Modelling with cellular automata: problem solving environments and multidimensional applications

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Chapter 4. Modelling 3D Tumour Growth

4.1 Introduction

Cancer is the second leading cause of death (after heart diseases) in developed countries [95]. Cancer represents a wide spectrum of pathologies, which can be commonly characterized by an uncontrolled cells division, caused by a series of DNA mutations. Normally, the balance between proliferation and programmed death (apoptosis) is strictly controlled to save the integrity and structure of a tissue. This harmony will be broken as soon as at least one cell will start to divide rapidly and unsupervised [96].

An effective cancer treatment is an objective for many scientists from different fields. Not only biologists and clinicians should participate in the fight against the disease. The role of mathematical modelling is gaining importance. Modern biological approaches are often unable to disentangle the underlying mechanisms of tumour growth. This is where statistics and mathematical modelling make their contribution [97, 98].

Modelling gives an opportunity to predict the progress and the impact of different treatment schemes. A large range of models for tumour growth in different tissues have been proposed [99–110]. As was said in Sect. 1.1, explanation is the way for validating the level of cognition. This “explanation” starts form the choice of the level of abstracting, while creating the conceptual model, and this where the key problem lies.

The phenomenon of tumour growth is extremely complex and consists of many tightly coupled multiscale subphenomena. Despite the overwhelming amount of information that is available on many of these relevant processes, it turns out that many details required for mathematical modelling (such as accurate values for model parameters) are just not known. In Sect. 4.2 we will present an introduction to the biology of tumour growth by enumerating and discussing the primary subphenomena.

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The second problem is validation of the models of tumour growth, which is due to the relative lack of high quality dynamical data on tumour growth. In case of humans, as soon a tumour has been detected treatment starts. That is why it is hard to find a detailed description of the dynamics of untreated tumour growth in time. The importance of validation as a final stage of the conformance between the computational experiment and the phenomenon (see Fig. 1.1) rise the value of this problem. On the other hand we do have a lot of in vitro data, and if we have then it is often contradictory (for example compare the opinion of the proliferating layer thickness in [111] and in [112,113]). Nevertheless researchers are still creating new models of fair validity, usually neglecting relevant subphenomena of the complex process of tumour growth. An overview of existing models is provided in Sect. 4.3.

Sometimes two models are simulating very similar kinds of tumour, but demonstrate different growth patterns. At the same time there are data sets, which validate each one. Moreover there still are several completely different sights on several subphenomena. This proves the fact that despite of the significant progress we are quite far from understanding the tumour growth underlying mechanisms.

In Sect. 4.4 we are presenting two three-dimensional cellular automata based models of tumour growth. With the help of these models we study the relevance of the tumour natural shrinkage and its influence on the growth regime. In addition we introduce several algorithmic substitutions to optimize computations and demonstrate the fact that this does not influence the resulting growth regime. Moreover, involving one of our models, we study the influence of the successful mitoses rate on the tumour growth dynamics.

4.2 Biology of Tumour Growth

Tumour growth can be decomposed into a wide range of interconnected subphenomena. In Fig. 4.1 we enumerate key subphenomena and group them within following scales:

**Microscopic** $[\mu]$, covering intracellular processes taking place on the molecular level.

**Mesoscopic** $[m]$, covering the cellular scale.

**Macroscopic** $[M]$, covering the tissue (tumour) scale.

We have listed most subphenomena, which are usually discussed in the field of tumour growth modelling. The allocation to a specific scale is sometimes debatable, Fig. 4.1 presents a possible view. Other authors have created a scale separation map of tumour growth, basically proposing a comparable division into intra-cellular, cellular and tissue level processes [114]. Subphenomena, which are usually not taken into account in mathematical models of tumour growth shown in grey.

We ignore any processes related to treatment. Taking those into account would significantly enlarge the figure.
4.2. Biology of Tumour Growth

Figure 4.1: Key subphenomena, grouped into micro, meso and macro scales. Processes, which are often ignored in models, shown in grey. Several subphenomena are represented by both microscopic and mesoscopic biological processes, so they are shown on the overlapping area.

In the following we will discuss each of these subphenomena in terms of microscopic (Sect. 4.2.1), mesoscopic (Sect. 4.2.2), and macroscopic (Sect. 4.2.3) scales. The processes, which are “shared” between two scales will be discussed twice.

4.2.1 Microscopic Subphenomena

Initial mutations. Tumour growth is assumed to start from a single malignant cell [111]. There is a theory that malignant cells appear in a body from time to time, but are usually killed by the immune system. As soon as the immune system fails to kill it the cancerous growth starts [115].
Each healthy cell can become a malignant one if it underwent at least two DNA mutations. The first one is a mutation of a proto-oncogene, which is a gene, involved in the cell cycle regulation process. If the mutation of the proto-oncogene results in an uncontrolled cell proliferation then the proto-oncogene is an oncogene [116].

For example, an oncogene can express the protein, which will implement a truncated receptor (without the exterior sensor, but with all the interior part). This receptor can act as if the signal molecule is permanently connected thus constantly promoting proliferation of the cell. This is one of many possible mechanisms, used by oncogenes.

The second mutation should affect a tumour suppressor gene (or anti-oncogene). Usually, proteins coded by such genes are able to slow down or even halt the proliferation or promote apoptosis in case of a cell disease. There are number of such proteins. The retinoblastoma protein (pRb) was the first tumour suppressor protein discovered. But growth of half of solid tumours starts from the mutation of the gene, coding for the p53 protein.

**Proliferation.** Cell proliferation is a series of internal processes, which take place in a cell finally leading to its duplication via mitosis. Four phases can be distinguished in the cell cycle: gap-1 (G1 phase), DNA synthesis (S phase), gap-2 (G2 phase) and mitosis (M phase) [116,117]. After division, both mother and daughter cell continue to proliferate entering the G1 phase. In Fig. 4.2 schematic representation of proliferation is presented.

Figure 4.2: Schematic representation of the cell cycle. Regulatory points in G1 and G2 phases are shown with small arrows. G0 phase corresponds to a quiescent state.
During G1 and G2 phases the cell accumulates and prepares resources for DNA synthesis (during G1) or mitosis (during G2). A key process during G1 and G2 is self-verification of conditions of health. For example, the p53 protein participate in DNA verification during the G1 phase. If errors are detected p53 will delay G1 until DNA has been repaired. It also is able to drive the cell into apoptosis (see “Apoptosis”), if the DNA cannot be repaired. During G2 the two copies of DNA are compared. Copy errors may also lead to the apoptosis. Even during the S phase a special mechanism prevents erroneous genes to be put into the synthesized DNA.

At the same time there are so called “regulatory points” [118] during the G1 and G2 phases. After having passed it during the G2 phase the cell will necessarily enter the M phase (even if the consequences can be fatal). The same applies to entering S after the G1 phase.

The cell cycle is controlled by cyclins and cyclin-dependent kinases (CDKs). Cyclins are special proteins, which are synthesized and destroyed by lysosomes. During G1 the activity of CDKs is low because the relevant cyclin partners are missing – their production is inhibited, and they are rapidly degrading. But then cyclin synthesis is promoted, hence the CDKs are activated, and their activity remains high during S, G2, and M phases, since this is necessary for DNA replication and other processes occurring during the final stages of the cycle. At the end of mitosis (in the anaphase) a protein complex is activated and marks specific target proteins (such as cyclins) for degrading with the help of the proteolytic machinery of the cell. This protein complex is composed of a dozen of polypeptides and two auxiliary proteins: CDC20 (cell division cycle protein 20) and CDH1 (CDC20 homologue 1). These two proteins present the target proteins for labelling. Together, they label cyclins for destruction at the end of the cycle, allowing the control system to return to G1. CDC20 and CDH1 activity is also controlled by CDK complexes. However, CDKs control each protein separately and while CDK activates CDC20 it inhibits CDH1 [115]. In Fig. 4.3 the dynamics of the concentration of several CDKs is shown.

Despite the large number of differences between the cell-cycle of cancerous and normal cells, some mechanisms are common. In particular, regulation of the transition through the check points is accomplished in both cases the CDK network.

Quiescence (see also “Hypoxia”). Cells need three kinds of resources for supporting its life: glucose and oxygen for metabolism, and aminoacids as building blocks [118]. A cell can enter a quiescent state called gap-0 phase (G0), for example, due to lack of supporting resources or bad external conditions (high concentration of toxins or physical pressure). In Fig. 4.2 the possible moment of entering this state is shown with a dashed line [103–106].

Quiescence is a bit similar to anabiosis – all internal metabolic processes are slowing down. Quiescent cells are much less susceptible to apoptosis than proliferating cells. Being quiescent, cells still exist in the morphological sense. They
still are able to migrate (see “Migration”) and still need nutrients, but much less than being proliferating (approximately, by a factor of ten) [99, 109]. This gives them the opportunity to survive during lack of resources. The ability of cancer cells to go into a quiescent state provides them with a remarkable resistance to unfavourable conditions.

The transition to the quiescent state is reversible and the cell can get back to proliferation. Many cells in human body are quiescent and some particular cells be quiescent permanently [116].

Quiescence becomes a weapon of cancer cells, which might wait, being protected from apoptosis, while normal cells are starving or dying, for example, from chemotherapy. This allows malignant cell to wait for better times, for example, until the local cell density will decrease and there will be more oxygen available, so they can come back to the proliferating state.

**Necrosis.** Necrosis is the first kind of cell death [116]. It is provoked by external causes such as lack of resources, spread of toxins, extreme pressure etc. As soon as the cell dies, its membrane breaks, the intracellular liquid is flowing away,
and all the consumable resources are absorbed by surrounding cells. Remaining material should be processed by phagocytes (see “Immune system response”) if they are able to find it. Otherwise the remains will start to degrade acting as toxins and tumour angiogenesis factors (TAFs) (see “Tumour Angiogenic Factors (TAF)”) simultaneously.

**Apoptosis.** In contrast to necrosis, apoptosis is a programmed cell death [116]. In early literature it was also called “shrinkage necrosis”. Apoptosis can be triggered by p53 in case of DNA damage, by hypoxia, by viruses etc.

One of the mechanisms of tumour invasion is that malignant cells are trying to trigger apoptosis of neighboring normal cells on the boundary of the tumour clot [108]. If the neighbourhood of a normal cell contains more normal cells than cancer cells, then the threshold p53 concentration will be fixed to a higher value than for the situation where there are more cancer cells than normal cells. As a result, normal cells are more resistant to apoptosis if they have more normal cells as neighbours.

