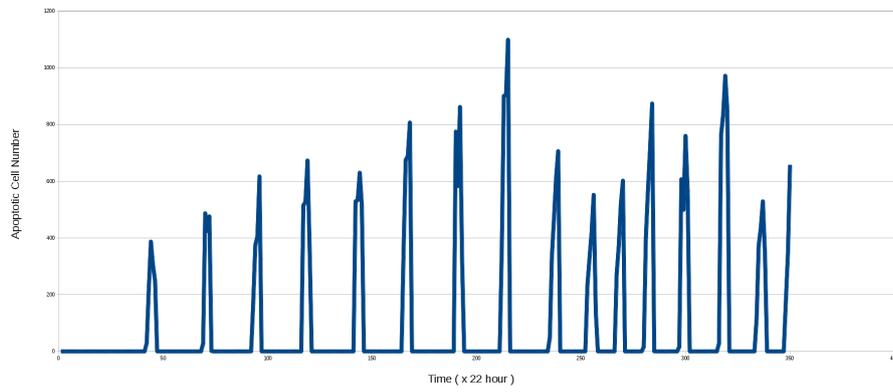


Figure 6.11: Apoptotic Cell Number for  $O_2=0.2$ , Glucose=1,  $m_r = 0.1$

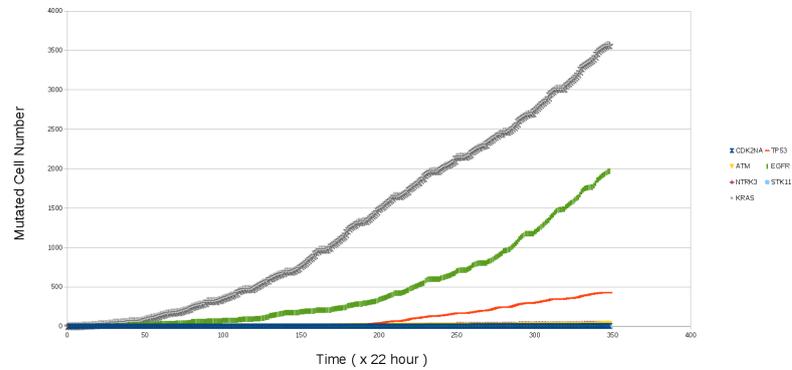


Figure 6.12: Mutated Cell Number for  $O_2=0.2$ , Glucose=1,  $m_r = 0.1$

Finally lets look at how tumor reacts if mutation system is shutdown completely.

