# Contents

# List of Figures

# List of Tables

# List of Abbreviation

| | |
|---|---|
| 2D | Two Dimension |
| CA | Cellular Automata |
| CC | Computational Cell |
| CM | Computational Model |
| CSC | Cancer Stem Cells |
| CSV | Comma Separated Value |
| DNA | Deoxyribo Nucleic Acid |
| ECM | Extracellular Matrix |
| EMT | Epithelial-mesenchymal transition |
| ES | ECM Site |
| FD | Fiber Density |
| GNU | GNU's Not Unix! |
| GUI | Graphical User Interface |
| HT | Hierarchical theory |
| IDE | Integrated Development Environment |
| MMP | Matrix Metallo Proteinase |
| ODE | Ordinary Differential Equations |
| SCA | Stem Cell Automata |
| SEM | Standard Error of Mean |
| SS | Simulation Steps |
| TAC | Transiently Amplifying Cells |
| TDC | Terminally Differentiated Cells |
| TIC | Tumor Initiating Cells |

# Chapter 1

# Introduction and Overview

## 1.1 Introduction

This chapter gives introduction to biological concepts, Cellular Automata(CA), motivation behind the project, list of existing systems and their limitations, the proposed system and organization of report.

## 1.2 Biological concepts

Various tissues, organs have different stiffness. Tissues are not made up solely of biological cells. A substantial part of their volume is extracellular space, which is largely filled by an intricate network of macromolecules constituting the Extracellular Matrix(ECM). This matrix is composed of a variety of proteins and polysaccharides that are secreted locally and assembled into an organized meshwork in close association with the surface of the cell that produced them. The ECM can influence the organization of a cell's cytoskeleton. This can be vividly demonstrated by using transformed (cancer like) fibroblasts in culture. Transformed cells often make less fibronectin than normal cultured cells and behave differently. [48].

A FSA is a machine that has finite set of states the machine can be in, reads input from a set of characters and transition occurs on condition of present state and input, a FSA is a restricted Turing machine [46]. Biological systems, having cell types and transitions between cell types based on cell and neighbourhood properties, can be modelled with FSA which can represent cell type change as state changes in FSA and cell and neighbourhood properties as input to FSA. This project considers four type of cells CSC, Transiently Amplifying Cell (TAC), Terminally Differentiated Cell (TDC), ECM Site (ES) as states of Automata, where CSC, TAC and TDC are considered BCs. Transition function is dependent on BCs and ES properties.

## 1.3 Motivation behind the project

CSCs have been shown to associate with different aggressive cancer phenotypes including drug resistance. CSCs are transformed cancer cells possessing the properties similar to stem cells. The project will contribute to our understanding of how CSCs contribute to cancer invasiveness.

## 1.4 Existing system

Existing systems have probed CSC dynamics as function of oxygen diffusion or only motility of BCs. Generation of intratumor heterogeneity as function of intrinsic motility of cell, exact role of motility has not been examined. The effects of increase in ECM confinement to tumor growth and cellular heterogeneity within tumor is not probed. Tumor progression and emergence of intratumor heterogeneity as function of ECM proteolysis is not well understood.

## 1.5 Proposed system

The proposed system integrates ECM confinement, BC motility and proteolysis propensity to explore the lacks existing system mentioned in 1.4. This project develops a Computational Model (CM) that combines discrete and continuous modelling approaches.

## 1.6 Organization of report

This thesis is divided into various chapters and each chapter deals with a specific topic. Chapter 2 contains the literature survey and the technologies that are needed for this work. Subsequently, Chapter 3 addresses the System Requirements Specification. Chapter 4 presents the design of the system in terms of modules and the approach used. Chapter 5 shows the implementation details of each of the major modules and analysis drawn from implementation. Chapter 6 explains the system testing. Chapter 7 explains results, type of files obtained from simulation. Finally, the conclusion of the work and future enhancements is presented in Chapter 8.

## 1.7 Conclusion

This chapter covers biological concepts of CSCs, overview on CSCs proliferation implementation using CA and using it to extend to proposed system of CSCs proliferation as a function of ECM, biological cell motility and ECM proteolysis propensity.

# Chapter 2

# Literature survey

## 2.1 Introduction

This chapter covers the literature in the field of CA applied to CSC proliferation. This covers existing systems and problems addressed, their drawbacks and proposed system to overcome the drawbacks, followed by definition of CA, biological terminologies and softwares used.

## 2.2 Literature survey

To explain the experimental findings that large number of cells ($> 10^6$) are required to initiate tumor, two theories viz. stochastic theory (ST) and hierarchical theory (HT) were proposed [1–4]. While ST predicts that the tumor population is homogeneous and all tumor cells have the equal capabilities to initiate tumor *but* with low probability, the HT predicts that tumor population is heterogeneous and only a fraction of whole population can initiate new tumor. These special cells are called Tumor Initiating Cells (TIC) or cancer stem cells (CSC). Presence of CSC in different tumors and their role in cancer progression have been demonstrated by several experimental studies [10–15]. Role of these cells in drug resistance has also been demonstrated [16, 17]. CSC undergo cell division process similar to a non-tumors stem cells i.e. they can either undergo symmetric division to increase the CSC contents or can perform asymmetric division to increase the non-CSC fraction of the tumor [18, 19]. This decision of undergoing symmetric versus asymmetric division have been shown to influence the population dynamics for both tumor populations cells as well as non-neoplastic tissue cells [20].

