

Developing a multi scale cellular automata simulation model of a vascularised brain tumour environment to predict angiogenic potential of brain endothelial cells.

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Personal Statement

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Abstract

Angiogenesis, the growth and formation of capillaries from pre-existing vessels, is involved in various disease pathologies including brain cancer. Brain tumours are highly vascularised as they require new blood vessels to maintain oxygen and nutrition supply for their growth and survival. In recent years, molecular biology as well as imaging techniques have uncovered several aspects of angiogenesis and the vessel assembly process, based on which novel agents have been developed to target and counteract tumour-induced angiogenesis. However, many the guiding principles and molecular processes guiding angiogenic processes remain unknown and reliant on quantitative, computational models. Multiscale cellular automata models of angiogenesis have previously been employed but as new data become available, these generalized models can be refined to investigate particular aspects of angiogenic processes in different tissue environments.

In this project, I developed multiscale multicellular models of angiogenic sprouting simulating cell migration and sprouting in response to VEGF and DLL4/NOTCH1 mediated endothelial tip cell selection in a neural tissue and vascularized tumour environment. The preliminary results I collected demonstrate that CompuCell3D is a viable tool to emulate angiogenic sprouting in neural tissue. I identified some parameters including cell adhesion and volume which dramatically affect qualitative cellular responses including cell migration in my model. Future work emerging from complementary projects in the host lab will aimed to refine our current SBML and include Jagged/NOTCH1-4 contribution. Our approach employs a mix of in vitro and in silico experimentation whereby experiments can be repeated in vitro and results can be compared to the synthetic data created through computational modelling and, if correlating, confirm and expand the theory behind cellular processes. The tool can be taken further in a translational context with the simulation of anti-angiogenic treatment as well as the consideration of variables including time frame, dosage and tumour progression to investigate treatment strategies and aid in drug efficiency as well as planning clinical trials. Further, patient cells can be linked to experimental data in the future, and be used to predict likely treatment response and outcomes.

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Abbreviations

1D	One-dimensional	LS	Lambda Surface
2D	Two-dimensional	LV	Lambda Volume
3D	Three-dimensional	MCS	Membrane contact site
6A5BU	6-Amino-5-Bromouracil	MMP	Matrix metalloproteinase
6AT	6- Aminothymine	MNG	Meningioma
7DX	7-Deazaxanthine	mRNA	Messenger Ribonucleic acid
aFGF	Acidic fibroblast growth factor	MVD	Microvascular density
ANG2	Angiopoietin 2	NGF	Neurotrophins nerve growth factor
BDNF	Brain-derived neurotrophine factor	NICD	Notch Intracellular Domain
bFGF	Basic fibroblast growth factor	PA	Plasminogen activator
CC3D	Compucell3D	PAI	Plasminogen activator inhibitor
CNS	Central nervous system	PBS	Phosphate buffered saline
COM	Centre of Mass	PDGF	Platelet-derived growth factor
COX	Cyclooxygenase	PEX	Peroxins
D	Delta	PIFF	Potts initialisation file format
D_	Average Delta form Neighbours	RGD	Arginine-Glycine-Aspartic Acid
DAPI	4',6-diamidino-2-phenylindole	SBML	Systems biology markup language
DLL4	Delta like canonical Notch ligand 4	TIMP	Tissue inhibitors of metalloproteinases
DN, dN	Delta Notch	TS	Target Surface
ECM	Extracellular matrix	TSP	Thrombospondin
ECPT	Endothelial Cell Profiling Tool	TV	Target volume
EGFR	Epidermal growth factor receptor	uPA	Urokinase-type plasminogen activator
FBS	Fetal bovine serum	uPAR	Urokinase-type plasminogen activator receptor
GBM	Glioblastoma multiforme	VEC	Vascular endothelial cadherin
HDMEC	Human dermal microvascular endothelial cell	VECBP	Vascular endothelial cadherin binding parameter
HIF1	Hypoxia-inducible Factor-1	VEGF	Vascular endothelial growth factor
HUVEC	Human umbilical vein endothelial cells	VEGFR	Vascular endothelial growth factor receptor
IFN- γ	Interferon gamma	VEGFR1, 2, 3	Vascular endothelial growth factor receptor 1, 2, 3
IFN- α	Interferon alpha	XML	Extensible markup language
iPSC	Induced pluripotent stem cell		
LED	Light emitting diode		

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1. Introduction

Angiogenesis, the growth and formation of capillaries from pre-existing vessels, is involved in various disease pathologies such as neurodegeneration, stroke and cancer (Qutub et al., 2009). Angiogenesis in the brain and neurovascularisation, are regulated by pro- and anti-angiogenic factors released by neurones and microglia, acting on brain endothelial cells (Karamysheva, 2008). In recent years, many molecules implicated in angiogenesis and its regulation have been identified, and novel agents have been developed to target and counteract angiogenesis in vascular disease, including brain cancers such as glioblastoma multiform (GBM) (Ahir et al., 2020). Central Nervous System (CNS) tumours and brain tumours are some of the most complex cancers due to their classification, location, microenvironment and genetic background (Guarnaccia et al., 2018). In addition to growth and metastasis, brain tumours rely on angiogenesis to infiltrate surrounding tissue. In the case of a brain tumour, this can lead to a loss of cognitive and motor function. Based on the above, GBM have an extremely poor prognosis with a 5-year survival rate of only 5% (Leon et al., 1996). An advanced understanding of angiogenic processes can help us develop better treatment approaches by suspending and reducing vascular supply and hereby preventing tumour growth and metastasis (Kim & Lee, 2009).

Where molecular biology as well imaging techniques have uncovered several aspects of angiogenesis and the vessel assembly process, the guiding principles remain unknown and reliant on quantitative, computational models (Czirok, 2013).

Within this project, a tool is developed to better understand angiogenesis by simulating brain endothelial cell behaviour and differentiation within a vascularised brain tumour environment as demonstrated in Figure 1.

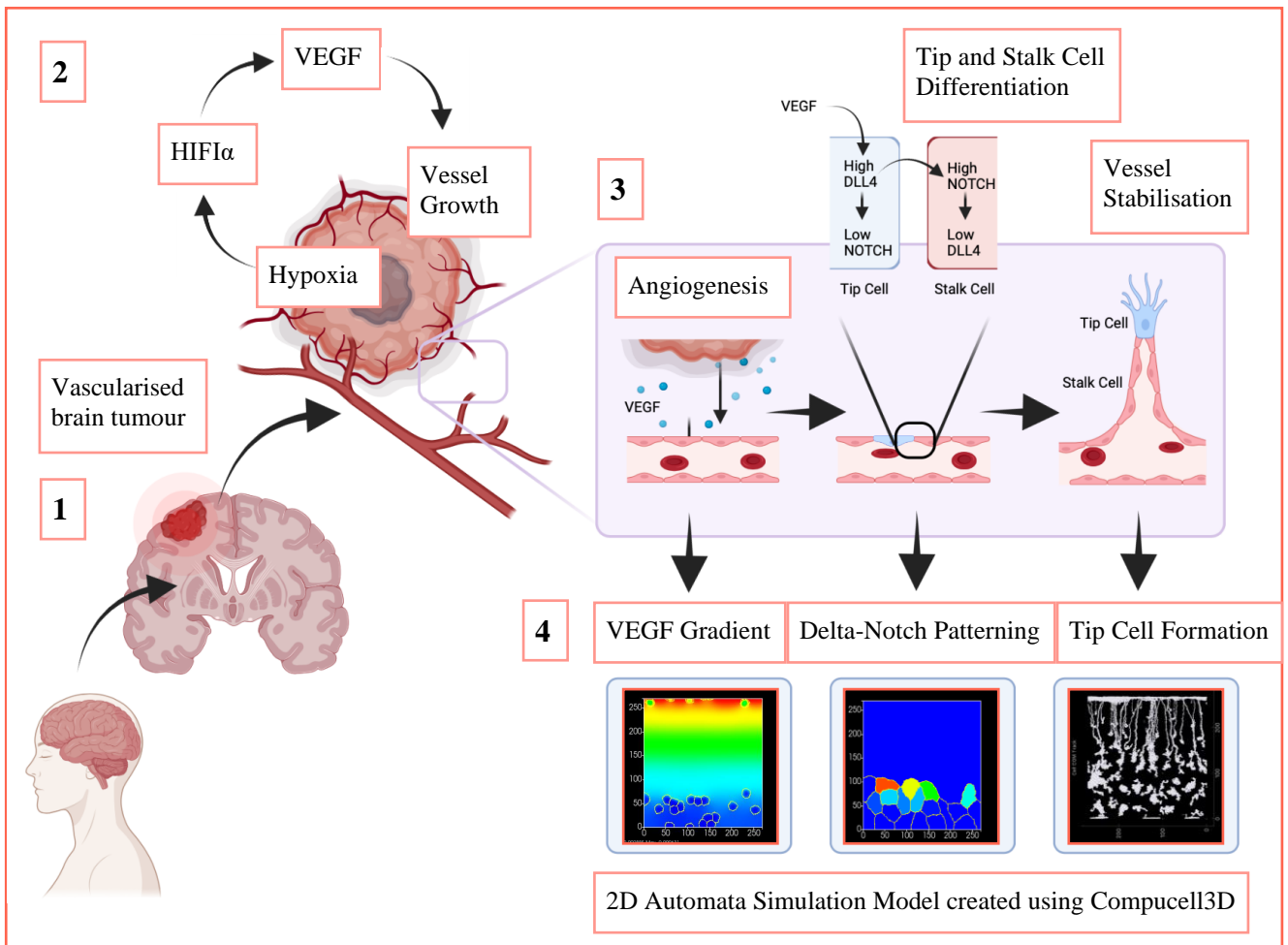


Figure 1. Modelling angiogenic potential in a vascularised brain tumour environment.

1) Vascularised brain tumour environment. 2) Inducing vessel formation: hypoxic cells secrete HIF1 α , inducing VEGF mediated vessel growth through angiogenesis. 3) Angiogenesis: VEGF mediates endothelial stalk- and tip cell formation through Delta-NOTCH (DN) signalling. 4) Translating angiogenic processes into a multi scale cellular automata simulation model: simulating VEGF secretion, DN patterning and tip cell formation and migration for vessel growth. (Created Using BioRender).

1.1 Angiogenesis

Angiogenesis, is regulated by activator and inhibitor molecules (Nishida, 2006). As demonstrated in Figure 2 angiogenesis occurs when hypoxia-inducible factor 1 (HIF1) is up-regulated in hypoxic cells and activates VEGF transcription factors. VEGF is then secreted by the cell, leading to VEGF-VEGFR binding on the capillary surface. This causes a change in vessel permeability and allows the tip cell, an activated endothelial cell, to break down the basement membrane whilst endothelial stalk cells proliferate underneath the tip cell. The leading edge of the living sprout then releases matrix metalloproteinases (MMPs), a family of enzymes which proteolytically degrade surrounding extracellular matrix (ECM) components (Rundhaug, 2005).

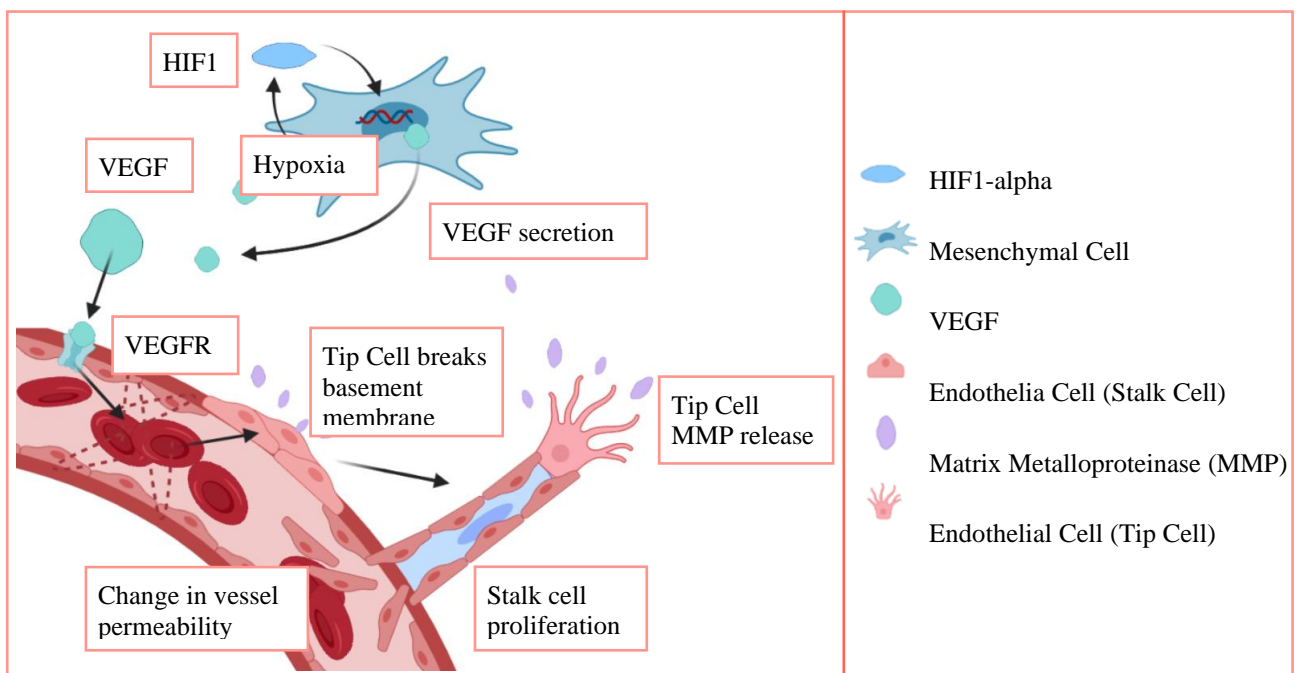


Figure 2. Angiogenesis (Adapted from Qutub et al., 2009). Hypoxic cells secrete HIF1, leading to VEGF secretion and VEGFR activation, and changes in vessel permeability. This allows endothelial tip cells to break the basement membrane, stalk cell proliferation and MMP release. (Created using BioRender).

Remodelling of the ECM through the degradation of the vascular basement membrane allows endothelial cells to migrate and access the surrounding tissue. MMPs further enhance angiogenesis by helping to detach pericytes from vessels undergoing angiogenesis; or inhibit angiogenesis through endogenous angiogenesis inhibitors; which has been implemented in early stages of clinical trials for anti-angiogenic drugs.

1.2 Endothelial Tip- and Stalk Cell Selection

Vascular networks are formed through the shuffling and fusing of endothelial tip and stalk cells, which can be influenced by inflammatory environments through inflammatory cytokines, ECM-related microenvironment and metabolic cell division, as seen in cancers, through delta-like 4 (DLL4) and NOTCH (Toomey et al., 2009). Angiogenic endothelial tip- and stalk cell selection is regulated by the balance between various pro- and anti-angiogenic factors; as well as their downstream signalling networks (Chen, 2019). EC specification into stalk and tip cells is a dynamic phenotype rather than permanent cell fate, where endothelial cells compete for tip cell phenotypes and undergo changes within their metabolism, gene expression and response to extracellular signalling, as demonstrated in Table 1 (Kim et al., 2011).

Table 1: Differential expression of genes and markers in endothelial cell phenotypes.

Adapted from Chen et al., 2019.

Phenotype	Expressed Genes and Markers									
Tip cell	Adm	Ankrd37	C1qtnf6	Cldn5	Col4a1	Col4a2	Cotl1	Dll4	Ednrb	Fscn1
	Gpihbp1	Hspg2	Igfbp3	Inhbb	Jup	Kcne3	Kcnj8	Lama4	Lamb1	Lxn
	Marcks11	Mcam	Mest	N4 bp3	Nid2	Notch4	Plod1	Plxnd1	Pmepa1	Ptn
	Ramp3	Rbp1	Rgcc	Rhoc	Trp53ill	Unc5B	VEGR2	VEGFR3		
Stalk Cell	Ackr1	Aqp1	C1qtnf9	Cd36	Csrp2	Ehd4	Fbln5	Hspb1	Ligp1	Il6st
	Jam2	Lgals3	Lrg1	Meox2	Plscr2	Sdpr	Selp	Spint2	Tgfbi	Tgm2
	Tmem176a	Tmem176b	Tmem252	Tspan7	VEGFR1	Vwf				

On a molecular level, the extracellular microenvironment and VEGF receptor (VEGFR) expression levels on the cell surface induce the tip phenotype in endothelial cells, where endothelial cells with high levels of VEGFR2 and lower levels of VEGFR1 are more likely to adapt leading

positions, as illustrated in Figure 3. (Bentley et al., 2008; De Smet et al., 2009). Here, extracellular VEGFC activates VEGFR3, highly expressed in the tip cell phenotype, which down-regulates the VEGFR2 pathway. VEGFR regulates DLL4 levels which impact the up-stream regulation of NOTCH, the receptor for DLL4. This is key to tip cell selection as this relies on the DLL4-NOTCH signalling pathway which regulates competitive interactions and lateral inhibitions among tip and stalk cells. Tip cells receive more extracellular VEGF through VEGFR2 or VEGFR3 on the membrane and express higher levels of DLL4 and are more susceptible to NOTCH. The NOTCH signalling pathway on neighbouring cells is then activated; whereby neighbouring cells express lower levels of VEGFR2 and VEGFR3, and high levels of VEGFR1, causing the cells to keep low levels of DLL4 and remain stalk cells, unable to activate NOTCH and change phenotype.