Table 6.13: Tumor Growth for  $O_2=0.2$ , Glucose=1,  $m_r = 0$

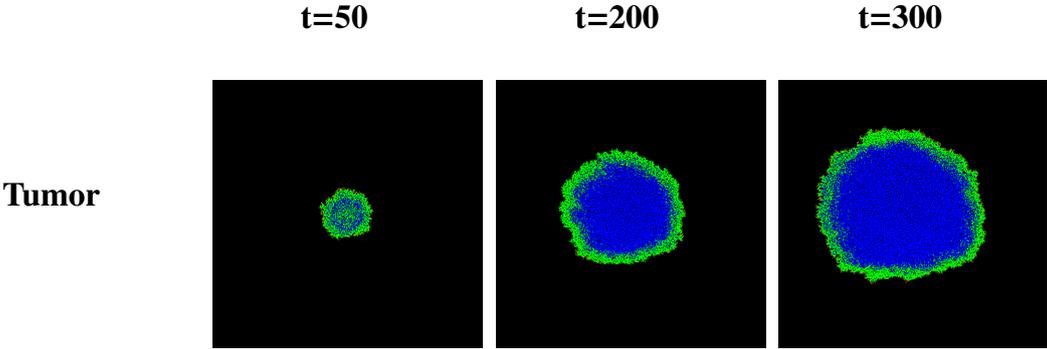


Table 6.14: Diffusion in Microenvironment for  $O_2=0.2$ , Glucose=1,  $m_r = 0$

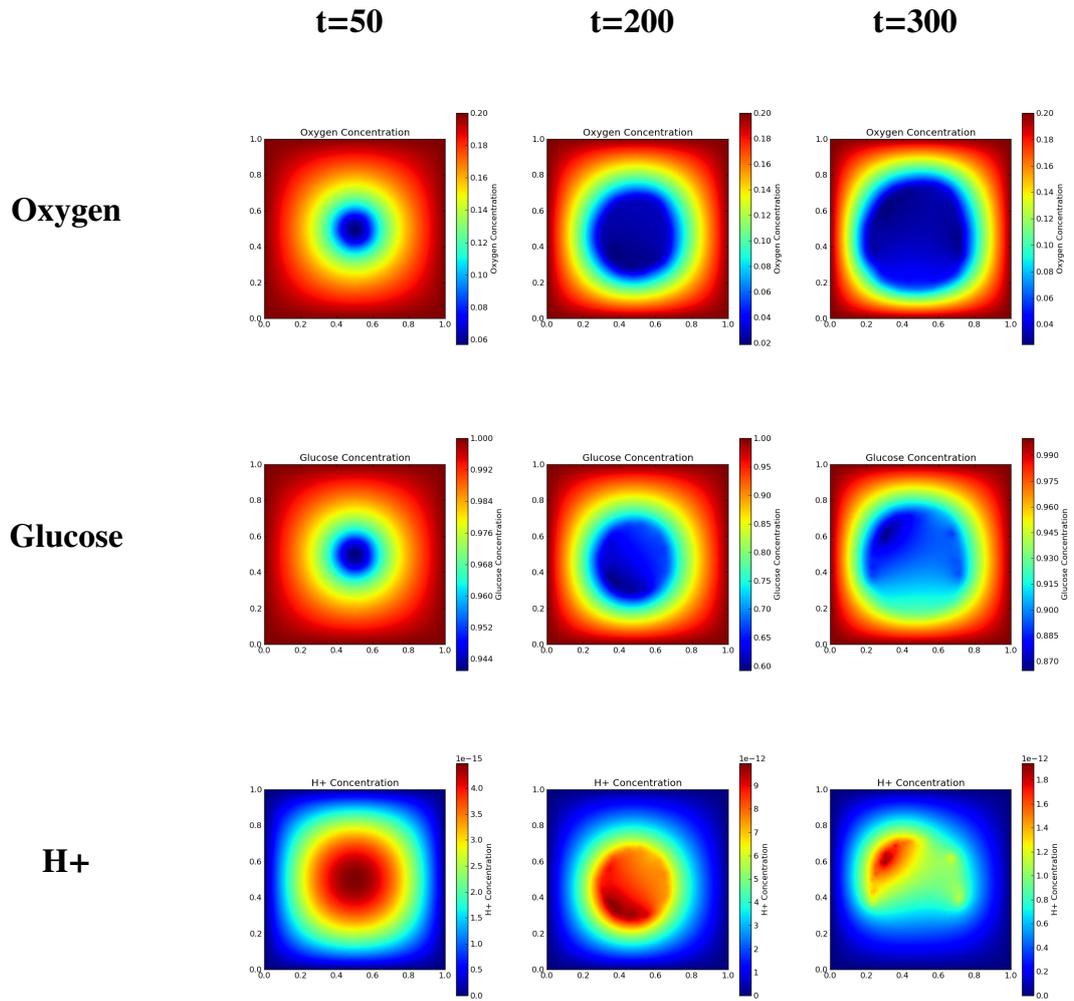
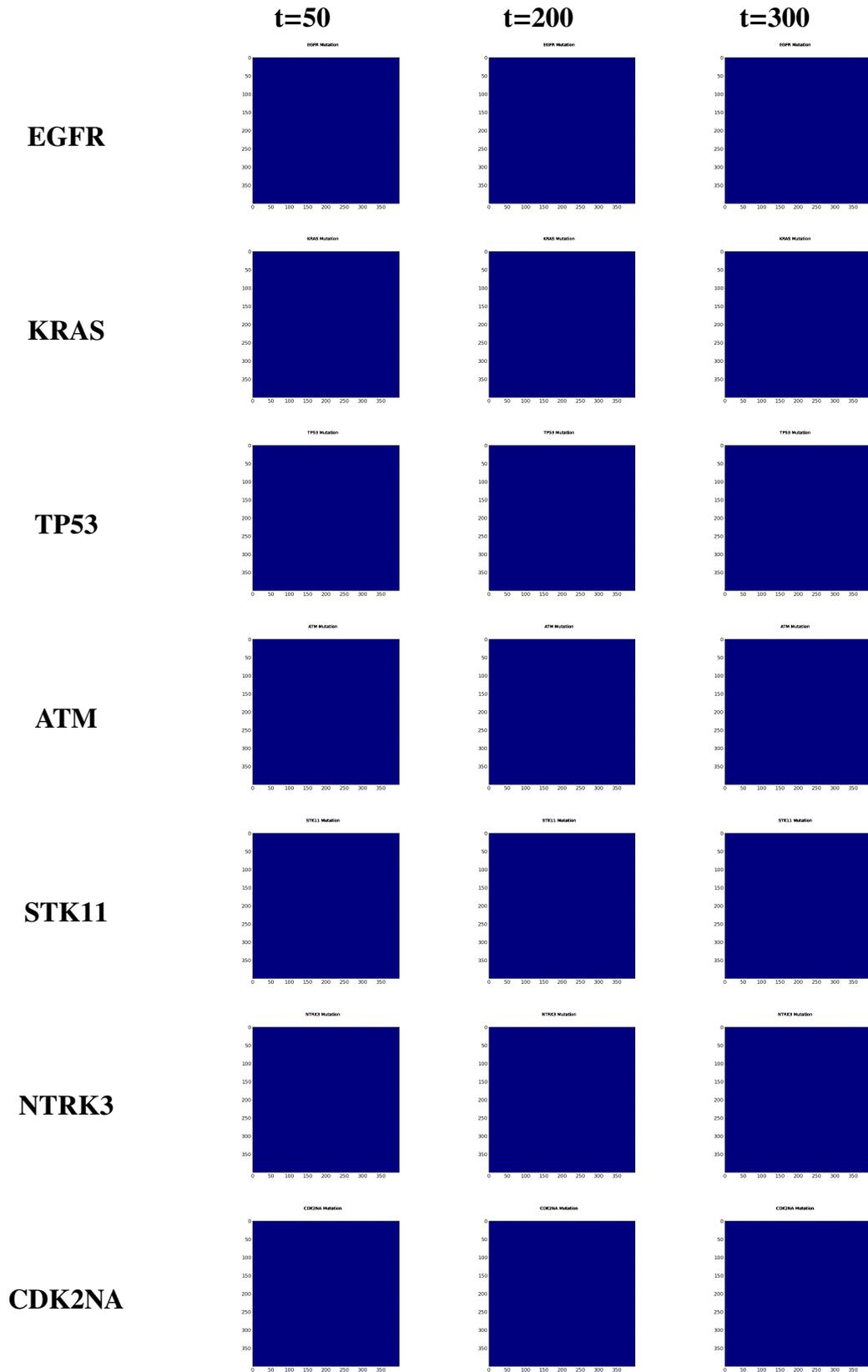