## 2.3 Cellular Automata

### 2.3.1 Biological roots

The notion of a CA originated in the works of John von Neumann (1903–1957) and Stanislaw Ulam (1909–1984). CA as discrete, local dynamical systems (to be formalized later in this chapter) can be equally well viewed as a mathematical idealization of natural systems, a discrete caricature of microscopic dynamics, a parallel algorithm, or a discretization of partial differential equations. According to these interpretations distinct roots of CA may be traced back in biological modelling, computer science, and numerical mathematics [50].

In the 1950s John von Neumann and Stanislaw Ulam proposed the concept of cellular automata. In recent years, the originally very restrictive definition has been extended to many different applications. In general, a cellular automaton (CA) is specified by the

following definition:
a regular discrete lattice (L) of cells (nodes, sites) and boundary conditions,

### 2.3.2 Biological terminologies

Stem cells are undifferentiated biological cells that can differentiate into specialized cells and can divide (through mitosis)

## 2.4 Technologies used

Following tools are required to implement this project.

### 2.4.1 GNU Octave

GNU Octave is a high-level interpreted language, primarily intended for numerical computations. Octave is normally used through its interactive command line interface (Figure 2.1).

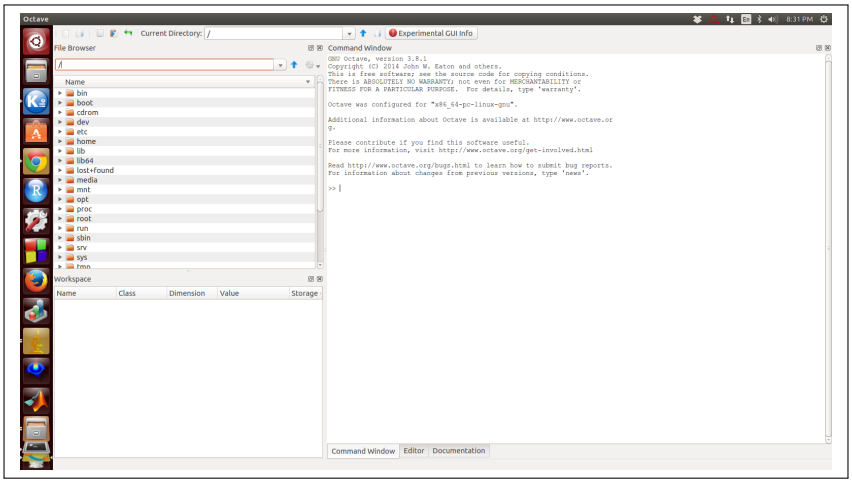


Figure 2.1: Octave Graphical User Interface

It can also be used to write non-interactive programs. The Octave language is quite similar to Matlab so that most programs are easily portable.

## 2.5 Conclusion

This chapter covers existing system and problems they address, their limitation followed by proposed system to overcome the limitations. The biological roots of CA, and also explanation of biological terminologies like CSC, TAC, TDC, ECM and proteolysis was covered. Various softwares required for project like C++, Code::Blocks, GNU Octave, ImageJ, RStudio, MATLAB® ,QtiPlot and Apache OpenOffice were described.

# Chapter 3

# System Requirements

## 3.1 Introduction

System requirements cover all of the requirements at the system level, which describe the functions the CA as a whole should fulfil to satisfy the requirements. It is expressed in an appropriate combination of textual statements, views and non-functional requirements; the latter expressing the levels of speed, reliability, scalability and standards to meet.

## 3.2 Software requirements

The softwares required to implement this project can be installed using sudo apt-get command or through Ubuntu Software Center, and usage of each has be mentioned in Table 3.1

| Software | Use |
|---|---|
| Ubuntu 14.04 | Operating System |
| C++ | Programming language to code biological properties and transitions. |
| Code::Blocks | IDE for C++ programming language |
| GNU Octave | Image generation |
| ImageJ | Video generation |
| RStudio | Plotting results |
| MATLAB® | Result quantification |
| QtiPlot | Plotting results |
| OpenOffice | Save quantifications |

Table 3.1: Softwares used

## 3.3 Hardware requirements

Hardware requirements define the minimal and optimal configurations for the system [54]. Following are the hardware requirements for this project

| | | |
|---|---|---|
| Processor | : | 1.5 GHz |
| Physical memory | : | 2 GB |
| Secondary memory | : | 47 GB |

## 3.4 Functional requirements

These are statements of services the system should provide, how the system should react to particular inputs, and how the system should behave in particular situations. In some cases, the functional requirements may also explicitly state what the system should not do [54]. In this project functional requirement describes the biological properties, constraints and operations that have to be satisfied by Mathematical Model, which should simulate biological cell division, spread of CSC as the function of intrinsic parameters, ECM, motility and proteolysis.

## 3.5 Non-functional requirements

Non Functional Requirements describe the constraints on development process and standards to be followed in development process.

1. Speed - on mentioned hardware requirement one simulation should run in around 5 minutes.
2. Reliability - simulation results should not vary drastically.
3. Scalability - simulation can be scaled to size of 10000 * 10000.
4. camelCase used for function names

   Empty List

## 3.6 Conclusion

The software, hardware, functional and non-functional requirements for the project was elicited covering various open source softwares, hardware requirements and nonfunctional requirements covering scalability, response time and biological constraints to be followed.