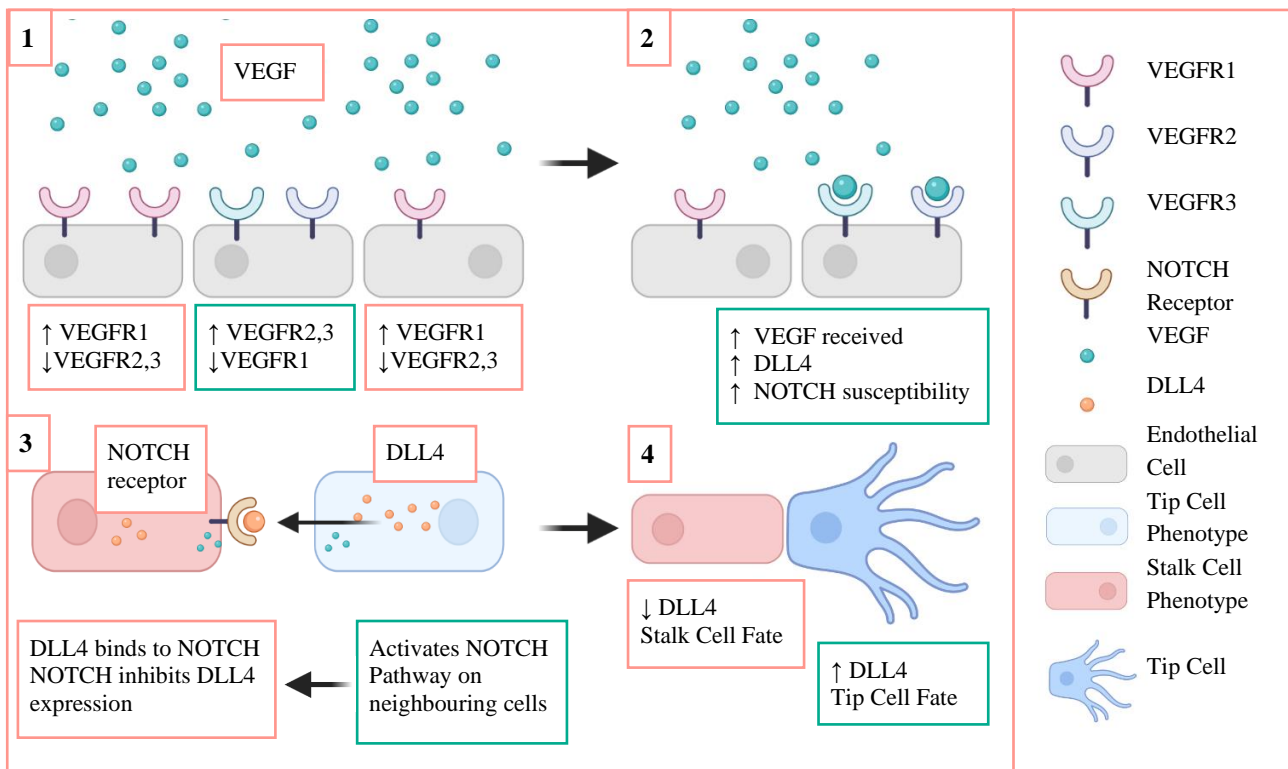


Figure 3. Lateral Inhibition and Delta-Notch Signalling in Angiogenesis. 1) VEGF secretion in the extracellular microenvironment. 2) Cells with high VEGFR3 and low VEGFR1 expression take up more VEGF leading to VEGFR2 and DLL4 Receptor (NOTCH) up-regulation. 3) The tip cell phenotype develops through high levels of DLL4, due to high NOTCH expression, and activate the NOTCH signalling on neighbouring cells inducing low levels of VEGFR2,3 causing those to keep low levels of NOTCH receptors, inhibit DLL4 and adapt the stalk cell phenotype. 4) Stalk and Tip cell fates through DLL4. Adapted from Koon et al., 2018. (Created Using BioRender.)

Concluding, a feedback loop pattern between VEGF, VEGFR, DLL4 and NOTCH patterns endothelial cell proliferation into tip- and stalk cell phenotypes by stabilising tip cells through NOTCH signalling in neighbouring cells (Weavers et al., 2014; Geudens et al., 2011). Additionally, the absence of NOTCH signalling causes a dysfunction of NOTCH-VEGFR feedback-mediated lateral inhibition and inhibits tip cell conversion.

1.3 Relevance Of Angiogenesis in Cancer Treatment

Brain tumours are highly angiogenic and can be classified into two types: primary brain tumours, which originate in the brain, and secondary brain tumours, which metastasise from other cancers (Kim & Lee, 2009). Brain blood vessels are generally tightly organised and involved in the blood and brain barrier tissue exchange (Guyon et al., 2021). Within brain tumour vasculature, two types of blood vessels exist. Those are either formed from pre-existing vessels absorbed by the tumour and neoangiogenic vessels, or created through angiogenesis (Lakka and Rao, 2008; Chinot et al., 2014). GBM is the most common, vascularised and deadly brain tumours. Postoperative survival is shorter in patients with high tumour microvascular densities (MVDs) compared to those with lower densities, illustrating the significance of tumour vasculature for tumour growth and potential in anti-angiogenic treatment (Leon et al., 1996). Solid tumours growing beyond a critical size, 1-2 mm in diameter respectively, require new blood vessels to maintain oxygen and nutrition supply for their growth and survival; known as tumour-induced angiogenesis. The pro- and anti-angiogenic factors controlling angiogenesis can be secreted by the ECM as well as cancer, stromal, endothelial and blood cells (Tandle et al., 2004). If pro- and anti-angiogenic molecules are well balanced, there is no angiogenic switch, meaning that angiogenesis is not induced. However, the balance of pro- and anti-angiogenic molecules is disrupted in a vascularised tumour environment and the enhanced expression of pro-angiogenic molecules leads to an uncontrolled promotion of angiogenesis (Fidler & Ellis, 2004). This is often triggered by hypoxia, low oxygen concentrations, which is caused by the fast pace of tumour growth. Tumour hypoxia is seen in the expression of hypoxia-inducible factor-1 (HIF1) which regulates angiogenesis as seen in Figure 2, as well as glucose metabolism related gene expression. Further, VEGF and other factors such as VEGF mRNA, VEGFR1,2 which play key roles in angiogenesis are over-expressed in high grade gliomas. Additionally, neurotrophins nerve growth factor (NGF) and brain-derived neurotrophin factor (BDNF) enhance endothelial cell survival and proliferation (Nakamura et al., 2006).

Due to the high vascularisation and of GBM compared to lower grade gliomas and benign expansive lesions such as meningiomas (MNGs), this model of a vascularised tumour environment is based on GBM. Guarnaccia et al. (2018) demonstrated that endothelial cells from primary GBM show higher efficiency when forming complex vascular architecture, impairing the blood brain barrier (BBB) and over-expressing pro-angiogenic mediators. It was further demonstrated that anti-angiogenic treatment, such as emozolomide, sunitinib or bevacizumab triggers different proliferative, apoptotic and angiogenic response depending on dosage and timeframe. The study provided a wealth of new data that can be integrated in computational models to gain mechanistic insights and to identify most promising molecular targets through in-silico screenings.

Based on the novel platform created by Guarnaccia et al. (2018), reproducing tumour vascularisation in a dish to allow the screening of drug resistance and sensitivity for targeted GBM therapy approaches, a computational model of a vascularised GBM environment is created to simulate changes in angiogenic potential.

1.4 Modelling Approaches Of Angiogenesis

Tumour angiogenesis is a biologically and physically complex process, and even though temporary vessel regression is a clinically well-known concept, few simulation models have succeeded in recreating it (Yanagisawa et al., 2021). Current tip- and stalk cell dynamics and formation model approaches range from compartment based models to cellular automata, cellular potts, stochastic differential equation and partial differential equation models.

Mathematical simulation modelling is often used to explore mechanisms within tumour angiogenesis and predict cancer progression and therapeutic responses (Metzcar et al., 2019). Here, three types of models have been proposed. Firstly, the sole simulation of angiogenesis which can be seen in a model by Anderson et al. (1998). Secondly, the simulation of tumour growth and invasion sustained by angiogenesis, which has been modelled by Jiang et al. (2005), Peng et al. (2017) or Shirinifard et al. (2009). Finally, simulations integrating angiogenesis and tumour growth have been created through 3D models. Examples here are the models by Macklin et al. (2009), created to research ECM decomposition surrounding the tumour, and Tang et al. (2014), investigating chemotherapeutic drug delivery. These models identified several factors which inhibit or promote angiogenesis within a tumour microenvironment, however, regulating pro- and anti-angiogenic factors and their receptors remain poorly understood (Yanagisawa et al., 2021).

1.5 Computational Modelling Of Biological Processes

Cellular modelling can range from studying a limited number of cells and specific morphologies in 2 dimensions (2D) to studying millions of cells in three dimensional (3D) simulated tissues (Metzcar et al., 2019). Individual cell modelling enables the direct translation of biological observations into simulation rules. This can be used to simulate oxygen, growth factor and treatment transport; used to connect microenvironmental conditions to cancer development. Computational modelling allows the generation of new insights and understanding, hypothesis testing and tracing chains of causation by merging different sets of information (Brodland, 2015). This can be done across various length scales such as multi-scale and multi-faceted models, which is often connected to gene expression, cell properties, tissue mechanics and cell phenotypes.

There are two broad categories of computational modelling of angiogenesis: continuum and discrete (Milde, 2008). Continuum models describe average, larger scale cell population behaviour through a system of partial differential equations whereby capillary networks are described as endothelial cell densities. Even though they provide valuable insights in angiogenic processes, continuum models are limited in their ability to predict the structure of vascular networks. Discrete models, such as agent based, cellular automata and cellular Potts models, allow the incorporation of nutrients, oxygen and drug throughput and can be used for the study of the morphology of vascular networks. A summary of existing computational modelling approaches of angiogenesis can be found in Table 2.

Table 2. Computational Modelling Approaches of Angiogenesis. Taken from Qutub et al. (2009)

Methodology	Property (Molecular)	Property (Cellular)	Property (Tissue)
Discrete			
Monte Carlo Steps	VEGF Interaction		
	VEGF-VEGFR		
2D Agent Based	FGF	Chemotaxis	Branching
	VEGF	Cell migration, proliferation	Capillary network formation
	PDGF		
Potts Model	VEGF	ECM interaction	Branching
	VEGF diffusion		Capillary formation
2-D random walk-based models	VEGF diffusion	Cell migration, persistence	Branching
	VEGF degradation	Chemotaxis	Capillary network formation
	VEGF production	Cell Density	
	FGF		
Hybrid			
3D-Agent based	VEGF	Endothelial cell dynamics	Branching
	VEGF gradient	Chemotaxis	Capillary formation
	DLL4		
2-D discrete	Angiostatin	EC migration	Branching
		Chemotaxis	Capillary formation
		Matrix degradation	
		Cell-surface receptor binding	
		Cell-matrix interactions	
		Pericytes/macrophages	
Continuous			
Reaction-Diffusion	VEFG-VEFGR, VEGF-neuropilin interactions	ECM interactions	VEGF
	HIF1-alpha degradation	MMP driven VEGF release	VEGF-VEGFR interactions
	MMP interactions	Matrix heterogeneity	Blood flow
		HIF1-alpha autocrine synthesis	O2 Transport
Wave equations		EC interaction, proliferation, migration	Capillary network formation
Fractals			Capillary network formation
			Comparison of tumour tissue and adjacent normal neurovascular network formation

1.4 Cellular Automata Modelling

Cellular automata and agent-based models have made major breakthroughs in natural sciences within the last two decades through their simplicity, flexibility, and ability to incorporate both temporal and spatial dimensions resulting in aggregated behaviour, which cannot be modelled in any other form (Santé et al., 2010; Clarke, 2013). Cellular automata modelling has been applied in various real life sciences including mathematics, computer graphics and biology (Czerniak et al., 2018). Cellular automata evolution takes place in a grid of identical cells, surrounded by each the same amount of neighbours (Burstedde et al., 2001). Grid and automata behaviour is influenced by three structural factors: space capacity, grid regularity and number of neighbouring cells. The first is dependent on the magnitude of the task at hand. The second is relies on the identity of the cells and therefore, directly influences the third as shown in Figure 4.

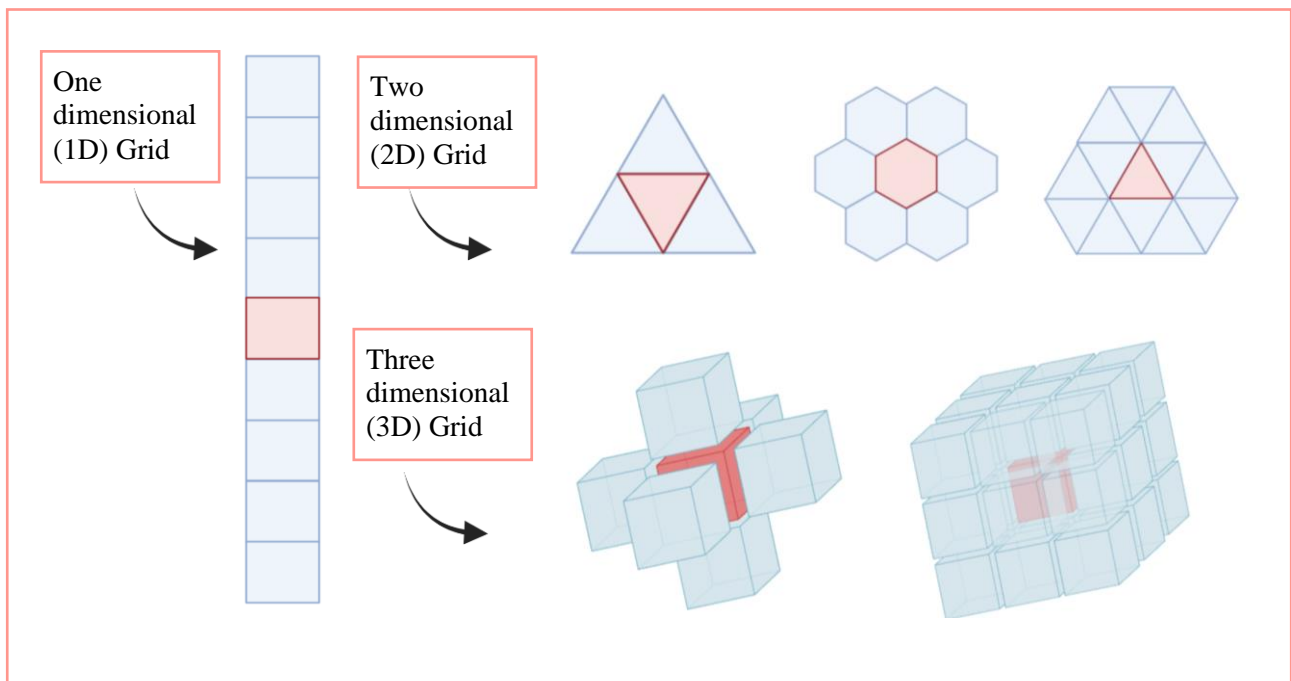


Figure 4. Types of Cellular Automata Grids.

Adapted from Czerniak et al. (2018). (Created Using BioRender)

A rule-based simulation in a lattice system can demonstrate cell behaviours and interactions, such as their migration or differentiation and their neighbours or environments, such as the ECM or microvasculature, respectively. Hereby the simulation of individual cell interactions and behaviour allows the observation of an emergent system's behaviour as demonstrated in Figure 4.

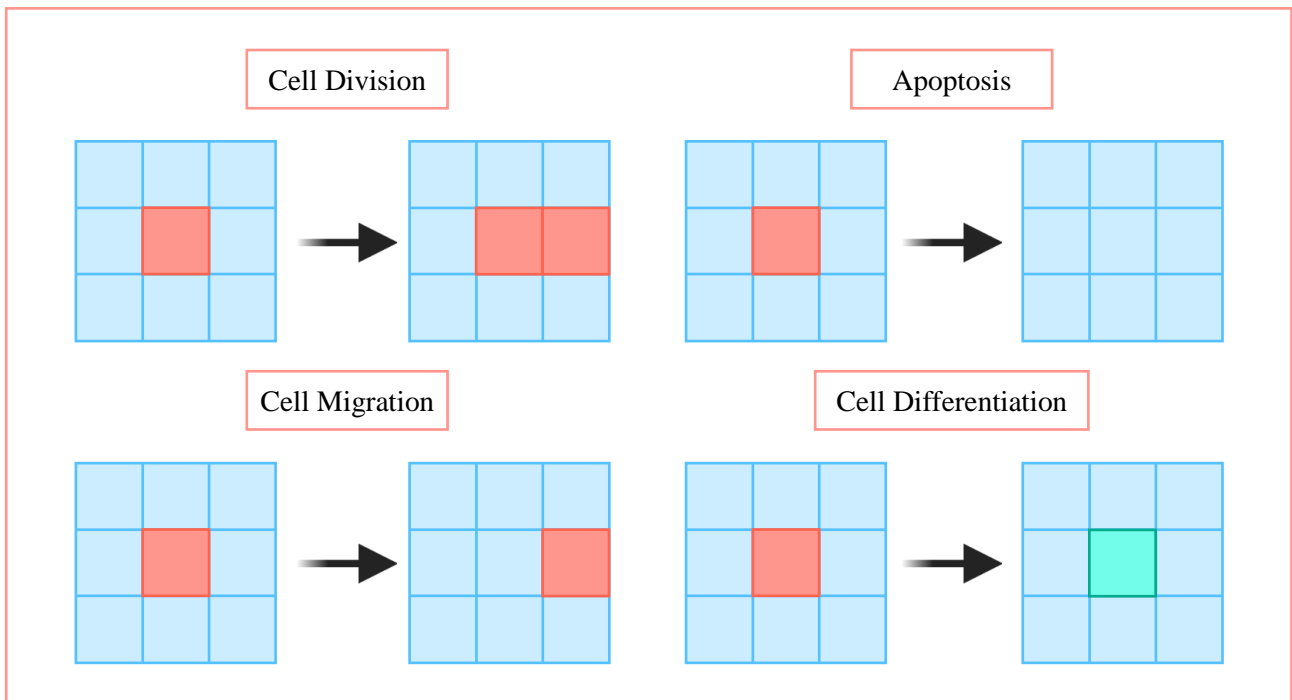


Figure 5. Cell behaviour in the lattice. Adapted from Hwang (2009).

