Phase-Field Models for Tumor Growth in the Avascular Phase

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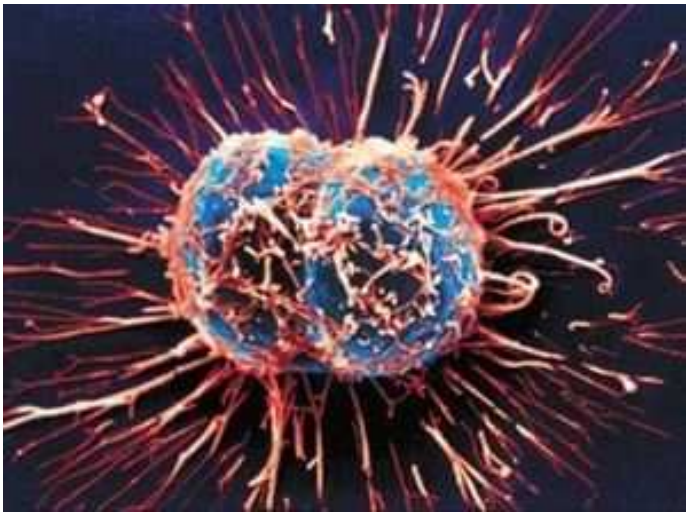

Figure 1: Cancer Cells Splitting [1]

"Growth for the sake of growth is the ideology of the cancer cell."

Edward Abbey (The Journey Home, 1977)

Abstract

Tumor is an abnormal growth of mass tissue resulting from several and successive cell divisions without control, caused by modifications in DNA genome of the triggering cell, i. e. this initial cell suffered several mutations.

To proliferate the tumor has to overcome several external factors such as the body's immune system that considers it a foreign body and tries to fight it, the pressure exerted by the extracellular matrix and adjacent normal cells and has yet to fully capture all the nutrient needed to feed its cells and thus enable them to be divided and the tumor can continue to proliferate.

However, the tumor does not grow indefinitely, there is a moment when the nutrient released from blood vessels and capillaries present within the body is no longer enough to feed all the tumor cells and some begin to die, usually is the center of the tumor that becomes necrotic.

In principle, at this time the tumor ceases to grow reaching an equilibrium state where the cells that die at each time offset the new cells formed.

This steady state occurs when the tumor reaches approximately 2 mm in diameter and at that time the tumor develops new skills that allows it to continue proliferating. May be through the induction of angiogenesis, the movement in the body or can become malignant and metastasize.

The work developed on this project aims to simulate the evolution of the tumor until the time it reaches the steady state and is able to produce VEGF to induce the formation of a new capillary network that will provide it all the support and necessary food.

The mathematical model used is continuous and multi-phase, taking into account that the tumor growth is nutrient limited.

The system has two cell phases, characterized by an order parameter ϕ, one phase is compound of tumor cells ($\phi = 1$) and the other is compound of normal cells ($\phi = -1$). This system has a blood vessel located at one end and provides nutrient at each time step, nutrient starting condition is linear.

For the tumor, the initial condition is circular and has a well defined interface that penalizes the spatial variations of phase, avoiding unwanted phase fluctuations around it. The model also considers the cell density, which reproduces more effectively the surrounding environment of the tumor.

The results present the study of the tumor's evolution according to the rates of growth and apoptosis, and depending on the mobility of the cellular matrix too. The goal was to verify that the tumor eventually cease their growth at a certain moment. What we observe is that at a given time the tumor began to stop growing and reach the steady state, but at that moment moves towards the capillary in order to get more nutrients and to grow again.

It was verified that the movement towards the capillary is an effective way for the tumor to continue proliferating.

Resumo

Tumor é o crescimento anormal de uma massa de tecido resultante de várias divisões celulares sucessivas sem controlo, originadas por alterações no genoma do DNA da célula precurssora, ou seja esta célula inicial sofreu várias mutações.

Para proliferar o tumor tem de vencer diversos factores externos, tais como o sistema imunitário do organismo que o considera um corpo estranho e tenta combatê-lo, a pressão exercida pela matriz extracelular e pelas células normais adjacentes e tem ainda de conseguir captar todo o nutriente necessário para alimentar as suas células e possibilitar deste modo que elas se dividam e o tumor possa continuar a proliferar.

Contudo o tumor não cresce indefinidamente, há um momento em que o nutriente libertado pelos vasos e capilares sanguíneos presentes naquela zona do organismo já não é suficiente para alimentar todas as células do tumor e algumas começam a morrer, geralmente o centro do tumor torna-se necrótico.

Em principio, nesta altura o tumor deixa de crescer atingindo-se um estado de equilibrio em que as células que morrem em cada instante compensam as novas células formadas.

Esse estado estacionário ocorre quando o tumor atinge cerca de 2 mm de diâmetro e nesse momento o tumor desenvolve novas características que lhe permitem continuar a proliferar. Pode ser através da indução de angiogénese, da deslocação no organismo ou pode tornar-se maligno e metastizar-se.

O trabalho desenvolvido neste projecto pretende simular a evolução do tumor até ao momento em que este atinge o estado estacionário e se encontra em condições de produzir VEGF para induzir a formação de uma nova rede de capilares que lhe fornecerão todo o suporte e alimento necessário.

O modelo matemático desenvolvido é contínuo e multi-fase, tendo em consideração que o crescimento tumoral é limitado pelo nutriente.

O sistema possui duas fases celulares, caracterizadas pelo parâmetro de ordem ϕ, uma fase composta pelas células normais ($\phi = -1$) e a outra pelas células tumorais ($\phi = 1$). Este sistema possui um vaso sanguíneo que se encontra num dos extremos e fornece nutriente em todas as iteracções, tendo uma condição inicial linear. Quanto ao tumor a sua condição inicial é circular e possui uma interface bem definida que penaliza as variações espaciais de fase, evitando flutuações de fase indesejadas em torno da mesma. O modelo considera também a densidade celular, reproduzindo assim de forma mais eficaz o ambiente circundante do tumor.

Os resultados apresentam o estudo da variação da evolução do tumor em função das taxas de crescimento e apoptose das células de tumor, bem como em função da mobilidade da matriz celular. O objectivo era verificar que o tumor acaba por cessar o crescimento num determinado momento. O que se observou foi que o tumor quando estava a parar de crescer e a atingir o estado estacionário se movia em direcção ao capilar de modo a conseguir mais nutriente e poder crescer novamente.

Foi verificado que a deslocação em direcçâo ao capilar é uma forma eficaz do tumor continuar a sua proliferação.

Aknowledgments

In first place I want to thank Dr. Rui Travasso that followed me more closely during this year and with an endless patience guided and helped me in this project. I also want to thank Dr. Fernando Nogueira that helped me whenever I needed and all the Center for Computational Physics colaborators that welcomed me in the best way possible.

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The purpose of studying in Coimbra is not only to get a degree, but also to meet people. Thanks to all those who have become my friends and deserve all my respect and friendship. Thanks also to all the friends that I did not meet in Coimbra but accompanied and supported me over these years.

Now a special thanks to the teachers of this graduation, particularly to Professor Dr. Miguel Morgado, that was always available to answer our questions and help with any problems that arose.

Lack only mention the misfortunes that taught me a lot and allowed me to evolve in personal and professional life.

If I forgot someone, here's a thank you as well.

List of Abreviations

DNA - Deoxyribonucleic Acid

VEGF - Vascular Endothelial Growth Factor

G_1 - Phase Gap 1

S - Sintesis Phase

G_2 - Phase Gap 2

M - Mitosis Phase

G_0 - Quiescent Phase

mm - Milimeters

CA - Cellular Automaton

ECM - Extracellular Matrix

List of Symbols

ϕ - Order parameter that describes the cell types

α_c - Cells consumption rate

D - Nutrient diffusion rate

n - Nutrient concentration

r - Radius of the tumor

ε - Interface width

α_r - Tumor proliferation factor

G - Tumor growth rate

A - Tumor apoptosis rate

M - Cells mobility (First Model)

ρ - Cell density

M_ϕ - Order parameter mobility (Second Model)

M_ρ - Density mobility

ρ_t - Tumor cell density

ρ_n - Normal cell density

t - time

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1 Introduction

1.1 General Aspects

All over the word millions of people live with cancer diagnosis, so it is unquestionable the need to pursue the research within this topic.

Cancer is responsible for one in eight deaths worldwide[2]. This includes more than 100 different diseases with high diversity of epidemiology and risk factors that reaches the majority of cell types and organs of the human body. Cancer is characterized by a disorderly proliferation that may exceed the limits of normal tissue and metastasize to distant organs. All cancers arise as a result of changes in DNA sequence of the cancer cells genome [3].

Drugs that aim to block the blood supply to tumors, known as angiogenesis inhibitors, have been blamed for being started a new era in the cancer therapy. Targeting the blood vessels that feed tumors was not the solution expected to fight tumors, but refinements in strategy may suggest important ways to treat the disease [4, 5, 6, 7].

Models that are purely *in vitro*, such as focus formation and anchorage-independent growth (term used to characterize cells that do not require a solid substratum for growth, i.e., the solid glass or plastic surface of a culture dish or micro-carrier beads. Such cells can be grown in suspension or soft media in which they float freely [8]), are not particularly adjustable to study the relationship and evolution of tumor cells in their biological microenvironment, the crucial game between cancer cells and different types of surrounding normal cells or even for testing the potential efficacy of therapies directed at the tumor microenvironment[9].

In vivo studies of tumors are challenging due to the small size of animal models and because most of the time what was observed is the interaction between the human tumor cells and the animal organism. There are some interesting *in vivo* platforms for studying the tumor growth within an human cellular microenvironment by developing a teratoma into an immunocompromised mouse[9]. But even in that cases the tumor requires time to grow naturally.

The tumor cell's characteristics could change and adapt themselves to the surrounding microenvironment in order to survive and proliferate [10, 11]. A computational model may not reproduce all the biological variables, but could help to characterize and quantify those variables.

These are some of the reasons that lead to the importance of designing a computational model that is able to simulate tumor growth before and after angiogenesis induction.

1.2 Objectives

Cancer may not be the most important cause of death in the world, but it is still on the top of those causes[2]. It is very important to understand how a tumor develops and induces angiogenesis to keep increasing its volume, since if it does not induce the promotion of its own capillary network it remains in a stationary state, and may even decreases its size.

The main objective of this work is to simulate the tumor's growth and movement from an initial state, when blood supply from a pre-existent vessel is enough, to its critical volume, when tumor releases Vascular Endothelial Growth Factor (VEGF) in order to induce angiogenesis and provide its own blood supply.

It is important to study and analyse the favorable conditions for tumor growth and what happens within its biological microenvironment in order to stop the growth. One of the most important stages of the tumor evolution is when it achieves a steady state and induces angiogenesis or develops new skills to continue growing, like migrating on the organism in order to arrive in a densely vascularized and well-perfused organ, such as brain, liver, lung or kidneys.

2 Theoretical Background

2.1 Introduction

The cells are the structural and functional units of all known life forms and are the smallest unit of an organism that is considered living.

The cell's renovation and repair involves proliferation and cell differentiation. Proliferation is the process of cell division, it is an adaptive mechanism inherent to body cell's replacement and occurs as a consequence of cell death or additional need of cells in the body. Differentiation is the specialization's process by which new cells acquire the structure and function of the cells that they will replace [12].

In adult tissues, the size of a cell population is determined by the rates of proliferation (process of cell division), differentiation (process of cell specialization/maturation) and death by apoptosis (process of cellular self destruction) [12, 13, 14, 15].

In normal tissue proliferation is a regulated process that allows the equilibrium between new cells formation and apoptosis [12], but in biology all the rules have exceptions, and tissue proliferation is not always perfect.

The cell cycle, or cell-division cycle, is characterized by four distinct phases: G_1 phase, S phase, G_2 phase (also known as interphase) and M phase. M phase is itself composed of two tightly coupled processes, that are mitosis, in which the cell's chromosomes are divided between the two daughter cells, and cytokinesis, in which the cell's cytoplasm divides forming two distinct cells [13, 16].

In G_1 phase cells increase in size. The mechanism of G1 checkpoint control verify and ensure that all conditions are favorable to the beginning of DNA synthesis. The DNA replication occurs during the S phase [13].

During the G_2 phase cells continue to grow. The mechanism of G2 checkpoint control determines if the cell is ready to begin dividing. In the mitotic phase, the cell growth ceases and starts an orderly division of the initial

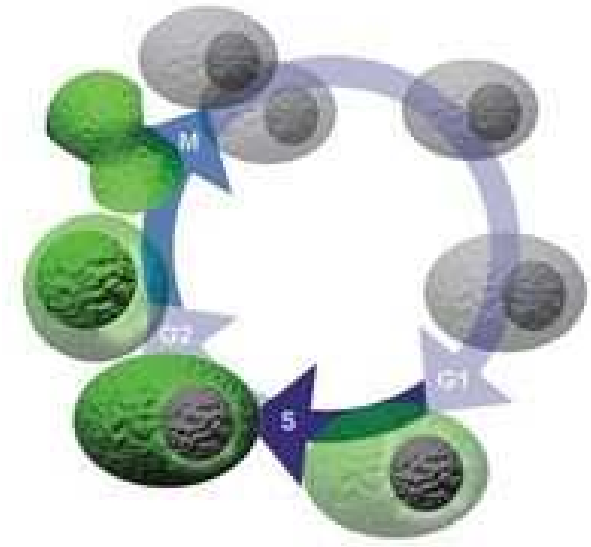

Figure 2: Cell Cycle Overview[17]

cell into two daughter cells. In the middle of mitosis is ascertained that cell division can be completed by a checkpoint [13].

Activation and begining of each phase is dependent on the proper progression and completion of the previous one.

Cells that have temporarily or reversibly stopped dividing, have entered in a quiescence state also called G_0 phase. That is a resting phase where the cell has left the cycle and stopped dividing. [13] (see Fig. 2).

The deregulation of several cell cycle components may lead to tumor formation. Many oncogenes regulate the cell cycle and a mutation of one of these may induce the cell to multiply uncontrollably, forming a tumor [12, 15].

Although the duration of cell cycle in tumor cells is equal to or longer than normal cell cycle, the proportion of cells that are in active cell division (versus quiescent cells in G_0 phase) in tumors is much higher than that in normal tissues [12, 13].

2.2 Cell Growth

The body tissues grow by increasing the number of cells that compose them. In an adult specimen most cells only reproduce themselves in order to replace others that have died or undergone damage, for example through injury or illness [14, 15, 18].