**Hypoxia** (see also “Quiescence”). The lack of oxygen (or hypoxia) can be considered as a special case, because the behaviour of the cell can differ from the situation of lack of other resources, mentioned above in the discussion of quiescence. Hypoxia may lead to slowing down of the cell cycle without getting quiescent [116]. Such state can be called “arrest”, because the most of cell’s functions, including proliferation, will be suspended. This mechanism is controlled by p27, whose production is stimulated by hypoxia. Lower p27 concentration will result in faster proliferation, lower apoptosis rate and lower normal cells mitotic activity [118].

Arrest is observed mostly in healthy cells. Malignant cells in conditions of hypoxia can demonstrate both – arrest or quiescence.

Hypoxia also stimulates vascular endothelial growth factor (VEGF) secretion to catalyze vessel growth and thus provide blood supply. Finally, hypoxia rises the p53 concentration and apoptosis probability [108].

**Senescence.** Even in fine conditions cell life cannot last forever. There is the so called Hayflick’s limit [119] for the maximum number of possible divisions a single cell can undergo. In 1961 Leonard Hayflick showed that no human cell divides more than 40–60 times. Later it was found that this is caused by the fact that telomeres is shortened each time during DNA replication. However, it can become too short to allow creation of a complete DNA copy. In addition, histons (alkaline proteins, which package the DNA into nucleosomes) are also loosing elasticity in time.

At the same time malignant cells do not suffer from senescence and usually are immortal due to the activation of their telomerase genes [120]. Mutation of such genes are involved in 85% of solid tumours (see “Initial mutations”). This means that reactivation of telomerase in healthy cell increases the risk that it can become malignant.
Mitosis suppression. Cell mitosis can fail even after the regulatory point due to external causes such as overcrowding, physical pressure, incoming intercellular signals or other reasons. A malignant cells which is situated deep inside the tumour has less chance to divide successfully than one on the periphery of the tumour [121,122]. Mitosis failure is discussed in literature, but there we no attempts to quantify it. This subphenomenon is always neglected in tumour growth models, because the rate of successful mitoses can hardly be observed and measured in vivo or in vitro. In Sect. 4.4.5 we will perform an in silico study of possible mitosis rates.

Roles of proteins (p16, p21, p27, p53, p57). Life of a cell is regulated by many proteins. Modelling a cell on the microscopic level will require the simulation of the dynamics of their concentrations [118]. Only five proteins, which are now considered as most important to tumour growth are discussed below.

P16 – CDK inhibitor 2A (see Fig. 4.3), a tumour suppressor protein, which plays an important role in the cell cycle regulation. Mutations in p16 may lead to miscellaneous cancers and often to melanoma [123]. Increased expression of the p16 gene as organisms age reduces the proliferation of stem cells. This will act as a cancer protection, but rises the chances for cellular senescence (see “Senescence”). A chain of chemical reactions puts p16 concentration in tight interdependence with concentration of p53.

P21 – CDK inhibitor 1. It plays a regulatory role of cell cycle progression during DNA synthesis. Most of the time the expression of the gene, which codes for p21, is controlled by the concentration of p53, but sometimes it is expressed independently.

P21 can promote the growth arrest and cellular senescence. Not being able to provoke cell death on its own, it inhibits apoptosis. This protein also participates in a the stress response [124]. In term of tumour growth this means that it is important for response to therapy. At the same time, mutations related to p21 do not correlate with cancer. In contrary, experiments on mice showed that the complete lack of p21 does not pose any problems in the organism development [125].

P27 – CDK inhibitor 1B, one of the most important proteins, which was already discussed (see “Hypoxia”). By blocking the activation of CDK2, p27 controls the cell cycle progression at G1 phase. It is also required early in the cell cycle for the assembly of cyclin D1/CDK4 complexes. This allows to conclude that in the beginning of the cell cycle p27 acts as a starter, but later it takes a restrictive function, so lower p27 concentration will result in faster cell cycle and lower apoptosis rate [108]. P27 is used by miscellaneous growth factors to slow down the proliferation [115].

But the function and influence of p27 seems to be even more complex and is not fully understood: “The current dogma has been that p27 is a classic tumour suppressor... Our studies suggest a more complex role for p27. We found that
p27’s role as a tumour suppressor requires two functional copies of the gene, since the loss of a single copy of p27 results in increased susceptibility to breast tumour formation. However, loss of both p27 alleles results in decreased growth of breast epithelium, and a reduced frequency of breast tumour formation [126].

In normal cells the production of p27 is controlled along the cell cycle, whereas no such control has been assumed in cancer cells. Although p27 mutations are quite rare in tumours, this plays significant role.

P53 – the most important tumour suppressor protein (see “Proliferation”, “Apoptosis”). It is also called “the guardian of the genome”. The p53 gene can activate DNA repair proteins, mediate apoptosis in various situations, such as DNA damage, lack of resources, presence of certain cytokines etc. Cells, which have lost p53 expression or have acquired a mutation in the p53 gene, can survive in unfavourable conditions for longer periods than their wild-type counterparts [116].

In normal cells, p53 expression stimulates apoptosis and may also inhibit VEGF production (see “Tumour Angiogenic Factors (TAF)”). Mutations in p53 may lead to an extremely dangerous scenario in which cells do not decrease VEGF production rate and stop triggering apoptosis [108].

P57 – CDK inhibitor 1C is a negative regulator of cell proliferation, having an ability to slow down or even arrest the cell in the G1 phase. On contrary, its mutations may lead to uncontrolled cell proliferation and are associated with sporadic cancers and the Beckwith-Wiedemann syndrome [118].

Tumour Necrosis Factor (TNF). From a chemical point of view, TNF is a group of cytokines produced mainly by monocytes and macrophages and is a special kind of an immune system reaction. TNF can induce cytolysis or apoptosis of the tumour cells [127]. The way it triggers apoptosis is not involving p53, because it is mutated in most of the tumour cells. TNF uses a special TNF receptor, which is associated with procaspases and is able to cleave it. In addition it is also able to trigger the caspase cascade leading to apoptosis.

The range of TNF functions is very wide. It is also able to interact with endothelial cell receptors to give leukocytes an opportunity to access an infection. Except death it can provoke tumour cells to proliferate, differentiate, mutate, depending on the conditions and tasks. But the primary role of TNF is in the regulation of the immune cells. Mammals have a special TNF gene, providing such a rich set of possibilities.

4.2.2 Mesoscopic Subphenomena

Proliferation. From the mesoscopic point of view we consider the cell as a closed box, which is characterized (among the others) by its phase. The duration of these phases is the key here. Assuming a total cell cycle duration of around 25 hours the actual division of a cell during mitoses will take 30–60 minutes. The
G1 phase requires at least 9 hours. Of course there is a large biological spread on these data, so in our models these durations will be of a stochastic character.

Another important mesoscopic note is that during M phase a cell is most fragile and sensitive to external factors like treatments. In contrast it is mostly resistant (excluding G0) during the S phase.

**Necrosis and Apoptosis.** The key question with regard to necrosis and apoptosis at the mesoscopic scale is the difference between these two kinds of cell death. First, usually necrosis happens with a group of cells, while apoptosis occurs with individual cells. When dying due to necrosis, the cell is breaking with a significant part dissolving. However, the rest remains until evacuated with blood. On the other hand, apoptotic cells break apart into several vesicles called apoptotic bodies, which are immediately phagocytosed, because apoptosis is the result of systematic processes and the immune system is informed about its occurrence. So, apoptosis leaves no remains, while necrosis does. This can be relevant for future dynamics as these remains can start acting as toxins [116].

**Hypoxia.** Similar to necrosis, hypoxia is a phenomenon acting on regions of cells. As was mentioned above, hypoxia triggers chemical pathways and promotes a number of mesoscopic responses, such as VEGF release (see “Tumour Angiogenic Factors (TAF)”), arrest, quiescence, and others [108].

**Quiescence.** A quiescent cell is even more resistant to external impacts than in the S phase (see “Proliferation”). This state seems to be very advantageous for malignant cells, but for most types of cells there is a principal limit of time, which cell can spent quiescent [103–106].

**Senescence.** In some sense senescence may look almost similar to quiescence. The only difference is that it is not reversible and necessarily leads to necrosis sooner or later.

**Tumour Necrosis Factor (TNF).** Leaving all the details on the microscopic level, TNF is a substance which is subject to diffusion. Moreover, two types of TNF should be distinguished: TNF-α (known as cachexin or just “TNF”) and TNF-β (known as lymphotoxin). They differ by their effect and concentration ranges [118].

**Adhesion.** Cellular adhesion is binding of a cell to a surface – extracellular matrix or another cell. Cell adhesion molecules (such as selectins, integrins, and cadherins) and proteins play an important role for living organisms [116]. For example, Alzheimer’s disease is caused by errors in the cells adhesion regulation. Cell adhesion proteins hold solid tissues together. When a cell is dividing it becomes round and looses connections with the substrate. There is a special mechanism (see “Intercellular communications”), which allows only a few cells to divide at the same moment in a local region of space. Otherwise this may break tissue integrity. At the same time, malignant cells are characterized with
much lower adhesiveness than their healthy counterparts, so this mechanism is
switched off for them. Moreover, if normal cells cannot divide without being
connected to the substrate prior to mitosis, tumour cells can hardly divide,
being tightly attached to the substrate.

Cancer can involve mutations in genes, corresponding to adhesion proteins. This
results in abnormal intercellular interactions [128].

Local pressure. The effect of local cell overcrowding influences all key processes
such as mitosis (it can fail due to high local pressure), apoptosis (it can be
triggered), necrosis (extreme pressure can result in necrosis), quiescence (cells
can try to survive by entering it). Local pressure depends on the tissue land-
scape: it is higher close to blood vessels, which bring resources to the cells. In
contrary, spread of toxins forces cells to migrate away. Cells can retrieve inform-
ation about the pressure via intercellular communications from neighboring
cells [99, 107].

Intercellular communications. Three types of intercellular information exchange
can be distinguished by distance: juxtacrine signalling is happening if cells are
immediately attached to each other, paracrine signalling is possible on short
distances, and endocrine signalling is a large range communication [116].