Cellular Automata Potts models, as seen in Figure 6, allow one biological cell to occupy more than one lattice site. This allows cells to change shape within the simulation. Multiple cell behaviours, such as division, necrosis, differentiation or migration, can be implemented on single cells, and implemented in the lattice and to observe single cell and cell population behaviour.

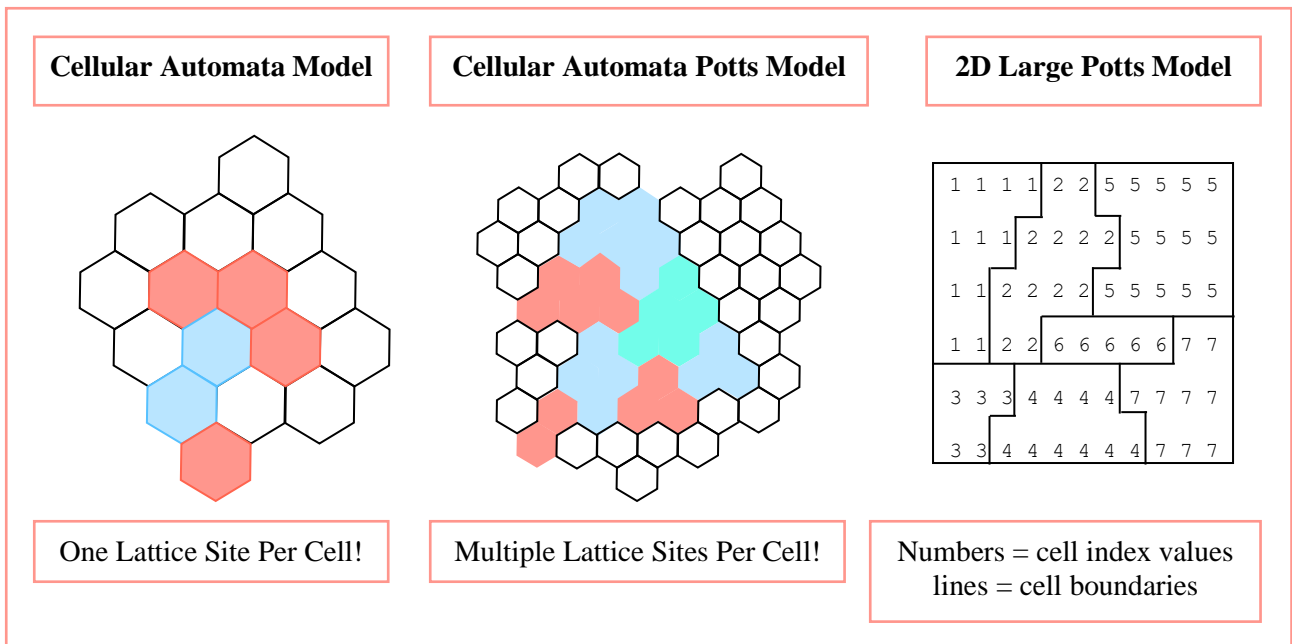


Figure 6. Cellular Automata, Potts and Large Potts Modelling.

Adapted from Metzcar et al. (2019) and Alber et al. (2003).

Cells can migrate at random to any of the neighbouring sites at multiple time points within the simulation (Checa & Prendergast, 2009). This can be implemented in a manner similar to a model by Ferreira et al. (2002), utilising the probability of migration as an increase factor of the amount of tumour cells, as the probability of migration increases with the level of nutrients received. Cell differentiation can be modelled by changing one cell type to another within its lattice site as seen in a mesenchymal stem cell differentiation model by Checa & Prendergast (2009). Here, differentiation relies on stimulus and local vascularity at maturation age. This can be taken further by modelling cell transitions based on cell arrangement and surrounding space as seen in a model of epithelial cell morphogenesis by Grant et al. (2006) where the extracellular environment is modelled through environmental factors such as oxygen concentrations or neighbours, and cellular responses are determined by their response network. Following the extensive opportunities in cellular modelling, a simulation model, which can be used as a tool to predict angiogenic potential based on interchangeable experimental data is developed.

1.4 Aims and Objectives

To fulfil the overarching aim of this project, (1) a simulated vascularised brain tumour environment including VEGF secretion is created, including DELTA-NOTCH signalling in response to VEGF secretion and endothelial tip cell formation. Further, (2) endothelial cell tip formation and migration is simulated and tracked, whilst implementing experimental data in form of previously obtained images of endothelial cells. The simulations will be created with CompuCell3D (CC3D), using the built in plugins for cell manipulation and cell tracking. The model can be expanded and changed within its parameters to simulate changes in intracellular signalling in response to anti-angiogenic treatment responsiveness within angiogenic potential and tip cell formation based on literature. Results can then be tested in vitro utilising primary or iPSC-derived EC. Results can then be tested in-vitro utilising iPSC. Further, the model can be connected to ECPT, an endothelial cell processing tool developed to profile individual EC in their monolayers whilst providing relational and spatial information regarding cell behaviours including NOTCH activation or cell proliferation (Chesnais et al., 2021).

1.5 Project Relevance, Gaps In Literature And Translational Potential

Today, we have limited tools to assess cell-to-cell interactions or measure their movements live in a strictly lab-based experimental environment. In a translational context, new anti-angiogenic treatment methods and enhanced efficacy could be achieved through a better understanding of angiogenesis through the simulation of influencing factors, mediators and drug and dosage schedules. By developing a tool to predict angiogenic potential for improved experimental design and prediction of treatment outcomes, the translational potential of understanding endothelial cell behaviour is a big step in the direction of personalised medicine and early treatment prognosis in the future. Currently, a third of cancer drugs are approved based on their response rate in small clinical trials, which is often only low or modest (Chen et al., 2019). Here, better understanding of endothelial cell behaviour, as well as cell-to-cell interactions under different conditions and across multiple levels of biological organisation in respect to space and time, could help eradicate early clinical trial errors and improve or even predict treatment outcomes. The flexibility of computational models enables quick testing of various hypotheses and experimental design changes, which could take months in strictly lab-based studies (Stepanova et al., 2021).

Modelling in CompuCell3D enables reusing, testing or adapting previous or published models to create synthetic data and build hypotheses. The computational approach can help avoid simple mistakes in early wet lab experimentation or clinical trials as well as further understand and develop the effectiveness of a drug in a specific context regarding its dosage, schedule, and targets.

2. Materials and Methods

2.1 Computational Model Selection

Where computational models cannot replace experiments or act as proof of mechanisms in given situations, they can demonstrate whether or not proposed mechanisms are sufficient to produce a phenomenon. Many of the aforementioned models can be implemented within specialised computational frameworks including CC3D and Morpheus. Where both CC3D and Morpheus work with SMBL (Systems Biology Markup Language) models, Morpheus' is declarative and models from numerical implementation, using a declarative domain-specific markup language in both biological and Mathematical terms in C++ (Starruß et al., 2014). CC3D was chosen as it runs on Python, which is straight forward and much easier to learn in the given time frame. Further, CC3D utilises SMBL rather than CellML, as seen in MatLab, which is uses a ranked retrieval system whereby similarity measures are applied and adapted to measure similarities with existing models (Henkel et al., 2010). SBML is declarative and oriented towards describing biological processes. When supporting SBML as an out/input format, different tools can be used to operate within the model which decreases the probability of translation errors (Hucka et al., 2018). The selection of model and experimental process used is summarised in Figure 7.

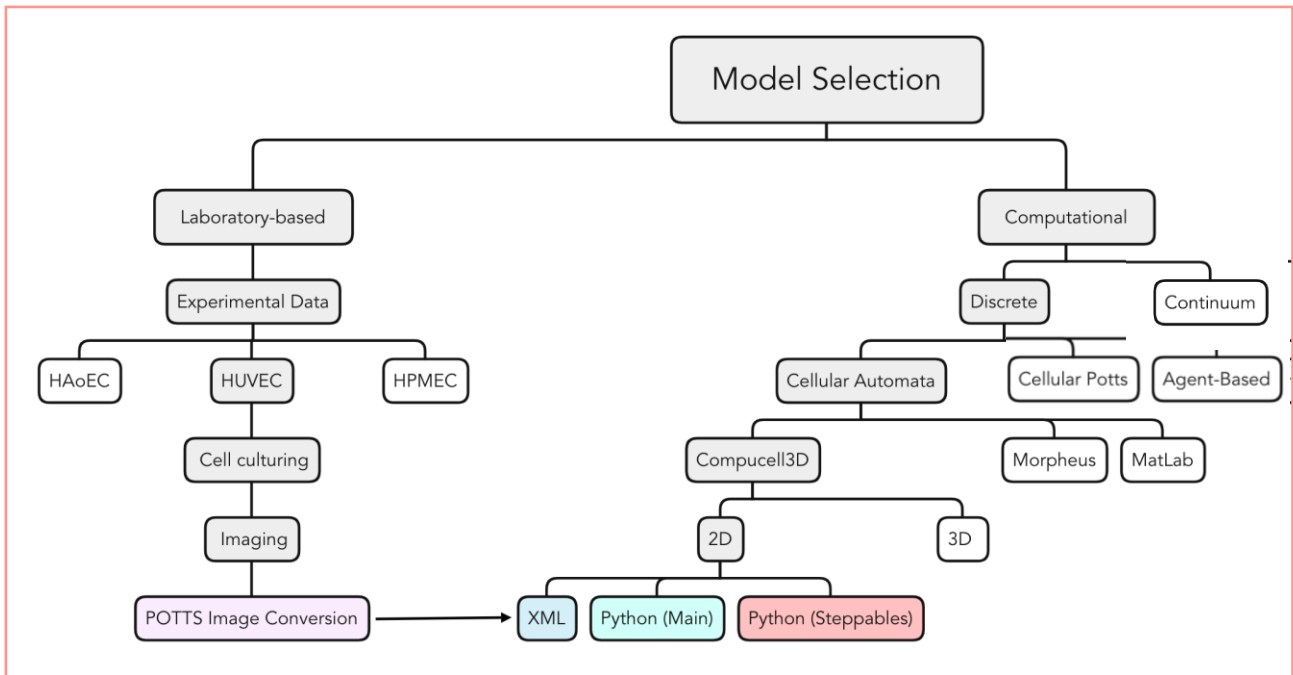


Figure 7. Model Selection and Experimental Data Implementation. (Creating Using XMind.)

CC3D is a multi-cell, multi-scale computational simulation method, which allows the study of multi-cell phenomena at the tissue scale through Monte Carlo multi-cell modelling and an open-source simulation environment. Models are based on biologically observed cell behaviours, interactions, and movements and allows rapid modelling and simulation within tissue formation and cellular dynamics (Swat et al., 2012).

2.2 Simulating Experimental Data

Where many previously created mathematical and computational models of angiogenesis and angiogenic potential have had great impact on our understanding of the molecular processes behind it, this model is taking the idea further by utilising experimental data obtained in the Vascular Cell Dynamics Lab. Hereby we can test the theory and simulate angiogenic potential using our own data in form of images. Images are converted into Potts Initialisation File Format (PIFF) and implemented the simulation. Initialising a PIFF in the simulation overrides earlier data. Cell IDs are be assigned to the cells contained in the PIFF file and may take up multiple lines in the code. They translate to locations within a 3D 'box' or 2D lattice, as illustrated in Figure 8. Lattice boundaries stated within the code and declared cell types need to match the boundaries of the PIFF image file to be implemented the simulations.

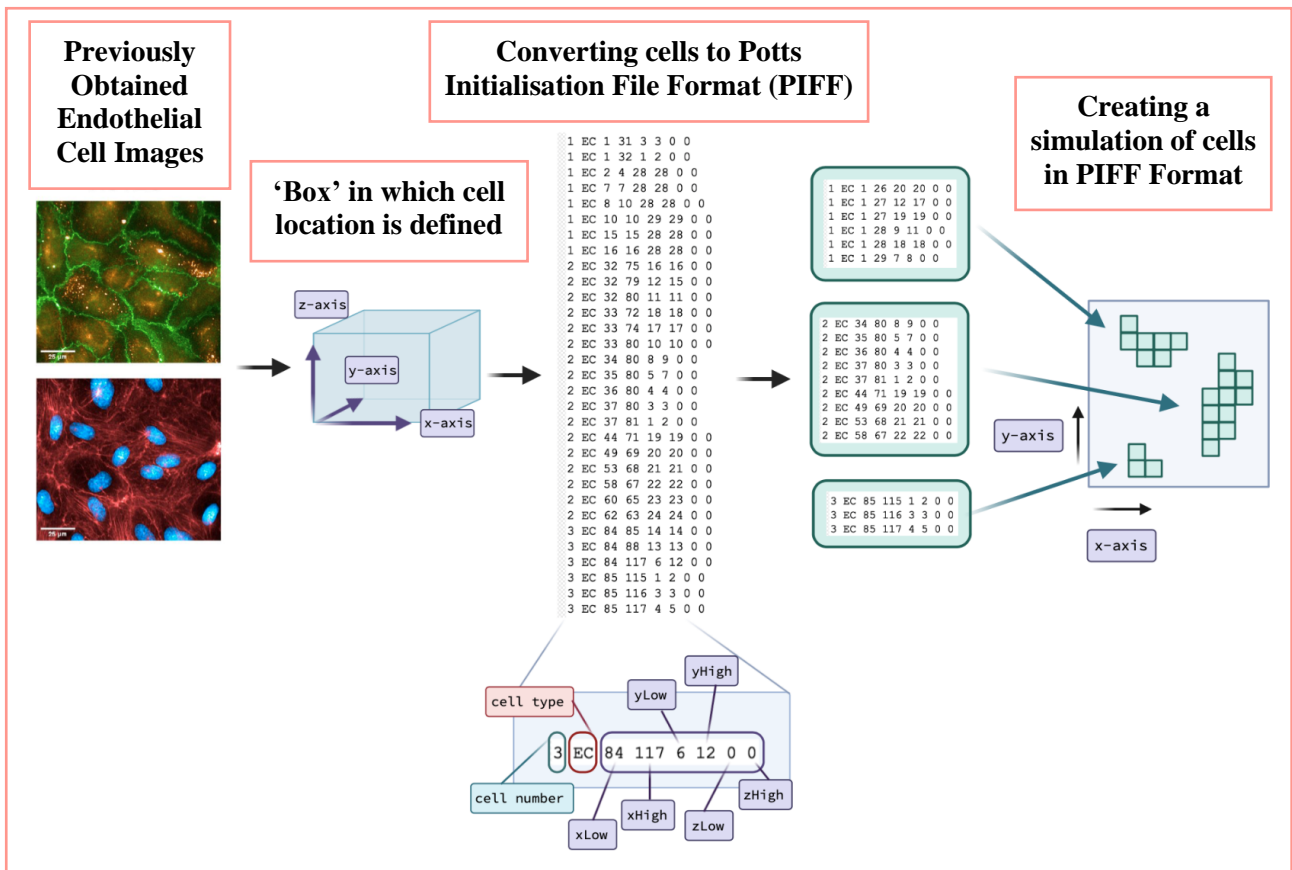


Figure 8: Translating experimental data into synthetic data. Cell locations in previously obtained images of endothelial cells can be converted into the Potts Initialisation File Format (PIFF) and located within the ‘Box’ cell through their code line. One cell can occupy multiple lines and be initialised and simulated in CompuCell3D. (Created Using BioRender.)

2.3 Cell Culture And Imaging

In-vitro experiments and imaging was performed as described in Chesnais et al., Biorxiv 2021. In brief, primary human umbilical vein endothelial cells (HUVEC, PromoCell), human aortic EC and human pulmonary microvascular EC (HUVEC, HAoEC, HPMEC, PromoCell) were cultured on 10 µg/mL fibronectin coated flasks (fibronectin from human plasma, PromoCell), grown in Endothelial Cell Growth Medium MV 2 (EGMV2, PromoCell) in absence of antibiotics and detached with Accutase (Thermo Fisher Scientific, Waltham, MA) used by passage 5. Further, 4×10^4 ECs were seeded in fibronectin-coated 96-well plates (µclear, Greiner), cultured for 48 hours under basal (EGMV2, Promocell) or activated (EGMV2 + 50 ng/mL VEGFA, Peprotech) conditions in triplicate where EC formed confluent monolayers at the time of image acquisition and immunostaining. For immunostaining, cells were fixed with 2% paraformaldehyde in phosphate buffered saline (PBS) for 10 minutes at room temperature, blocked one hour with PBS supplemented with 1% fetal bovine serum (FBS), permeabilised with 0.1% Triton X 100 and incubated for another hour at room temperature with activated NOTCH (NICD, Abcam ab8925, 1 µg/mL final) and primary antibodies against CDH5 (Ve-Cadherin Novusbio NB600-1409, 1 µg/mL final). EC lineage was tested utilising CDH5 and showed that all cells were CDH5+; NICD punch were released through anti-activated NOTCH1. Finally, plates are washed and incubated for one hour using secondary Alexa 488-conjugated antibody and Alexa-555-conjugated antibody (Thermo), Phalloidin-Atto 647N (Sigma) and Hoechst 33342 (1 µg/mL, Sigma).

Images were obtained using Operetta CLS system (PerkinElmer, Waltham, MA) equipped with a 40×water-immersion lens (NA 1.1). 3 areas were acquired in each well, whereby each area is composed of nine microscopic fields at 40× magnification. Acquisition parameters such as exposure time and LED power were standardised, and 3 intraexperiment replicates were conducted for each experiment.

2.4 Creating A Vascularised Tumour Environment

After the image has been converted to PIFF format (2.2) it can be implemented, initialised and defined according to its cell properties as demonstrated in Figure 9. Further, a vascular wall of endothelial cells can be created within the XML code to simulate tumour angiogenesis within the vascular wall environment through VEGF secretion as a diffusion Steppable in XML and Python.