Not all cells have the ability to reproduce, most of them became mature and specialize in their particular job in the body. These mature cells loose the ability to reproduce as they develop. However there will always be enough stem cells around, that are undifferentiated, to replace cells that are damaged or killed [18, 19].

The normal cells growth and healing is a very regulated and precise process, regulated by chemical factors and intrinsic factors of cells.

In opposition to tumors, normal cells have a natural ability to stick together in the right place; this phenomenon is called cell adhesion. Molecules on the cell surface, are in contact with the molecules of the neighboring cells surfaces [19].

If the genes of a cell are very badly damaged, or if it becomes dissociated from its proper place, the cell will self destruct by the apoptosis process. A deeper understanding of the cell apoptosis mechanism and of its possible shortcomings will help the development of cancer treatments in the future [5, 19].

The tumor cells may develop the ability to avoid apoptosis, in which case the cells do not self destroy [14, 20, 21]. However they can not prevent necrosis, which is the premature cell death, but when caused by external factors such as lack of blood supply [21].

2.3 Tumors

The common definition for Tumor is an abnormal growth of mass tissue, due to several cell's divisions without control after an initial cell mutation (DNA genome modification) [21, 22, 23]. Tumors can be cancerous (malignant) or non-cancerous (benignant).

However, a tumor is not a homogeneous mass composed of the same type of cancer cells multiplied indefinitely. It consists of cancer stem cells, mature, metastatic, stromal, endotheliais, etc..all embedded in an extracellular matrix [10]. In tumor cells two or more of those characteristics are absent [21].

The normal body cells have some important characteristics, like the ability to control and regulate their proliferation, stick together, become specialised or 'mature' and self destruct if any of these mechanisms is malfunctioning. In tumor cells two or more of these characteristics are not present. A tumor cell is not the result of a single genetic mutation but of several ones in genes regulating pivotal processes for the good functioning of the cell [14, 21, 24].

Tumor cells can survive if they move to another part of the body, and may not stop reproducing after they had double 50 or 60 times. The cancer cells are normally able to prevent themselves from apoptosis, or may self destruct more slowly than they reproduce, so that their numbers continue to increase [20, 24, 25, 26].

Cancer cells do not self destruct because the genes that regulate apoptosis are damaged or lost. So long as there is space and nutrients, the tumor cells continue to replicate itself, regardless of the extra damage that they can cause to the tissue where the tumor is located [21].

In addition cancer cells don't stick together, since they often lose the signaling molecules on their surface that keep normal cells in the right place. So they can become detached from their neighbours and in part this explains how cancer cells spread to other parts of the body, forming metastasis (see figure 3) [20, 25, 27].

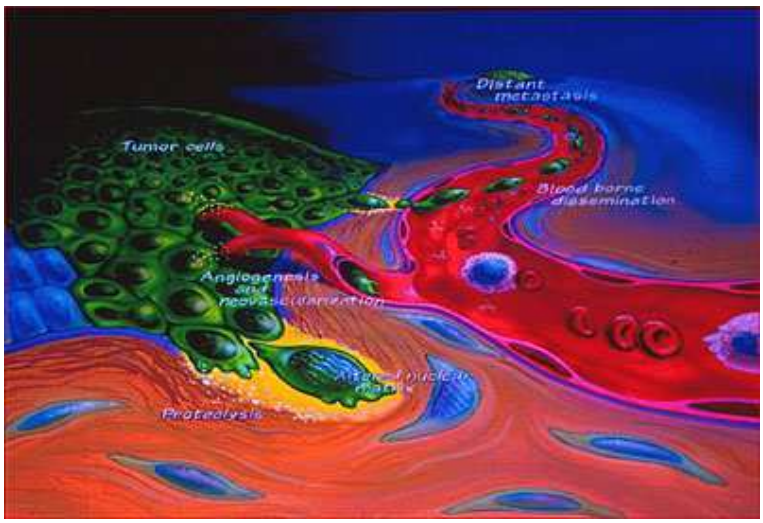

Figure 3: Tumor cells proliferation [28]

Initially, tumors do not have their own blood supply and need to be close to a blood vessel that provides them with oxygen, nutrients and allows waste products removal [23, 29].

As the tumor grows it demands more nutrients and the existing blood supply is not enough for all tumor mass requirement. So some cells become necrotic and counterbalance the tumor cells proliferation. It stops to grow and achieves a steady state, that corresponds to its critical size [21, 30, 31, 32].

However, tumors as defensive action may either move towards the capillary or start to release VEGF, which promotes the new capillary formation by a process called angiogenesis, check the angiogenesis induction in figure 3 [25, 30, 31, 33, 34].

Malignant tumors can be classified into two types with respect to the pattern of growth: expansive and infiltrative lesions [35].

The expansive, not infiltrative, are tumors dependent on angiogenesis to get nutrients and oxygen, because this type of growth pattern is characterized by intercellular adhesion molecules that keep cancer cells together to avoid migration. Infiltrative tumors may exploit pre-existing vasculature

through a process called co-option and thus grow without need of angiogenesis if they migrate to a densely vascularized, well perfused organ. However, they may also induce angiogenesis [35, 36].

We can sort the overview in figure 3 as an expansive tumor that induced angiogenesis to be more efficient in their proliferation.

2.4 Angiogenesis

Angiogenesis is the process that allows the formation of new blood vessels from pre-existing vessels and occurs in different stages of our life. The blood vessels inside wall is formed by vascular endothelial cells. These cells rarely divide, doing that only about once every 3 years on average. However, when the situation requires it, angiogenesis can stimulate them to divide [30, 31, 37].

Angiogenesis is not only a process induced by tumors, being normal both in children and in adults. In addition to its role in tumors, angiogenesis occurs normally in the human body at specific times in development and growth [37, 38].

Angiogenesis is the result of a delicate balance between Anti and Pro angiogenic factors [31]. When this equilibrium is disturbed, then an abnormal capillary network is formed being characterized by capillaries of variable width, not organized in an hierarchical network and with very permeable walls (see Fig. 4) [30, 31, 32, 39].

For example, a developing child in the mother's womb must create the vast network of arteries, veins, and capillaries that are found in the human body. A process called vasculogenesis creates the primary network of vascular endothelial cells that will become major blood vessels. Later on, through angiogenesis sprout out from the existing vessels, new vessels or capillaries that complete the child's circulatory system [38].

Creation of new blood vessels also takes place in adults, although it is a relatively infrequent event. In women, angiogenesis is active a few days each

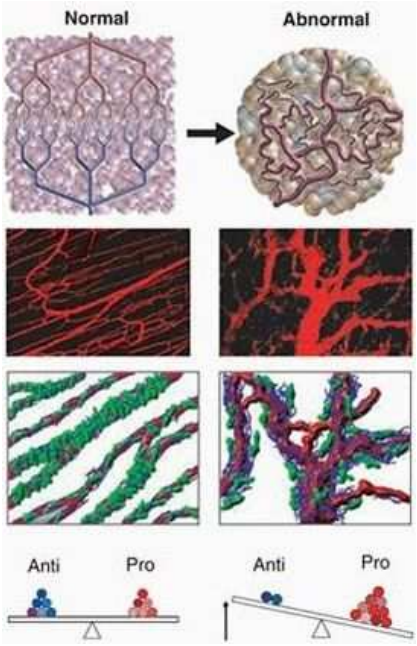

Figure 4: Angiogenesis: Anti and Pro angiogenic factors balance [41]

month as new blood vessels form in the lining of the uterus during the menstrual cycle. Also, angiogenesis is necessary for the repair or regeneration of tissue during wound healing [37].

Tumor angiogenesis is the proliferation of a network of blood vessels that penetrates into the cancerous mass, supplying nutrients and oxygen and removing waste products. Tumor angiogenesis actually starts with cancerous tumor cells releasing molecules that send signals to surrounding normal host tissue [6, 30, 31, 40].

When the solid tumor reaches a critical proportion (about 2 mm diameter) and is still nonvascular, it starts to produce and release VEGF. This factor is a chemical factor (polypeptide) produced by tumor cells, as a result of hypoxia, that interacts with a nearby vessel and promotes the creation of the network of new blood vessels [6, 21, 31, 33].

2.5 Mathematical Model Overview

Many aspects of tumor and tissue growth have been studied using mathematical models.

Mathematical model can be classified in *continuum* and *discrete* approaches, as well as so called *hybrids*, which combine both the techniques in one form or another [42, 43].

Since much of the information about cells is collected at the cellular and sub-cellular level, ideally, what is needed is a rigorous derivation of large-scale *continuum* models from individual-based models that may more easily be based on well-founded experimental information. In this way it should be possible to ensure that small-scale properties relevant for the large scales are retained and to quantify the validity of the continuum equations for finite population sizes [44, 45].

However, the mathematical techniques that allow rigorous derivations of continuum models from agent based models exist in only a very limited number of cases [44, 46].

In those cases for which it is not possible to relate the agent-based and continuum models at intermediate population sizes, some progress can be made by comparing their spatial-temporal dynamics. While normally focusing on compact monolayers, growing on a flat substrate, the models can readily be extended to three dimensions [44, 46, 47].

The main features of a *discrete* model is based on its ability to investigate the dynamics of a tumor at the cellular and subcellular level. The cells are autonomous and have a set of rules that characterize their behavior. However these models are limited to a comparably smaller scale due to prohibitive computational costs [45].

Discrete models are generally represented by a cellular automaton (CA), since they describe a spatial matrix in which the dynamics between cells and their "neighbors" are defined by a set of local rules that may also decide the transition and communication between the grid points. Normally each point on the grid represents an individual agent or a cluster of agents [44].

Continuum models in turn describe the tumor tissue as a continuous medium, being able to capture larger scales of volumetric tumor dynamics and are computational cost efficient. However have limitations in the implementation of interactions between heterogeneous cell-microenvironment and cell-cell dynamics, and even to describe the dynamics at the molecular level [44, 45].

Therefore, *hybrid* models have become popular due to their ability to allow for tumor simulations across multiple scales in space and time [42].

The *hybrid* models span a large range of scales but are rather complex to program and have many fitting parameters [45].

However, when we consider several cellular phases and we want to describe the interface dynamics, the phase-field method is particularly useful. The phase-field is a mathematical tool that converts the problem of having a mobile boundary at the sharp interface in a set of easily treatable differential equations for an order parameter ϕ that is continuous in space but has different constantvalues at each phase [48, 49, 50].

The correct interface dynamics is obtained when the interface width becomes small compared with its curvature radius. Phase-field models are very tailorable because allow the change of the energy related to the interface, as well as the way the partial differential equations are written in order to describe a vast range of systems [48, 50].

3 Description of Computational Model

3.1 Nutrient Diffusion and Model General Aspects

The computational model that has being developed is a continuum model. The program design is developed using Fortran 90 and the model simulates the tumor's growth before it induces angiogenesis.

Tumor growth is nutrient limited, the tissues get nutrients and oxygen through the blood vessels walls and diffusing in the extracellular matrix (ECM) in order to arrive into the cells. When the tumor cells cluster and form a multicellular spheroid, they receive the nutrients through the boundary and the nutrient diffuses toward the center of the tumor [23, 49].

The model considers nutrient diffusion from a blood vessel, located in the far right, the concentration of nutrients at the vessel is kept equal to 1 throughout the simulation, which means that there is a continuous flow of blood in the capillary and nutrient concentration at the vessel is not a function of how much nutrients are consumed at each iteraction by the tissue cells. The type of cells at a point in the tissue is described through an order parameter ϕ. Tumor cells have $\phi = 1$ and other cells $\phi = -1$.

This is a multiphase model, considering the tumor cells as one phase and the surrounding tissue (normal cells) as another phase. It doesn't consider a separate phase for the extracellular matrix, which is where the nutrient diffuses to achieve the cells, so in the model the nutrient concentration is independent of the cell concentration. This approximation is also used in models which consider the ECM as a separate phase.

The diffusion equation (1) considers the nutrient diffusion in the tissue and nutrient consumption by the tissue cells at rate $\alpha_c(\phi)$ (2). Since ϕ is the order parameter that describes the type of cells, this means that the rate of nutrient consumption depends on the type of cell.

$$\partial_t n(\overrightarrow{r}, t) = D\nabla^2 n(\overrightarrow{r}, t) - \alpha_c(\phi) \tag{1}$$

$$\alpha_c(\phi) = \begin{cases} 0.0014 & , \phi \leq 0 \\ 0.14 & , \phi > 0 \end{cases} \tag{2}$$

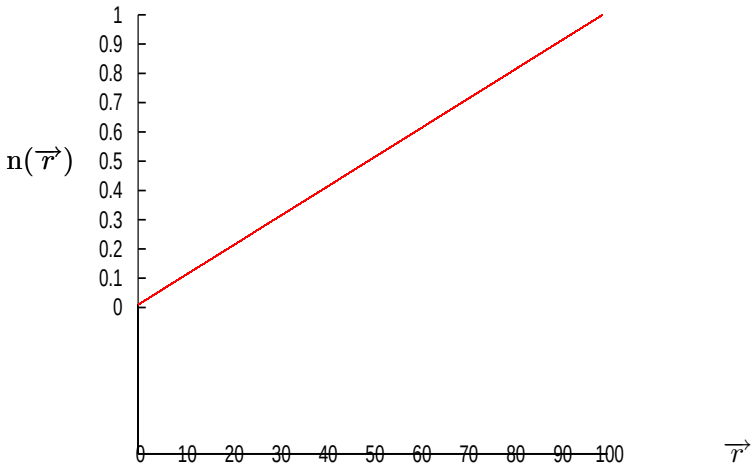

Figure 5: Nutrient Initial Condition

At each iteration the blood vessel provides nutrients to all the tissue surface. The starting condition for the nutrient is linear, from 0 to 1 at the capillary (see Fig. 5), which avoid that a large number of tumor cells die at the onset of the simulation. When the nutrient conditions are propitious the tumor proliferates.

In Figure 5 we see the distribution of nutrient on the basis of cartesian coordinates, and the outlook for $\overrightarrow{r} = (x; 0)$.

If the nutrients concentration falls below a threshold value then the cells are unable to survive and undergo necrosis, generating a necrotic core. From *in vitro* experiments is concluded that avascular tumors possibly reach an equilibrium around 2 mm in diameter [21, 31], at which the rates of proliferation and necrosis averaged over the tumor volume are identical. Typically, at that stage the tumor contains an outer rim of proliferating cells, a central necrotic core and an intermediate region of quiescent cells, that are alive but not proliferating due to deprivation of nutrient [23].