In most of cases cells are interchanging information with the help of signalling
molecules which can bind to receptors. Endocrine signalling is performed by
hormones.

Among others, cells are exchanging information concerning the conditions in
which they stay. A cell can be informed about less crowded places and the
amount of resources in some neighbourhood (even not immediately close) and
make a decision about migratory actions (see “Migration”). Growth factors –
special proteins, which are capable of stimulating cellular proliferation, are often
triggered by signalling molecules interchange. Malignant cells can utilize this
feature if oncogenes code growth factors.

Tumour Angiogenic Factors (TAF). Solid cancers cannot grow beyond a limited
size. The only way in which glucose and oxygen can reach inner cells in isolated
tumours is by diffusion through outer malignant cells. In such situation only the
outermost layers of cells are supplied adequately, when the rest are suffering from
lack of resources. The lack of glucose and oxygen leads avascular tumour mass
to produce and release substances called TAF. These substances are diffusing
through the surrounding tissue. On reaching the vasculature TAF triggers a
cascade of events which initiates the growth of vascular structures into the
tumour (see “Vasculogenesis and angiogenesis”) to restore the supply of the
resources.

Vascular endothelial growth factor (VEGF) [129] is one of the best known chem-
ical signal for blood vessels growth stimulation. TAF is a general class of sub-
stances, when VEGF is a subclass – the platelet-derived growth factors family
of cystine-knot growth factors. The functioning of VEGF consists in binding to
tyrosine kinase receptors on the cell surface, activating them through transphosphorylation. As a result not all VEGF, which is diffused through the tissue, is necessarily triggering vessel formation. When modelling this process free VEGF and assimilated VEGF should be distinguished. The probability of contact with a receptor defines the assimilation. VEGF degradation also plays key role in the process [108].

In healthy tissue VEGF is secreted to generate the circulatory system during embryonic development and restore vessels after they were damaged for some reasons. VEGF is interacting not only with vascular endothelium (as one may consider from its name), but is also involved into the stimulation of monocytes and macrophages migration. VEGF secretion may also lead to the migration or further differentiation of a cell. At the same time it can contribute to a disease when it is overexpressed. Producing VEGF cancer is able to grow over the avascular limit and produce metastasis (see “Three stages of growth”). Moreover, VEGF overexpression can result in vascular diseases and malignant tissue is often subjected to them.

Drugs based on VEGF secretion suppressors (like ranibizumab or bevacizumab) are often used in cancer treatment.

Vasculogenesis and angiogenesis. The constructive evolution of the circulatory system involves two major processes: vasculogenesis (the formation of the new embryonic circulatory system) and angiogenesis (the growth of vessels from existing vasculature). Angiogenesis may lead to anastomosis – the reconnection between previously branched vessels, which leads to a kind of loops formation. Due to anastomosis pathological fistulas can be formed [130].

In the vascularized tissue vessels form a high density network: for each cell there should be a vessel at a distance of no more then five cells [108]. The formation of this network is in tight connection with the tumour itself: vessels influence tumour shape (by pushing cells, while growing into and by bringing resources), while the tumour influence vessels (by secreting TAFs). In addition, vessels in malignant tissue are very unstable (much less stable than in healthy tissue) and can undergo a dematuration process after being assimilated into the tumour. As a result, newly formed vessels of a previously avascular tumour clot can easily collapse. At the same time the concentration of VEGF will remain high enough to trigger angiogenesis, so the process will be restarted [108].

Influence of pH-factor. From the point of view of metabolism malignant cells behave similar to their healthy counterparts, consuming both: glucose and oxygen. Glycolisis always results in a rise of acidity, and this becomes a weapon for a cancer, because the resistance of tumour cells is much higher. There is a theory that cancer may promote the glycolytic phenotype (see “Phenotypical mutations”) in order to increase the invasiveness [108].

The value of the pH-factor influences the cell cycle duration (the speed of proliferation) and cell survival probabilities.
Influence of $\text{H}^+$. Another one side-effect of glycolytic phenotype is the rise of $\text{H}^+$ ions concentration. $\text{H}^+$ can also catalyze angiogenesis [108].

Natural shrinkage (see also “Necrosis”). Usually shrinkage is associated with tumour treatment, but in fact any neoplasm, where cells are subject to necrosis, show shrinkage. After a cell dies due to necrosis its membrane disintegrates, then all the fluid content flows out and diffuses away or is consumed by neighboring cells. As a result the remains occupy nearly one third of volume of the living cell [103–107].

This may have relevance for determining the tumour growth regime, because the necrotic core usually occupies more than half of the tumour volume (see “Three layered structure”). Most models do not take natural shrinkage into account [131]. That is why in Sect. 4.4 we present several mesoscopic models of tumour growth with and without such shrinkage in order to illustrate its relevance and influence to the growth regime.

Immune system response (see “Tumour Necrosis Factor (TNF)”). The immune system is a complex of processes and biological structures aiming to protect an organism against malfunctioning and invasions by identifying and killing pathogens. The immune system governs leukocytes (white blood cells), which behave like independent units, travelling inside the organism and taking care of abnormalities. Leukocytes can be grouped into phagocytes (macrophages, neutrophils, and dendritic cells), mast cells, eosinophils, basophils, natural killer cells, and lymphocytes (B cells and T cells) [132].

One of the basic functions of the immune system is garbage collection and evacuation of cells remains (see “Necrosis” and “Apoptosis”). In addition, VEGF acts as a macrophages attractor. While modelling this subphenomena one should account that macrophages and other immune system facilities are travelling with the blood flow and thus are able to access only the neighbourhood of vascularized areas.

Due to their mutations malignant cells express antigens which are considered by the immune system as foreign. Tumour antigens are presented in a way similar to viral antigens and the response of the immune system is to destroy the malignant cells by means of killer T cells. The immune system may also involve natural killer cells into the tumour cell destruction if malignant cells express fewer major histocompatibility complex (MHC) class I molecules on their surface than their healthy counterparts [116].

Some tumours are trying to hide from the immune system. One of the possible mechanisms a cell can use is trying to reduce the number of MHC class I molecules on its surface. This can hide the cell from T killers, but will attract natural killers [132].

Some malignant cells are able to release immune response inhibitors such as the TGF-$\beta$ (transforming growth factor), which suppresses the activity of macrophages and lymphocytes.
Migration. Even when forming a tumour clot, malignant cells are moving inside it, according to information from it’s receptors. The main reason for cells to move is looking for better conditions. This means that all cells are trying to get close to the surface of the clot, where the pressure and toxins concentration is less, and the concentration of resources is larger. Important to note that not only proliferating cells, but also quiescent cells are subjected to such migration (see “Quiescence”).

At the same time experiments show that cells are migrating not only towards the periphery. A significant number is migrating towards the center of the tumour (necrotic core) [133,134]. This inward flow is a requirement for saturation of avascular growth. Without it, a constant flow of nutrients from the perfectly vascularized tissue around the tumour will result in unbounded growth [99].

Two major types of migration should be distinguished. The first is chemotaxis – motility according to chemical signals, in the direction of the largest concentration of an attractor (glucose, for example) or the smallest concentration of an repeller (toxins or the products of degradation, for example). The second one is haptotaxis – motility under the influence of an extracellular matrix. The key role in this process belongs to fibronectin, which enhances cell adhesion to the matrix. According to haptotaxis, a cell is trying to adopt its shape and to move to minimize an energy of adhesion (see “Adhesion”).

The only time when cell cannot migrate is during mitosis, because all the internal systems are focused on the division and ignore incoming information from the receptors. Obviously, even in the M phase cells can still change their position due to pressure (see “Local pressure”) if they are pushed towards neighbourhood with lower cell density.

Phenotypical mutations. The term “phenotype” designates some characteristic of a cell. Changes in phenotype are originated by the expression of definite genes or influence of environmental factors. So, mutations which turn the cell into the malignant state lead to phenotype changes.

Usually viable malignant cells have modified phenotype, which boosts their invasiveness and resistance [135,136]. That is why, as was mentioned above (see “Influence of pH-factor”) tumour cells are promoting glycolytic metabolism. Shifting of the proliferating cycle regulatory points is also observed (see “Proliferation”). They also try to overcome the Hayflic’s limit on the number of divisions.

A healthy cell is not dividing all the time, but is waiting for a signal to divide via the intercellular communications mechanism (see “Intercellular communications”). Malignant cells have a phenotype in which this mechanism is switched off and they are dividing persistently.

Tumour phenotypical mutations cannot be neglected, because they influence such properties as the shape of a tumour (because of the influence on invasiveness), speed of growth and the growth regime [137].
Cancer Stem Cells (CSC). The theory of a CSC-driven tumour growth represents a relatively new insight on the phenomenon [138]. Phenotypical mutations makes malignant cells strong enough to win the competition against other cells. This phenomenon was called “tumour Darwinism” [139]. But recent experimental evidence demonstrates the diversity in differentiation grade of malignant cells. Cells with an immature phenotype expression seem to be the main vehicle of tumour growth, having unique opportunities to renew themselves and to differentiate. Such features are known as attributes of normal stem cells. That is why malignant cells with such abilities were named as CSCs.

The CSC concept sheds new light on the process of the malignancy formation, but is still involved in a very few models of the growth [109,110]. But even several decades ago it was observed that malignant tumour cells can demonstrate abnormal mitosis, producing three or more daughter cells [131]. Such “wild division” is a typical feature of not differentiated cells.

4.2.3 Macroscopic Subphenomena

Three layered structure. From the macroscopic point of view, three layers can be distinguished in an avascular tumour spheroid (see Fig. 4.4). The outermost consists principally of the proliferating cells, which are close enough to the nutrients supply from the healthy tissue. Then comes a quiescent layer. Cells of this layer turn to a non-proliferating state because of lack of nutrients and spread of toxins. The Necrotic core is a clot of remains of the dead cells, which were suffering from lack of resources too long, while being quiescent [99].

The external supply of nutrients on the avascular phase of tumour growth often results in a radially symmetric shape. Nevertheless, such growth is limited, because the inward flux of nutrients is proportional to the area of the surface of the spheroid. In other words is is proportional to the squared radius of the spheroid, while the volume is proportional to the radius cubed. So, avascular growth will stop, unless angiogenesis starts.