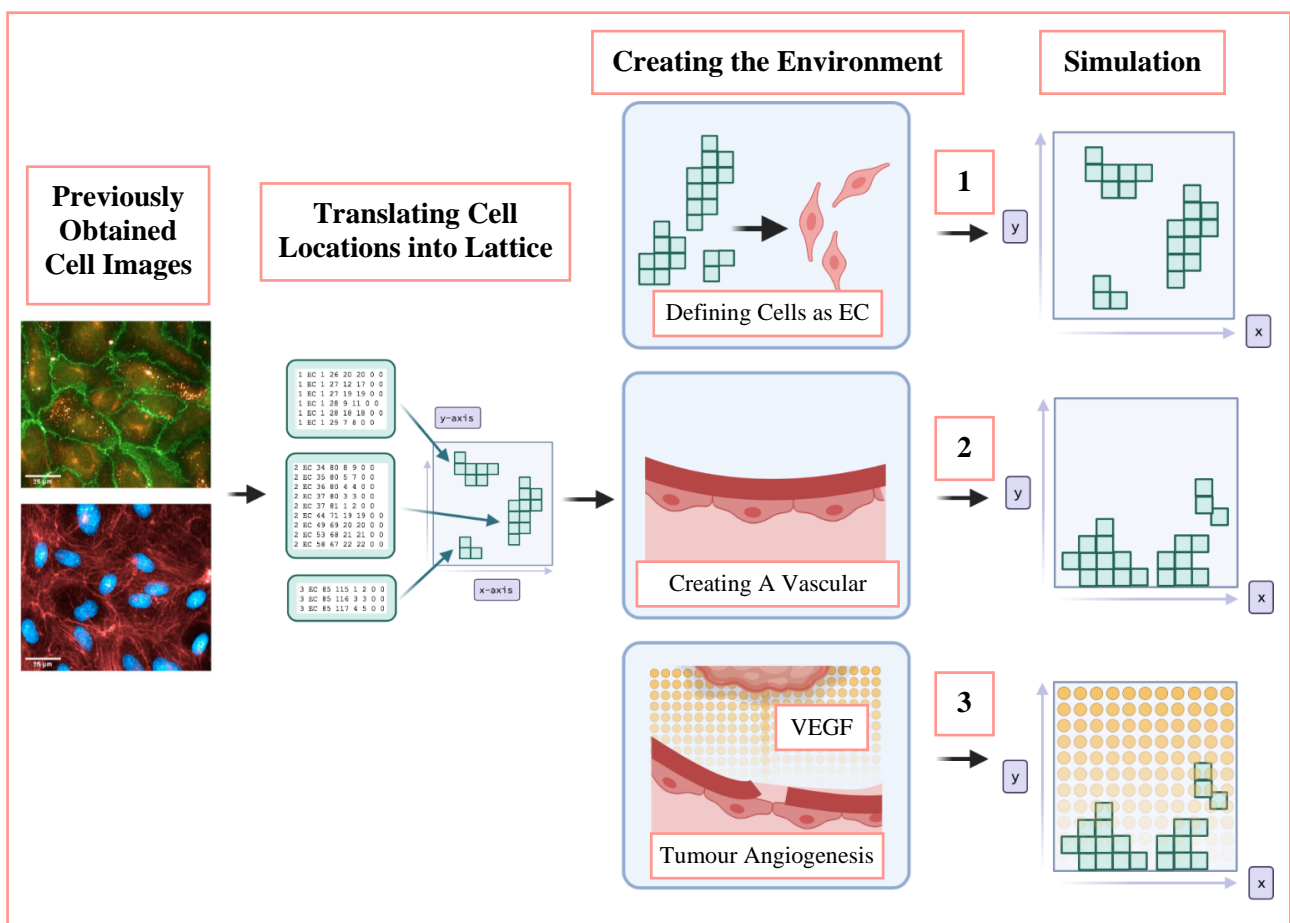


Figure 9. Creating a Vascularised Tumour Environment in CC3D. 1) Defining cells from the image as Endothelial Cells (EC) in XML. 2) Creating the vascular wall in the simulation. 3) Simulating a vascularised tumour environment and tumour angiogenesis through VEGF secretion as VEGF diffusion. (Created Using BioRender.)

2.5 Simulating Delta-Notch Signalling in Response to VEGF

As demonstrated in 1.2, Delta-Notch signalling plays a key role in angiogenesis and tip- and stalk cell formation, leading to the creation of new vasculature. The contact mediated lateral inhibition as seen in Delta-Notch signalling can be constructed within a mathematical model, and applied to existing cells in CC3D. Collier et al. (1996) developed a mathematical model in accordance with experimental data, presupposing that receiving inhibition through NOTCH activation decreases the ability to produce Delta; and hereby inhibit Delta production in other cells. This introduces a feedback loop, amplifying differences between adjacent cells, generating patterns similar to living systems though initial and boundary conditions, as demonstrated in Figure 10.

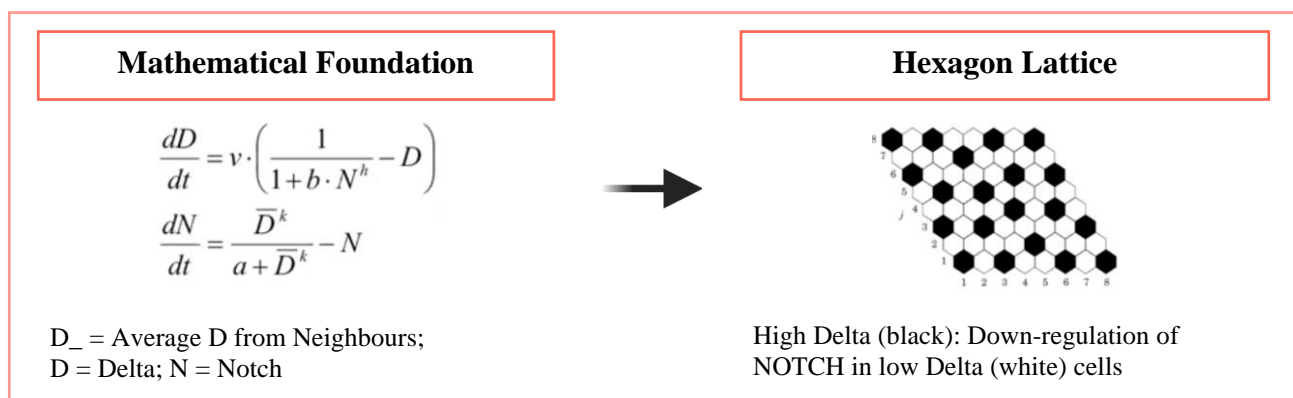


Figure 10. Mathematical Modelling of Delta-Notch signalling. Adapted from Collier et al. (1996)

Based on the model by Collier et al. (1996), the parameters in the formula can be expanded mathematically to include the impact VEGF secretion and the intensity thereof. As demonstrated in Figure 11, this can be implemented within this simulation and applied to experimental data in PIFF format within the simulation, whereby Delta-NOTCH patterning and the following cell behaviour, such as stalk and tip cell formation can be observed.

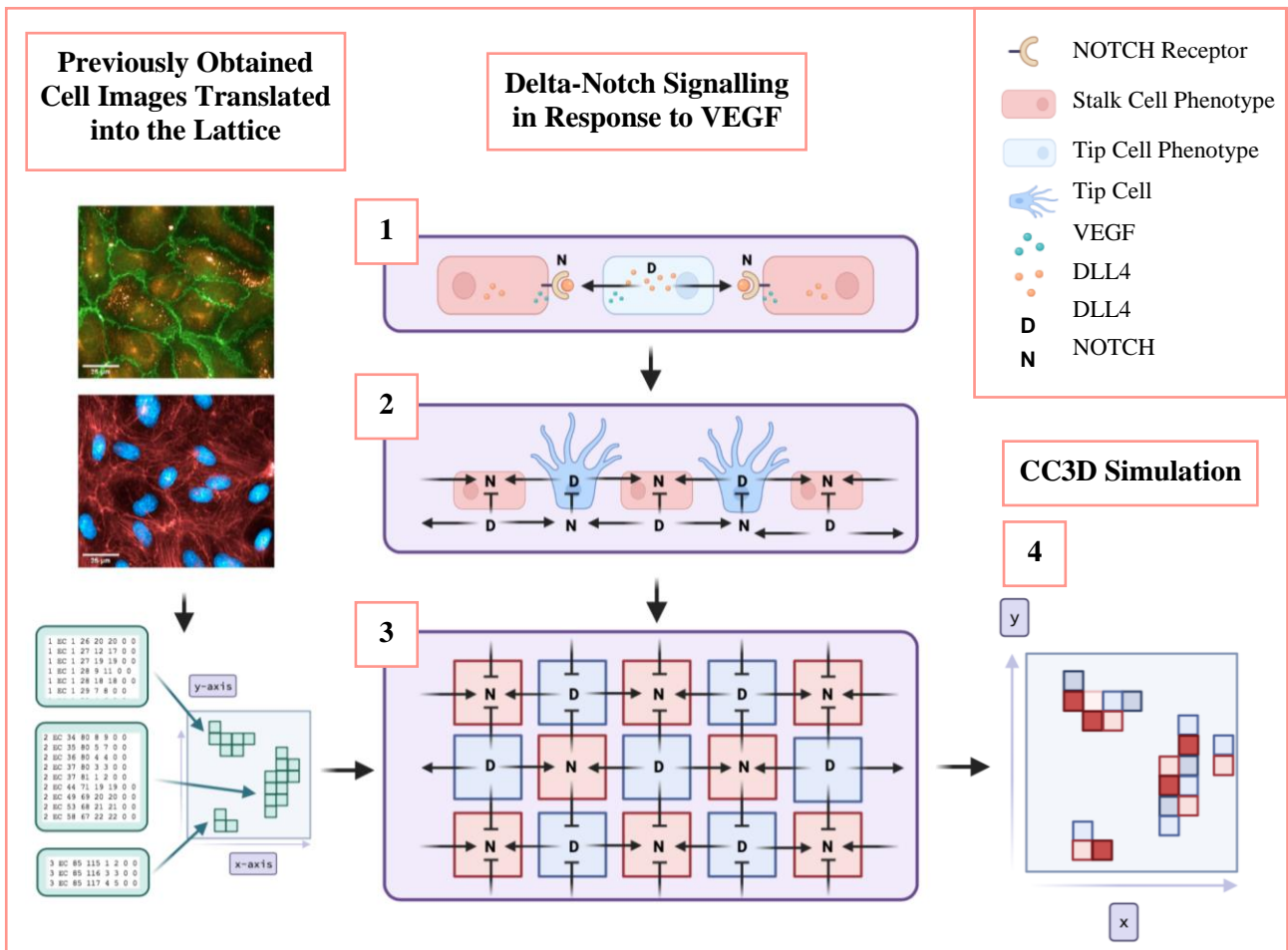


Figure 11. Simulating Delta-Notch Signalling In Experimental Data. Previously obtained images are translated into PIFF format and initialised in simulation, Delta-Notch signalling in response to VEGF is implemented onto cells: 1) Lateral Inhibition and Delta-Notch Signalling in Angiogenesis: Cells with high VEGFR3 expression (later tip cells) take up more VEGF from their extracellular microenvironment, causing high DLL4 Receptor (NOTCH) expression and hereby high DLL4 uptake, activating the NOTCH signalling pathways and inhibiting DLL4 in neighbouring cells, forcing them to adapt the stalk cell phenotype. 2) Simplifying stalk-tip cell Delta-Notch Signalling. 3) Implementing DLL4-NOTCH signalling and inhibition in the simulation lattice through Steppables affecting neighbouring cells. 4) Implementing the Delta-Notch steppable in this simulation using previously obtained images in PIFF format. Adapted from Koon et al., 2018. (Created Using BioRender.)

2.6 Simulating And Tracking Tip Cell Formation And EC Migration

Tracking endothelial cell migration through tip cell formation caused by the Delta-NOTCH-VEGF feedback loop is indicative of angiogenic potential and vessel stabilisation and can be simulated in CC3D. Here, cells can be tracked by their centre of mass through a plot whereby each tracking pixel is assigned a MCS value. The first, original location is coloured blue, and the following locations, a yellow and green gradient with a red tip, as demonstrated in Figure 12.

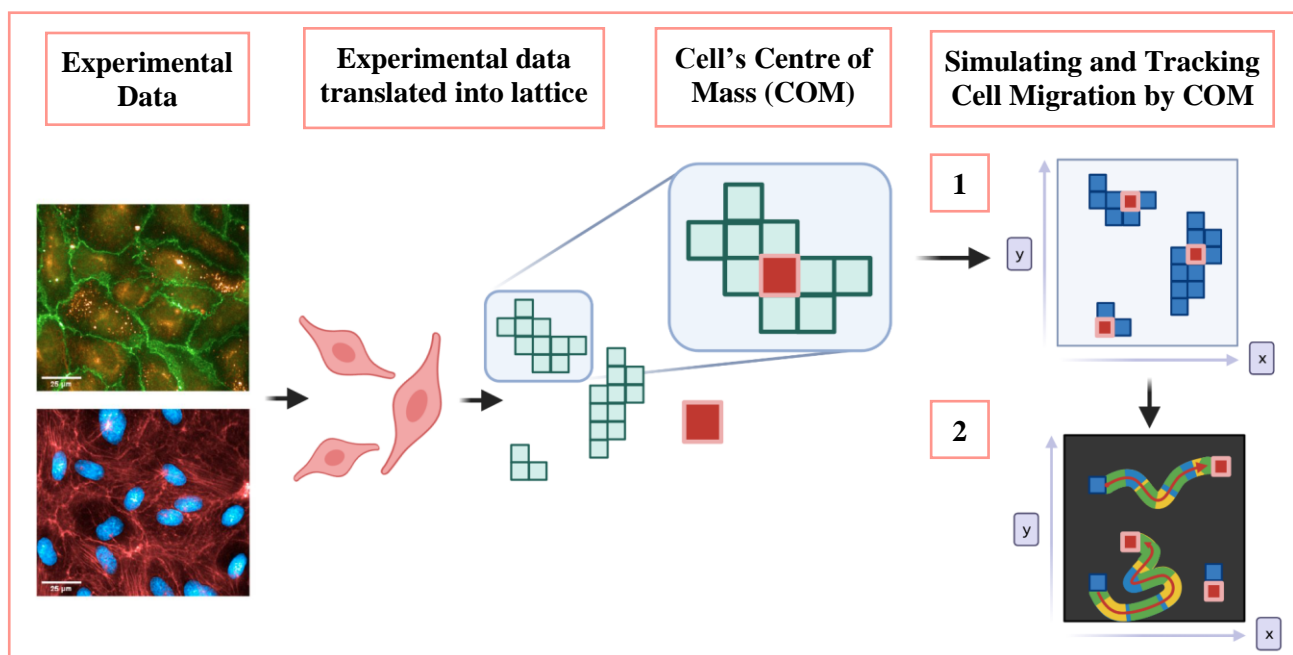


Figure 12. Tracking Cell Migration Through A Cell's Centre Of Mass. Experimental data is translated into the lattice through conversion and implementation of the image in PIFF format. 1) Creating a field 'Centre of Mass' (COM) for the cell (red). 2) Tracking COM movement in a field where blue represents the cell's original location, red the most recent. (Creating Using BioRender.)

2.7 Implementing Theory In CC3D Code

CC3D modelling is declared in Main (Python), which is used to import CC3D and Steppables into the Simulation; Steppables (Python) and XML (C++) files. When simulating the vascularised tumour environment and endothelial cells in the lattice, environmental condition such as VEGF secretion, cell adhesion and contact energy, cell size and surface, lattice and boundary conditions are defined in Plugins, Steppables and Potts in XML, as illustrated in Figure 13.

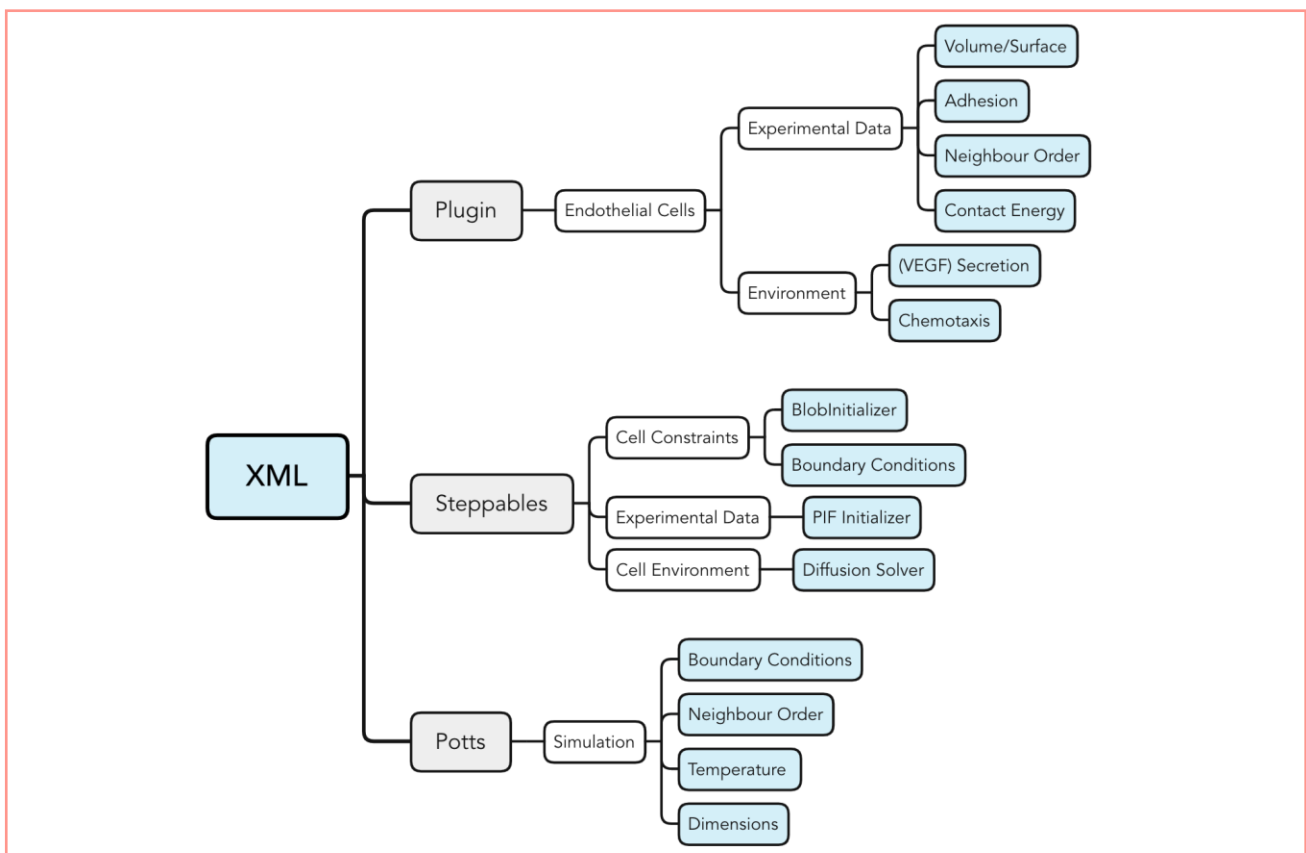


Figure 13. Relevant components in modelling a vascularised tumour environment in XML.