The tumor cell's consumption rate is an hundred times superior to the normal cells consumption and it is imposed that nutrients concentration can not be negative. In the model the nutrient threshold for the tumor cells death is the absence of nutrient.

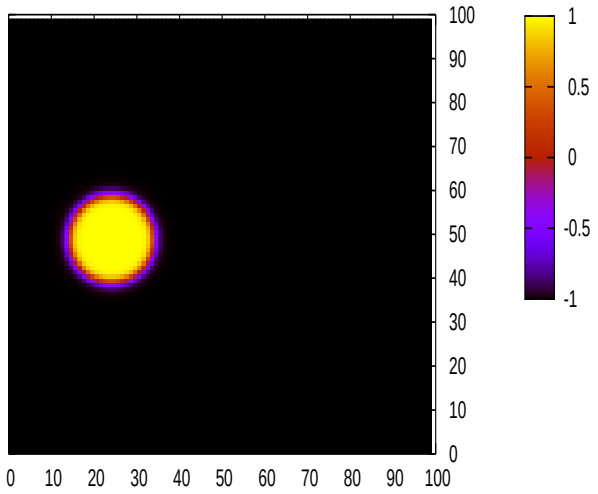

Figure 6: Cell Phases Initial Condition

3.2 Cell Proliferation: First Model

Initially the model did not consider the effects of pressure exerted by the interaction between cells, because the total cell density was kept constant.

The starting condition consists of a small circular tumor (radius (r) is set equal to 10 lattice units) at a distance from the existent vessel (see Fig. 6).

The equation that describes the location of the tumor and the shape of the interface between the two cell phases:

$$\phi(\overrightarrow{r}, t) = -\tanh(\sqrt{(x-25)^2 + (y-50)^2} - r) \tag{3}$$

In Fig.7, the red curve is obtained by the simulation, the blue curve is a representation of the previous equation and the green line ($\phi = 0$) intersects the curves at the point of phase separation.

The interface between two cell types costs energy $\sqrt{2}\epsilon/3$ per unit length, where ϵ is the interface width. This interface ensures that spatial variations

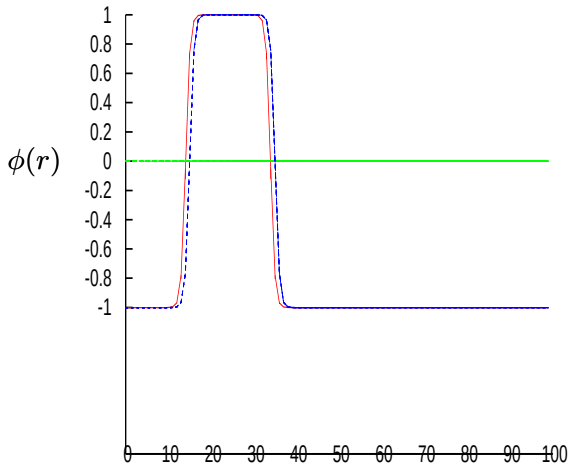

Figure 7: Interface Profile

of phase, different phases next to each other, are penalized, avoiding undesirable fluctuations around the interface. The equations for the evolution of ϕ in time are the following:

$$\partial_t \phi(\overrightarrow{r}, t) = \nabla M(\phi) \cdot \nabla \mu(\phi) + \alpha_r(n, \phi) \tag{4}$$

where the chemical potential is given by:

$$\mu(\phi) = -\phi(\overrightarrow{r}, t) + \phi^3(\overrightarrow{r}, t) - \varepsilon^2 \nabla^2 \phi(\overrightarrow{r}, t) \tag{5}$$

The equation of the chemical potential is derived in Appendix A.

In the fourth equation the parameter $\alpha_r(n, \phi)$ is introduced to describe the cells evolution, it is a proliferation factor. With favorable nutrient conditions, the tumor grows at rate G otherwise cells die at rate A. Normal cells have this reproduction rate kept equal to zero because it is assumed that they reproduce and die at the same rate, maintaining the equilibrium.

$$\alpha_r(\phi, n) = \begin{cases} G & , \phi \geq 0 \quad , n > 0 \\ -A & , \phi > -1 \quad , n = 0 \\ 0 & , \phi < 0 \quad , n > 0 \end{cases} \tag{6}$$

Factor M(ϕ), describes the cells mobility and was introduced to reproduce the fact that normal tissue cells form an ordered lattice, while tumor cells, being more spatially disorganized, may have a larger mobility.

$$M(\phi) = \begin{cases} 1 & ,\phi \geq 0 \\ 0.1 & ,\phi < 0 \end{cases} \tag{7}$$

In order to conserve the total ϕ, that are imposed mirror conditions at the simulation box walls.

3.3 Cell Proliferation: Second Model

The model describes the type of cells at a point in the tissue through an order parameter ϕ like in the first model, but in this case we introduce a new equation for total cell density $\rho(\overrightarrow{r}, t)$ (see equation 8 and following).

The following equations describe the evolution of ϕ and ρ in time (deductions are in Appendix B):

$$\partial_t \rho(\overrightarrow{r}, t) = M_\rho \nabla^2 \rho(\overrightarrow{r}, t)) + L(\phi, \rho, n) \tag{8}$$

$$\begin{aligned} \partial_t \phi(\overrightarrow{r}, t) \quad = \quad & \frac{-M(\rho)}{\rho(\overrightarrow{r}, t)} \phi(\overrightarrow{r}, t) \nabla^2 \rho(\overrightarrow{r}, t) + \frac{M_\phi}{\rho(\overrightarrow{r}, t)} \nabla^2 \mu(\phi) + \\ & + \frac{M(\rho)}{\rho(\overrightarrow{r}, t)} \nabla^2 (\phi(\overrightarrow{r}, t) \rho(\overrightarrow{r}, t)) + G(\phi, \rho, n) \end{aligned} \tag{9}$$

$$\partial_t (\phi(\overrightarrow{r}, t) \rho(\overrightarrow{r}, t)) = M_\phi \nabla^2 \mu(\phi) + M_\rho \nabla^2 (\phi(\overrightarrow{r}, t) \rho(\overrightarrow{r}, t)) + H(\phi, \rho, n) \tag{10}$$

To see the deductions of equations 10 and 11 appeal to Appendix C.

Factor M_ϕ, describes the cells mobility and their ability to phase separate. we can make it ϕ dependent in order to reproduce the fact that normal tissue cells form an ordered lattice, while tumor cells being more spacially disorganized, may have a large mobility. The M_ρ factor is kept equal to 1 but could also be made ϕ dependent.

In the equation 11 the parameter $H(\phi, \rho, n)$ is introduced to express the cells evolution, it is a proliferation factor, and is described by:

$$H(\phi, \rho, n) = \rho G(\phi, \rho, n) + \phi L(\phi, \rho, n) \tag{11}$$

Where $G(\phi, \rho, n)$ and $L(\phi, \rho, n)$ are given by:

$$G(\phi, \rho, n) = \begin{cases} G\phi(\overrightarrow{r}, t)(1 - \phi(\overrightarrow{r}, t))(1 - \rho(\overrightarrow{r}, t)) & , \phi \geq 0, n > 0 \\ -A(1 - \phi^2(\overrightarrow{r}, t)) & , \phi > -1, n = 0 \\ 0 & , \phi < 0, n > 0 \end{cases} \tag{12}$$

$$L(\phi, \rho, n) = \begin{cases} G\phi(\overrightarrow{r}, t)\rho(\overrightarrow{r}, t)(1 - \rho(\overrightarrow{r}, t)) & , \phi \geq 0, n > 0 \\ -A\rho(\overrightarrow{r}, t)(1 + \phi(\overrightarrow{r}, t)) & , \phi > -1, n = 0 \\ 0 & , \phi < 0, n > 0 \end{cases} \tag{13}$$

These equations represent the growth (and death) factors, respectively for the order parameter and for density parameter (equation 12 is derived in Appendix B). G and A are respectively the growth and necrosis rate of the cells. Normal cells have the proliferation rate kept equal to 0, because it is assumed that they reproduce and die at the same rate, maintaining the equilibrium.

We define also the parameters ρ and ϕ in order to the tumor cells density (ρ_t) and the normal cells density (ρ_n), through the equations:

$$\rho = \rho_t + \rho_n \tag{14}$$

and:

$$\phi = \frac{\rho_t - \rho_n}{\rho_t + \rho_n} \tag{15}$$

The density at each point in space corresponds to the total density of normal and tumor cells. The expression for the order parameter ϕ ensure that the amount of normal cells over time, in the total space, remains constant.

In order to conserve the total ϕ, that are imposed mirror conditions at the simulation box walls like in First Model.

Parameters	Values
x	100
y	100
r	10
D	35
α	0.014
ε	1
M_ρ	1
$\Delta(t)$	$1.28{\cdot}10^{-4}$

Table 1: Values for the simulations.

4 Simulation's Results/Discussion

4.1 Nutrient Diffusion

Both models assume that the initial condition of the distribution of nutrients in the system is linear, but despite this condition some cells initially die before the regular growth profile emerges. Until the system stabilizes, the consumption of tumor cells is always higher than the diffusion of nutrients in the space.

The values for the diffusion (D) and consumption (α) rates were defined to assure that after a time t=50 (equivalent to 390000 iterations) the nutrient concentration value at half tissue surface is about 0.5 (see Fig. 8) like in the initial condition (see Fig. 5). After that time we can consider that nutrient diffusion in the system is stable and the changes at the tumor are due to the dynamics between growth and death rates and also the dynamics between the two cell phases (normal cells and tumor cells).

As we can see at figure 9 and at all the figures regarding the distribution of nutrients on the space, the distribution has a darker region corresponding to a low nutrient concentration at the location of the tumor, which consumes more nutrients than the rest of the tissue in each time step.

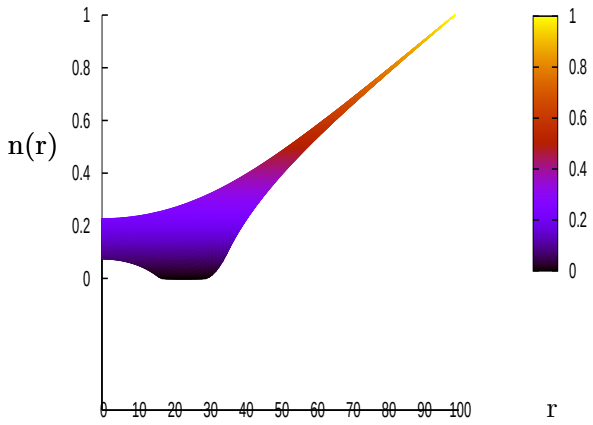

Figure 8: Nutrient diffusion at t=50: $A = 0.05$, $G = 0.2$ and $M_\phi = 10$.

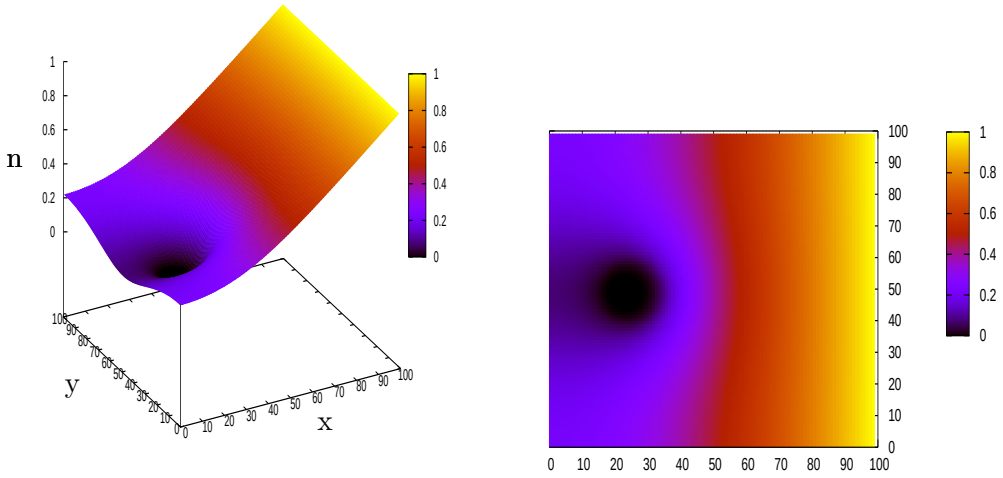

Figure 9: Nutrient diffusion at t=50: $A = 0.05$, $G = 0.2$ and $M_\phi = 10$.

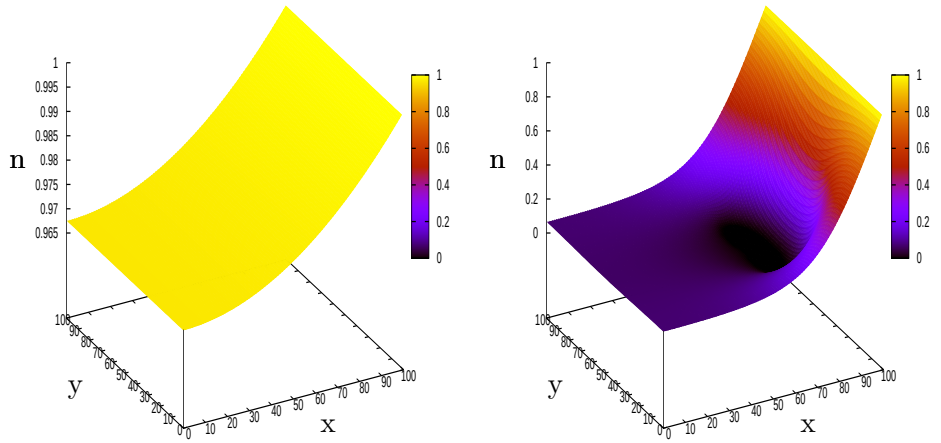

Figure 10: Nutrient diffusion at t=2000, $M_\phi = 10$: $A = 0.1$, $G = 0.01(left)$ and $A = 0.01$, $G = 0.1(right)$

Normal cells do not grow or die in the presence or absence of nutrient, respectively, however the tumor cells reproduce if there is enough nutrients and die in the absence of nutrient.

The relationship between the rates of necrosis (A) and growth (G) determines the levels of nutrients that may exist in phase space at the differents simulations.

From one simulation to another, the variables that can be changed are the mobility and the proliferation rates.

If A is too high (and G is preferably low) in the absence of nutrient tumor cells die very quickly, which may disappear, so that the nutrient level is virtually constant throughout the space and close to 1 (see Fig. 10 (left)).

If instead the growth rate is higher and A is reduced, then it appears that the tumor proliferates and nutrient levels remain low in the tumor, close or equal to 0 (see Fig. 10 (right)), which leads to tumor cells death and allows that the nutrient levels rise back above the threshold. This balance between tumor cell death and nutrient levels will be explained in more detail later on.