# Chapter 4

# System Analysis and Design

## 4.1 Introduction

Systems analysis is a problem solving technique that decomposes a system into its component pieces for the purpose of the studying how well those component parts work and interact to accomplish their purpose [51]. Systems design is the process of defining the architecture, components, modules, interfaces and data for a system to satisfy specified requirements. This chapter explains the modules of read configuration, initialize CA, update state of CA, properties of ES and BCs, save results, quantify and generate images.

## 4.2 Objectives of the system

The system should read the parameters from a configuration file, initialise CA with ECM, place one CSC in center and start simulation. The results should be saved, which should contain BC count, FD in each zone at every simulation step. Every ten simulation step entire CA state should be saved. For results of every ten simulation step there should be hassle free transformation to biological equivalent image.

## 4.3 System design

The proposed system is a Computational lattice consisting of cancer cell (C), ECM site (E) and free space (F).

## 4.4 Modules design

### 4.4.1 Get configuration

Get configuration module reads parameter name and the value from configuration file. Parameter types are integer or real.

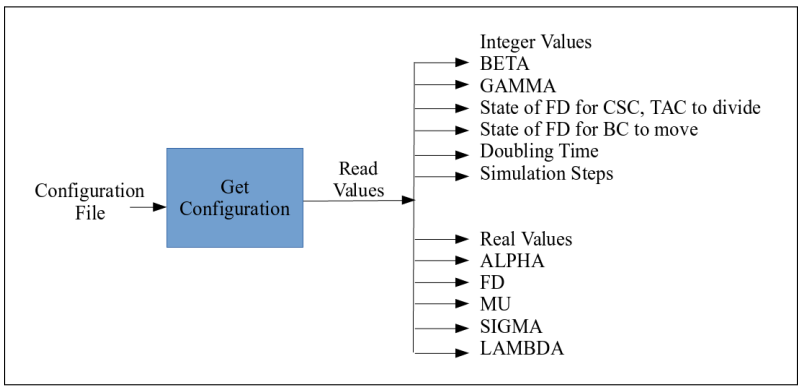


Figure 4.1: Get configuration

Integer parameters to be read are $\beta$, $\gamma$, state of fiber density for CSC, TAC to divide, state of fiber density for BC to move, Doubling Time, Simulation Steps. Real valued parameters to be read are $\alpha$, FD, $\mu$, $\sigma$, $\lambda$. Get configuration module then forwards the value to initialization module.

### 4.4.2 Initialization of Cellular Automata

Initialization module takes input from get configuration module and sets properties of CA, and places one CSC in center, surrounded by ES with FD as fiber density and about $\sigma$ number of ES (Figure 4.2 and Figure 4.3). It then calls simulation module.

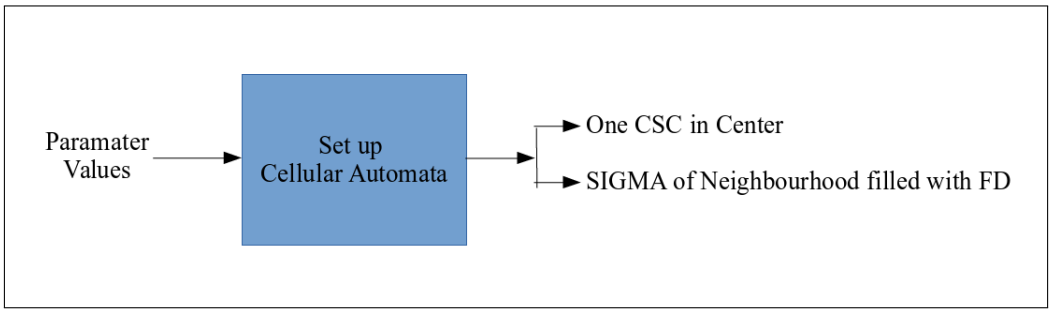


Figure 4.2: Initialization of Cellular Automata

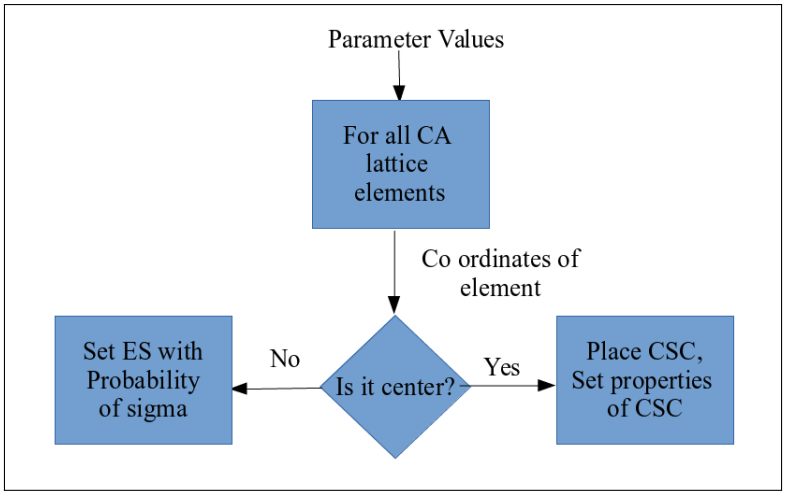


Figure 4.3: Flow for initialization of Cellular Automata

## 4.5 Conclusion

This chapter explains about different units like read configuration, initialization, update CA, properties of ES and BC, quantify results and generate images modules in detail that are used to design the system.