Three stages of growth. Three stages of cancer growth may be distinguished: avascular growth, angiogenesis, and vascular growth. On the first stage the tumour is growing in the absence of blood supply. As was mentioned above, growth during this stage is limited by the amount of nutrients, which could be obtained via the tumour surface. It cannot grow over few millimetres in diameter, so the tumour starts to produce TAFs to promote vascularization. As soon as the creation of a circulatory subsystem inside the malignant clot is completed, the tumour enters the vascular stage and gets access to virtually unlimited resources. It gets the opportunity to grow beyond the limit of the avascular stage and produce metastasis. Metastasis represents a minimalistic tumour clot. It can be brought by the blood flow to another location inside the organism and is able to initiate the growth of another tumour there. So, while the avascular phase of tumour growth is harmless, once it become vascular, it is potentially fatal [108].
Chapter 4. Modelling 3D Tumour Growth

Figure 4.4: Schematic representation of the three layered tumour structure.

Being in vascular stage, tumour can release great number of metastasis into the circulation, but only less than 0.1% can survive and initiate a tumour growth in another area of organism [140, 141].

From the medical point of view two main properties allow to classify the tumour: the grade and stage of the growth. Unfortunately, there is no precise system of tumour grading. It was agreed that it should be based on the degree of malignant cells differentiation. Thus there is no gauge for the measure of this parameter, it is empirical and subjective. Moreover, different parts of the same specimen can vary in the degree of differentiation. So a biopsy in a random point of a neoplasm cannot characterize the tumour as a whole.

On practice, medics use three grades: well differentiated, moderately differentiated, and poorly differentiated. One quarter of tumours falls into the first group, one half into the second, and the remaining quarter into the third.

Circulatory system (see “Vasculogenesis and angiogenesis”). All processes, which are related to the blood flow and angiogenesis should take an origin from the preexisting circulatory subsystem, which exists in the tissue, surrounding the neoplasm. The structure of the vessels is quite complex. Their radius is not
constant, they are bifurcating and reconnecting. Simulation of these processes requires knowledge, that is not yet available.

Moreover, the actual blood flow brings not only glucose and oxygen, with the red blood cells, but also wide variety of white blood cells (see and “Immune system response”).

**Physical pressure.** Local pressure, which appears inside a cellular mass should be accounted for together with the pressure, which can be produced by macroscopic objects around the tumour, such as bones and neighbouring organs.

These lists is not are by no means complete. Nevertheless we have included the main subphenomena, which are involved into tumour growth. The effect of some of them is underestimated (see grey nodes in Fig. 4.1). So, in Sect. 4.4 we are going to present studies of two of them: natural shrinkage and mitosis suppression.

### 4.3 Overview of Existing Models

A lot of tumour growth models were proposed in last four decades [137,142]. The biological basis of the phenomenon was permanently being studied and refined during that time. This process is still not finished. So, each new finding as suggested by experiments shed new light on the process of tumour growth. Conceptual models therefore were subjected to revisions, extensions and renovations based on new experimental evidence.

The choice of scales, methods, and subphenomena to be included, multiplied by the number of possible computational modelling vehicles results in many simulation approaches. We will briefly describe some models, which played a significant role in the history of the field. In the following overview models will be presented more or less in chronological order, and named by the first author. The aim of this overview is to show the progress in the fields, but also to point to relevant subphenomena that have not been included into the state-of-the-art models for tumour growth.

In 1986 Adam offered [143] his by now classical one-dimensional continuous mathematical model of tumour growth. This model is based on the generalized mitotic inhibitor (chalone) dynamics simulation. Based on [144] assumes that the growth of a tissue is regulated by a threshold mechanism: there is a value of chalone concentration, above which mitosis is not possible. Among others, chalone provides a limit to the tissue dimensions limiting. Overcoming this limit, the growth becomes uncontrolled*.

In comparison with model of Glass [144], which was offered 13 years earlier, model of Adam requires much greater chalone production rate to achieve tissue stability, but this stability is achieved at much larger limiting dimensions. At that time, which can be named as the initiation of the field, models were competing on the level of gaps of validity and stability.

*Compare these reasoning with the phenotypical mutations and cancer stem cells concept.
Next year Adam generalized the model to three dimensions [145, 146]. This model was able to provide a qualitative description of the spheroid growth, but it was not compared with the experimental data. An important note is that the approaches of Glass [144] and Adam [143, 145, 146] did not incorporate any volume loss mechanisms such as necrosis.

In 1993 Qi et al. [100] proposed a two-dimensional model of tumour growth, which was accounting such microscopic subphenomena as cells proliferation, cytotoxic behavior of the immune system, local pressure inside the tumour and some more. This model is able to reproduce phenomenological Gompertz growth [147, 148]. The model is based on a cellular automaton with the grid of squares and von Neumann neighbourhood. Each cell contains one of four objects: a healthy cell, a malignant cell, a dead tumour cell or a complex, produced by phagocytes as a product of the immune system reaction. The timestep equals one day. A cross of five tumour cells is taken as the initial condition. Proliferation is modelled in a probabilistic way. Moreover, a tumour cell is able to proliferate only if there is at least one vacant cell nearby.

An automaton cell containing a complex can become unoccupied with a distinct probability. A dead cell is also able to dissolve. This mimics important subphenomena, like infiltration of healthy tissue into the cancerous one due to local pressure or migration and evacuation of cell’s remains with the blood flow. Even more recent models are often neglecting this.

To compute the pressure, a large enough neighbourhood is taken into consideration to compute the number of malignant cells.

In the same year, Chaplain et al. [131] proposed a model of tumour growth based on continuous inflation under the mechanical pressure from the environment using results and techniques from nonlinear elasticity theory. Conditions and restrictions for the processes of tumour invasion are formulated in terms of the strain-energy function. Metastasis are interpreted as bifurcations of the spherical shell, which is used to essentially model the tumour.

This formulation in terms of the strain-energy function exposes the difference between two important classes of tumours: benign and malignant. The degree of benign tumour cells differentiation is much higher. So, a being tumour has a close resemblance to the originating tissue. The growth rates and the way of extension are different. Malignant tumours start with the exponential growth, but as the tumour enlarges, the cell loss increases and the rate of growth gradually slow down to linear. Benign tumours proliferate locally and grow by expansion, compressing the surrounding tissue, and thus causing its atrophy, while malignant tumours grow by both expansion and infiltrating the surrounding tissue.

The fact that malignant cells differ from the healthy cells from the mechanical point of view at least by their adhesiveness to one another is also taken into account.
Elasticity theory describes the basic relationship between forces in a continuous body and its shape. The deformation of the body can be described in terms of the strain, which is directly related to the stress. Authors assume that the underlying deformations, involved in the growth of solid tumours, are large and thus they are using finite deformation elasticity theory to model the growth.

Initially, the tumour is assumed to be a sphere with a necrotic core inside and a layer of proliferating cells around it. All cells are supposed to be identical and presented as incompressible structures of constant volume. The layer of proliferating cells is modelled as the membrane of a balloon being inflated. Such membrane is characterized by a strain-energy function. The key misconception of such modelling is caused by the fact that the membrane of the balloon should physically grow (the amount of material increases, because of cells replication), when it is not met in traditional balloons.

Chaplain et al. consider a thick layer of living cells and bifurcations into non-spherical configurations, abnormal mitoses (which can be considered as mimicking the phenotypical mutations).

Being interpreted from the oncological point of view, the physical model parameters have significant medical meaning: the strain-energy function represents the degree of malignancy of the tumour and, at the same time, the degree of differentiation of the tumour cells. “Gaussian curvature” represents the local growth rate of the surface of the tumour, “mean curvature” – the local variation of the tension force over the tumour surface, which indicates the degree of spread of the tumour versus invasion and metastasis. Classification of real tumours using these three parameters will give the ability to determine all of the main factors by grading and staging of simulated tumours.

Moving focus to the mechanical properties of cancer was quite novel at that time. The model retains many important features of previous models, while introducing new qualitative measures of both tumour and malignant cells (macroscopic and microscopic).

In 2000 Kansal et al. [101] created a three-dimensional cellular automata model of tumour growth with an adaptive grid. It focused on malignant neuroepithelial tumours such as glioblastoma multiforme. In this model growth starts from a few malignant cells, then forms a multicellular spheroid, and proceeds to macroscopic stages. Only on this latest stage a tumour can be detected with clinical methods.

The authors consider the tumour as a self-organizing complex dynamic system with Voronoi tessellation, initially generated according to random sequential addition. Traditionally, healthy cells are considered as vacancies, which can be occupied by the malignant cells. Proliferation of cells is only possible if there is at least one such “vacancy” nearby.

The resulting tumour represents an idealized spheroid with fluctuations on the boundaries between proliferating, quiescent and necrotic layers. The approach fits experimental data.
In the same year *Kocher et al.* [102] presented a simulation of the cytotoxic and vascular effects of radiosurgery in brain tumours. Radiosurgery is a kind of cancer treatment, based on necrotizing of definite tissue regions.

This model is based on a three-dimensional cellular automaton with Moore neighbourhood. Each tumour cell occupies single grid position, with a size equal to 20 $\mu$m along all axis. The cell cycle duration is 5 days. A single tumour cell is placed in the center of the grid as an initial state for the simulation. Cells are dividing once per cycle duration. In order to desynchronize them a random value from +2 to −−2 days is added. Daughter cell takes place in one of randomly chosen neighboring positions. If the chosen position is occupied, then all tumour cells and vessels are shifted.

The model of the circulatory systems assumes that capillaries can be positioned at every seventh grid cell in any space directions (so, there will be no less than 140 $\mu$m between vessels). Tumour cells which are farther than 100 $\mu$m from a blood supply are considered to be quiescent. Cells on a distance more than 140 $\mu$m are regarded as necrotic.

Irradiation simulation is based on a linear-quadric model [117,149], which is usually used for this. Survival probabilities are computed for proliferating and quiescent cells separately. Cells hit by irradiation undergo mitotic death at their next attempt to divide. Quiescent cells are dieing after one cell cycle if they were lethally hit.

For simulation of angiogenesis, new capillaries are generated as soon as the capillary density in some area fell below 90% of “normal”. A model also accounts for vessels occlusions. If a vessel was seriously hit by irradiation, occlusion may occur at a random time point between the moment of irradiation and a maximum time of one year. After occlusion the vessel stops to deliver nutrients. The model demonstrates the agreement with medical data, taken from 90 patients with one to three brain tumours.