4.2 Cells proliferation for the First Model

At this stage the model consider that the mobility of normal cells is very small (M=0.1), so that they do not have large freedom of movement and hinder the intrusion of the tumor cells, although the latter have high mobility (M=1). In this way we ensure that there is not spreading of tumor cells in normal tissue, but they proliferate as a whole.

These differences in mobility between the two phases were introduced in a way to reproduce the effects of pressure that are observed in biological systems.

During the simulations, regardless of the values chosen for the variable parameters (A and G), it was observed that as expected, the tumor tries to occupy space and moves to places with higher concentration of nutrients in the direction of the blood vessel.

Another interesting fact observed at figures 11, 12 and 13 is that the cells of the center of the tumor are the first to suffer necrosis. That creates a necrotic core, cells no longer behave like tumor cells and thus do not require such a high amount of nutrients allowing the tumor cells nearby have more food and proliferate.

At this first situation the apoptosis rate is higher than growth rate, so despite having a linear initial condition of nutrient, the tumor has not enough nutrients to feed its cells at the begining and disappear after t=50.

Reducing the A we can see in figures 14, 15 and 16 that the tumor grows and even move slightly but as A is also low it does not move a lot. Another interesting aspect is that as the rates of proliferation are low and balance themselves, the first cells to die are those that are further away from high concentrations of nutrients.

However at figures 17, 18 and 19 we increase the growth rate and because the death rate is low, the tumor grows very quickly and reaches the capillary. At this point it can get all the nutrients it needs and occupies almost all the space.

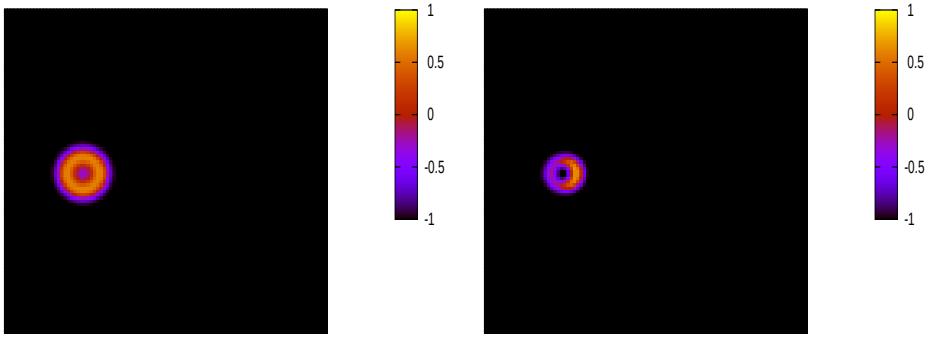

Figure 11: Cells Phases at t=10 (left) and t=20 (right), G=0.01 and A=0.1

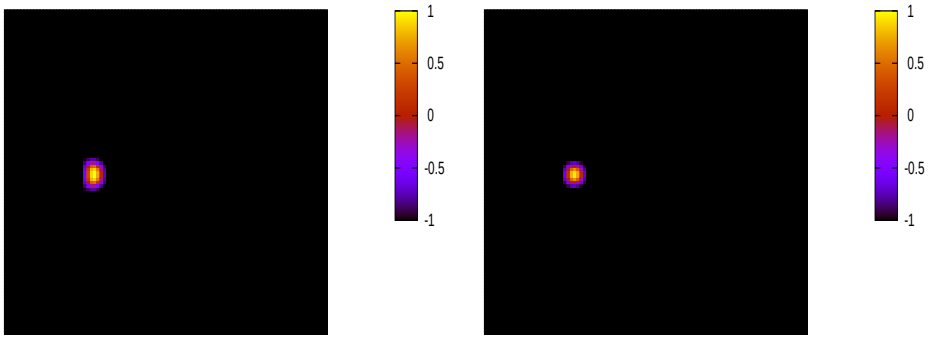

Figure 12: Cells Phases at t=30 (left) and t=40 (right), G=0.01 and A=0.1

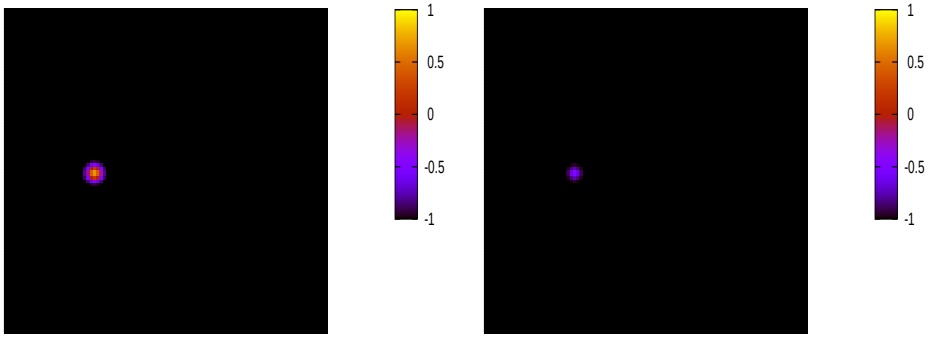

Figure 13: Cells Phases at t=50 (left) and t=60 (right), G=0.01 and A=0.1

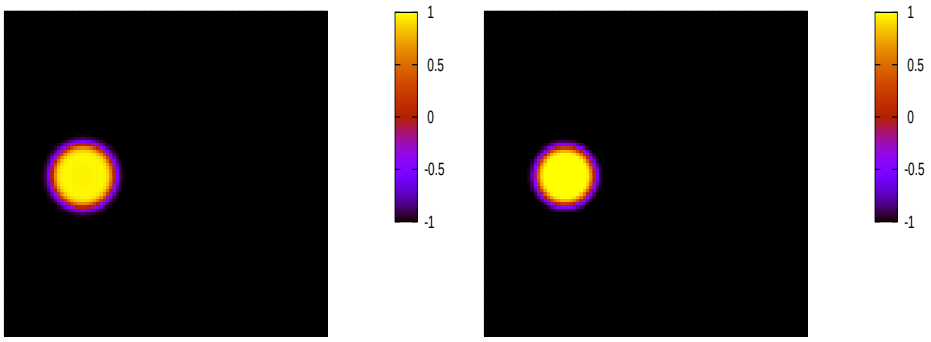

Figure 14: Cells Phases at t=10 (left) and t=100 (right), G=0.01 and A=0.01

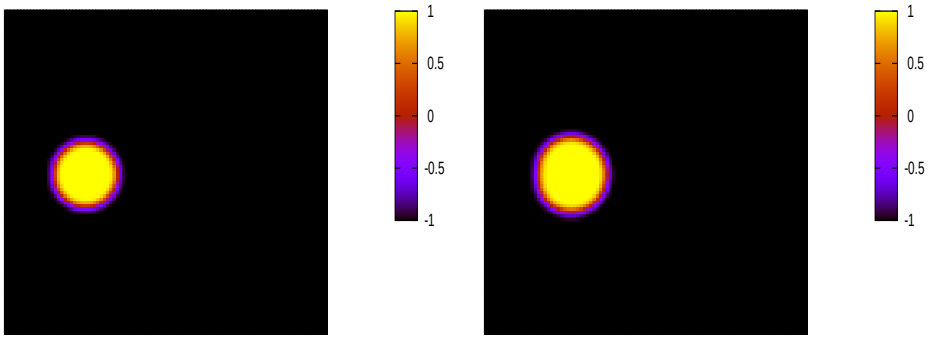

Figure 15: Cells Phases at t=200 (left) and t=500 (right), G=0.01 and A=0.01

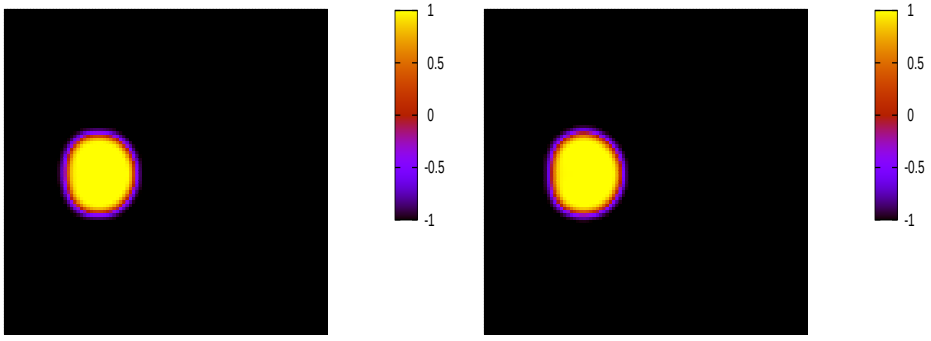

Figure 16: Cells Phases at t=800 (left) and t=1000 (right), G=0.01 and A=0.01

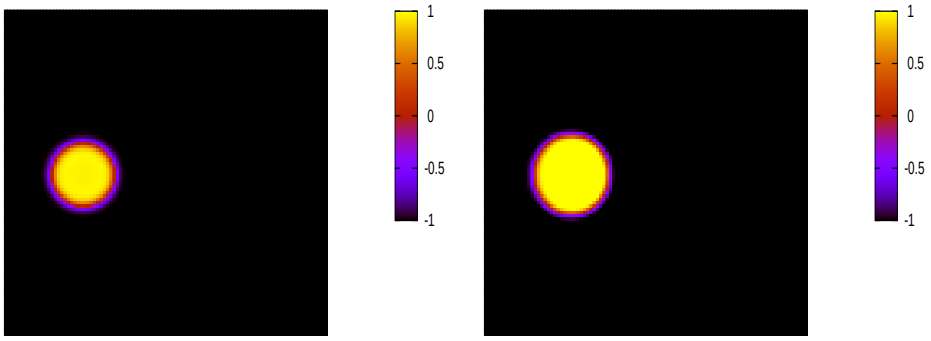

Figure 17: Cells Phases at t=10 (left) and t=50 (right), G=0.1 and A=0.01

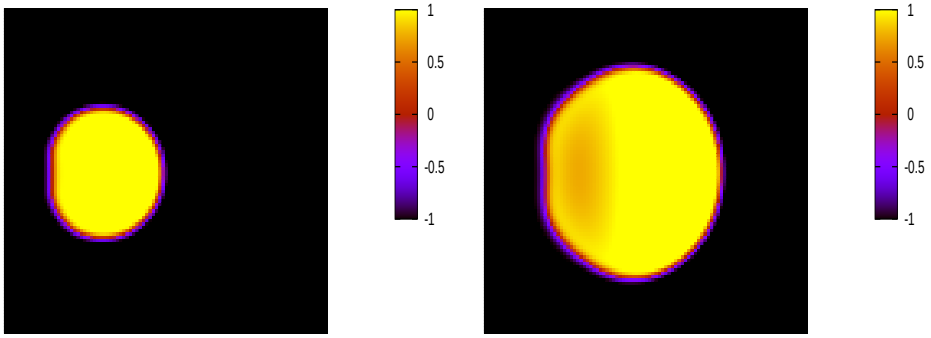

Figure 18: Cells Phases at t=100 (left) and t=200 (right), G=0.1 and A=0.01

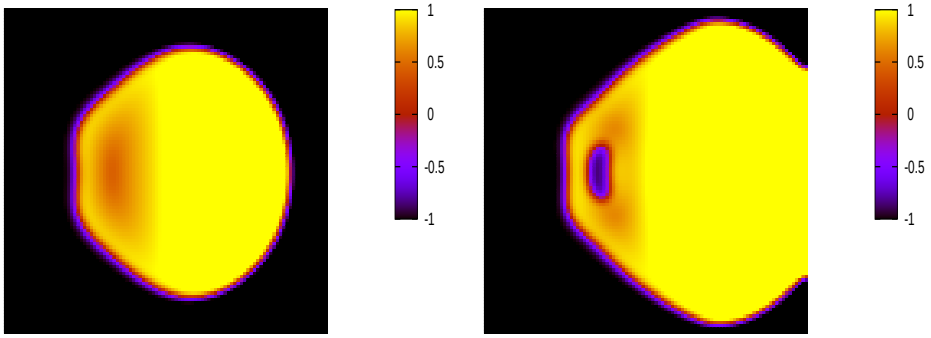

Figure 19: Cells Phases at t=250 (left) and t=300 (right), G=0.1 and A=0.01

4.3 Cells proliferation for the Second Model

At this stage the model considers the cell density. The initial condition is that the cell density in all space is equal to 0.5, regardless of whether they are normal or tumor cells.

In areas where only normal cells coexist density remains equal to 0.5, but at the space occupied by the tumor the cell density may increase and even reach its maximum value $\rho = 1$.

To calculate the density of tumor cells in all the space we use the equation:

$$\langle \rho_t \rangle = \frac{\langle \rho\phi \rangle + \langle \rho \rangle}{2} \tag{16}$$

The graph that is obtained from (12) gives us a temporal evolution of the tumor cells density in space. What allows us to infer more clearly if the tumor proliferated or not.

The parameters that are the variables at this stage are the mobility of the phase-separating cells (M_ϕ), the necrosis (A) and the growth (G) rates.

If A is very high, regardless of G be large or small, the tumor cells start to die at the begining (before nutrient diffusion stabilizes) and the tumor can not recover and eventually disappears.

If both rates are relatively low and similar, the tumor maintains the initial shape during its proliferation and does not grow or move much.

However, if A is low and G is very high, the tumor grows quickly, occupying adjacent spaces and moves in space in order to achieve higher concentrations of nutrients, moving in the direction of the blood vessel and eventually achieving it.

About how the tumor develops according to the rates of proliferation we had already talked in section 4.2 so in this section we will focus more on the analysis of mobility.