# Chapter 5

# System Implementation

## 5.1 Introduction

This chapter explains the algorithmic procedure of the implementation of CSC and TAC division, TDC update, biological cell migration, ECM proteolysis by biological cell and an algorithm of the entire system.

## 5.2 Biological properties and its implementation

CSC driven tumor growth follows a stem-cell like hierarchical cell division and specification program, a hierarchical cell division program was considered to implement cell division in the model. In this model, a CSC can either divide symmetrically (where it generates 2 CSC) or asymmetrically (where a single CSC generates 1 CSC and 1 TAC). TAC generated through asymmetric division of CSC possess a proliferation potential of $\beta$ i.e. it can divide maximum $\beta$ number of times [19].

## 5.3 Pre settings for Cellular Automata

This CA model is based on a discrete model of hierarchically structured cells. Biological cells are modelled as matrix element in a 200 x 200 two dimensional matrix. Two extra rows above and below, and to left and right enclose the CA. One of them to provide cell to migrate, and one to provide the neighbourhood for the migrated cell. Every Simulation Step, cell is selected in random to apply update function, as to avoid any bias in the way that cells are chosen [9]. Each cell can be in only one of four states CSC, TAC, TDC or ES.

In CA, we are considering properties of age, doubling time, size, fiber degradation potential, sensing radius, remaining rounds of amplification and motility potential for BCs and Fiber Density(FD) for ES.

1. Type to represent if cell is BC(CSC, TAC or TDC) or ES.
2. Identity of cell $\in \mathbb{Z}^+$, which is unique for each cell and identifies it.

### 5.3.1 Properties of biological cell

(a) Age $\in \mathbb{N}$, holds age of BC.

(b) Doubling time $\in \mathbb{N}$, is time after which CSC or TAC divide.

(c) Size $\in \mathbb{N}$, decides how many matrix cells the BC occupy, for all the simulations we set it to 1. 1 BC or ES occupies one matrix cell.

(d) Proteolysis propensity $\in$ (0,1), $\lambda$ is factor proteolysis of neighbourhood ES by BC, initially set to 0.5 for BCs.

(e) Sensing radius $\in \mathbb{N}$ is number of immediate neighbours properties a cell can sense, we use 1 which gives eight cells surrounding the given cell or forms Moore neighbourhood. Eight neighbours of a cell are the cells surrounding the cell one each above and below, to left and right and four diagonally.

(f) Remaining rounds of amplification $\in \mathbb{N}$ is still how many divisions can TAC undergo before it stops dividing.

(g) Motility potential $\in$ (0,1), $\mu$ is P(BC will move).

This CA model investigates the growth dynamics of CSCs with different parameters and compare population of BCs, to predict BC heterogeneity, saturation as a function of $\alpha$, $\beta$, $\gamma$, $\sigma$, $\mu$ and $\lambda$. All simulation is seeded with one CSC in the center of CA, and varying are parameters ($\alpha$, $\beta$, $\gamma$), $\sigma$, $\mu$, $\lambda$ and assuming uniform nutrient supply.

## 5.4 Cell division

In this project the model considers CSCs and TACs as the only BC that divide. Every BC age. CSCs and TACs divide into present and a randomly selected neighbouring ES only when their age $\geq$ Doubling Time and the ES has no fibers, analogous to cells growing for some time before dividing again.

Algorithm: Cell Division, checks the type of cell and initiate respective divide.

**Data:** present cell and neighbourhood ES details
**Result:** initiate CSC or TAC division if conditions hold
**if** cell.type == CSC **then**
  cell.divideCSC();
**else if** cell.type == TAC **then**
  cell.divideTAC();
**end if**

## 5.5 Simulation

Simulation algorithm: At each iteration of the simulation, a CA cells is randomly selected and evolved based on some mechanistic rules (Algorithm of Simulation). If the selected site is of type S (free space) or E (ECM site) then nothing happens. Else (if selected site is C (cancer cell)) the age of the cell is increased by an amount $\delta$ which is determined based on fiber density of the neighbourhood [31, 32]. After increasing cell age, the cell select a

neighbouring ECM_SITE or FREE_SITE randomly. BCs can move into or divide in neighbourhood ES only if FD = 0. If there is no ECM_SITE or FREE_SITE in neighbourhood of cell, cell undergo transformation where it can either convert to a TDC. If the type of cell is TAC and its current $\beta$ value is greater than 0, or die out and if the type of cell is TDC and its age is equal to $\gamma$ value.

Algorithm of Simulation

**for** 1 to NUM_OF_ITERATIONS **do**
- **for** ROWS * COLUMNS times **do**
  - cell ← Randomly select an automata cell; **if** (cell.type == E OR cell.type == S) **then**
    - // No action is performed
  - **else**
    - // Increase cell age by $\delta$, $\delta = 1 + 1.125 * \frac{[FD]}{[FD]+1}$; $\delta$ = 1 for TDC;
    - cell.age ← cell.age + $\delta$
    - **if** There is no ECM_SITE or FREE_SITE in neighbourhood **then**
      - **if** cell.type == TAC AND cell.$\beta$ = 0 **then**
        - cell.type = TDC
      - **end**
      - **if** cell.type == TDC AND cell.age = $\gamma$ **then**
        - cell.type = ECM_SITE
      - **end**
    - **else**
      - neighbourES ← randomly select one ECM_SITE from neighbourhood;
      - **if** neighbourES.FiberDensity = 0 **then**
        - **if** cell.age $\geq$ DOUBLING_TIME and cell.type == CSC or TAC **then**
          - **if** cell.type == CSC **then**
            - Perform symmetrical division of cell with probability $\alpha$ or asymmetrical division with probability (1-$\alpha$).
          - **else**
            - Generate two daughter cells with $\beta$ less than the mother $cell$.
          - **end**
        - **else**
          - Move $cell$ to neighbourES free site with probability $\mu$.
        - **end**
      - **else**
        - $\lambda = 0.5625 \times \frac{[FD]}{[FD]+1}$;
        - neighbourES.FiberDensity = neighbourES.FiberDensity - $\lambda$ ;
      - **end**
    - **end**
  - **end**
- **end**
- Save state of system;