In 2002 *Mansury et al.* [122] offer an agent-based multiscale model of brain tumour growth. The behaviour of the system is controlled by both tumour cells self-organization and interactions between them and the environment. This approach combines a mesoscopic and macroscopic look onto the system. Growth stimuli like the presence of vessels, resources supply, and suppressors like toxic metabolites are taken into account. Migratory behavior is modelled simultaneously by local and global search to emulate the spread of information via receptors. Proliferation is simulated as a stochastic process with a dependency from the local nutrients supply.

On the macroscopic level the model is evolving on a two-dimensional grid. Each cell of the grid is characterized by the number of biological cells located in it and its area. Biological cells can migrate, divide or die with some probabilities. Proliferation can occur only on the surface of the neoplasm, so mitoses is assumed to be totally suppressed inside the tumour. Such view can hardly be correct alongside with the assumption that all cells of proliferating layer are
dividing. Nevertheless, the same assumption was made as early as in 1961 in the model of Eden [150].

Dorman et al. [99, 107] presented a model for pattern formation of multicellular tumour spheroids with the help of the two-dimensional hybrid lattice-gas cellular automaton. The model explicitly simulates mitosis, apoptosis and necrosis as well as nutrient consumption. A rare feature is modelling a diffusing chemotactic signal, which is emitted by necrotic cells. This signal induces migration of tumour cells towards its maximal signal concentrations. Local pressure also influences migration.

The chemotactic motility induces an antagonistic process to tumour expansion and this is why the lattice gas looks very advantageous. As was mentioned in Sect. 4.2, if there would be no cell flow towards the necrotic core, but only motionless cells and cells moving to the periphery, it would result in the unbounded tumour growth. That is why the two opposing populations are considered.

The lattice-gas automaton has four velocity channels and one resting channel. Mitosis, apoptosis and necrosis rates depend on the local nutrient concentration and the degree of overcrowding. Redistribution of cells is defined by rules which account for adhesion, pressure (cells are pushed towards neighbouring cell with lower density) and chemotactic motility (tumour cells desire to move into the direction of the maximal signal gradient).

One malignant cell always occupies the rest channel, if there is another malignant cell in the neighbourhood. The other cells are distributed via channels which point to less crowded neighbors. The density of a node is defined as the number of tumour cells plus one third of the number of necrotic cells, which represents a three times smaller volume. This density simulates the local pressure and represents a unique accounting of natural shrinkage for the mesoscopic model.

In order to demonstrate the impact of active and passive cells motion, velocity channels are associated with priorities, depending on the chemotactic signal. The density and the priority-based ordering of velocity channels defines an interval from which a preference weight is randomly chosen.

The model reproduces realistic tumours with complex geometry (not just spheroids) and correlates with experimental data.

Stamatakos et al. [103–106] present a macroscopic cellular automata model of tumour growth and response to radiation therapy, which extends some ideas from Kocher’s model [102], mentioned above, but without angiogenesis.

Each cell state (except necrosis and apoptosis) is associated with a maximum duration and transitions probabilities. Authors use cytokinetic model offered by Duechting et al. [117]. Nevertheless the conceptual model does not consider individual biological cells. They are united into so called “geometrical cells” and each of these geometrical cells corresponds to a cell of the automaton.
Geometrical cell stores the amounts of biological cells for each state. If any of these amounts become larger then a threshold, a new geometrical cell will be created nearby and biological cells will be divided between the old and the new geometrical cell. Chain shifting along a straight line is performed if needed. In this way the tumour expands. Inversely, if any of the amounts of biological cells becomes smaller than a given threshold, a geometrical cell will be collapsed and remaining biological cells will be distributed between its neighbors. Chain shifting may be used if needed. In this way the tumour can shrink.

Radiation therapy is modelled based on linear-quadric model [117, 149]. Fatally damaged biological cells undergo two final divisions before death.

The model uses patient specific imaging data in combination with a detailed description of cells evolution. Initial numbers of biological cells for each geometrical cell can be obtained from positron emission tomography (PET) images, single photon emission computer tomography (CT) or functional magnetic resonance imaging [105]. As a result the model allows to predict the results of radiation therapy for a given patient and create individualized treatment schema.

The geometrical cells-based approach is very opportune for such study, because imaging technologies cannot detect cells colonies smaller than a millimeter in diameter. The amount of biological cells in each phase can be estimated according to the position of the corresponding geometrical cell within the tumour, based on estimated metabolic activity in the local area.

The model is easily extensible. As an example, reference [106] explains how the status of p53 was taken into account in addition to basic functionality.

In 2005 Alarcon at al. [108] developed a multi-scale model, accounting a wide range of subphenomena. To be precise, it simulates the blood flow and structural adaptation of the vasculature, transport of oxygen, competition between malignant and healthy tissue, cell division, apoptosis, VEGF release, and the coupling between these processes.

On the top layer of the model there is a hexagonal vascular network. Each individual vessel is assumed to undergo structural adaptation (changes in radius) in response to different stimuli until the network reaches a stationary state. The radius is assumed to be a parameter of vascular cells, and vessels thickening and thinning does not result in additional cells. Nevertheless, the model accounts for vascular adaptation, complex blood rheology (plastic deformations and fluidity) and the red blood cell distribution at bifurcations.

On the intermediate layer (the cellular layer) there are intercellular interactions and spatial distribution of cells. Authors distinguish healthy and malignant cells, which are modelled as individuals. These two populations compete for space and resources and malignant cells are usually better competitors. This competition is introduced by simple rules, which connect intermediate layer with the intracellular layer of the model.

On this bottom layer intracellular processes, like division, apoptosis, and VEGF
secretion are modelled using ordinary differential equations and a cellular automaton with a two-dimensional grid with von Neumann neighborhood. The state of each cell is an element of 13-dimensional space. Semantically, each cell can represent one of four: empty space, malignant cell, healthy cell or vessel.

At the same time authors focused attention on how external conditions modulate the dynamics of intracellular phenomena: how the level of extra oxygen affects the division rate, the expression of p53 and the production of VEGF. Since the spatial distribution of oxygen depends on both the spatial distribution of cells (taken from cellular level) and the distribution of haematocrit (taken from vascular layer), these processes at the intracellular layer are tightly linked to the behavior of the other two layers: cell proliferation and apoptosis influence the spatial distribution of the cells. The cellular and the intracellular layers modulate the process of vascular adaptation through diffusion of VEGF and its absorption by the endothelial cells lining the vessels.

The authors also offer some implications for therapy, but do not account migration and immune system response.

In 2006 Escudero [151,152] proposed a model of a tumour as a competing process between the tumour and the host. Malignant cell diffusion at the neoplasm boundary demonstrates the strategy of minimizing the pressure and promoting tumour development. Stochastic partial differential equations are used for the model. Starting from Mullins-Herring equations [153,154] to describe molecular beam epitaxy Escudero derives an expression for diffusive drift and growth laws. The model predicts the constant velocity growth regime. Linear stability analysis of radial solutions allowed to estimate the density distribution of new cells. The fact that on later stages growth is characterized by deceleration of the growth rate is not taken into account.

The model uses an assumption that the difference in local pressure is caused mainly by geometric effects. This is just one of the simplifications. In [152] an example of flower-like initial conditions in discussed. The model correctly reproduces the geometry of tumour with spherical symmetry.

Khain et al. [155] offered a continuum model, which, in contrast to Escudero [151,152], takes other subphenomena into account. The model assumes one nutrient metabolism and uses two coupled reaction-diffusion equations for simulation of cells and nutrient concentrations. When the amount of the nutrient and cells diffusion coefficient exceeds some critical value, the propagating front becomes unstable with respect to transversal perturbations. One of the goals was to determine this instability threshold.

This work is inspired by in vitro experiments, where spheroids were put into collagen-I gel and allowed to grow. The key task of this model is to satisfy the following experimental observations: within the inside region rapid proliferation takes place, whereas in the invasive region cells have high migration rate, but slower proliferation. The switch of phenotype is modelled by introducing
a function, reflecting the dependency of the proliferation rate from the local pressure.

The scope of macroscopic continuum models remain the same as the original model by Adam [143,145,146]. That language of “proliferation coefficients”, “consumption rates”, “densities”, and “spreads” belongs to a completely different level of abstraction than real experimental data. Conceptual model creation should account for this, otherwise it can evolve into the computational model, which will depend on parameters, which cannot be determined precisely enough.

Ayati et al. [156] offered a multiscale continuous tumour growth model, which takes molecular, cellular, and tissue level subphenomena into account. The model is focused on the invasion of tissue by a tumour, under the assumption that the surrounding tissue is an origin of vasculature.

The authors study functions, which describe the dynamics of the proliferating and quiescent cells densities. These functions are considered versus sequences of mutated phenotypes, surrounding tissue density, concentrations of oxygen and toxins.

The numerical simulation in two dimensions shows that the interior core of the tumour mass becomes necrotic, because of its increasing distance from the oxygen supply provided by the exterior tissue. This was observed, while classifying cells into proliferating and quiescent only by their ability to diffuse and to haptotactical motility.

The approach to simulation is worth special discussion. Authors apply Galerkin methods of moving-grid, which provide higher-order accuracy, to age-, time-, and space-dependent equations. Then authors solve each of these equations by a combination of step-doubling and alternating directions implicit methods. The model is considered as a template for handling a broader range of biological problems, which can be easily extended by additional equations to account for more subphenomena.

In 2007 Zhang et al. [157] proposed an agent-based model, including epidermal growth factor receptor activation, TGF-α, glucose and oxygen tensions, migration and many other subphenomena. Vessels are modelled as trivial nutrient source points. This work continues the one made by Mansury et al. [122] described above.

Each virtual tumour cell has four layers: external space (source of nutrients and growth factors), cell membrane, cytoplasm and nucleus. A simplified cell cycle model is used: it includes only mitosis, but despite this the influences of hypoxia, p27 and phosphorylated retinoblastoma proteins expression on the cell division are taken into account. Models for these subphenomena were taken from [108].

†Proliferation inhibitor.
Simulation is performed on the level of each single biological cell. Correlation with experimental data is not discussed in the paper. The model covers a wide range of subphenomena and has a huge number of parameters.

In 2010 Sottoriva et al. [109, 110] presented a two-dimensional cellular automata model, based on the emergent cancer stem cells hypothesis [138]. Cancer stem cells (CSCs) have unlimited replicative potential and self-renewal abilities, while differentiated cancer cells (DCCs) are subjected to Haiflick’s limit. So discussed model distinguishes CSCs and DCCs among the malignant cells. Authors assume that only CSCs contribute to tumour enlargement in the long run.