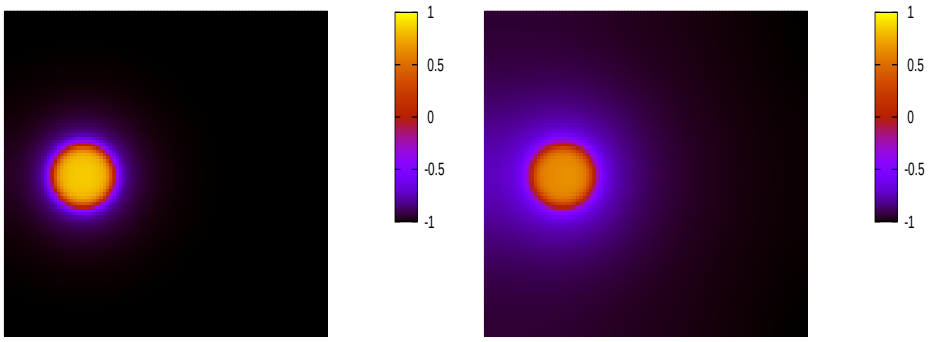

Figure 20: Cells Phases at t=10 (left) and t=100 (right), M_ϕ=1, G=0.1 and A=0.01

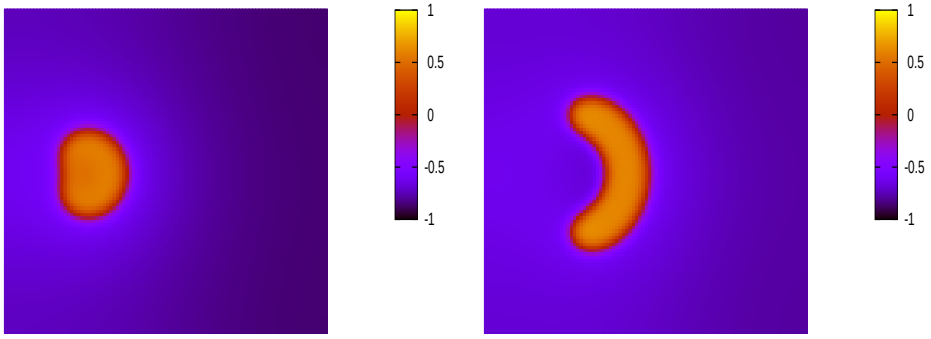

Figure 21: Cells Phases at t=500 (left) and t=1000 (right), M_ϕ=1, G=0.1 and A=0.01

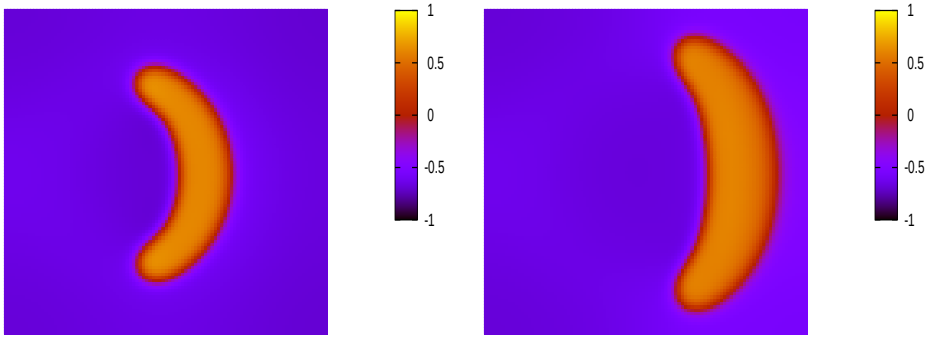

Figure 22: Cells Phases at t=1500 (left) and t=2000 (right), M_ϕ=1, G=0.1 and A=0.01

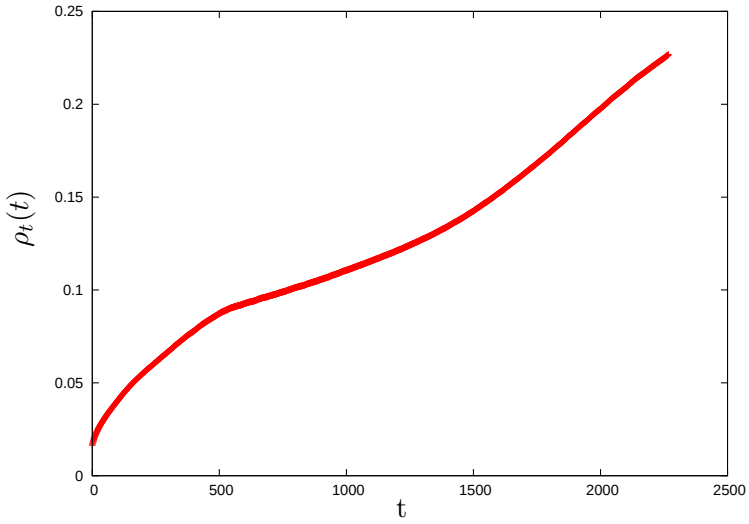

Figure 23: Evolution of ρ_t in time: M_ϕ=1, G=0.1 and A=0.01

The graphs sequence of figures 20, 21 and 22 show the evolution of cell phases when M_ϕ is small (equal to 1). We notice that the phase separation with the progress in time is not very clear, i. e. the interface between tumor cells and normal cells is not sharp and tumor cells get out of the tumor region.

In accordance with the rates of proliferation, and as expected, the tumor grows rapidly and moves towards the capillary. Because with this mobility the cells have great freedom of movement, the tumor acquires the shape that allows it to keep the largest number of cells in contact with high concentrations of nutrients.

Increasing the mobility factor, we can observe at figures 24, 25 and 26 where M_ϕ=10, that there is a clearer phase separation, because the the phase separation is faster than the density homogeneization. Due to this reason, and despite the tumor growing and moving towards the blood vessel, its shape remains similar to the original shape.

The way that the tumor found to combat the lack of nutrient was to move in space.

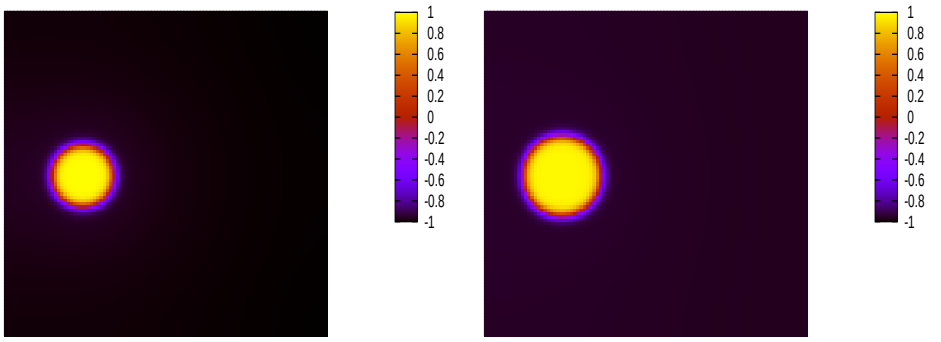

Figure 24: Cells Phases at t=10 (left) and t=100 (right), M_ϕ=10, G=0.1 and A=0.01

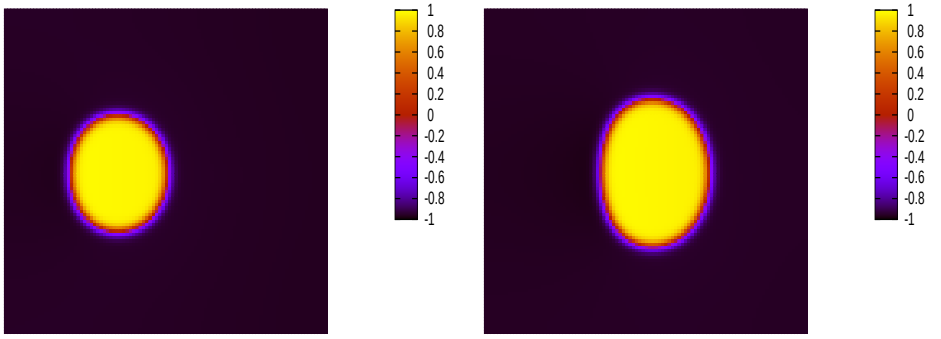

Figure 25: Cells Phases at t=500 (left) and t=1000 (right), M_ϕ=10, G=0.1 and A=0.01

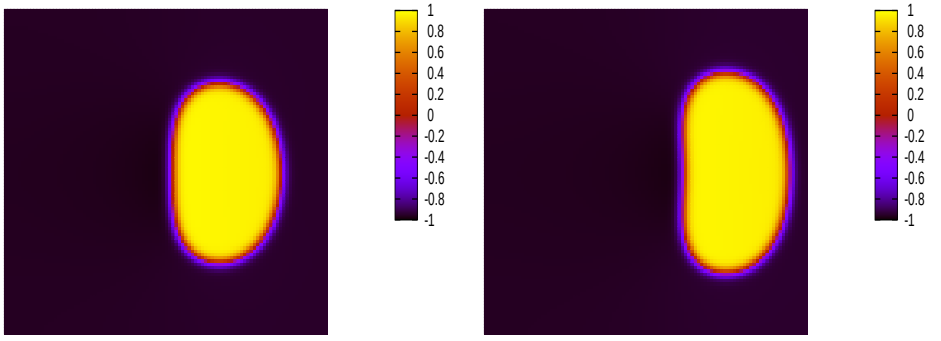

Figure 26: Cells Phases at t=1500 (left) and t=2000 (right), M_ϕ=10, G=0.1 and A=0.01

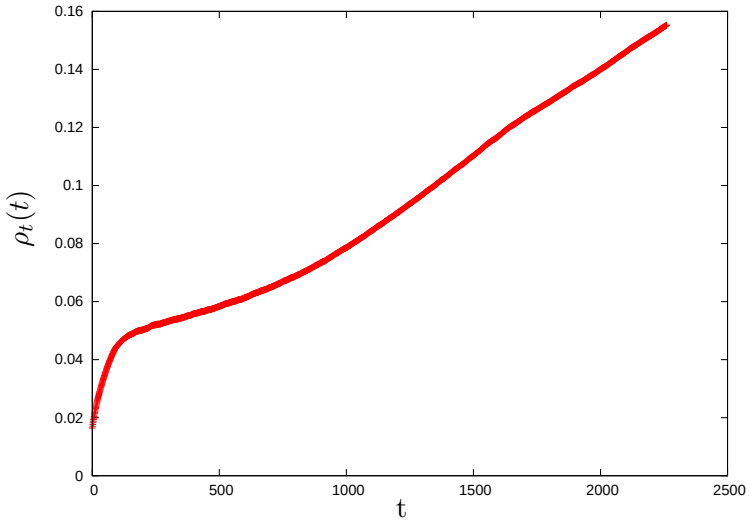

Figure 27: Evolution of ρ_t in time: M_ϕ=10, G=0.1 and A=0.01

In the two previous situations A is very low and G is high so the tumor cells have the need to proliferate and as the death rate is slow, the density of tumor cells increase significantly.

In figures 23 and 27, we can check that ρ_t increases in the time, for both previous situations. For a smaller M_ϕ there is a higher density of tumor cells in space during all the time, since the tumor is not confined to the tumor region it can reach the capillary earlier and grow faster.

In these conditions, tumor will not stop growing how is natural in avascular tumors, because it can move fast and achieve always the amount of nutrient it needs to feed the cells and to continue moving until reaches the blood vessel.

For this set of results, we only can conclude that a higher cell motility represent better the biological conditions of pressure exerted by the external environment of the tumor.

It also characterizes better the behavior of the cells by defining the cellular phase separation.

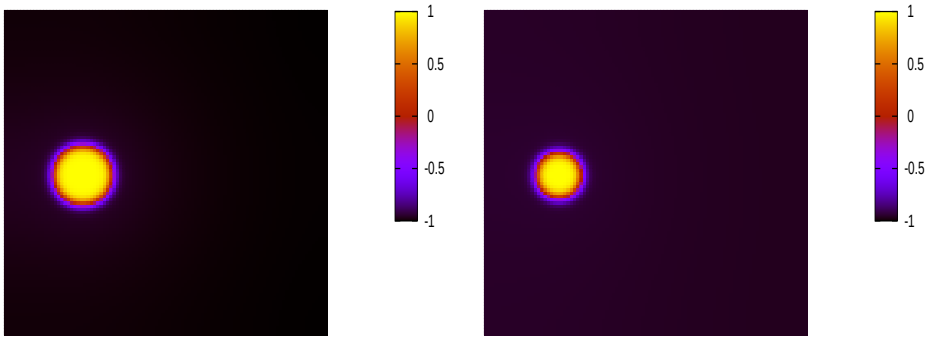

Figure 28: Cells Phases at t=10 (left) and t=100 (right), M_ϕ=10, G=0.01 and A=0.1

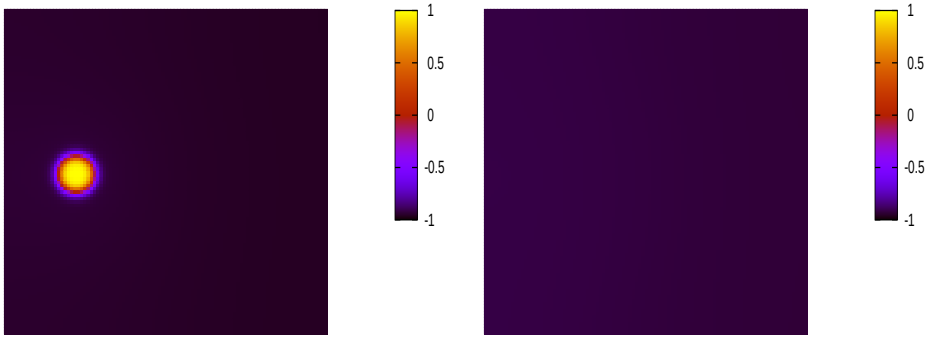

Figure 29: Cells Phases at t=200 (left) and t=300 (right), M_ϕ=10, G=0.01 and A=0.1

Maintaining high cell motility, which focuses on the representation of biological conditions, if we study the case of having small G and large A (see figures 28 and 29), what we notice is that the tumor disappears after an estimated time t=250.

Since the tumor cells have a limited freedom of movement and normal cells precludes the tumor growth, and the growth rate is reduced, the tumor never recovers from the initial phase in which the nutrient diffusion has not been yet stabilized (for t<50) and the cells will die until the tumor disappears.

For high motility (like $M_\phi \geq 10$) if death rate is large, even if we have a growth rate high too, the tumor cells disappear because they can not overcome the death at the early stage.