(Created Using XMind.)

Following the creation of the extracellular environment and cell boundaries, Python Steppables are used to model cell-to-cell interactions such as Delta-NOTCH signalling,

environmental changes within the extracellular matrix such as VEGF secretion and the visualisation of this simulation model and cell tracking (Figure 14).

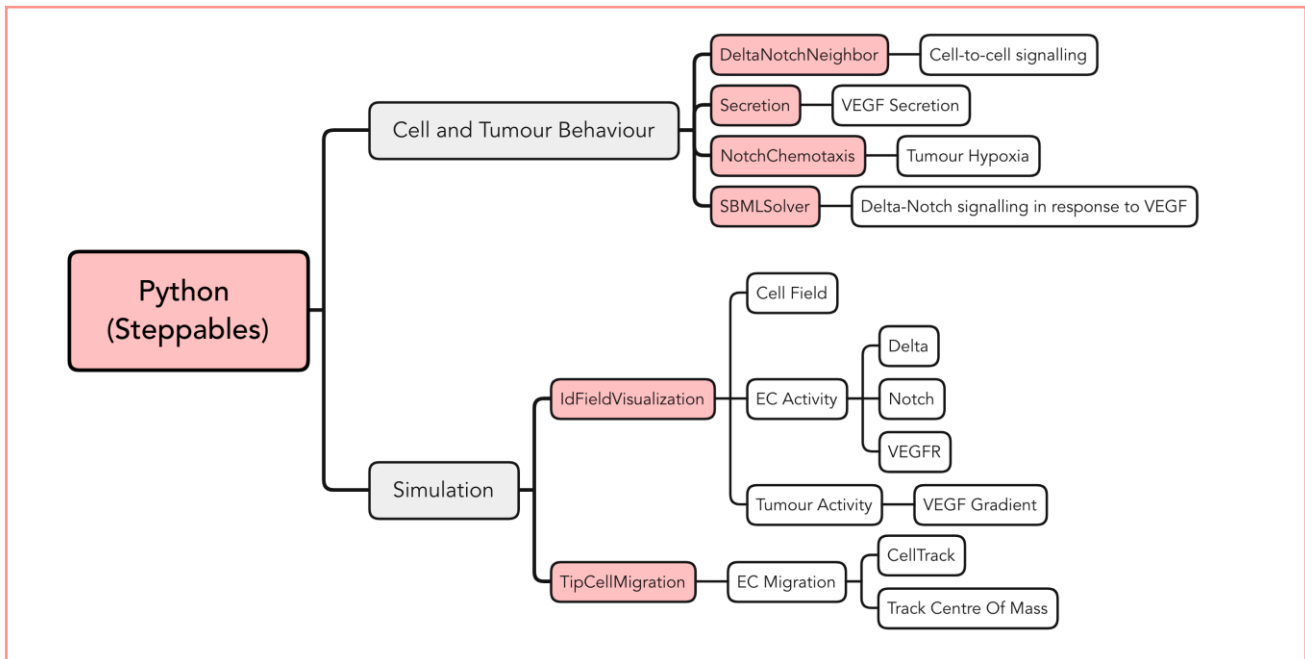


Figure 14. Intent Of Selected Steppables (Python). (Created Using XMind.)

Whilst creating the code, CC3D demo models were modified and implemented in order to model Delta-NOTCH signalling, VEGF secretion and Delta-NOTCH signalling in response to VEGF secretion leading to tip cell formation. Further demos were used to track cell migration and therefore endothelial tip cell formation. Demo models were imported from CC3D and modified according to cell types, simulation boundaries and other values.

3. Results

In order to develop a tool to predict angiogenic potential in endothelial cells based on experimental data, a workflow has been developed. This includes the acquisition, imaging and conversion of experimental data in conjunction with the computational simulation of a vascularised tumour environment and visualisation of signalling pathways as illustrated in Figure 15.

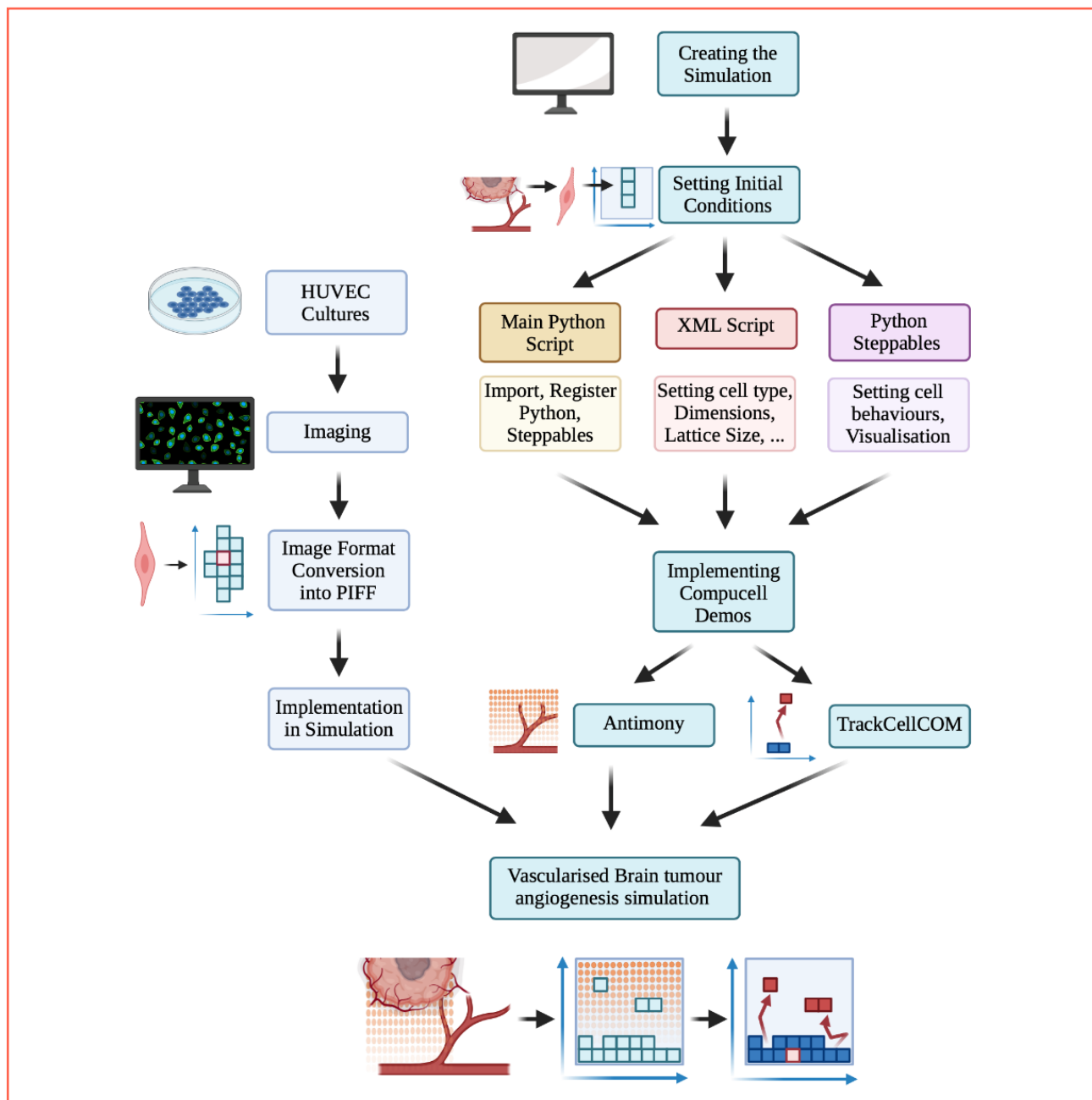


Figure 15. Project Workflow. (Created Using BioRender.)

3.1 Experimental Data

HUVEC were seeded under low fetal bovine serum (FBS) conditions for 48 hours in the presence or absence of supra-physiologic VEGF. Cells reached confluence after 24 hours upon spreading and adhesion, and were cultured for an additional 24 hours to enable stable inter-endothelial adherens junction (IEJ) formation. Monolayer stabilisation was confirmed through a uniform cobblestone morphology under low magnification and proliferation, junctions and NOTCH activation were considered when analysing the culture. As seen in Figure 16, the culture was stained for activated NOTCH (NICD), VE cadherin (VEC), nuclei and actin cytoskeleton which demonstrated a smaller and more disorganised cytoskeleton in HUVEC when compared to HAoEC (Primary Human Aortic Endothelial Cells) and HDMEC (Primary Human Dermal Microvascular Endothelial Cells).

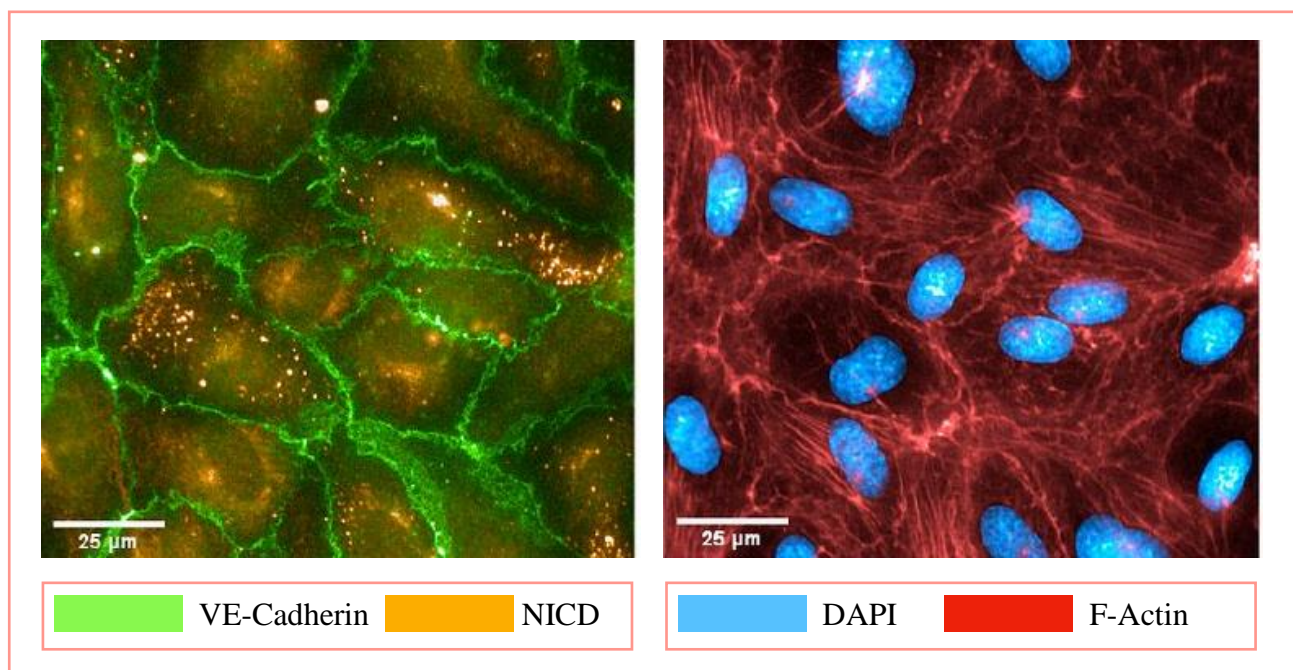


Figure 16. Microphotographs of HUVEC. HUVEC untreated immunostained for VE-cadherin (green), NICD (orange), F-actin (red) and DAPI (blue); (scale bar: 25µm).

As morphological features are organ specific in EC, interactions between EC and other cell types can differ by their source (Uwamori, 2019). HUVEC were selected based on their ability

to form more extensive microvascular networks and low permeability coefficient in microvasculature and high NOTCH signalling, similar to brain endothelial cells. In order to implement experimental data into the simulation, the HUVEC images are translated into PIFF format utilising ImageJ and R script. In the process of building a functional simulation with the desired outcome, 3 models were created implementing various approaches, based on existing CC3D demos simulating delta-notch signalling, delta-notch signalling in response to VEGF and angiogenesis, cell tracking and working simulations provided by Dr Veschini.

3.2 Model 1: Delta-NOTCH Signalling in Experimental Data

Model 1 tackles first steps in simulating Delta-NOTCH signalling in experimental experimental data using CC3D. A simulation was created, implementing experimental data defined as cell types 'Medium' and 'EC'. Code errors were solved correcting lattice dimensions matching boundary conditions of the experimental data image frame, relocating the experimental data image in the model simulation folder and rephrasing the image initialiser code. Cells imploded upon initial simulation. Cell volume, binding parameter, target and lambda volume and surface and lastly temperature were adjusted to successfully stabilise the cell count and attain cell contact needed for Delta-NOTCH signalling. Results show that changes in the AdhesionFlex plugin within the XML code, the VEC Binding Parameter (adhesion molecule VE-Cadherin (VEC)) and TargetVolume and TargetSurface significantly impacted cell contact and movement. Results shown in Figure 17 show changes in temperature, surface and target constraint demonstrated little to no impact in cell behaviour, contact and inventory. Low target and lambda volume led to small appearance and loss of contact in cells, high volumes led to enhanced cell contact and cell expansion toward the edges of the lattice.

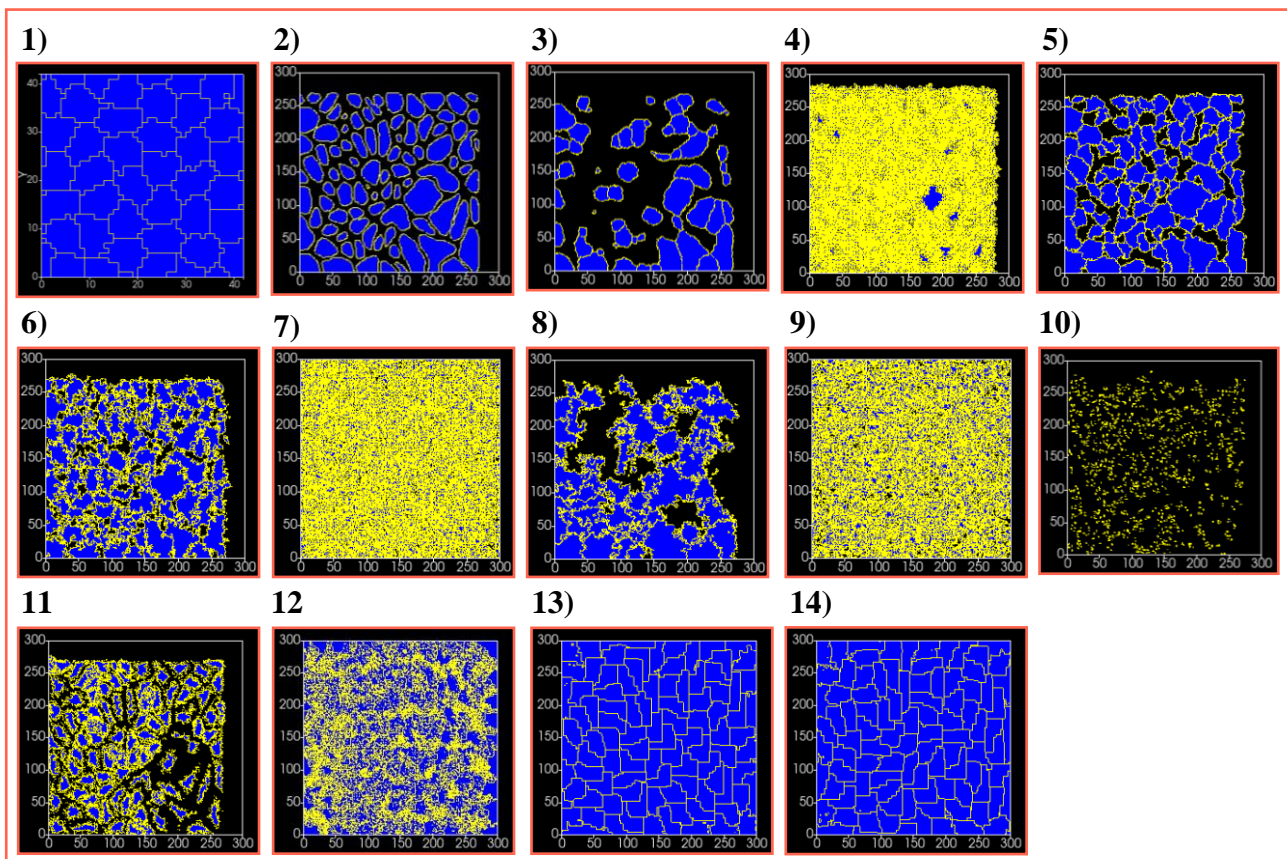


Figure 17. Model 1 in Cell_Field View. 1) DeltaNotch Template. 2) Including Experimental Data: Cells disappear. 3) VEC Binding Parameter (VECBP) = 3. Cells Implode slower. 4) VECBP = 15. Cells Multiply. 5) VECBP 0.5 -> 8.5. Cells Implode Slower. 6) VECBP 0.5 -> 9. Cell Count Near Consistent. 7) VECBP = 9.5, Consistent Cell Count. 8) VECBP = 9. Cell Inventory Decreases. 9) VECBP = 9.25. Cell Count Consistent. 10) Target Volume (TV) = 10, No Contact, Cells Shrink. 11) TV, Lambda Volume (LV); Target, Surface constraint (TS, LS) = 300. Cells appear larger, do not move. 12) TV, TS = 1000, LV, LS = 200. Cells Expand. 13) TV = 1000, LV = 10, TS = 150, LS = 10. Cells Expand toward edges. 14) Changes in Temperature: Temperature = 1.