Model is implemented by a two-dimensional cellular automaton with von Neumann neighbourhood. A single biological cells is represented by a single automaton cell, which is assumed to correspond to $10 \, \mu m \times 10 \, \mu m$ area of tissue. The cell can be in one of four states: healthy, proliferating, quiescent, or necrotic.

Oxygen metabolism is considered, while glycolysis is neglected. Model is based on the assumption that proliferation concentrates in the proximity of the tumour surface. Mitosis suppression is simulated by the probability of division, which is distributed linearly from 1 (on the surface of the neoplasm) to 0 (at the depth of 60 cells or 600 $\mu m$). If there is no vacant place for a daughter cell after division, an outward chain shifting in used.

Oxygen around the tumour is kept constant by the vascular system. There are oxygen concentration thresholds for proliferating cells to turn quiescent and for quiescent cell to turn dead. In contrast to models, described in [103–106], there is no quiescence duration limitation in this one. So, in principal, cell can stay quiescent forever.

The model gives special attention to the invasiveness study. During the tumour progress, malignant cells lose cell-to-cell attachment and that is why they become able to invade surrounding tissues [158]. This subphenomenon is modelled using the hybrid discrete-continuum technique [128]. According to this technique cells dispersion is characterized by the random motion coefficient. The cell adhesion is simulated in the following manner: for each malignant cell the number of tumour cells in the neighbourhood is calculated and compared with the adhesion coefficient, which is in the range from 0 to 4. So cells are moving according to both a random motion coefficient and an adhesion coefficient.

CSCs divide symmetrically with probability $P_S$ and asymmetrically with probability $1 - P_S$. So, classical approach to tumour growth modeling, which neglects CSCs, corresponds to $P_S$ equal to 1.

One kind of experiments with the discussed model consists in varying $P_S$ value to study tumour dynamics and invasiveness for different CSC frequencies, while the maximal number of DCCs divisions is limited (to 5 in [109]). Results of such study are presented in Fig. 4.5.

Model is also able account tumour phenotypical mutations by assuming that CSC division has a chance $P_{MUT}$ to acquire a genetic hit and generate a daugh-
Figure 4.5: Invasive behavior in the CSC model. A shows that different $P_S$ values result in different CSC fractions. B demonstrates growth curves for different $P_S$ values. In C quantitative measure for invasiveness shows increasing invasive behavior with declining $P_S$. D shows how the hierarchical organization affects tumour morphology. Color legend for three left columns of D: cells which have divided within the last 48 h are shown in dark grey (depicted larger); nondividing cells – in light grey; necrotic core – the dark grey formation in the center (if any). Rightmost column reproduce the same formation as its left neighbour, but in different color scheme to demonstrate the localization of CSCs: tumour mass – in light grey; CSCs – in darker grey (depicted larger). All figures represent 6 mm × 6 mm of tissue.

ter cell with a different phenotype selected from a randomly generated pool of 30 phenotypes. Authors demonstrate that under an equal mutation rate ($P_{Mut} = 0.1$), the CSC model demonstrates a slower acquisition of new phe-
4.3. Overview of Existing Models

Figure 4.6: Tumour evolution and phenotypical selection. Phenotypes are generated randomly. A corresponds to equal mutation rates ($P_{\text{Mut}} = 0.1$). The cumulative amount of phenotypes is higher in the classical model ($P_S = 1$) compared with the CSC model ($P_S = 0.03$). In B authors adapted $P_{\text{Mut}}$ to obtain an equal rate of emergence of phenotypes. In C, under equal conditions, the phenotypes in the CSC model are more diverse compared with the classical model.

notypes, due to its smaller effective population size, but also shows a radically different selection process. Simulation results are shown in Fig. 4.6.

The work demonstrates that hierarchical organization of malignancies significantly contributes to the invasive morphology and increased heterogeneity of tumours. Therefore it is a crucial issue for better understanding tumour biology and to improve current cancer treatments.

The major properties of the models discussed above are summarized in Table 4.1. Our model, which will be discussed in Sect. 4.4, is also included as the “Naumov et al. [159, 160]” row.

Only eight classes of subphenomena were used to illustrate the differences between the models. The task was not to cover the whole spectrum of subphenomena, discussed in Sect. 4.2, but to show different directions in which models are tending to move. If one model accounts cell senescence or adhesion, while others do not, this is not a reason to put them into different classes. At the same time, accounting blood flow or any kind of treatment may be such a reason.

Two modelling trends can be distinguished. Assuming that the table contains most significant models, in period between 2000 and 2005 cellular automata were
<table>
<thead>
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<th>Authors [references]</th>
<th>Dimensions</th>
<th>Type</th>
<th>Subphenomena</th>
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<td>Vessels</td>
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<td>Chaplain et al. [131]</td>
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<td>Kansal et al. [101]</td>
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4.4. Several Models of Tumour Growth

Table 4.1: A comparative survey of discussed tumour growth models. The first column contains the name of the first author with reference, the second column stores the dimensionality of the modelling realm, the third column presents the applied formalism, used for model description and execution (“math” for continuous equations; “CA” for cellular automata; “agents” for agent-based systems). Eight remaining columns list the information about subphenomena. Column “Cycle” shows, which biological phases of malignant cells are distinguished (“−” means none; “M” means mitosis only; “M+A” means mitosis and apoptosis; “full” means the full cell cycle). For the rest columns “+” means that subphenomenon is accounted (or at least mimicked) and “−” designates that it is not. Column “Vessels” shows if circulatory system related subphenomena are included (“⊕” designates that vessel growth is “really modelled”, not just imitated, for example, in probabilistic manner). Column “Positives” shows the accounting of subphenomena, which promote tumour growth (like favourable cells conditions). Column “Negatives” shows the accounting of subphenomena, which inhibit tumour growth (like spread of toxins). Note that "Positives" are not the negation of "Negatives" and vice versa. Column “Treatment” shows if any cancer treatment is modelled (here the type of treatment is mentioned instead of “+”). Column “Migration” demonstrates if migration and locations comparison mechanisms are taken into account. Column “Phenotypes” shows if any phenotype distinguishing is simulated. Column “Immunity” designated if immune system response (cytotoxic effects, elimination of tumour cells, white blood cells flow etc.) is taken into account.

mostly used as computational vehicles. Then the attention has moved to continuous models. Now attention returns to cellular automata.

4.4 Several Models of Tumour Growth

As was already mentioned in Sect. 4.1, here we will present two three-dimensional cellular automata based models of tumour growth, with special attention to natural tumour shrinkage simulation. Then we will study the influence of the successful mitoses rate on the tumour growth dynamics, involving one of these models. There was no three dimensional mesoscopic cellular automata model, which has payed special attention to it. Some involve shrinkage implicitly [161], but a distinct rate of shrinkage was not determined. In [107] it was accounted for two dimensional case, but its influence was not studied.

Cellular automata [10, 19, 21, 84, 162] were chosen as basic simulations vehicle, because they have proven their suitability and advantageous usability in the field of biomodelling [99, 163].

The scientific community has produced significant results and software [32, 48] based on cellular automata. The model was implemented with the help of the CAME&L software environment [48, 49]. Results of the simulation were validated against in vitro growth of the LoVo cells spheroid [164].
4.4.1 General Considerations

On the level of conceptual models we choose a level of abstraction, which will give us an opportunity of creating an approach to incorporate subphenomena on the module basis. The aim is to be able to perform simulations with and without inclusion of distinct modules, as it was recommended in Sect. 2.1 speaking about “extensibility”.

Traditionally, tumour growth models are based on the assumption that the process starts from a single malignant cell [111]. In the current version of the model one nutrient metabolism is considered. While neglecting angiogenesis, the healthy tissue around the tumourous clot is supposed to be perfectly vascularized. Nutrient is diffusing from the healthy area to the clot of malignant cells. As a result, normal cells act as nutrition sources for the cancerous tissue. We model the tumour during the early stage, when its size is not larger than several millimeters along each axis. We assume homogeneous pressure, and neglect obstacles such as, for example, bones.

The simulation is always based on the number of assumptions. Tumour expansion due to cell division or its shrinkage due to cell death are smooth processes in real biological systems, but would be modeled with a set of volumetric jump (or number of jumps) in silico. A schematic representation of the cell volume along the cell cycle in vivo is shown in Fig. 4.7.a [165]. When modelling a biological cell with a single automaton cell, this process is substituted with the burst-like expansion (Fig. 4.7.b). At the same time, when using cellular Potts models [166] a single biological cell is represented by a number of automaton cells and the expansion is performed smoother, but still discreetly, of course.

The main question in cellular automata models of tumour growth is how to find a place for a newborn cell. Two approaches are mostly used for this. The first one consists in looking for a vacancy (a healthy cell) in the immediate neighbourhood of the mother cell. Only the outermost tumour cells are considered to be able to divide in this case. Such models reproduce observations that the proliferating rim should be “several” cells thick. This is a trivial case of the mitosis inhibition due to overcrowding and physical pressure inside the tumour [121].

The second way of expansion modelling in cellular automata is a chain shifting algorithm [101, 103–106, 121, 167–169]. Cells are pushed outwards one by one along a certain trajectory, starting from one of the mother cell’s neighbours. In most cases this trajectory represents a straight line [103–106] (Fig. 4.8.a) or a random zigzag [109]. A newborn cell is placed to the newly freed vacancy (Fig. 4.8.b).

The same algorithm can be used for shrinkage modelling, but in this case cells are shifted along the trajectory in the inward direction (Fig. 4.9).

The chain shifting approach has a number of disadvantages:

- This algorithm is not local and this affects its parallelization.
- Strictly speaking, this algorithm cannot be implemented as a synchronous cellular automaton, prohibiting the use of corresponding benefits, which can be provided by an optimized cellular automata problem solving environment.
- The invasiveness of the tumour depends on the type of phenotypical mutations, which occurred with its cells. Plane chain shift along straight lines will result
4.4. Several Models of Tumour Growth

Figure 4.7: Schematic representation of the cell’s volume dynamics along the cell cycle (a) in biological system and (b) in its in silico model.

in a fussy boundary of the clot unless additional routines to maintain pattern integrity will be involved [169].