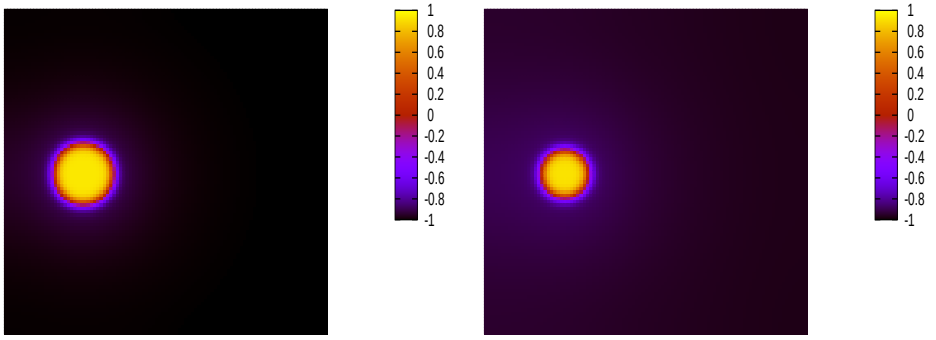

Figure 30: Cells Phases at t=10 (left) and t=100 (right), M_ϕ=5, G=0.05 and A=0.05

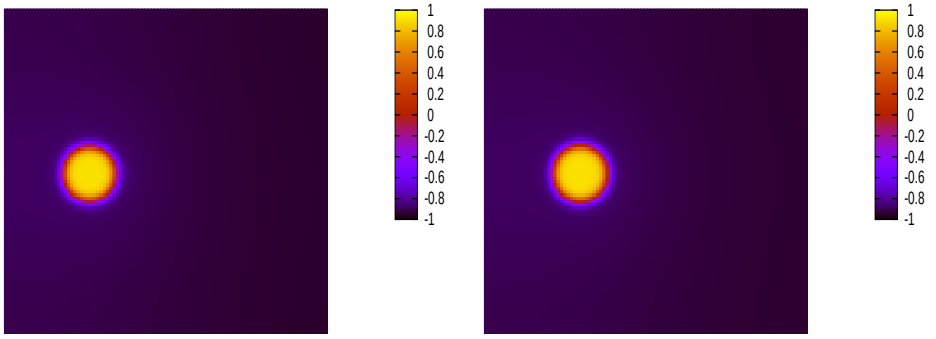

Figure 31: Cells Phases at t=500 (left) and t=1000 (right), M_ϕ=5, G=0.05 and A=0.05

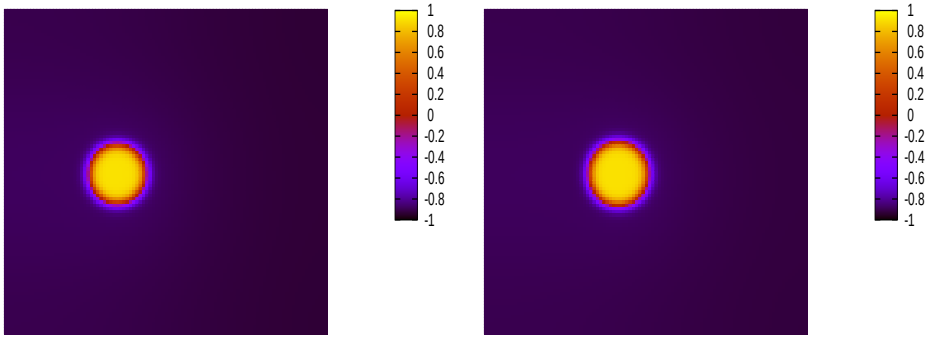

Figure 32: Cells Phases at t=1500 (left) and t=2000 (right), M_ϕ=5, G=0.05 and A=0.05

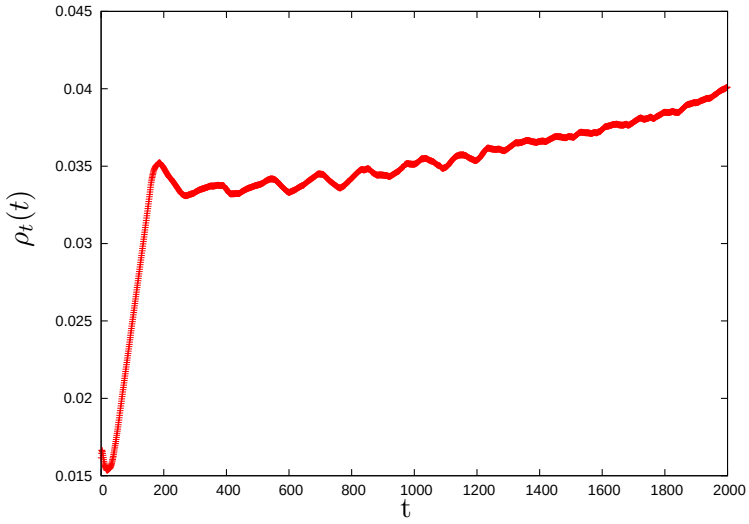

Figure 33: Evolution of ρ_t in time: M_ϕ=5, G=0.05 and A=0.05

Choosing an intermediate value for the mobility (M_ϕ =5) and in the case of figures 30, 31 and 32 if we define that the rates of proliferation and death are both small and equal, we obtain that the tumor almost achieves the steady state.

Initially the tumor suffer a reduction in its size, for the reasons already outlined above, but as A is low the tumor can recover. We can also notice that as the mobility is not very high, there is not a total separation of cells phases because the color of the normal tissue is not black any longer, however these changes are minimal.

To corroborate what we observed in the previous figures we will focus now on the figure 33.

The graph of the density of tumor cells confirms the initial reduction in tumor size and the consequent recovery. There is a sharp increase between t=50 (after the diffusion stabilization) and t = 200. From that point the curve increases slightly, is not linear and shows some fluctuations, each rise corresponds to the time when the tumor has moved towards the capillary.

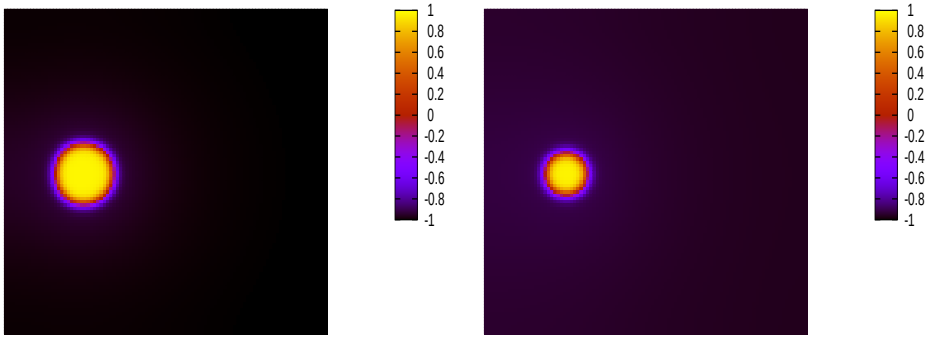

Figure 34: Cells Phases at t=10 (left) and t=100 (right), M_ϕ=5, G=0.05 and A=0.2

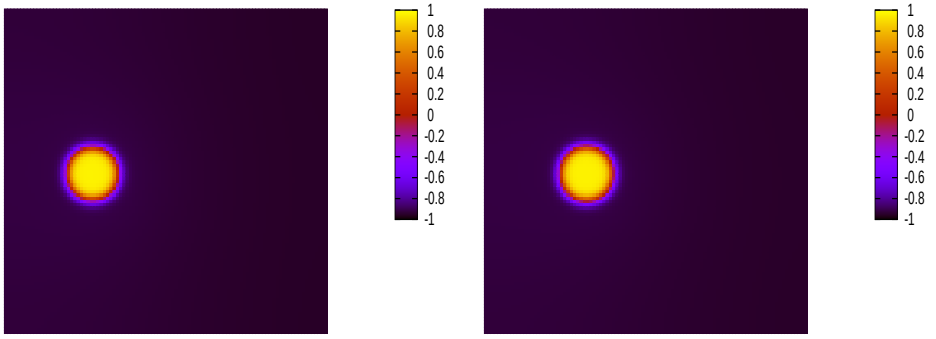

Figure 35: Cells Phases at t=500 (left) and t=1000 (right), M_ϕ=5, G=0.05 and A=0.2

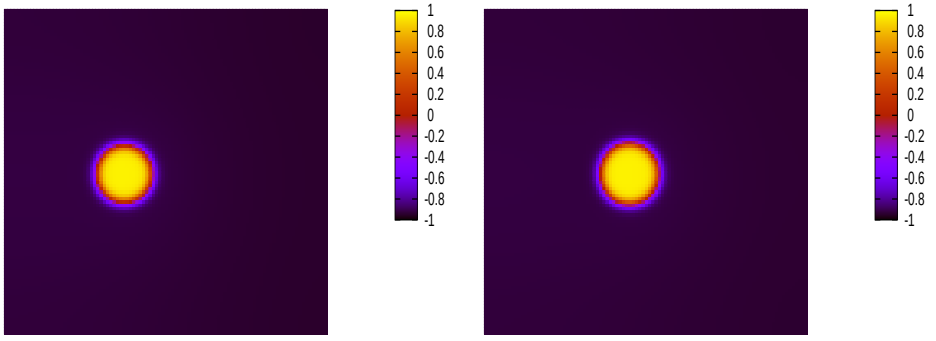

Figure 36: Cells Phases at t=1500 (left) and t=2000 (right), M_ϕ=5, G=0.05 and A=0.2

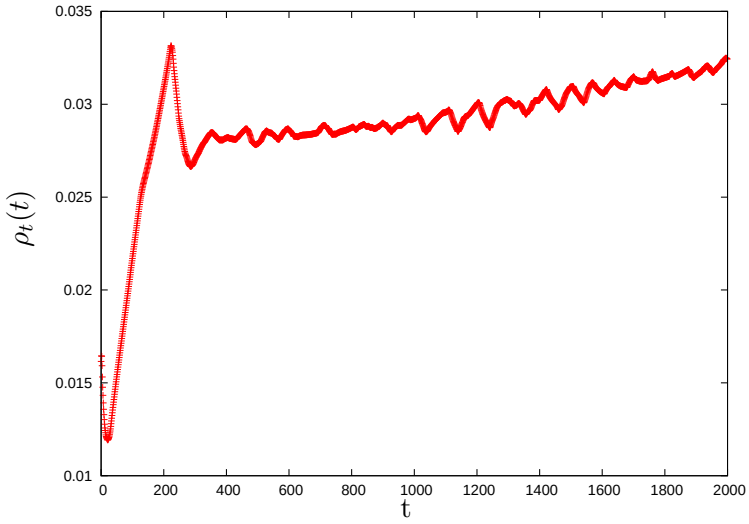

Figure 37: Evolution of ρ_t in time: M_ϕ=5, G=0.05 and A=0.2

By increasing the death rate we wanted to check if the tumor was able to recover after the initial reduction of the size and proliferate in the same way as above and verify what its influence on the fluctuations of the density graph.

Checking the figures 34, 35 and 36 we can note that tumor is able to recover, despite its size has reduced more than the previous case. The tumor does not grow so much, as we expected as A is greater, but behaves similarly.

Comparing the figures 37 and 33 we can notice that after t=50 both start to grow again, however, the density of tumor cells in the first graph have the minimum on $\rho_t \simeq 0.015$ while in the second is on $\rho_t \simeq 0.012$, which corroborates the fact that the death rate of it cells is higher. The peak in figure 37 is more acentuated than in figure 33 and the system stabilizes at t=300 rather than ate t=200. We observe the same fluctuations, corresponding to the movement of the tumor within the space, but in fact the fluctuations decrease with time.

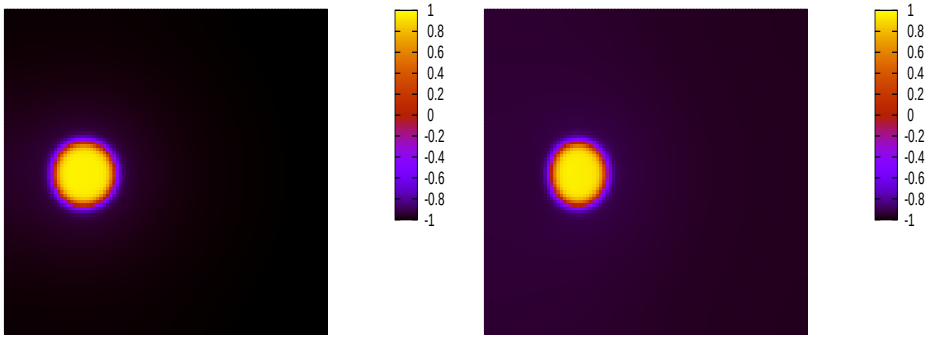

Figure 38: Cells Phases at t=10 (left) and t=100 (right), M_ϕ=5, G=0.2 and A=0.2

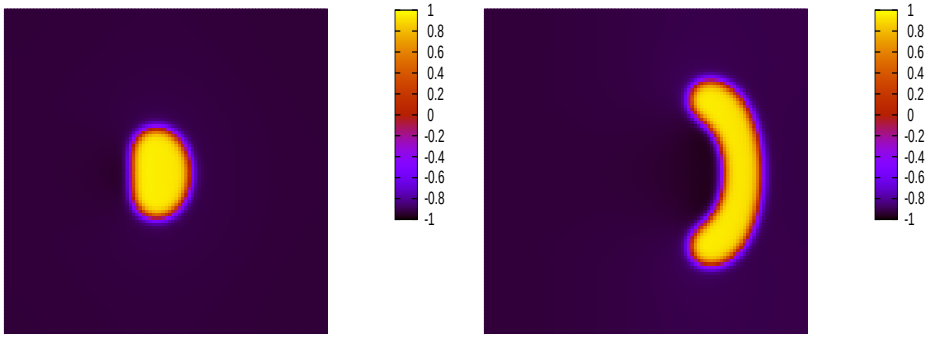

Figure 39: Cells Phases at t=500 (left) and t=1000 (right), M_ϕ=5, G=0.2 and A=0.2

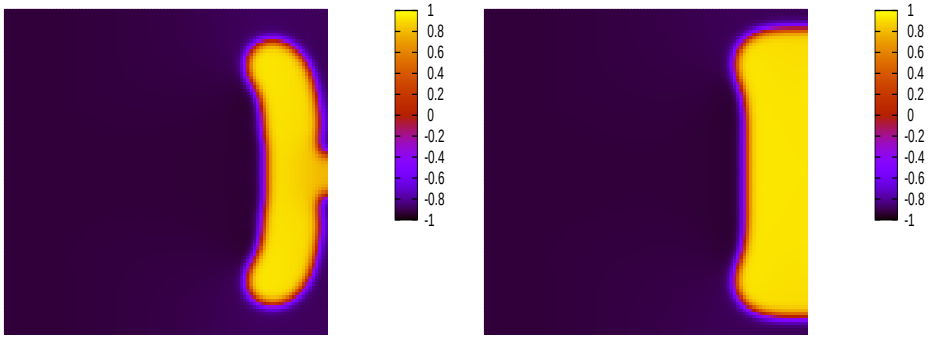

Figure 40: Cells Phases at t=1500 (left) and t=2000 (right), M_ϕ=5, G=0.2 and A=0.2

In order to verify if the tumor always behave in the way described earlier when M_ϕ=5, we decided to increase the growth rate while maintaining the A high.