**end**

## 5.6 The main results

Four main conclusions that can be drawn from implementation are as follows (Color code to used to represent BCs in images are CSC as red, TAC as green, TDC as blue, ES as grey and free space as white. TAC with higher remaining rounds of amplification as higher green values. ES with higher fiber content with as higher grey value):

**ECM confinement can check tumor growth in absence of ECM proteolysis** : Availability of free space is one of the main prerequisites for cell division. In tissue environment, presence of other type of cells and ECM fibers creates a space constraints for dividing cells.

## 5.7 Conclusion

This chapter covered detailed algorithmic procedures for the biological properties of divide, move and mathematical formulae applied to update proteolysis propensity and FD in ES. The analysis of results suggests CSC symmetric division rate fosters tumor growth and controls intratumor cellular heterogeneity, ECM confinement can check tumor growth in absence of ECM proteolysis, increased cell motility facilitates cancer progression in confined ECM by enhancing TAC content of tumor population and ECM confinement enhances tumor progression in presence of ECM proteolysis and increased cell motility.

# Chapter 6

# System Testing

## 6.1 Introduction

System testing of software is testing conducted on a complete, integrated system to evaluate the system's compliance with its specified requirements. This chapter covers unit and integration testing. Unit testing can be done by checking each biological property like division, motility and proteolysis. Integration testing combines all biological properties and checks if it still satisfies the set Mathematical Model.

## 6.2 Unit testing

In unit testing the smallest testable parts of an application, called units, are individually and independently scrutinized for proper operation. Unit test were conducted on units of read configuration, initialization of CA, divide, move, proteolysis, properties of ES and BC, save results and quantify. Each unit performing its task as expected.

### 6.2.1 Read configuration

The read configuration module should read values from configuration file, which is being carried out.

## 6.3 Integration testing

Integration testing is a level of software testing where individual units are combined and tested as a group.

### 6.3.1 Test cases

Common settings FD Threshold = 0, FD = 1, Doubling Time = 50, Simulation Steps = 1400

1. Asymmetric Division as $\alpha = 0$, $\beta = 1$, $\gamma = 100$, $\sigma = 0$, $\mu = 0$, $\lambda = 0$.
2. Symmetric Division as $\alpha = 1$, $\beta = 1$, $\gamma = 100$, $\sigma = 0$, $\mu = 0$, $\lambda = 0$.
3. With Confinement as $\alpha$ 0.5, $\beta = 5$, $\gamma = 200$, $\sigma = 0.5$, $\mu = 0$, $\lambda = 0$.
4. With Motility as $\alpha = 0.5$, $\beta = 5$, $\gamma = 200$, $\sigma = 0.5$, $\mu = 0.5$, $\lambda = 0$.
5. With Proteolysis as $\alpha = 0.5$, $\beta = 5$, $\gamma = 200$, $\sigma = 0.5$, $\mu = 0.5$, $\lambda = 0.5$.

### 6.3.2 Results and snapshots

Results obtained for test cases mentioned in 6.3.1:

| | Asymmetric | Symmetric | Confinement | Motile | Proteolysis |
|---|---|---|---|---|---|
| CSC in Zone 1 | 1 | 2179 | 20 | 28 | 1330 |
| CSC in Zone 2 | 0 | 0 | 0 | 0 | 1054 |
| CSC in Zone 3 | 0 | 0 | 0 | 0 | 807 |
| CSC in Zone 4 | 0 | 0 | 0 | 0 | 576 |
| CSC in Zone 5 | 0 | 0 | 0 | 0 | 448 |
| TAC in Zone 1 | 0 | 0 | 28 | 175 | 4678 |
| TAC in Zone 2 | 0 | 0 | 0 | 0 | 5079 |
| TAC in Zone 3 | 0 | 0 | 0 | 0 | 5112 |
| TAC in Zone 4 | 0 | 0 | 0 | 0 | 4312 |
| TAC in Zone 5 | 0 | 0 | 0 | 0 | 2655 |
| TDC in Zone 1 | 38 | 0 | 20 | 515 | 2087 |
| TDC in Zone 2 | 0 | 0 | 0 | 1 | 1893 |
| TDC in Zone 3 | 0 | 0 | 0 | 0 | 2250 |
| TDC in Zone 4 | 0 | 0 | 0 | 0 | 2276 |
| TDC in Zone 5 | 0 | 0 | 0 | 0 | 1661 |
| Total CSC | 1 | 2179 | 20 | 28 | 4215 |
| Total TAC | 0 | 0 | 28 | 175 | 21836 |
| Total TDC | 38 | 0 | 20 | 516 | 10167 |
| Total Cells | 39 | 2179 | 68 | 719 | 36218 |
| CSC Percentage | 0.0256 | 1 | 0.2941 | 0.0389 | 0.1163 |
| ES in Zone 1 | 0 | 0 | 4065 | 4126 | 0 |
| ES in Zone 2 | 0 | 0 | 3982 | 3997 | 0 |
| ES in Zone 3 | 0 | 0 | 4168 | 4204 | 0 |
| ES in Zone 4 | 0 | 0 | 4074 | 3976 | 186.349 |
| ES in Zone 5 | 0 | 0 | 3826 | 3759 | 1138.28 |
| Total ES | 0 | 0 | 20115 | 20062 | 1324.629 |

Table 6.1: Quantification of test cases

### 6.3.3 Asymmetric division

Only asymmetric division should result in only one CSC, rest of BC will be TAC or TDC, which is verified in (Table 6.1 Asymmetric) and (Figure 6.1 A).