In order to simulate Delta-NOTCH signalling within the above lattice of experimental data, DeltaNotch.cc3d was taken from the CC3D_3.7.5 simulation folder and amended to fit the experimental data model's cell type and boundary conditions. Cell visualisation fields were created for Delta, NOTCH and NICD, implementing mathematical modelling replicated from DeltaNotch.cc3d. Initial code output errors including missing attributes and local variables were

solved by amending values in the Steppable class code according to the experimental data, and implementing the SMBL solver. Finally, Delta-NOTCH signalling was simulated and visualised as illustrated in Figure 18.

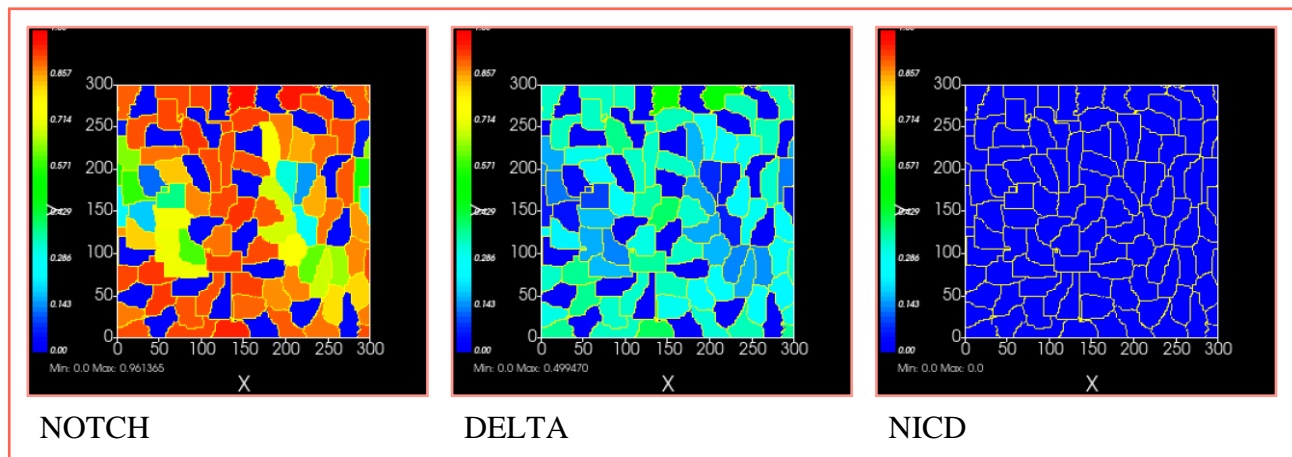


Figure 18. Delta-NOTCH Patterning Simulation Visualised in Model 1.

A graph detailing NICD values was created to allow for a more detailed analysis of NICD values in conjunction with DELTA and NOTCH values over time. The graph was constructed from the EC_Connect_V7 simulation model provided by Dr Veschini. A separate visualisation field was created, indicating NICD values per field over time defined by MCS, illustrated in Figure 19.

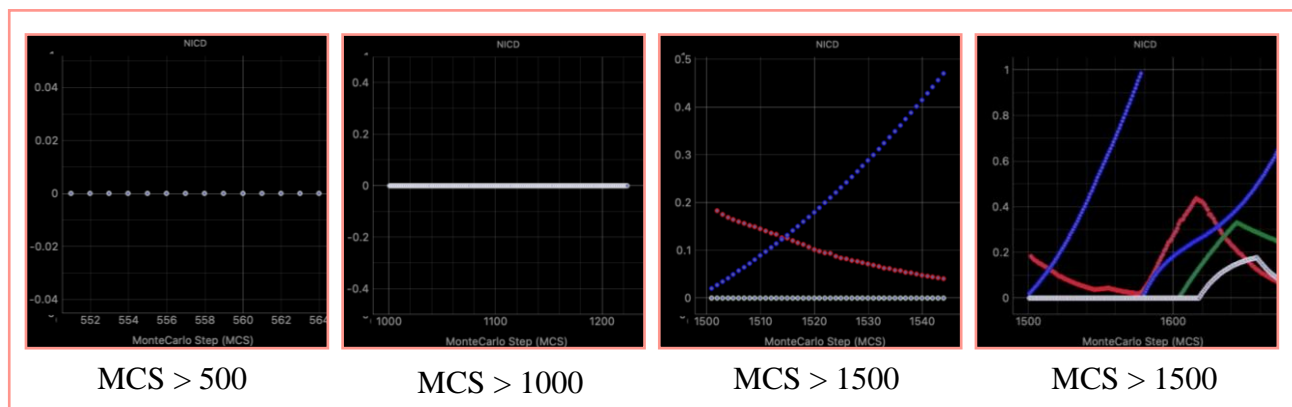


Figure 19. Implementing And Visualising The NICD Graph By Time In MCS.

3.2 Model 2: Visualising Signalling Pathways

Model 2 expands the study of further angiogenic factors by implementing fields for VEGF, JAGGED1, both implicated in inducing angiogenesis, through additional visualisation fields and the GeneExprMach Steppable. The GeneExprMach Steppable focuses on mRNA transcription, protein translation and NICD dependent regulation of gene expression under appropriate conditions. The field creation was successful but no data was displayed (Appendix 1). DeltaNotch.cc3d was replaced with SBMLSolverAntimony3.cc3d, simulating Delta-NOTCH signalling and VEGF diffusion. Cell types, boundary conditions and values were amended to suit the experimental data boundaries and definitions, illustrated in Figure 20. Here, the vascular wall is simulated through an endothelial cell layer on the left, and VEGF secretion is simulated as a diffusion Steppable on the right.

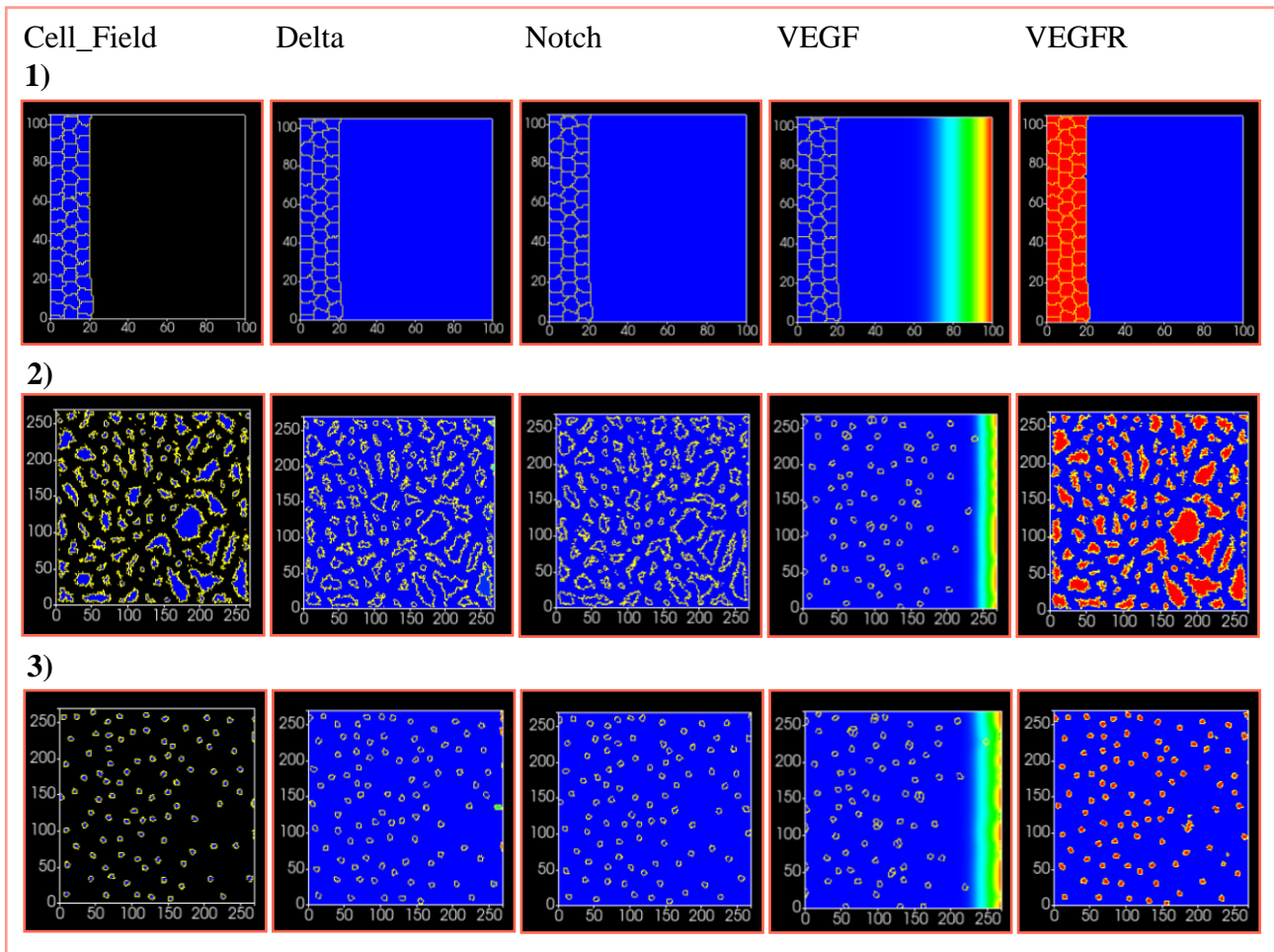


Figure 20. Implementing Experimental Data In Antimony 3 Simulation. 1) Antimony model before implementation experimental data. 2, 3) Implementation of experimental data and modification of lattice dimensions and cell property definitions at time = 6s and 9s.

3.4 Model 2: Visualising Tip Cell Formation And Cell Migration

Assuming that the VEGF-Delta-NOTCH signalling pathway induces tip cell formation whereby tip cells migrate towards the VEGF secreting agent, a vascularised brain tumour, we can track endothelial cell differentiation and tip cell migration to determine angiogenic potential in given experimental data of endothelial cells. TrackCellCOM.cc3d imported and amended according to the model's existing cell types and boundary conditions to track cell behaviour and migration by centre of mass, shown in Figure 21.

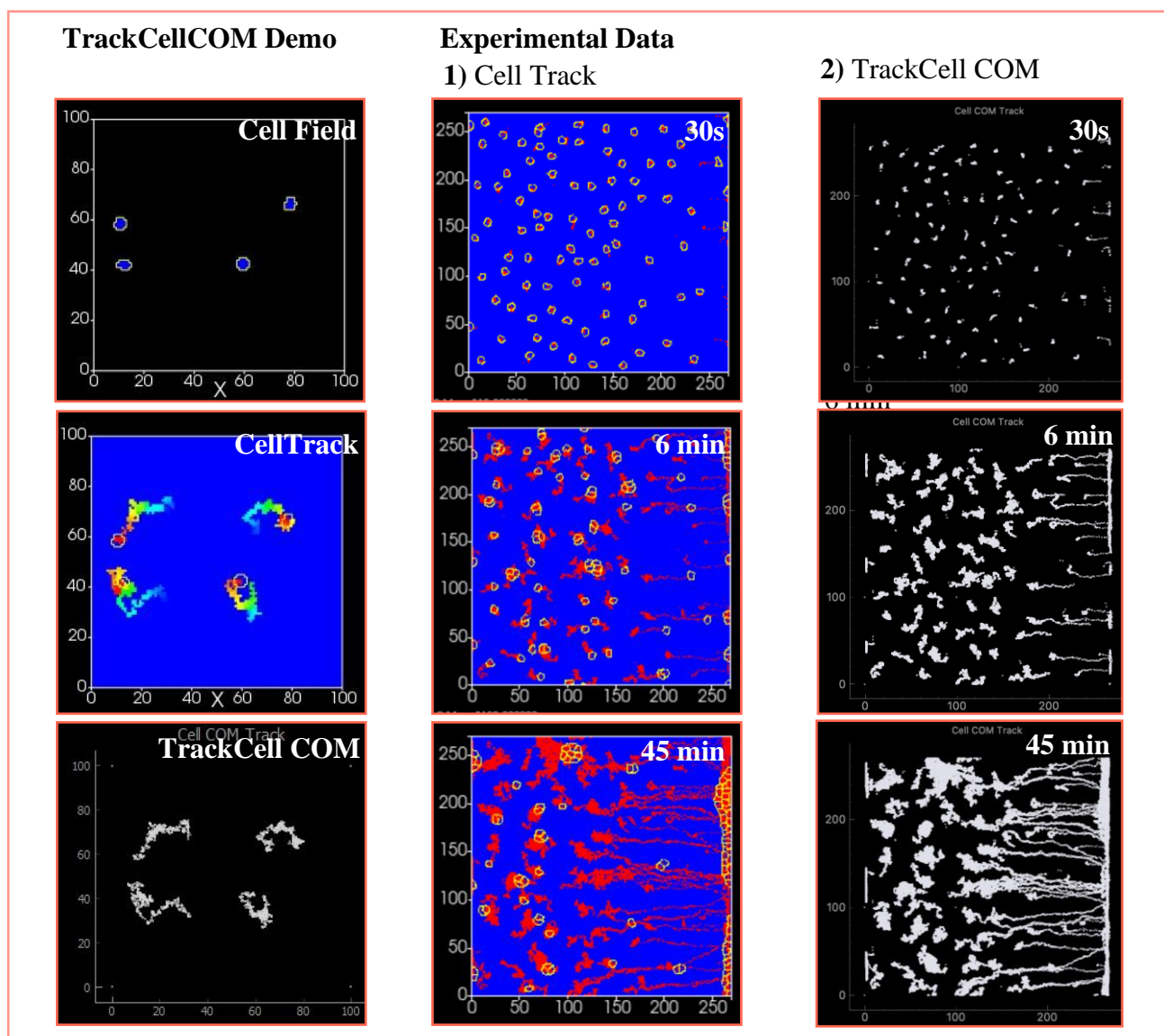


Figure 21. Simulating and tracing Endothelial Cell Migration and Tip Cell Formation.

3.5 Model 2: Simulating VEGF Secretion And The Vascular Wall

A vascular wall environment of endothelial cells was created along the lower x-axis, and the VEGF gradient was displaced to the upper x-axis. Cells were enlarged to enhance contact energy and adhesion, a cell wall was created to constrain cells within the simulation (Figure 22).

Further, cell inventory was reduced due to cell size, Delta-NOTCH signalling and minimal cell migration was observed. However, enhanced cell adhesion and contact resulted in a loss of cell movement and tip cell formation and migration could not be observed (Appendix 2). Cell movement could slightly be restored when removing the wall constraint, causing diffusion and mirroring of cells at the lattice wall, and a loss of the vascular wall environment (Appendix 3).

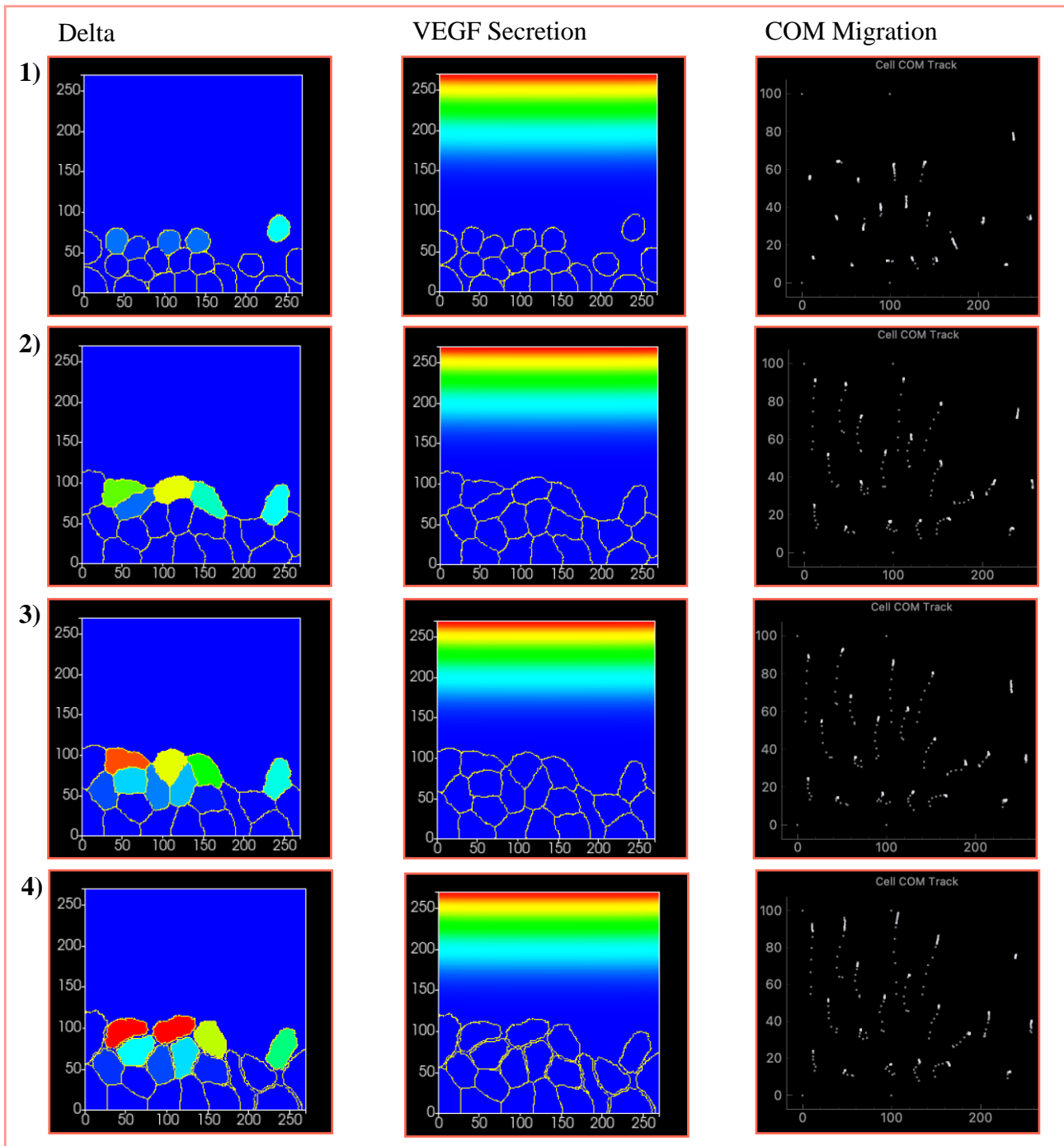


Figure 22: Simulating Delta-Notch Signalling In various cell volumes. 1) Creating and freezing a wall. 2) Enhancing contact energy. 3) Enhancing DiffusionCoefficient in DiffusionSolverFE. 4) Reducing Contact energy between all cells and medium.