In Sect. 4.4.2 we present an algorithm, which simulates shrinkage and expansion using chain shifting. In Sect. 4.4.3 we describe an alternative to the chain shifting algorithm, which represents a computational workaround. Validation of both algorithms is discussed in Sect. 4.4.4.

4.4.2 Algorithm 1. Basic

The algorithm is implemented with a three dimensional cellular automaton with Cartesian metrics and Moore’s neighbourhood [26]. The timestep is equal to one hour. This value was chosen as an appropriate compromise to represent the various phases in the cell cycle, and to allow for some cell to cell variability in the duration of the cell cycles.

Each automaton’s cell represents a single biological cell, with size $10 \mu m \times 10 \mu m \times 10 \mu m$. Each cell can be in one of the following biological states

- $N$ corresponds to a normal tissue cell;
Figure 4.8: Chain shifting along a straight line after cell division. The mother cell is shown in black, the daughter cell is hatched. Cells are enumerated to illustrate the order of the shift. Cell positions (a) before the shift and (b) after the shift are shown.

Figure 4.9: Chain shifting along a random trajectory due to cell necrosis. A dead cell is shown in black. Cells are enumerated to illustrate the order of the shift. Cell positions (a) before the shift and (b) after the shift are shown.

- $T_M$, $T_{G1}$, $T_S$, and $T_{G2}$ designate different phases of the proliferating malignant cell’s cycle;
- $T_{G0}$ denotes a quiescent malignant cell;
- $D$ represents the mortal remains of dead cells.

Initially all cells of the grid are normal biological cells, except the center one, which
4.4. Several Models of Tumour Growth

holds a malignant cell ready for mitosis (in $T_M$ state). The cell cycle is modeled as follows: in good conditions a malignant cell is continuously transiting through phases of the cycle – $T_M$, then $T_{G1}$, then $T_S$, then $T_{G2}$ and again $T_M$. The amount of timesteps which a new phase has to last is randomly generated at the moment of the cell’s transition to a new phase, according to known statistical data [170]. To determine the moment of a transition the generated duration is compared with the amount of steps spent in the current phase.

The duration of the $T_M$ phase is always assumed to be one timestep. As mentioned in Sect. 4.4.1 mitosis inside the tumour can be prohibited due to overcrowding and physical pressure. A cell, which is situated deep inside the tumour, has less chance to divide successfully than an outer one [121]. This can be modeled in a probabilistic manner, however the exact “rate of abandoned divisions” can hardly be measured in vitro or in vivo. In our approach the inner cells fail to divide with a probability of 30%. This is an average probability of a mitosis failure, which was calculated using algorithm 2 described in Sect. 4.4.3. We will further study the influence of the mitoses rate on growth dynamics in Sect. 4.4.5.

Being in the mitotic phase, in case of a successful mitosis, a chain shift in random direction along a straight line is performed starting from the mother cell. A newborn cell in the $T_{G1}$ phase is placed to the disengaged cell, which is a mother cell’s neighbour. As was mentioned in Sect. 4.4.1, such kind of chain shift will result in a very fussy boundary of the growing clot. That is why we introduced one more operation: move the outermost malignant cell of the trajectory to one of its vacant neighbours (neighbours in $N$ state), if it is closer to the center of the tumour. This imitates the pressure between the healthy tissue and the cancerous formation.

One nutrient metabolism is considered, which is diffused into the clot of malignant cells from the outside. Cancer cells act as sinks, and the healthy tissue acts as source. Diffusion is much faster than the time step used to model the cell cycle. Therefore, at each time step we compute a steady state field of concentration of the nutrient. Metabolism is simulated for malignant cells only, with normal tissue acting as a nutrition source, due to the assumption that it is perfectly vascularized.

The key parameter of the model related to the metabolism is the consumption rate per hour, which defines the amount of nutrient sufficient for the proliferation. When the level of nutrient gets below this threshold the cell turns quiescent. In this state the cell needs much less nutrient. Quiescent cells do not change their states unless they return to proliferation or die [108, 115]. The cell can stay quiescent no longer then a fixed amount of time [103–106]. The cell returns from quiescence to proliferation (transits to the phase $T_S$) if the local level of nutrient is above the consumption rate for the proliferating state, otherwise it dies.

Simulation of shrinkage concerns necrosis. Once a cell dies it transits to the $D$ state with probability $1/3$, otherwise it collapses due to chain shift in a random direction along a straight line from the tumour center to the died cell. All cells along the line should be moved one by one in the inward direction.

Both outward and inward shifts should be performed after all cells of the grid have been considered on the current timestep. In was implemented by providing two lists, which store the coordinates of cells, which initiated the shifts. During the timestep,
the first list accumulates coordinates of all mother cells for the outward shifts and the second collects 2/3 of died cells coordinates for inward shifts. After all cells were considered, the shifts are executed.

4.4.3 Algorithm 2. Optimized

In this section an alternative for the chain shifting algorithm is proposed. In this approach we do not look for a place for a newborn cell near the mother cell, but on the surface of the tumour. This substitutes chain shifting of cells one by one in the outward direction by placing the daughter cell directly to the outer end of the chain. This purely computational trick can be used when such local cellular phenomena as adhesion and influence of pressure are neglected. Taking Hayflick’s phenomenon [115] into account also needs to abandon the usage of such approach, but during the avascular stage of tumour growth cells are dying due to the lack of resources earlier than after 50 divisions.

The destination point, where a newborn cell appears, is found with the help of a random walk from the mother cell.

Such “teleportation” of newborn cells was used in [121], but in that work all malignant cells are equal, because the authors did not distinguish the phases of a cell cycle and the division had a probabilistic character. Our algorithm distinguishes cell phases and will therefore result in concentration of cells in the $T_{G1}$ phase closer to the surface, but this is not critical due to the fact that cells on the surface would not die immediately, having sufficient supply of nutrient.

For tumour shrinkage modelling, each third cell, which is subjected to death via necrosis, turns to the $D$ state. The other two thirds turn to a new state $H$, which corresponds to “holes” – virtual empty spaces. Holes have a volume equal to that of a single cell. They should be eliminated from the modeled mass due to reshuffling. Their life cycle consists in getting out of the tumour by flowing to the surface. As soon as they reach the normal tissue they turn into state $N$. Holes are changing their position according to a random or direct walk in the outer direction. On each timestep a hole is swapped with one of its neighbouring cells, chosen randomly or according to a walking strategy.

In other words, if according to algorithm 1, described in Sect. 4.4.2, the chain shift should be performed immediately (see Fig. 4.9), here we offer to execute this process step by step as shown in Fig. 4.10. A number of such holes walking steps in succession will lead to total elimination of holes from the tumour. If the total elimination would be performed after each iteration, this would be the same as the shrinkage simulation, utilizing the chain shifting along a random trajectory.

The fact that holes are moving at the speed of one cell per timestep gives the newborn cells a chance to occupy holes before they get out. This will improve effectiveness of both: newborn cells placing and holes walking.

Tumour shrinkage and expansion can now be implemented with the help of a synchronous cellular automaton. Random walking is also not a local operation (as the chain shifting), but it can be implemented, having read only access to one data plane and write only access to another if the set of data fields associated with the
4.4. Several Models of Tumour Growth

cell will be enlarged by one boolean flag. This flag is significant only for holes and normal cells. It is "true" if on the current timestep they have already found their inhabitants (newborn malignant cells) and cannot be considered as candidates for any other transitions. A better way is to create a separate data plane for this flag, because there is no need to store two copies of it. One more reason to implement it as a separate plain is the fact that this is the only data field, which will require a synchronized access in case of parallel implementation. The same could be also achieved utilizing just a synchronized list of the coordinates of newly occupied cells.

Figure 4.10: Hole random walk step by step in the outer direction, assuming that the resulting random trajectory is the same as on Fig. 4.9. The hole is hatched.


4.4.4 Experiments

Gompertz Law Based Validation

One of the key approaches to the validation of the in silico tumour growth consists in the volumetrical comparison with in vivo or in vitro data. The kinetic behavior of a tumour growth process can be reproduced by the Gompertz law [147, 148, 164, 171–173] which is widely used for these purposes.

The law can be written in the following form:

\[ V(t) = V_0 \exp\left(\frac{a}{b} \left(1 - \exp(-bt)\right)\right), \quad (4.1) \]

where \( V(t) \) is the tumour volume in time; \( V_0 \) the initial volume; \( a \) the initial instantaneous tumour growth rate; \( b \) the growth retardation factor.

Knowing \( V_0 \) it is possible to describe the growth with two parameters: \( a \) and \( b \). The validation procedure consists in the comparison of these two parameters for the in silico data with the same values known for real in vitro or in vivo specimen.

Demicheli et al. [164] report the following values for the in vitro growth of the mice LoVo cells spheroids:

\[ a = 0.04 \pm 0.01 \text{ h}^{-1}, \]
\[ b = 0.0022 \pm 0.0007 \text{ h}^{-1}. \quad (4.2) \]

Moreover, they postulate a strong linear correlation between both parameters:

\[ a = (7 \pm 1) b + (0.023 \pm 0.002). \quad (4.3) \]

Experimental Setup

Before starting experiments it is necessary to tune the model to reflect the in vitro tumour composed of LoVo cells. In Table 4.2 parameters of the model are presented with their values.