### 6.3.4 Symmetric division

Only symmetric division should result in all BC being CSC, which is verified in (Table 6.1 Symmetric) and (Figure 6.1 B).

### 6.3.5 With confinement

With ECM confinement should result in less proliferation, number of BC and zones proliferated to is lesser than with motility case ( 6.3.6), which is verified in (Table 6.1 Confinement) and (Figure 6.1 C). No change in ES, and around 20000 number of ES as $\sigma$ = 0.50

### 6.3.6 With motility

BC with motility but without proteolysis propensity, but BCs will have higher proliferation so BCs found in outer zones also as compared to with confinment case ( 6.3.5), which is verified in (Table 6.1 Motility) and (Figure 6.1 D). No change in ES, and around 20000 number of ES as $\sigma$ = 0.50

### 6.3.7 With proteolysis

There should be change in ES count as BC now have ECM proteolysis capabilities as compared to confinement( 6.3.5) and motility case( 6.3.6) where ES count does not change, This is verified in (Table 6.1 Proteolysis) and (Figure 6.1 E).

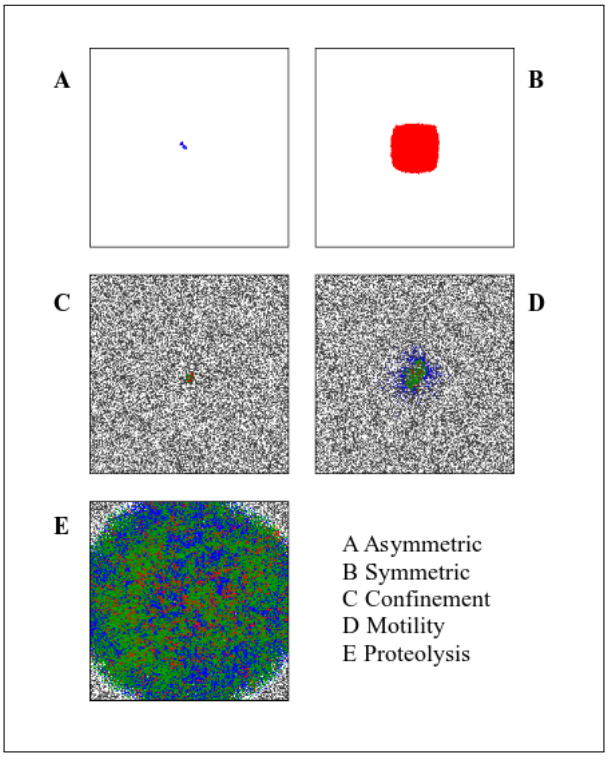


Figure 6.1: Test case results

## 6.4 Conclusion

Unit test cases covered testing of basic biological properties and integration testing of collection of the basic biological properties as one system. Test cases are matching expected results of biological settings of intrinsic parameters, with confinement, with motility and with proteolysis.

# Results

## Introduction

This chapter covers assumptions made for parameters values to run simulations and format of files that save results of simulations. Configuration file consists the setting values for which simulation has to be run, consisting of Fiber Threshold, FD, Doubling Time, Number of Simulation Steps, $\beta$, $\gamma$, $\lambda$, $\alpha$, $\sigma$, $\mu$.

## Parameter values and model assumptions

Due to limited understanding of CSC dynamics and availability of only some of the experimental data, there are several assumptions made to construct the current version of the model.

## Conclusion

The .csv result file help in faster quantification when used with MATLAB®. Only one single txt file is being used to save the entire state of CA which includes FD, FS, cell types, remaining rounds of amplification of TAC and age of TDC. This helps in quicker generation of images of biological equivalent state while reducing space requirements to save results.

# Conclusion and Future work

This project provides a comprehensive study of collective role of ECM confinement, cell motility and ECM proteolysis on hierarchical theory based tumor development mediated by CSC. In existing systems CA have been modelled to explore CSCs proliferation as factor of only oxygen diffusion, motility or ECM proteolysis. Leading to proposed system which overcomes the limitations by integrating all of ECM architecture, motility and ECM proteolysis in one single CA model.

While current study provides many new insights about CSC driven tumor development and generation of intra-tumor heterogeneity. Major conclusions that can be drawn from results are CSC symmetric division rate fosters tumor growth and controls intra-tumor cellular heterogeneity, ECM confinement can check tumor growth in absence of ECM proteolysis, Increased cell motility facilitates cancer progression confined ECM and enhances TAC content of tumor population, Cell motility and ECM proteolysis suppresses the effect of ECM confinement and enhances tumor progression.