Table 6.15: Mutations for  $O_2=0.2$ , Glucose=1,  $m_r = 0$



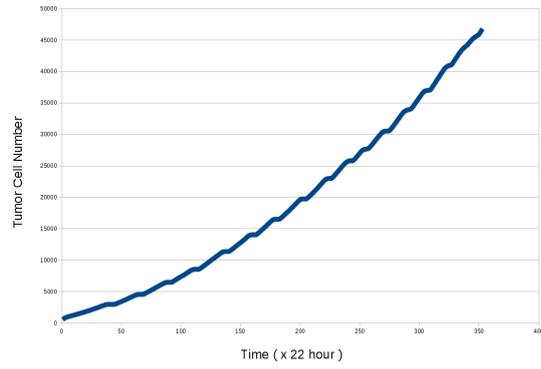


Figure 6.13: Tumor Cell Number for  $O_2=0.2$ , Glucose=1,  $m_r = 0$

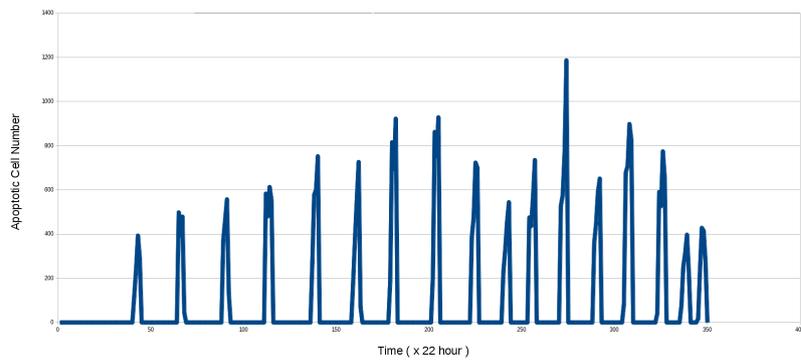


Figure 6.14: Apoptotic Cell Number for  $O_2=0.2$ , Glucose=1,  $m_r = 0$

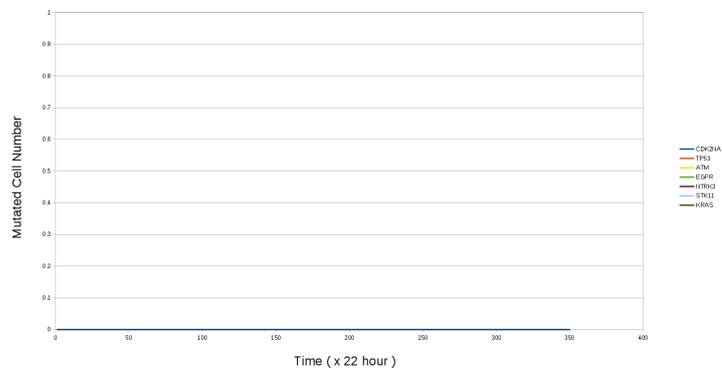


Figure 6.15: Mutated Cell Number for  $O_2=0.2$ , Glucose=1,  $m_r = 0$



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