In this particular case as we note at figures 38, 39 and 40 the tumor behaves like the other situations in which it had high growth rate, regardless of the mobility. Its shape is closer to the case where mobility was equal to 1, but phase separation is more visible like in the case where M_ϕ=10.

The objective of ensuring that the tumor growth cease occurred only in the case of graphs of figures 33 and 37, and only partially.

The growth and apoptosis rates could vary between 0 and 1, but above 0.1 is considered a high rate. For M_ϕ we tested values between 0 and 20, but the results more presentable are placed in the interval [1;10].

5 Final Remarks/Further Work

The tumor cells arise in our body as a result of successive cell divisions after an initial set of mutations which target important regulatory processes of a single cell [15, 18]. This abnormal growth of a mass of cells requires space to develop and grow. Because the cells of the body are in an organized lattice, these abnormal cells have to overcome several factors to get developed.

The tumor has to overcome the immune system so that its cells can survive, must overcome the external pressures exerted by the extracelullar matrix and the body's own cells to be able to proliferate and has yet to develop mechanisms in order to acquire the amount of nutrient needed to feed its cells and to produce new cells by cell division [51, 52, 53, 54, 55, 56].

That is the reason why tumor cells need more nutrients then normal cells, because they are always fighting to survive in an environment in which they are strange.

Attempting to reproduce the biological microenvironments through a computational model is really a great challenge because we can always add more variables, more interactions, to attempt a description closer to the real problem.

During this project we built the computational model by steps, starting with a very simple model, then adding parameters and variables until we reach the final model, which can always be improved.

At this point we can include the factor VEGF, produced at the tumor and diffusing in the ECM, in order to include angiogenesis. Thus the model would examine the tumor from its avascular phase until the time it becomes vascular.

Now the model only studies the tumor's avascular stage, being nourish by a blood vessel present in the system.

Tumor cells proliferate in a normal tissue that is deformable and let the tumor grow and even move. But normal cells do not allow the tumor to

have total freedom of movement, becuse they form an ordered lattice. Furthermore, the cellular matrix hinders the proliferation of the tumor while keeping the cell density in the space of study, constant over time.

We observe that the duplication of tumor cells through cell division, increasing the cell density of the tumor results on the compression of normal tissue.

After numerous simulations we verified that the tumor adapts it shape to achieve the maximum nutrient possible and be able to proliferate.

In the graphs of tumor cell density we could note that ρ_t do not stay constant how we expect, at a certain point. Instead of that, it have some ripples due to an interesting behavior. When the tumor stops having enought nutrient and would have to arrests its growth remaining in a steady state of proliferation, it moves towards the vessel. There it can grow a bit more until it reaches a new steady.

Hence, due to the anisotropy of nutrient distribution, we do not verify the arrest of tumor growth.

In a biological environment when the tumor reaches a steady state, it develops new features that allow it to continue increasing and proliferate. The most common is that tumor induces angiogenesis, but in the cases it is treated with angiogenesis-suppressors tumor often find other ways to proliferate such as developing mechanisms to adapt to the now changed environment or metastize.

In this system the tumor moved towards the vessel to continue increasing its size and would not, in principle, require angiogenesis at this step to proceed its development.

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Appendix

A Deduction of Equation (5)

Using the Ginzburg-Landau free energy to describe the ordered phase [50]:

$$F[\phi(\overrightarrow{r})] = \int (V(\phi(\overrightarrow{r})) + \frac{\varepsilon^2}{2}|\nabla\phi(\overrightarrow{r})|^2) d\overrightarrow{r} \tag{17}$$

Where V($\phi(\overrightarrow{r})$)is a potential and has a double-well structure. In the model presented:

$$V(\phi(\overrightarrow{r})) = -\frac{\phi(\overrightarrow{r})^2}{2} + \frac{\phi(\overrightarrow{r})^4}{4} \tag{18}$$

In order to have the minimal potential located in $\phi = \pm 1$.

$$F[\phi(\overrightarrow{r})] = \int (-\frac{\phi(\overrightarrow{r})^2}{2} + \frac{\phi(\overrightarrow{r})^4}{4} + \frac{\varepsilon^2}{2}|\nabla\phi(\overrightarrow{r})|^2) d\overrightarrow{r} \tag{19}$$

When the order parameter is conserved [50]:

$$\frac{\partial\phi}{\partial t} = \nabla^2 \frac{\delta F}{\delta\phi} = \nabla^2 \mu(\phi) \tag{20}$$

Where $\mu(\phi)$ is the chemical potential, and:

$$\mu(\phi) = \frac{\delta F}{\delta\phi} \tag{21}$$

$$\delta F[\phi(\overrightarrow{r})] = \int \frac{\delta F[\phi(\overrightarrow{r})]}{\delta\phi(\overrightarrow{r})} \delta\phi(\overrightarrow{r}) d\overrightarrow{r} \tag{22}$$

$$F[\phi + \delta\phi] = \int (-\frac{(\phi + \delta\phi)^2}{2} + \frac{(\phi + \delta\phi)^4}{4} + \frac{\varepsilon^2}{2}|\nabla(\phi + \delta\phi)|^2) d\overrightarrow{r} \tag{23}$$

$$\Leftrightarrow \quad F[\phi + \delta\phi] = \int (-\frac{\phi^2 + 2\phi\delta\phi}{2} + \frac{\phi^4 + 4\phi^3\delta\phi}{4} + \frac{\varepsilon^2}{2}(|\nabla\phi|^2 + |2\nabla\phi\nabla\delta\phi|)) d\overrightarrow{r}$$

$$\Leftrightarrow \quad F[\phi + \delta\phi] = \int (-\frac{\phi^2}{2} + \frac{\phi^4}{4} + \frac{\varepsilon^2}{2}|\nabla\phi|^2) d\overrightarrow{r} +$$

$$
\begin{aligned}
&+ \int (-\phi\delta\phi + \phi^3\delta\phi + \varepsilon^2|\nabla\phi\nabla\delta\phi|)d\overrightarrow{r} \\
\Leftrightarrow \quad & F[\phi+\delta\phi] = F[\phi] + \int (-\phi\delta\phi + \phi^3\delta\phi + \varepsilon^2|\nabla\phi\nabla\delta\phi|)d\overrightarrow{r}
\end{aligned} \tag{24}
$$

Integrating $|\nabla\phi\nabla\delta\phi|$ by parts:

$$
\begin{aligned}
\Leftrightarrow \quad & F[\phi+\delta\phi] = F[\phi] + \int (\delta\phi(-\phi + \phi^3 - \varepsilon^2|\nabla\phi|^2))d\overrightarrow{r} \\
\Leftrightarrow \quad & F[\phi+\delta\phi] - F[\phi] = \int ((-\phi + \phi^3 - \varepsilon^2|\nabla\phi|^2)\delta\phi)d\overrightarrow{r} \\
\Leftrightarrow \quad & \delta F = \int (\mu(\phi)\delta\phi)d\overrightarrow{r}
\end{aligned} \tag{25}
$$

Where the chemical potential is given by:

$$
\begin{aligned}
& \mu(\phi) = -\phi + \phi^3 - \varepsilon^2|\nabla\phi|^2 \\
\Leftrightarrow \quad & \mu(\phi) = -\phi + \phi^3 - \varepsilon^2\nabla^2\phi^2
\end{aligned} \tag{26}
$$

B Deduction of Equation (8), (9) and (12)

Considering the following equations for the dynamics of tumor and normal cells, that include phase-separation (first term), diffusion (second term) and tumor cells proliferation (parameter L):

$$\partial_t \rho_t = \frac{M_\phi}{2} \nabla^2 \mu + M_\rho \nabla^2 \rho_t + L \tag{27}$$

$$\partial_t \rho_n = -\frac{M_\phi}{2} \nabla^2 \mu + M_\rho \nabla^2 \rho_n \tag{28}$$

We can derivate the equations for ρ and ϕ defined by:

$$\rho = \rho_t + \rho_n \tag{29}$$

$$\phi = \frac{\rho_t - \rho_n}{\rho_t + \rho_n} \tag{30}$$

$$\Leftrightarrow \phi = \frac{\rho_t - \rho_n}{\rho} \tag{31}$$

For ρ we obtain:

$$\partial_t \rho = \partial_t \rho_t + \partial_t \rho_n \tag{32}$$

$$\Leftrightarrow \quad \partial_t \rho = M_\rho \nabla^2 \rho_t + M_\rho \nabla^2 \rho_n + L$$

$$\Leftrightarrow \quad \partial_t \rho = M_\rho \nabla^2 \rho + L \tag{33}$$

And finally for ϕ we get:

$$\partial_t \phi = \frac{\partial_t(\rho_t - \rho_n)(\rho_t + \rho_n) - \partial_t(\rho_t + \rho_n)(\rho_t - \rho_n)}{(\rho_t + \rho_n)^2} \tag{34}$$

$$\Leftrightarrow \quad \partial_t \phi = \frac{\partial_t(\rho_t - \rho_n)\rho - \partial_t \rho(\rho_t - \rho_n)}{\rho^2}$$

$$\Leftrightarrow \quad \partial_t \phi = \frac{(\partial_t \rho_t - \partial_t \rho_n)\rho - \partial_t \rho(\phi\rho)}{\rho^2}$$

$$\Leftrightarrow \quad \partial_t \phi = \frac{\partial_t \rho_t - \partial_t \rho_n - \partial_t \rho(\phi)}{\rho}$$

$$\Leftrightarrow \quad \partial_t\phi = \frac{\frac{M_\phi}{2}\nabla^2\mu + M_\rho\nabla^2\rho_t + L + \frac{M_\phi}{2}\nabla^2\mu - M_\rho\nabla^2\rho_n - \phi(M_\rho\nabla^2\rho + L)}{\rho}$$

$$\Leftrightarrow \quad \partial_t\phi = \frac{\frac{M_\phi}{2}\nabla^2\mu + M_\rho\nabla^2\rho_t + L + \frac{M_\phi}{2}\nabla^2\mu - M_\rho\nabla^2\rho_n - \phi(M_\rho\nabla^2\rho + L)}{\rho}$$

$$\Leftrightarrow \quad \partial_t\phi = \frac{M_\phi\nabla^2\mu + M_\rho\nabla^2\rho_t - M_\rho\nabla^2\rho_n - M_\rho\phi\nabla^2\rho + L(1-\phi)}{\rho}$$

$$\Leftrightarrow \quad \partial_t\phi = \frac{M_\phi\nabla^2\mu + M_\rho\nabla^2(\phi\rho) - M_\rho\phi\nabla^2\rho + L(1-\phi)}{\rho}$$

$$\Leftrightarrow \quad \partial_t\phi = \frac{M_\phi}{\rho}\nabla^2\mu + \frac{M_\rho}{\rho}\nabla^2(\phi\rho) - \frac{M_\rho}{\rho}\phi\nabla^2\rho + L(\frac{1-\phi}{\rho})$$

$$\Leftrightarrow \quad \partial_t\phi = \frac{M_\phi}{\rho}\nabla^2\mu + \frac{M_\rho}{\rho}\nabla^2(\phi\rho) - \frac{M_\rho}{\rho}\phi\nabla^2\rho + G \tag{35}$$

Where:

$$G = L\frac{(1-\phi)}{\rho} \tag{36}$$

$$\Leftrightarrow \quad G = \begin{cases} G\phi\rho(1-\rho)\frac{(1-\phi)}{\rho} & ,\phi \geq 0, n > 0 \\ -A\rho(1+\phi)\frac{(1-\phi)}{\rho} & ,\phi > -1, n = 0 \\ 0 & ,\phi < 0, n > 0 \end{cases}$$

$$\Leftrightarrow \quad G = \begin{cases} G\phi(1-\rho)(1-\phi) & ,\phi \geq 0, n > 0 \\ -A(1-\phi^2) & ,\phi > -1, n = 0 \\ 0 & ,\phi < 0, n > 0 \end{cases} \tag{37}$$

C Deduction of Equation (10) and (11)

$$\partial_t(\phi\rho) = \rho\partial_t\phi + \phi\partial_t\rho \tag{38}$$

$$\begin{aligned}
\Leftrightarrow \quad \partial_t(\phi\rho) &= \frac{M_\phi\rho}{\rho}\nabla^2\mu + \frac{M_\rho\rho}{\rho}\nabla^2(\phi\rho) - \frac{M_\rho\rho}{\rho}\phi\nabla^2\rho + G\rho + M_\rho\phi\nabla^2\rho + \\
&+ L\phi \\
\Leftrightarrow \quad \partial_t(\phi\rho) &= M_\phi\nabla^2\mu + M_\rho\nabla^2(\phi\rho) - M_\rho\phi\nabla^2\rho + G\rho + M_\rho\phi\nabla^2\rho + \\
&+ L\phi \\
\Leftrightarrow \quad \partial_t(\phi\rho) &= M_\phi\nabla^2\mu + M_\rho\nabla^2(\phi\rho) - M_\rho\phi\nabla^2\rho + M_\rho\phi\nabla^2\rho + H
\end{aligned} \tag{39}$$