There are several aspects like BCs properties like contractility, higher sensing radius and ECM cross linking not taken into account in this work and requires further extension of this model. Contractility influences motility of biological cells, higher sending radius would enlarge neighbourhood to be considered for ES and BC properties and ECM cross linking in fibers gives additional stiffness to ECM. Understanding the role of mutation and other cellular transformation processes like EMT are the future directions of this work.

# Outcome of the thesis

Paper submitted for review in PLOS journal with the title “ECM confinement, cell motility and ECM proteolysis collectively regulate CSC driven tumor growth and intra-tumor heterogeneity” with authors Author1, Author2 and Author3.

# Bibliography


[1] Southam CM, Brunschwig A, “Quantitative studies of autotransplantation of human cancer. preliminary report”, Cancer 14: 971–978, 1961

[2] Reya T, Morrison SJ, Clarke MF, Weissman IL, “Stem cells, cancer, and cancer stem cells”, nature 414: 105–111, 2001

[3] Dick JE, “Breast cancer stem cells revealed”, Proceedings of the National Academy of Sciences 100: 3547–3549, 2003

[4] Schatton T, Murphy GF, Frank NY, Yamaura K, Waaga-Gasser AM, et al, “Identification of cells initiating human melanomas”, Nature 451: 345–349, 2008

[5] T. Stiehl and A. Marciniak-Czochra, “Mathematical Modeling of Leukemogenesis and Cancer Stem Cell Dynamic”, Math. Model. Nat. Phenom. Vol. 7, No. 1, 2012, pp. 166-202 DOI: 10.1051/mmnp/20127199, 2012

[6] KA. Moore, IR. Lemischka, “Stem cells and their niches”, Science, 311, No. 5769,1880-1805, 2006

[7] Von Neumann, J. and A. W. Burks, “Theory of self-reproducing automata”, Urbana, University of Illinois Press, 1966

[8] Jiao Y, Torquato S, “Emergent Behaviors from a Cellular Automaton Model for Invasive Tumor Growth in Heterogeneous Microenvironments”, PLoS Comput Biol 712: e1002314, 2011

[9] Scott JG, Hjelmeland AB, Chinnaiyan P, Anderson ARA, Basanta D, “Microenvironmental Variables Must Influence Intrinsic Phenotypic Parameters of Cancer Stem Cells to Affect Tumourigenicity”, PLoS Comput Biol 101: e1003433, 2014

[10] Al-Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF, “Prospective identification of tumorigenic breast cancer cells”, Proceedings of the National Academy of Sciences 100: 3983–3988, 2003

[11] Al-Hajj M, Becker MW, Wicha M, Weissman I, Clarke MF, “Therapeutic implications of cancer stem cells”, Current opinion in genetics & development 14:43–47, 2004

[12] Singh SK, Clarke ID, Hide T, Dirks PB, “Cancer stem cells in nervous system tumors”, Oncogene 23: 7267–7273, 2004

[13] Singh SK, Clarke ID, Terasaki M, Bonn VE, Hawkins C, et al, “Identification of a cancer stem cell in human brain tumors”, Cancer research 63:5821–5828, 2003

[14] Kondo T, Setoguchi T, Taga T, “Persistence of a small subpopulation of cancer stem-like cells in the c6 glioma cell line”, Proceedings of the National Academy of Sciences of the United States of America 101: 781–786, 2004

[15] Setoguchi T, Taga T, Kondo T, “Cancer stem cells persist in many cancer cell lines”, Cell cycle 3: 412–413, 2004

[16] Dean M, Fojo T, Bates S, “Tumour stem cells and drug resistance”, Nature Reviews Cancer 5: 275–284, 2005

[17] Donnenberg VS, Donnenberg AD, “Multiple drug resistance in cancer revisited: the cancer stem cell hypothesis”, The Journal of Clinical Pharmacology 45: 872–877, 2005

[18] Scott JG, Hjelmeland AB, Chinnaiyan P, Anderson ARa, Basanta D, “Microenvironmental variables must influence intrinsic phenotypic parameters of cancer stem cells to affect tumourigenicity”, PLoS computational biology 10:e1003433, 2014

[19] Poleszczuk J, Hahnfeldt P, Enderling H, Rzhetsky A, “Evolution and phenotypic selection of cancer stem cells”, PLoS computational biology 11:e1004025–e1004025, 2015

[20] Morrison SJ, Kimble J, “Asymmetric and symmetric stem-cell divisions in development and cancer”, Nature 441: 1068–1074, 2006

[21] Caussinus E, Gonzalez C, “Induction of tumor growth by altered stem-cell asymmetric division in drosophila melanogaster”, Nature genetics 37: 1125–1129, 2005

[22] Hanahan D, Weinberg RA, “The hallmarks of cancer”, cell 100: 57–70, 2000

[23] Hanahan D, Weinberg RA, “Hallmarks of cancer: the next generation”, cell 144: 646–674, 2011

[24] Levental KR, Yu H, Kass L, Lakins JN, Egeblad M, et al. “Matrix crosslinking forces tumor progression by enhancing integrin signaling”, Cell 139:891–906, 2009

[25] Lu P, Weaver VM, Werb Z, “The extracellular matrix: a dynamic niche in cancer progression”, The Journal of cell biology 196: 395–406, 2012

[26] Sottoriva A, Verhoeff JJC, Borovski T, McWeeney SK, Naumov L, et al, “Cancer stem cell tumor model reveals invasive morphology and increased phenotypical heterogeneity”, Cancer research 70: 46–56, 2010