3.6 Model 3: Simulating And Tracking Angiogenic Potential

Model 3 combines progress and understanding of the software and cell behaviour in simulations from previous models. A new simulation was created, only implementing experimental data and cell tracking (Figure 23, Appendix 4).

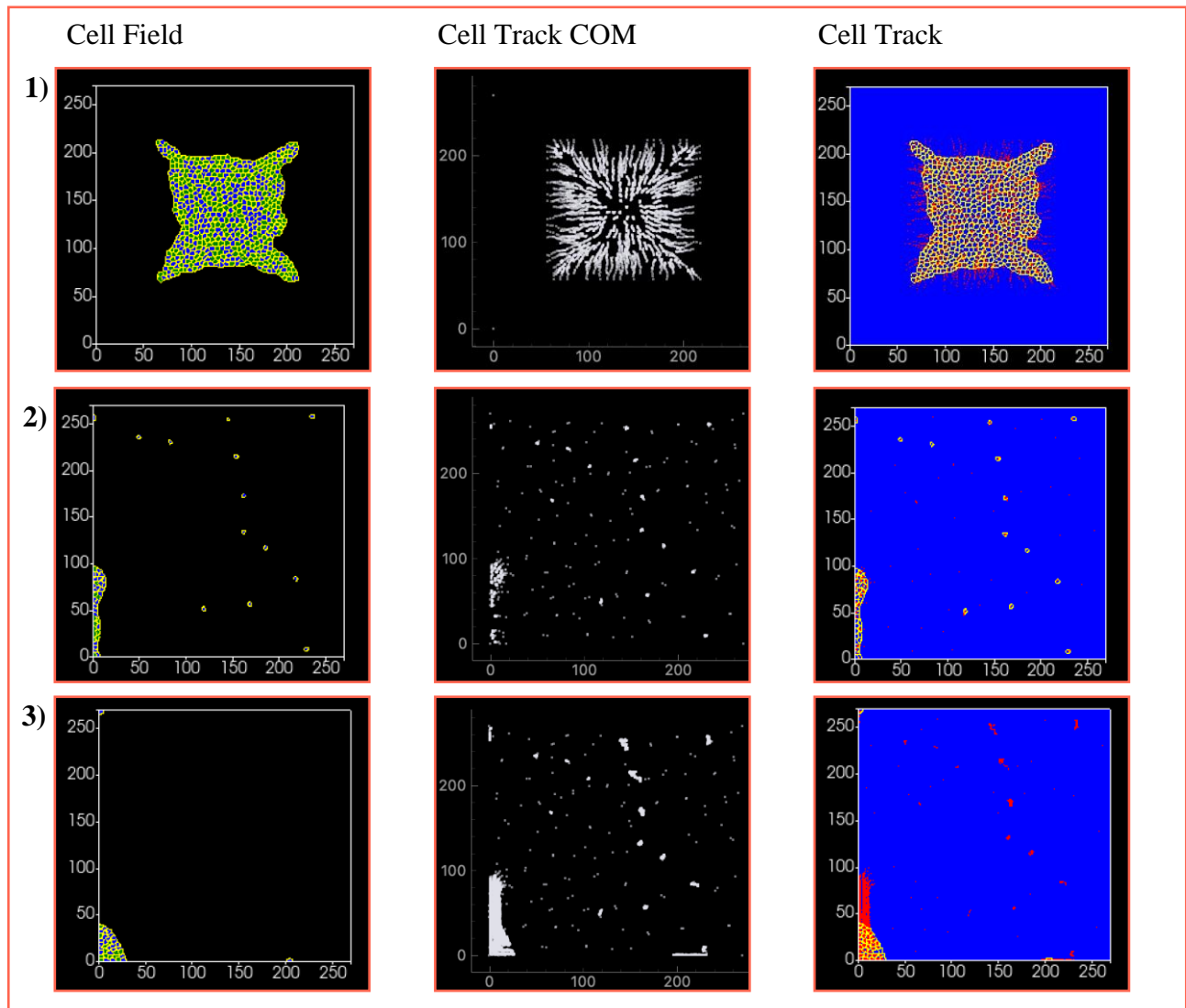


Figure 23. Model 3. 1) Cell track simulation at MCS = 200. 2) Implementation if experimental data at MCS = 212. 3) Implementation if experimental data at MCS = 10012.

Cell size was expanded by amending volume, target and surface constraints, and Antimony3 from CC3D_3.7.5 demos was implemented and amended according to simulation cell

and boundary values. The amount of cells in the lattice left no extracellular space to observe migration, however, Delta-NOTCH signalling in response to the VEGF gradient form the right end of the x-axis, seen in Figure 24. This can be taken further by amending cell size and cell inventory in future experiments to observe cell migration across the extracellular matrix.

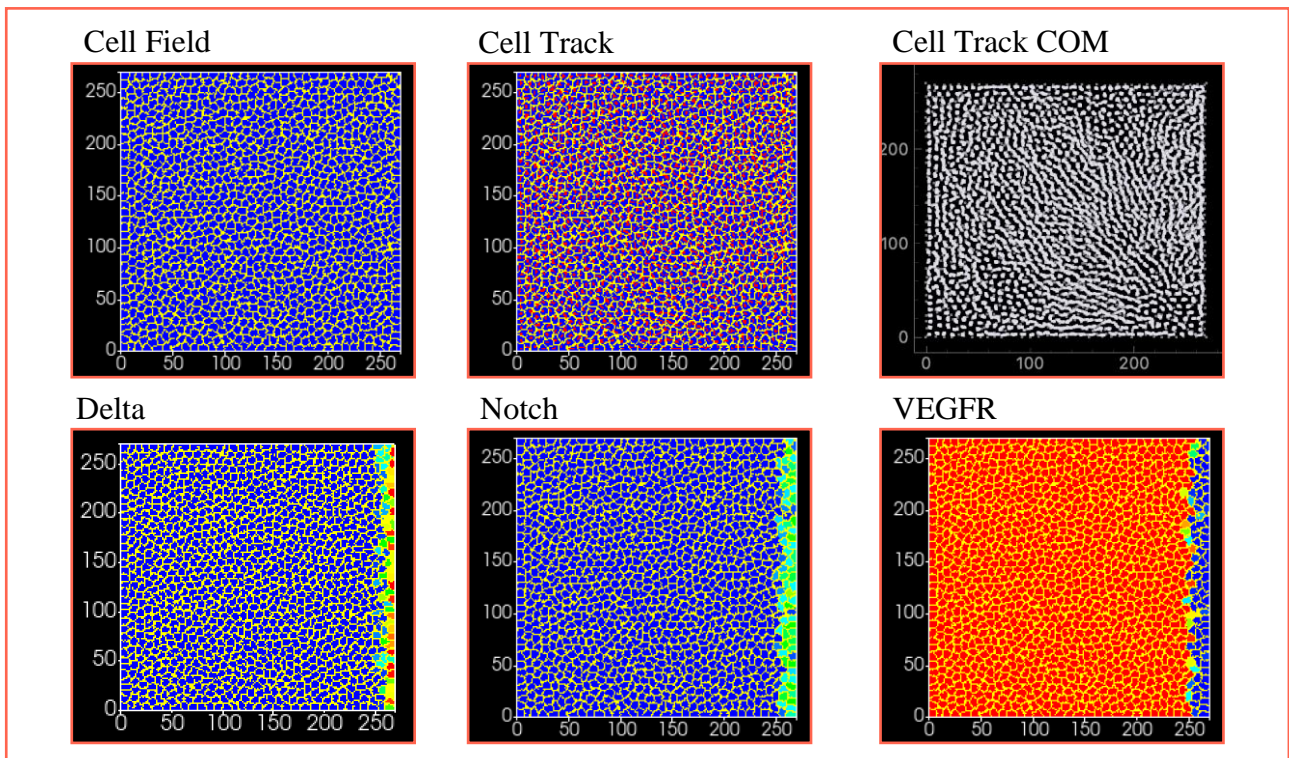


Figure 24. Implementation of experimental data and antimony at MCS = 200.

Finally, a summary of intent and selection of implemented demo file components within XML and Python Steppables utilised in model 3 can be found in Figure 25.

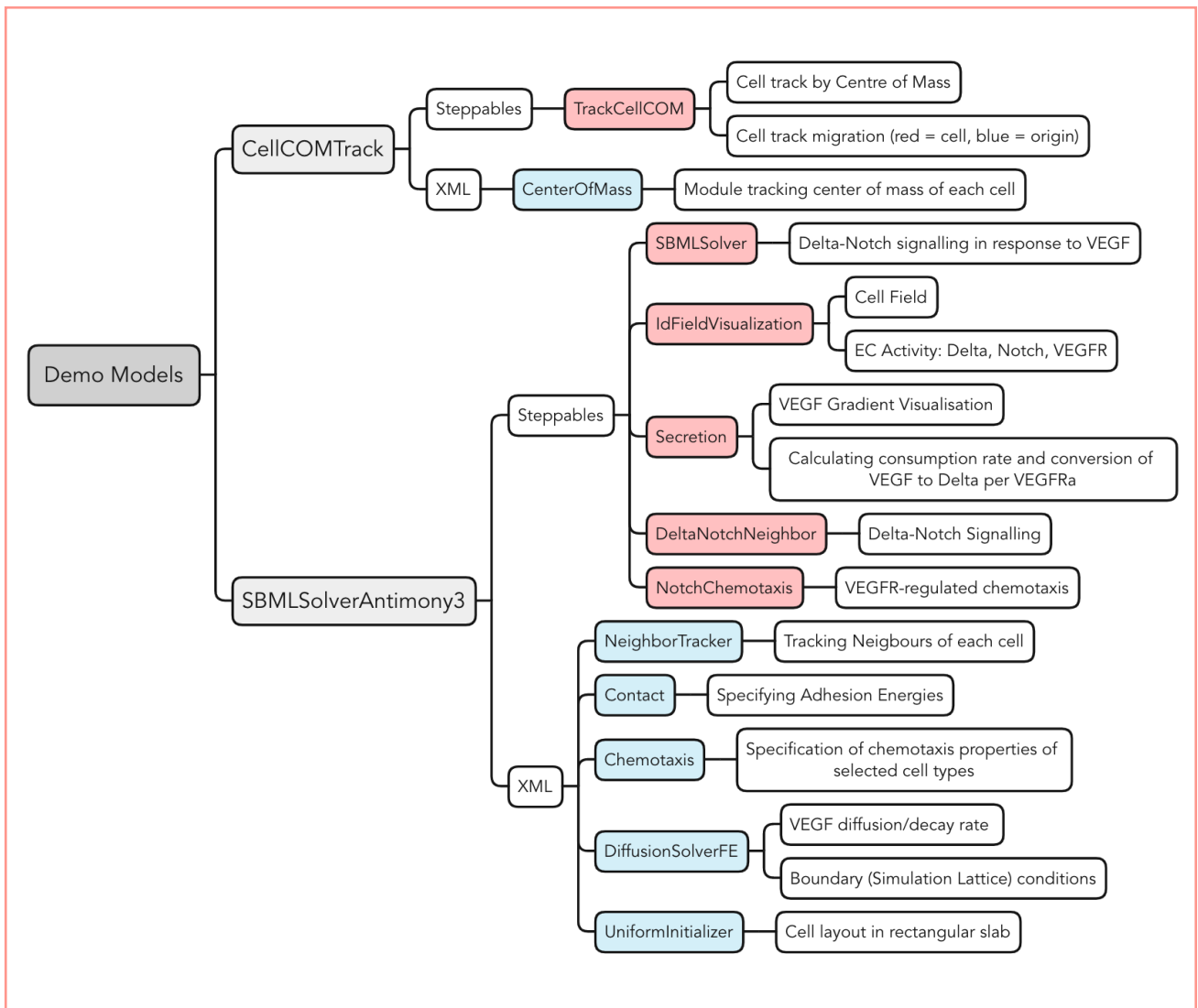


Figure 25. Summary Of Intent And Final Selection Of Model Components. (Created Using XMind.)

4. Discussion

4.1 Relevance of Results In Translational Potential

In a clinical context, deficient angiogenesis can be induced through angiogenic inhibitors or anti-angiogenic factors (Yoo et al., 2013). The quantification of angiogenesis in a biopsy specimen can be used to predict metastasis and recurrence risks. Therefore using biopsy data within this simulation model could simulate metastasis and recurrence; and gain a deeper understanding of underlying mechanisms. Furthermore, high microvessel density can be indicative of metastatic risk as they can facilitate the migration of cancer cells into the circulation. Implementing vessel density as a parameter within this model can help further understand tumour recurrence and cancer spread.

Additional translational application can be found in retinal vascular diseases often causing severe vision loss in developed countries through ocular neurovascularisation or leakage of retinal vessels (Campochiaro, 2013). Ocular vascularisation can also be found in proliferative diabetic retinopathy, neovascular glaucoma or age-related macular degeneration and is often treated through VEGF inhibition (Yoo et al., 2013). Retinal hypoxia and elevated levels of HIF-1 stimulating VEGF secretion, placental growth factor, platelet-derived growth factor-B (PDGF-B), stromal-derived growth factor-1 and their receptors and further hypoxia-regulated gene products including angiopoietin-2 are key features of the disease. The disease specific hypoxia induced VEGF secretion is similar to the components used whilst modelling angiogenic potential in a vascularised tumour environment, which could be modified and used to simulate angiogenic potential in a disease environment to further study the underlying disease mechanisms and VEGF-induced treatment efficacy.

4.1 Future Applications In Cancer Medicines Research

As we know, tumours secrete transmitters to stimulate angiogenesis. Hereby the study of anti-angiogenic agents is key to preventing and slowing down cancer metastasis and growth (Yoo et al., 2013). GBMs manifest as a focal lesion with central necrosis (Giese & Westphal, 1996). They are surrounded by an angiogenic tumour rim and invade their surrounding extracellular matrix through white matter tracts and blood vessels. Migrating glioma cells within the brain parenchyma often complicate surgery and radiotherapy, however, the mechanisms behind their migration remain unknown (Hoelzinger et al., 2007). Today, a cure is still extremely rare and GBM are often treated surgically, followed by radiotherapy and chemotherapy; and new agents including angiogenesis inhibitors such as Bevacizumab (also known as Avastin) which binds to VEGF and inhibits VEGFR activation for angiogenesis (Verhoeff et al., 2009). Bevacizumab is often administered alongside chemotherapeutic agents as demonstrated in Figure 26 (Ferrara et al., 2005).

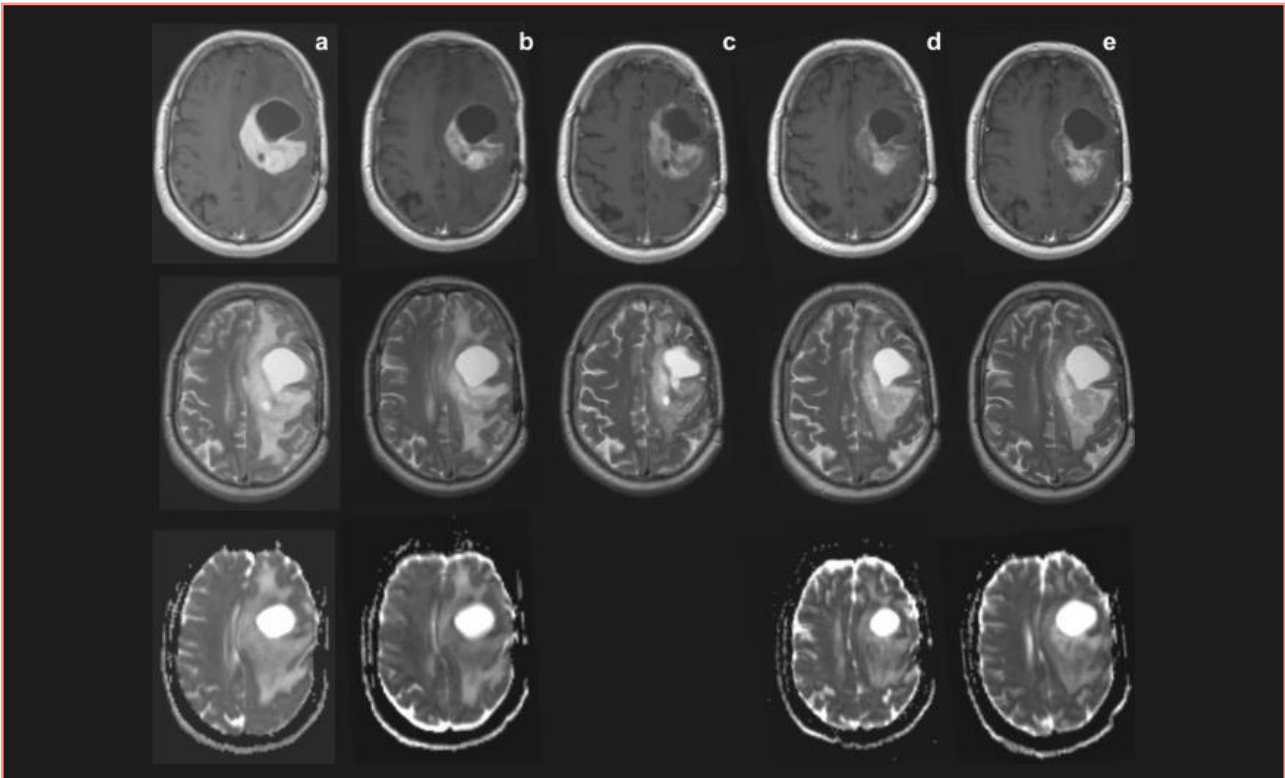


Figure 26. MRI Scans of bevacizumab treatment of recurrent GBM. Dosage: 10 mg/kg every 3 weeks, 50 mg/m² temozolomide daily. Description by columns: a) Day 0: cystic and tumour component pre-treatment, midline shift and vasogenic oedema (extracellular accumulation of fluid, occurring through blood-brain barrier disruption (Michinaga & Koyama, 2015). b) reduced midline shift. c) Day 21: no tumour progression, reduced midline shift and oedema. d) Day 88: decreased tumour and cystic size, normalised midline shift, light increase of oedema. e) Day 188: increased tumour size and cystic component, increased midline shift and oedema.