Where we introduce:

$$H = L\rho + G\phi \tag{40}$$

D First Computational Model

```
!Continuous Model with multiple points
PROGRAM ContModel
  IMPLICIT NONE
  !Initialize variables
  INTEGER :: x, y,  w, r, z
  REAL :: D, a, Dt, t, H, St, Gr, Dr, s, V2MU, MV2U, UV2M
  !Initialize matrices
  REAL, ALLOCATABLE :: F(:,:), N(:,:), U(:,:), M(:,:)
  REAL, ALLOCATABLE :: V2N(:,:), V2F(:,:)

  !Program Structure
  CALL ini_arrays()
  DO w = 1, int(t/Dt)
     CALL nut_dif()
     CALL grow_tum
  END DO

CONTAINS
  !Program Contents (Sub-Routines)
  SUBROUTINE ini_arrays()
    INTEGER :: i,j
    !Insert Constant's Values
    PRINT *, 'Enter x dimension of box: '
    READ *, x
    PRINT *, 'Enter y dimension of box: '
    READ *, y
    PRINT *, 'Enter tumour radius: '
    READ *, r
    PRINT *, 'Enter Diffusion Cte: '
    READ *, D
    PRINT *, 'Enter Alfa: '
    READ *, a
    PRINT *, 'Enter Interface Energy: '
    READ *,H
    PRINT *, 'Enter Growth Rate: '
```

```
READ *, Gr
PRINT *, 'Enter Death Rate: '
READ *, Dr
PRINT *, 'Enter Final Time: '
READ *, t
PRINT *, 'Enter Step for Print: '
READ *, St
Dt = 1/(4*16*(3.141592**4))
PRINT *, 'Dt =',Dt
PRINT *, int(St/Dt), 'Step for save (N of Iterations)'
PRINT *, int(t/Dt), 'Total of Iterations'

!Allocate all matrices to use in other sub-routines
ALLOCATE (F(x,y))
ALLOCATE (N(x,y))
ALLOCATE (V2N(x,y))
ALLOCATE (V2F(x,y))
ALLOCATE (U(x,y))
ALLOCATE (M(x,y))

OPEN (15, FILE='Cn.dat', ACTION='write')
OPEN (17, FILE='Cg.dat', ACTION='write')
write(15,*)
write(17,*)
CLOSE (15)
CLOSE (17)

!Fill in cell and nutrient matrices
DO j=1, y
   DO i=1, x
      N(i,j)=(i/100)
      IF (i==x) THEN
         N(i,j)=1
      END IF

   END DO
END DO

!Create tumor with radius r
DO j=1, y
```

```
      DO i=1, x
         F(i,j)=-tanh((sqrt(real(((i-(x/4))**2+(j-(y/2))**2)))-r)/1.0)
      END DO
    END DO
  END SUBROUTINE ini_arrays

  FUNCTION fx(i)
    IMPLICIT NONE
    INTEGER, INTENT (IN) :: i
    INTEGER :: fx
    IF (i>x) THEN
       fx=2*x-i
    ELSE IF (i<1) THEN
       fx=2-i
    ELSE
       fx=i
    END IF
  END FUNCTION fx

  FUNCTION fy(j)
    IMPLICIT NONE
    INTEGER, INTENT (IN) :: j
    INTEGER :: fy
    IF (j>y) THEN
       fy=2*y-j
    ELSE IF (j<1) THEN
       fy=2-j
    ELSE
       fy=j
    END IF
  END FUNCTION fy

  SUBROUTINE nut_dif()
    INTEGER :: i,j
    !Represent Nutrient diffusion
    DO j=1, y
       DO i=1 , x
          !Laplacian
          V2N(i,j)=N(fx(i+1),j)+N(fx(i-1),j)+N(i,fy(j+1))+N(i,fy(j-1))
-4*N(i,j)
```

```
          !Diffusion's Equation
          IF (F(i,j)>=0) THEN
             z=10
          ELSE
             z=0.1
          END IF

          N(i,j)=N(i,j)+(V2N(i,j)*D-z*a)*Dt

          IF (N(i,j)<=0) THEN
             N(i,j)=0
          ELSE IF (i==x) THEN
             N(i,j)=1
          END IF
       END DO
    END DO

    ! Save diffusion's plot for several time's steps
    IF (MOD (w ,int((St+0.00001)/Dt))==0) THEN
       OPEN (15, FILE='Cn.dat', STATUS='old', POSITION='append')
       DO j=1, y
          write (15,*) N(:,j)
       END DO
       write (15,*)
       CLOSE (15)
    END IF
  END SUBROUTINE nut_dif

  SUBROUTINE grow_tum
    INTEGER :: i,j

    !Represent Tumour Growth
    DO j=1, y
       DO i=1,x
          V2F(i,j)=F(fx(i+1),j)+F(fx(i-1),j)+F(i,fy(j+1))+F(i,fy(j-1))
-4*F(i,j)
          U(i,j)=F(i,j)*(F(i,j)*F(i,j)-1)-V2F(i,j)*(H**2)

          !Define cell's Mobility
```

```
          IF (F(i,j)>=0) THEN
             M(i,j)=1

          ELSE
             M(i,j)=0.1
          END IF
       END DO
    END DO

    DO j=1, y
       DO i=1, x

          V2MU=M(fx(i+1),j)*U(fx(i+1),j)+M(fx(i-1),j)*U(fx(i-1),j)
+M(i,fy(j+1))*U(i,fy(j+1))+M(i,fy(j-1))*U(i,fy(j-1))
-4*M(i,j)*U(i,j)

          MV2U=M(i,j)*(U(fx(i+1),j)+U(fx(i-1),j)+U(i,fy(j+1))
+U(i,fy(j-1)))

          UV2M=U(i,j)*(M(fx(i+1),j)+M(fx(i-1),j)+M(i,fy(j+1))
+M(i,fy(j-1)))

          IF (F(i,j)>=0 .AND. N(i,j)>0.0000001) THEN
             s=Gr
          ELSE IF (F(i,j)<=-1) THEN
             s=0
          ELSE
             s=-Dr
          END IF

          F(i,j)=F(i,j)+(0.5*(V2MU+MV2U-UV2M)+s)*Dt
       END DO
    END DO

    IF (MOD (w ,int((St+0.00001)/Dt))==0) THEN
       OPEN (17, FILE='Cg.dat', STATUS='old', POSITION='append')
       DO j=1, y
          write (17,*) F(:,j)
       END DO
       write (17,*)
```

```
      CLOSE (17)
    END IF

  END SUBROUTINE grow_tum
END PROGRAM ContModel
```

E Second Computational Model

```
!Continuous Model with multiple points
PROGRAM ContModel
  IMPLICIT NONE
  !Initialize variables
  INTEGER :: x, y,  w, r, z
  REAL :: D, a, Dt, t, H, St, Gr, Dr, L, G, M1, M2
  REAL :: AvRo, AvRoF
  !Initialize matrices
  REAL, ALLOCATABLE :: F(:,:), N(:,:), U(:,:), Ro(:,:), V2R(:,:), V2U(:,:)
  REAL, ALLOCATABLE :: V2N(:,:), V2F(:,:), Mf(:,:), Mro(:,:), V2RF(:,:)

  !Program Structure
  CALL ini_arrays()
  DO w = 1, int(t/Dt)
     CALL nut_dif()
     CALL grow_tum
  END DO

CONTAINS
  !Program Contents (Sub-Routines)
  SUBROUTINE ini_arrays()
    INTEGER :: i,j

    OPEN (13, FILE='val.txt', ACTION='read')
    READ (13,*) M1
    READ (13,*) Gr
    READ (13,*) Dr
    CLOSE(13)
    Dt = 1/(5*16*(3.141592**4))
    x=100
    y=100
    r=10
    t=2000
    St=1
    D=35.0
```

```
    a=0.014
    H=1.0
M2=1.0
    OPEN (14, FILE='Paramet.dat', ACTION='write')
    write(14,*) 'x =',x
    write(14,*) 'y =',y
    write(14,*) 'r =',r
    write(14,*) 'D =',D
    write(14,*) 'a =',a
    write(14,*) 'H =',H
    write(14,*) 'M1 =',M1
    write(14,*) 'M2 =',M2
    write(14,*) 'Gr =',Gr
    write(14,*) 'Dr =',Dr
    write(14,*) 't =',t
    write(14,*) 'St =',St
    write(14,*) 'Dt =',Dt
    write(14,*) int(St/Dt), 'Step for save (N of Iterations)'
    write(14,*) int(t/Dt), 'Total of Iterations'
    CLOSE (14)

    !Allocate all matrices to use in other sub-routines
    ALLOCATE (F(x,y))
    ALLOCATE (N(x,y))
    ALLOCATE (V2N(x,y))
    ALLOCATE (V2F(x,y))
    ALLOCATE (U(x,y))
    ALLOCATE (Mf(x,y))
    ALLOCATE (Ro(x,y))
    ALLOCATE (Mro(x,y))
    ALLOCATE (V2R(x,y))
    ALLOCATE (V2RF(x,y))
    ALLOCATE (V2U(x,y))
    OPEN (15, FILE='Cn.dat', ACTION='write')
    !OPEN (16, FILE='Cro.dat', ACTION='write')
    OPEN (17, FILE='Cg.dat', ACTION='write')
    OPEN (18, FILE='Ct.dat', ACTION='write')
    OPEN (19, FILE='RoT.dat', ACTION='write')
    OPEN (20, FILE='RoS.dat', ACTION='write')
    write(15,*)
```

```
  !write(16,*)
  write(17,*)
  write(18,*)
  write(19,*)
  write(20,*)
  CLOSE (15)
  !CLOSE (16)
  CLOSE (17)
  CLOSE (18)
  CLOSE (19)
  CLOSE (20)

  !Fill in cell and nutrient matrices
  DO j=1, y
     DO i=1, x
        N(i,j)=(i*1.0)/x

        IF (i==x) THEN
           N(i,j)=1
        END IF
     END DO
  END DO

  !Create tumor with radius r
  DO j=1, y
     DO i=1, x
        Ro(i,j)=0.5

        F(i,j)=-tanh((sqrt(real(((i-(x/4))**2+(j-(y/2))**2)))-r)/1.0)
     END DO
  END DO

END SUBROUTINE ini_arrays

FUNCTION fx(i)
  IMPLICIT NONE
  INTEGER, INTENT (IN) :: i
  INTEGER :: fx

  IF (i>x) THEN
```

```
      fx=2*x-i
    ELSE IF (i<1) THEN
      fx=2-i
    ELSE
      fx=i
    END IF
  END FUNCTION fx

  FUNCTION fy(j)
    IMPLICIT NONE
    INTEGER, INTENT (IN) :: j
    INTEGER :: fy

    IF (j>y) THEN
      fy=2*y-j
    ELSE IF (j<1) THEN
      fy=2-j
    ELSE
      fy=j
    END IF
  END FUNCTION fy

  SUBROUTINE nut_dif()
    INTEGER :: i,j
    !Represent Nutrient diffusion
    DO j=1, y
      DO i=1 , x

        !Laplacian
        V2N(i,j)=N(fx(i+1),j)+N(fx(i-1),j)+N(i,fy(j+1))+N(i,fy(j-1))
-4*N(i,j)
      END DO
    END DO

    DO j=1, y
      DO i=1 , x

        !Diffusion's Equation
        IF (F(i,j)>=0) THEN
          z=14
```

```
          ELSE
             z=0.1
          END IF

          N(i,j)=N(i,j)+(V2N(i,j)*D-z*a)*Dt

          IF (N(i,j)<=0) THEN
             N(i,j)=0
          ELSE IF (i==x) THEN
             N(i,j)=1
          END IF
       END DO
    END DO

    ! Save diffusion's plot for several time's steps
    IF (MOD (w ,int((10*St+0.00001)/Dt))==0) THEN
       OPEN (15, FILE='Cn.dat', STATUS='old', POSITION='append')
       DO j=1, y
          write (15,*) N(:,j)
       END DO
       write (15,*)
       CLOSE (15)
    END IF

  END SUBROUTINE nut_dif

  SUBROUTINE grow_tum
    INTEGER :: i,j

    !Represent Tumour Growth
    DO j=1, y
       DO i=1,x
          V2F(i,j)=F(fx(i+1),j)+F(fx(i-1),j)+F(i,fy(j+1))+F(i,fy(j-1))
-4*F(i,j)
          U(i,j)=F(i,j)*(F(i,j)*F(i,j)-1)-V2F(i,j)*(H**2)

          !Define Mobilities
          Mf(i,j)=M1
          Mro(i,j)=M2
       END DO
```

```
    END DO

    AvRo=0
    AvRoF=0

    DO j=1, y
       DO i=1, x
          V2U(i,j)=U(fx(i+1),j)+U(fx(i-1),j)+U(i,fy(j+1))+U(i,fy(j-1))
-4*U(i,j)

          V2R(i,j)=Ro(fx(i+1),j)+Ro(fx(i-1),j)+Ro(i,fy(j+1))+Ro(i,fy(j-1))
-4*Ro(i,j)

          V2RF(i,j)=Ro(fx(i+1),j)*F(fx(i+1),j)+Ro(fx(i-1),j)*F(fx(i-1),j)
           +Ro(i,fy(j+1))*F(i,fy(j+1))+Ro(i,fy(j-1))*F(i,fy(j-1))
       END DO
    END DO

    DO j=1, y
       DO i=1,x
          IF (F(i,j)>=0 .AND. N(i,j)>0.0000001) THEN
             G=Gr*F(i,j)*(1-F(i,j))*(1-Ro(i,j))
             L=Gr*F(i,j)*Ro(i,j)*(1-Ro(i,j))
          ELSE IF ((F(i,j)<=-1) .OR.(F(i,j)<0 .AND. N(i,j)>0.0000001))
 THEN
             G=0
             L=0
          ELSE
             G=-Dr*(1-F(i,j)**2)
             L=-Dr*(1+F(i,j))*Ro(i,j)
          END IF

          IF (Ro(i,j)<0.001) THEN
             F(i,j)=F(i,j)+((-F(i,j)*Mro(i,j)*V2R(i,j)+Mf(i,j)*V2U(i,j)
+Mro(i,j)*V2RF(i,j))/0.001+G)*Dt
          ELSE
             F(i,j)=F(i,j)+((-F(i,j)*Mro(i,j)*V2R(i,j)+Mf(i,j)*V2U(i,j)
+Mro(i,j)*V2RF(i,j))/Ro(i,j)+G)*Dt
          END IF
```

```
            Ro(i,j)=Ro(i,j)+(Mro(i,j)*V2R(i,j)+L)*Dt
            AvRo=AvRo+Ro(i,j)
            AvRoF=AvRoF+Ro(i,j)*F(i,j)
         END DO
      END DO

      AvRo=AvRo/(x*y)
      AvRoF=AvRoF/(x*y)
      IF (MOD (w ,int((St+0.00001)/Dt))==0) THEN
         OPEN (19, FILE='RoT.dat', STATUS='old', POSITION='append')
         OPEN (20, FILE='RoS.dat', STATUS='old', POSITION='append')
         write (19,*) (AvRoF+AvRo)/2
         write (20,*) (AvRo-AvRoF)/2
         CLOSE (19)
         CLOSE (20)
      END IF
      IF (MOD (w ,int((10*St+0.00001)/Dt))==0) THEN
         !OPEN (16, FILE='Cro.dat', STATUS='old', POSITION='append')
         OPEN (17, FILE='Cg.dat', STATUS='old', POSITION='append')
         OPEN (18, FILE='Ct.dat', STATUS='old', POSITION='append')
         DO j=1, y
            !write (16,*) Ro(:,j)
            write (17,*) F(:,j)
            write (18,*) (F(:,j)+1)*Ro(:,j)/2.0
         END DO
         !write (16,*)
         write (17,*)
         write (18,*)
         !CLOSE (16)
         CLOSE (17)
         CLOSE (18)
      END IF

    END SUBROUTINE grow_tum
  END PROGRAM ContModel
```