[27] Friedl P, Wolf K, ”Tumour-cell invasion and migration: diversity and escape mechanisms”, Nature Reviews Cancer 3: 362–374, 2003

[28] Wolf K, Wu YI, Liu Y, Geiger J, Tam E, et al, “Multi-step pericellular proteolysis controls the transition from individual to collective cancer cell invasion”, Nature cell biology 9: 893–904, 2007

[29] Friedl P, Gilmour D, “Collective cell migration in morphogenesis, regeneration and cancer”, Nature reviews Molecular cell biology 10: 445–457, 2009

[30] Enderling H, Alexander NR, Clark ES, Branch KM, Estrada L, et al, “Dependence of invadopodia function on collagen fiber spacing and cross-linking:computational modelling and experimental evidence”, Biophysical journal 95:2203–2218, 2008

[31] Klein EA, Yin L, Kothapalli D, Castagnino P, Byfield FJ, et al, “Cell-cycle control by physiological matrix elasticity and in vivo tissue stiffening”, Current Biology 19: 1511–1518, 2009

[32] Ulrich TA, de Juan Pardo EM, Kumar S, “The mechanical rigidity of the extracellular matrix regulates the structure, motility, and proliferation of glioma cells”, Cancer research 69: 4167–4174, 2009

[33] Alexander NR, Branch KM, Parekh A, Clark ES, Iwueke IC, et al, “Extracellular matrix rigidity promotes invadopodia activity”, Current Biology 18:1295–1299, 2008

[34] Das A, Kapoor A, Mehta GD, Ghosh SK, Sen S, “Extracellular matrix density regulates extracellular proteolysis via modulation of cellular contractility”, J Carcinogene Mutagene S 13, 2013

[35] Schneider CA, Rasband WS, Eliceiri KW, “Nih image to imagej: 25 years of image analysis”, Nature methods 9: 671–675, 2012

[36] Ricci-Vitiani L, Lombardi DG, Pilozzi E, Biffoni M, Todaro M, et al, “Identification and expansion of human colon-cancer-initiating cells”, Nature 445:111–115, 2007

[37] Friedl P, Wolf K, “Plasticity of cell migration: a multiscale tuning model”, The Journal of cell biology 188: 11–9, 2010

[38] Wolf K, Friedl P, “Mapping proteolytic cancer cell-extracellular matrix interfaces”, Clinical & experimental metastasis 26: 289–98, 2009

[39] Zaman MH, Trapani LM, Sieminski AL, Mackellar D, Gong H, et al, “Migration of tumor cells in 3D matrices is governed by matrix stiffness along with cell-matrix adhesion and proteolysis”, Proceedings of the National Academy of Sciences of the United States of America 103: 10889–10894, 2006

[40] Carey SP, Kraning-Rush CM, Williams RM, Reinhart-King CA, “Biophysical control of invasive tumor cell behavior by extracellular matrix microarchitecture”, Biomaterials 33: 4157–4165, 2012

[41] Stein RL, “Mechanism of Enzymatic and Nonenzymatic Prolyl cis-trans Isomerization”, Adv. Protein Chem., 44, 1–24. 1993

[42] Schmid FX, Mayr LM, Mücke M and Schönbrunner ER, “Prolyl Isomerases: Role in Protein Folding”, Adv. Protein Chem., 44, 25–66. 1993

[43] Fischer G, “Peptidyl-Prolyl cis/trans Isomerases and Their Effectors”, Angew. Chem. Int. Ed. Engl., 33, 1415–1436. 1993

[44] Hopcroft, John E.; Motwani, Rajeev; Ullman, Jeffrey D, “Introduction to Automata Theory, Languages, and Computation”, 2 ed.. Addison Wesley. ISBN 0-201-44124-1. Retrieved 19 November 2012

[45] Lodish H, Berk A, Zipursky SL, Matsudaira P, Baltimore D, et al, “Molecular Cell Biology”, 4th ed, Section–22.3. WH Freeman, 2000

[46] Black, Paul E, “Finite State Machine. Dictionary of Algorithms and Data Structures”, U.S. National Institute of Standards and Technology, 12 May 2008

[47] von Neumann, John, “First Draft of a Report on the EDVAC”, archived from the original on March 14, 2013, retrieved August 24, 2011, 1945

[48] Alberts B, Johnson A, Lewis J, et al, “Molecular Biology of the Cell”, 4th edition. New York: Garland Science, 2002

[49] Ira Pohl, University of California, Santa Cruz, “C++ By Dissection”, Addison-Wesley, 18 Oct 2001

[50] Philip Maini, Andreas Deutsch, Sabine Dormann, Germany, “Cellular Automaton Modeling of Biological Pattern Formation Characterization, Applications, and Analysis”, Birkhauser, 2005

[51] Lonnie D. Bentley, “Systems Analysis and Design for the Global Enterprise”, p.160 7th edition

[52] Walker, CBE, FRSE, Peter M. B., “Cambridge Dictionary of Science and Technology” reprint ed., Edinburgh: Press Syndicate of the University of Cambridge. p. 658. ISBN 0521394414, ed. 1990

[53] Pauling L, “The Nature of the Chemical Bond”, 3rd. ed, Cornell University Press. ISBN 0-8014-0333-2, 1960

[54] Ian Sommerville, “Software engineering”, 9th ed, ISBN-13: 978-0-13-703515-1, ISBN-10: 0-13-703515-2, 2011