<table>
<thead>
<tr>
<th>Name</th>
<th>Comment (Measure)</th>
<th>Used Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1DurationMean</td>
<td>Tumour cell’s gap-1 phase mean duration (in hours). In [170] durations of all phases of the murine sarcoma 180 cell’s cycle are reported. The cycle duration for that type of tumour cells is 19.8 hours, while for mice LoVo cells it equals to 29.3 hours [174]. Values for the cell cycle phases for LoVo cells were obtained by proportional scaling from the values for murine sarcoma 180 cells</td>
<td>12.3 h</td>
</tr>
<tr>
<td>SDurationMean</td>
<td>Tumour cell’s DNA synthesis phase mean duration (in hours). For details see the G1DurationMean description</td>
<td>12.5 h</td>
</tr>
</tbody>
</table>
### Table 4.2: Model parameters and their values used for the experimentation.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$G2DurationMean$</td>
<td>Tumour cell’s gap-2 phase mean duration (in hours). For details see the $G1DurationMean$ description</td>
<td>3.5 h</td>
</tr>
<tr>
<td>$G0MaxDurationMean$</td>
<td>Tumour cell’s gap-0 phase mean duration (in hours). This value represents not the exact prolongation of the cell’s quiescence, but the maximal possible duration of the dormancy. Value obtained from data published in [175]. Such order of magnitude is also presented in [176].</td>
<td>147.5 h</td>
</tr>
<tr>
<td>$G1DurationSD$</td>
<td>The standard deviation for tumour cell’s gap-1 phase duration (in hours). For details see the $G1DurationMean$ description</td>
<td>12.3 h</td>
</tr>
<tr>
<td>$SDurationSD$</td>
<td>The standard deviation for tumour cell’s DNA synthesis phase duration (in hours). For details see the $G1DurationMean$ description</td>
<td>3.75 h</td>
</tr>
<tr>
<td>$G2DurationSD$</td>
<td>The standard deviation for tumour cell’s gap-2 phase duration (in hours). For details see the $G1DurationMean$ description</td>
<td>1.4 h</td>
</tr>
<tr>
<td>$G0MaxDurationSD$</td>
<td>The standard deviation for tumour cell’s maximum gap-0 phase duration (in hours) [175].</td>
<td>30 h</td>
</tr>
<tr>
<td>NutrientConsumptionProlif</td>
<td>The amount of nutrient consumed by proliferating cell per hour (in grams per hour) [107]</td>
<td>$7.2 \cdot 10^{-11}$ g/h</td>
</tr>
<tr>
<td>NutrientConsumptionQuiescent</td>
<td>The amount of nutrient consumed by quiescent cell per hour (in grams per hour) [107]</td>
<td>$9.38 \cdot 10^{-12}$ g/h</td>
</tr>
<tr>
<td>NutrientDiffusion</td>
<td>Nutrient diffusion coefficient (in square meters per hour) [111]</td>
<td>$1.52 \cdot 10^{-7}$ m²/h</td>
</tr>
</tbody>
</table>

**Validation and Discussion**

Experiments were performed with the setup, described in Sect. 4.4.4, on a grid of $201 \times 201 \times 201$ cells. This size (and, consequently, the maximum dimensions of an in silico tumour) was chosen to stay well in the avascular growth regime.

Four variants of algorithms were tried. Namely,

- algorithm 1 with shrinkage (Algorithm 1);
Figure 4.11: Number of cells as a function of time is shown for the run of algorithm 2 (one datapoint per 10 hours of model time). We also draw the best fitting Gompertz curve with $a=0.0367$ and $b=0.00230$.

- algorithm 1 without shrinkage (Algorithm 1NS);
- algorithm 2 with shrinkage (Algorithm 2);
- algorithm 2 without shrinkage (Algorithm 2NS).

For every experimental run values of $a$ and $b$ were calculated. One example of the correspondence between the in silico data obtained using algorithm 2 and the best fitting Gompertz curve is shown in Fig. 4.11.

Average values of parameters calculated from 15 runs for all four algorithms are summarized in the Table 4.3, as well as the $a/b$ ratio.

All simulation results agree with the experimental values reported in Eq. 4.2. Moreover, all four sets demonstrate the linear correlation between parameters (Eq. 4.3).

Fig. 4.12 shows growth curves corresponding to average results, achieved utilizing all variants of algorithms. This figure together with Table 4.3 shows that algorithm 1 (with and without accounting shrinkage) reproduces the same spectrum of growth regimes as algorithm 2. So, despite the fact that the second algorithm has a number of procedures, which are not realistic from a biological point of view, it reproduces the same dynamics as the approach which is commonly used.

At the same time Fig. 4.12 shows a significant difference between the results with and without shrinkage. Taking shrinkage into account influences not only the volume values, but also the growth pattern (see the difference between $a/b$ ratios in the Table 4.3). Being a compromise between linear and exponential processes, growth without accounting shrinkage has a stronger exponential character. Moreover, it
Algorithm 1

Algorithm 1NS

Algorithm 2

Algorithm 2NS

Table 4.3: Mean values and standard deviations for $a$, $b$ and $a/b$ ratios for results, achieved with both algorithms with and without shrinkage.

saturates later and weaker. This illustrates the fact that exclusion of shrinkage from the model can have significant impact on the growth dynamics.

At the same time, algorithm 2 has avoided several shortcomings of algorithm 1 listed in Sect. 4.4.1 and has several more benefits, which are listed bellow.

- Parallel implementation of algorithm 1 requires global synchronization of data access and the control of the shifting trajectories crossing. This will lead to an unbalanced load on different nodes. Algorithm 2 is also not local, but it can be implemented, providing synchronized access to a field of single boolean flags, as explained earlier.

- In contrary to an algorithm 1, algorithm 2 could be implemented by a synchronous cellular automaton.
Algorithm 2 will produce a tumour with a smooth boundary, providing a good starting point for accounting the invasiveness, involving special routines for this.

Fig. 4.13 shows a comparison of execution time until a distinct timestep. Nutrient diffusion is a very time-consuming part for both algorithms. It takes 44% of the total time. At the same time, parallel implementation of algorithm 2 should demonstrate much better performance than parallelized algorithm 1 due to reasons mentioned above.

The spread of the results achieved with the help of all algorithms is much more compact than for the in vitro data. This agrees with the fact that the amount of simulated biological subphenomena is marginally small. Excluding other influential processes means less uncertainty of the resulting values.

4.4.5 The Influence of Mitoses Rate on Growth Dynamics

The fact is that mitosis inside the tumour can be prohibited due to overcrowding, physical pressure, incoming intercellular signals or other reasons. A cell, which is situated deep inside the tumour, has less chance to divide successfully than one on the periphery of the tumour [121]. The rate of successful mitoses can hardly be observed
4.4. Several Models of Tumour Growth

Figure 4.14: Amount of timesteps, needed for the modeled tumour to reach $201 \times 201 \times 201$ cells size versus the successful mitoses rates.

Figure 4.15: Maximal, minimal, and average values of $a$ (shown in black) and $b$ (shown in grey) versus the successful mitoses rates.
and measured in vivo or in vitro. At the same time it can be easily simulated in silico. Our objective here is to study the dependency of growth dynamics on this parameter.

We assume that the mitosis rate is uniformly distributed over the inner cells of the proliferating tumour layer. In vivo or in vitro this is different and there is a dependency between a chance to divide and the depth of the cell disposition. While the exact character of this dependency is questionable, we assume that the successful mitosis rate is the same for all cells inside the tumour. This assumption is not critical, accounting that a proliferating rim is usually several cells thick [111, 177].

Using the same experimental setup as above, we will validate the results against in vitro growth of the LoVo cells spheroid [164], trying to draw conclusions concerning the realism of distinct successful mitosis rates.

For these experiments algorithm 2 without shrinkage was used as a most traditional one, to study pure mitosis rate influence. In Sect. 4.4.1 we used a constant rate of 70%. Here we consider a range of values.

We have performed five experiments for each of the following successful mitoses rates: 10%, 20%, 30%, 40%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, and 100% (despite the last value not being realistic). The step is shortened between 50% and 90%, because, as we will demonstrate, this region represents the major interest. Again a grid of $201 \times 201 \times 201$ cells was used.

We measured the time (in cellular automaton steps) needed for the tumour to reach a $201 \times 201 \times 201$ cells size as a function of the successful mitoses rates. The
results, averaged over the five experiments, are shown in Fig. 4.14.

The maximal, minimal, and average values of \( a \) and \( b \) for a given successful mitoses rate are presented in Fig. 4.15. Starting with a successful mitoses rate of 20%, both Gompertzian growth parameters fall into the range of values as observed in vitro (see Eq. 4.2). At the same time, only starting from 60% almost all pairs of these parameters satisfy the linear correlation, given by Eq. 4.3. Only 2 of 40 experiments produce \( a \) and \( b \) values, which do not comply with it. At the same time, for a successful mitoses rate of 55% and below no single set of parameters pairs is in agreement with the linear correlation. This allows to make an assumption that at least 55% of inner proliferating tumour LoVo cells are dividing (see Fig. 4.16).

The global trends for \( a \) and \( b \) as function of the successful mitoses rate are as follows: \( a \) is growing and stabilising, while \( b \) is descending. At the same time, both parameters are tightly interdependent. This is illustrated by the fact that their spreads (relative magnitude of difference between minimal and maximal values) and relative positions of the average values look similar for any given successful mitoses rate. Their interdependency results in the fact that the average \( a/b \) ratio is monotonously growing as one may see in Fig. 4.16. This conforms with the expectations: the role of the exponential component of growth is rising with the successful mitoses rate, when the role of intrinsic saturation becomes less meaningful. Nevertheless, there is a limit in the avascular exponential growth, so \( a \) is stabilising.

4.5 Conclusions

This chapter described two models of avascular tumour growth, including the phenomenon of tumour shrinkage. Despite its simplicity, the results demonstrate a strong compliance with biological data in terms of the parameters of the Gompertzian growth. Moreover it is illustrated that ignoring shrinkage may have consequences not only for the final volumetrical results, but also for the observed growth pattern.

We propose two algorithms for tumour shrinkage modelling. One is based on the traditional chain shifting algorithm, while the second offers several novel computational adaptations. Agreement between both algorithms with biological data and with each other allows to conclude that the second algorithm can be used as a substitute for the first one.

Moreover, having no ability to measure such microscopic parameter as the successful mitoses rate in real biological system, we can draw some conclusions about its magnitude, based on the simulation presented in Sect. 4.4.5 and in vitro experiments of larger scale [164]. Our results suggest that at least 55% of inner proliferating tumour LoVo cells are dividing (see Fig. 4.16). Detailed study conforms with the expectations that the growth obtains more exponential character if the proportion of mitotic cells inside a tumour is increasing.

In Sect. 4.4.2 we use a value of the successful mitoses rate, which was computed during the simulations as a rate of the successful random walks from an inner malignant cell outside. A length of the walking trajectory was limited by a squared distance from the cell to the geometrical center of the tumour. Note, that the artificially com-
puted result was 70%, which falls into the range of possible rates.

At the same time, we also studied another way of approaching tumour growth modeling. Among the overviewed models we have described the method based on the cancer stem cells concept [109,110]. Our work demonstrates that hierarchical organization of malignancies significantly contributes to the invasive morphology and increased heterogeneity of tumours. As a result we may conclude that taking the sub-phenomenon of stem cells into account is important for better understanding tumour biology.