Figure taken from Verhoeff et al., 2009.

Based on a 3D model developed by Yanagisawa et al. (2021), the model developed within this study can further be applied in the simulation of treatment dosage and timing. The model encompasses angiogenesis and tumour growth through angiopoietin, regulating endothelial cell and vascular wall adhesion and migration, needed for angiogenesis initiation. Anti-angiogenic therapeutics such as bevacizumab, sunitinib and aflibercept can be modelled in CC3D via their

effective pathways, such as VEGF's inhibition due to bevacizumab. Theory on how certain therapeutics work can be tested using computational models and aid in treatment selection in the context of different grade vascular tumour environments and prognosis. If simulations use experimental data that could be connected to cell banks and linked to patient data, the model could be used to predict treatment efficacy. Medicines specific simulations can be added to existing models and experimental data in Steppable files, whereby pathways and response to defined extracellular factors can be amended and edited based on dosage, tumour grade and other parameters. As anti-angiogenic treatment in cancer is often given in conjunction with other medicines, multiple treatment agents can be implemented as steppable files and used to determine the most effective combinations, timings and dosages.

Other angiogenesis inhibitors include sorafenib and sunitinib, which bind to various endothelial cell receptors and other downstream signalling proteins to block angiogenic activity. Other common targets for angiogenesis inhibition in clinical trials include epidermal growth factor receptor (EGFR), VEGFR, VEGFR2,3 Integrin α -V- β -3 and various other factors, expanded and summarised in Table 3. As multiple cell-to-cell interactions can be simulated in computational models, various of the above factors can be implemented and interchanged within the model created within this study and used to find new potential treatment strategies and can help eradicate early errors in clinical trials.

Table 3. Angiogenic potential in individual compounds. Taken from Yoo et al., 2013

	Antiangiogenic compounds	Mechanism of action
Inhibitors of ECM remodeling	<ul style="list-style-type: none"> • Batimastat, Marimastat, AG3340, Neovastat, PEX, TIMP-1,2,3,4 • PAI-1,2, uPA Ab, uPAR Ab, Amiloride • Minocycline, tetracyclines, cartilage-derived TIMP 	<ul style="list-style-type: none"> • MMP inhibitors, block endothelial and tumor cell invasion • uPA inhibitors, block ECM breakdown • Collagenase inhibitors, disrupt collagen synthesis and deposition
Inhibitors of adhesion molecules	<ul style="list-style-type: none"> • α v β 3 Ab: LM609 and Vitaxin, RGD containing peptides, α v β 5 Ab • Benzodiazepine derivatives 	<ul style="list-style-type: none"> • Block EC adhesion, induce EC apoptosis • Antagonist of α v β 3
Inhibitors of activated ECs	<ul style="list-style-type: none"> • Endogenous inhibitors: endostatin, angiostatin, aaAT • IFN-α, IFN-γ, IL-12, nitric oxide synthase inhibitors, TSP-1 • TNP-470, Combretastatin A-4 • Thalidomide • Linomide 	<ul style="list-style-type: none"> • Block EC proliferation, induce EC apoptosis, inhibit angiogenic switch • Block EC migration and/or proliferation • Block EC proliferation • Inhibits angiogenesis in vivo • Inhibits EC migration
Inhibitors of angiogenic inducers or their receptors	<ul style="list-style-type: none"> • IFN-α, PF-4, prolactin fragment • Suramin and analogues • PPS, distamycin A analogues, bFGF Ab, antisense-bFGF • Protamine • SU5416, soluble Flt-1, dominant-negative Flk-1, VEGF receptor, ribozymes, VEGF Ab • Aspirin, NS-398 • 6AT, 6A5BU, 7-DX 	<ul style="list-style-type: none"> • Inhibit bFGF, Inhibit bFGF-induced EC proliferation • Bind to various growth factors including bFGF, VEGF, PDGF, inhibit EC migration and proliferation • Inhibit bFGF activity • Binds heparin, inhibits EC migration and proliferation • Block VEGF activity • COX inhibitors
Inhibitors of EC intracellular signalling	<ul style="list-style-type: none"> • Genistein • Lavendustin A • Ang-2 	<ul style="list-style-type: none"> • Tyrosine kinase inhibitor, blocks uPA, EC migration and proliferation • Selective inhibitor of protein tyrosine kinase

The model created within this study can further be applied in the study of treatment outcomes and response prediction can be found in a better understanding of the influence of anti-angiogenic treatment and environmental factors. As tumour growth through vascular supply is linked to VEGF over-expression, the VEGF pathway is often targeted in cancer treatment through

the use of bevacizumab, sunitinib and aflibercept (Vasudev & Reynolds, 2014). VEGF inhibition however is not effective in all cancers and is in need of further investigation of its use within different disease stages, duration, interaction with chemotherapy, proposed biomarkers and mechanisms of resistance such as enhanced cancer aggressiveness as illustrated in Figure 27.

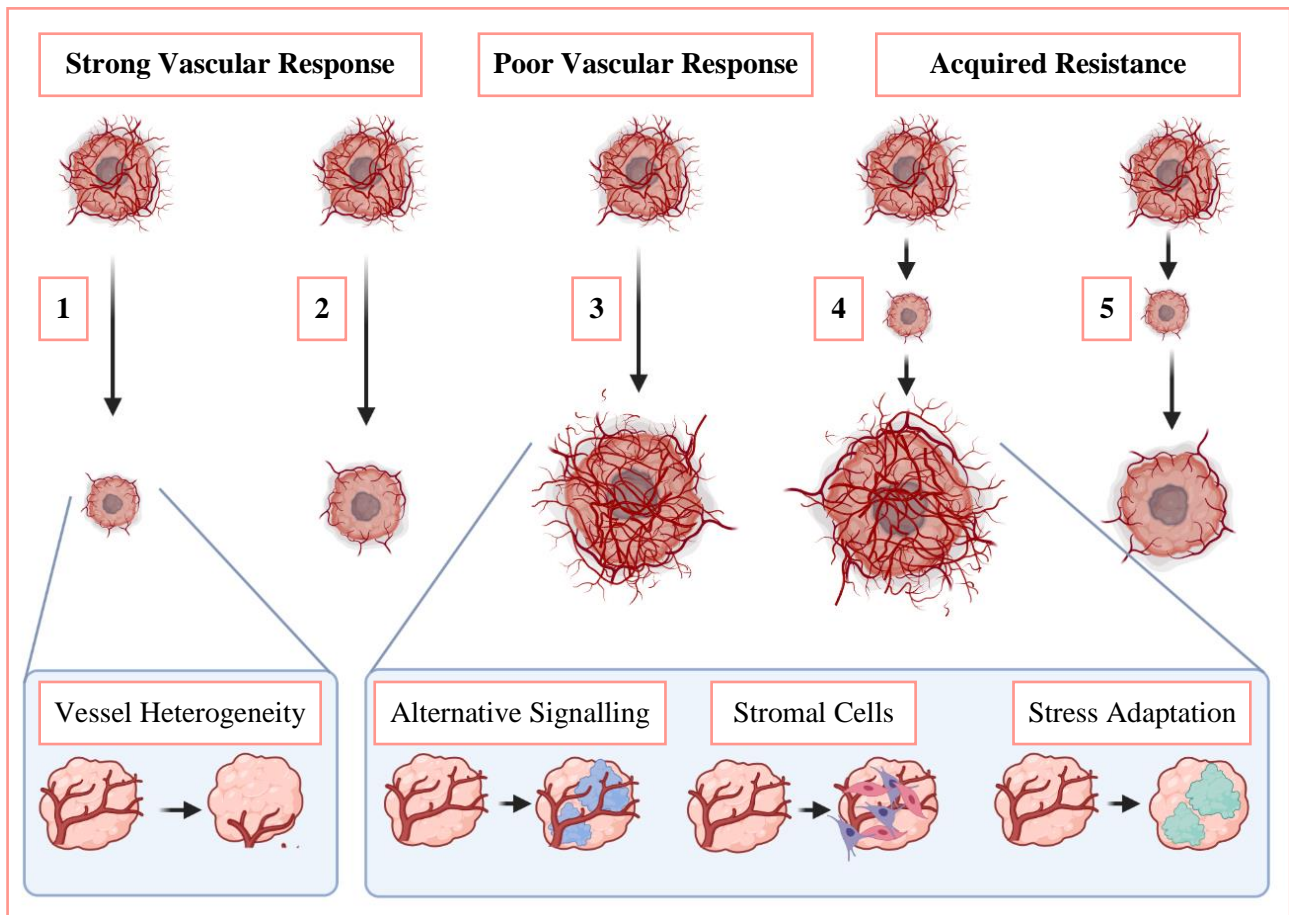


Figure 27: Angiogenic Therapy Response And VEGF targeted therapy resistance. 1) Strong vascular response, tumour shrinkage, strong vessel reduction. 2) Strong vascular response, tumour stabilisation. 3) Poor vascular response, minimal vessel reduction, tumour progression. 4, 5) Acquired resistance, alternative pathways are activated in response to lack of vascular supply leading to tumour progression and new vascular supply. Vessel Heterogeneity: Therapy insensitive vessels remain. Alternative Signalling Pathways: Up-regulation of alternative pro-angiogenic factors for vessel growth. Stromal Cells: Myeloid cells, fibroblasts infiltrate tumour and mediate therapy resistance through pro-angiogenic factors. Stress Adaptation: Tumour cells adapt to hypoxia and nutrient shortage. Adapted from Vasudev & Reynolds (2014). (Created Using BioRender.)

Other challenges in anti-angiogenic cancer treatment include drug resistance and impaired delivery through general genomic instability and tumour mass, as well as challenges in reaching cancer cells as the abnormally high interstitial fluid pressure in the tumour mass interstitium acts as barrier. Further simulation models involving experimental data could be developed utilising a combination of existing tumour progression models, such as AVascularTumour.cc3D in CC3D_4.3.7, and models of angiogenic potential and treatment simulations. This can be expanded into considering the influence of migrating glioma cells in the brain parenchyma (Hoelzinger et al., 2007). Cell migration can be investigated through cell migration models in CC3D and could better our understanding of mechanisms behind their migration.

4.3 Limitations And Future Directions

Cell based models are well suited to build computational experiments concerning the effects of a single cell on higher hierarchies and reactions such as stem cells or tumour, immune reactions (Mezcar et al., 2019). The benefit of working with a lattice based model is its speed and simplicity, however, the realism behind lattice based models is limited in their ability to incorporate off-lattice cell-to-cell biomechanics and interactions. As all values and cellular interactions in CC3D are declared, existing literature can simply be modelled to test and confirm a theory. Hereby both computational and mathematical models are framed within restricted conditions such as a fixed developmental stage, fixed temperature or number of cells which is not the reality in nature (Bray, 2014). Furthermore, each mechanism has the ability to adapt to stress and morph its molecular makeup to survive. Therefore learning, in an evolutionary sense, meaning how to respond appropriately to changes in the environment, requires further advances in computational modelling, possibly through machine learning and AI. Computational models can tell us if hypothetical mechanism could work as proposed and reveal flaws in current thinking, which can be aid us in better understanding molecular mechanisms and use these to develop new treatment strategies. However, any features that aren't explicitly stated in the simulation code won't be included in the simulation and may lead to incorrect results when modelling complex processes.

In the case of this simulation model, the accuracy of the biological processes occurring in angiogenesis and a vascularised tumour environment is enhanced through implementing experimental data and by modelling only processes which can be replicated within the Vascular Cell Dynamics Lab using induced pluripotent stem cells (iPSC) in vitro. Finally, this simulation model can be added to the ECPT, a EC profiling tool built within the Vascular Cell Dynamics Lab. The tool is aiming to enable single cell profiling with monolayers and provide regional as well as spatial information where this model can simulate regional environments and observe changes in spatial correlations as well as cell-to-cell signalling.

4.4 Conclusion

Concluding, this study demonstrates the potential of combining experimental data with computational simulations in understanding angiogenesis and angiogenic potential. Here, we can apply theory from literature, such as Delta-NOTCH signalling in response to VEGF and other extracellular factors, to experimental data and observe cell-to-cell interactions and behaviour on a single cell and larger scale basis. Experiments can be repeated in-vitro whereby results can be compared to the synthetic data created through computational modelling and experimental data and, if correlating, confirm and expand the theory behind cellular processes. The model of angiogenic potential in a vascularised tumour environment can be taken further in simulating anti-angiogenic treatment pathways, e.g. VEGF inhibition. Other parameters, such as time frame, dosage and tumour progression, can be simulated to investigate treatment strategies and aid in drug efficiency as well as planning clinical trials. Computational models of treatment responses based on experimental data can further be seen as a huge step towards personalised medicine if patient cells can be linked to experimental data via e.g. cell banks, and used to predict likely treatment response and outcomes.

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Reference

- Alber, M., Kiskowski, M., Glazier, J., & Jiang, Y. (2003). On Cellular Automaton Approaches to Modeling Biological Cells. *Mathematical Systems Theory in Biology, Communications, Computation, and Finance*.
- Ahir, B. K., Engelhard, H. H., & Lakka, S. S. (2020). Tumor Development and Angiogenesis in Adult Brain Tumor: Glioblastoma. *Molecular neurobiology*, 57(5), 2461–2478. <https://doi.org/10.1007/s12035-020-01892-8>
- Anderson, A. R., & Chaplain, M. A. (1998). Continuous and discrete mathematical models of tumor-induced angiogenesis. *Bulletin of mathematical biology*, 60(5), 857–899. <https://doi.org/10.1006/bulm.1998.0042>
- Benedito, R., Rocha, S. F., Woeste, M., Zamykal, M., Radtke, F., Casanovas, O., Duarte, A., Pytowski, B., & Adams, R. H. (2012). Notch-dependent VEGFR3 upregulation allows angiogenesis without VEGF-VEGFR2 signalling. *Nature*, 484(7392), 110–114. <https://doi.org/10.1038/nature10908>
- Brodland G. W. (2015). How computational models can help unlock biological systems. *Seminars in cell & developmental biology*, 47-48, 62–73. <https://doi.org/10.1016/j.semcdb.2015.07.001>
- Burstedde, C., Klauck, K., Schadschneider, A., & Zittartz, J. (2001). Simulation of pedestrian dynamics using a two-dimensional cellular automaton. *Physica A: Statistical Mechanics and Its Applications*, 295(3–4), 507–525. [https://doi.org/10.1016/S0378-4371\(01\)00141-8](https://doi.org/10.1016/S0378-4371(01)00141-8)
- Carrier, A., Geris, L., Bentley, K., Carmeliet, G., Carmeliet, P., & Van Oosterwyck, H. (2012). MOSAIC: a multiscale model of osteogenesis and sprouting angiogenesis with lateral inhibition of endothelial cells. *PLoS computational biology*, 8(10), e1002724. <https://doi.org/10.1371/journal.pcbi.1002724>
- Campochiaro P. A. (2013). Ocular neovascularization. *Journal of molecular medicine (Berlin, Germany)*, 91(3), 311–321. <https://doi.org/10.1007/s00109-013-0993-5>
- Checa, S., & Prendergast, P. J. (2009). A mechanobiological model for tissue differentiation that includes angiogenesis: a lattice-based modeling approach. *Annals of biomedical engineering*, 37(1), 129–145. <https://doi.org/10.1007/s10439-008-9594-9>
- Chen, W., Xia, P., Wang, H., Tu, J., Liang, X., Zhang, X., & Li, L. (2019). The endothelial tip-stalk cell selection and shuffling during angiogenesis. *Journal of cell communication and signaling*, 13(3), 291–301. <https://doi.org/10.1007/s12079-019-00511-z>
- Chesnais, F., Le Caillec, J., Roy, E., Danovi, D., & Veschini, L. (2020). High content Image Analysis to study phenotypic heterogeneity in endothelial cell monolayers [Preprint]. *Cell Biology*. <https://doi.org/10.1101/2020.11.17.362277>
- Clarke, K. C. (2014). Cellular Automata and Agent-Based Models. In M. M. Fischer & P. Nijkamp (Eds.), *Handbook of Regional Science* (pp. 1217–1233). Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-642-23430-9_63
- Chinot, O. L., Wick, W., Mason, W., Henriksson, R., Saran, F., Nishikawa, R., Carpentier, A. F., Hoang-Xuan, K., Kavan, P., Cernea, D., Brandes, A. A., Hilton, M., Abrey, L., & Cloughesy, T. (2014). Bevacizumab plus radiotherapy-temozolomide for newly diagnosed glioblastoma. *The New England journal of medicine*, 370(8), 709–722. <https://doi.org/10.1056/NEJMoa1308345>
- Czirok A. (2013). Endothelial cell motility, coordination and pattern formation during vasculogenesis. *Wiley interdisciplinary reviews. Systems biology and medicine*, 5(5), 587–602. <https://doi.org/10.1002/wsbm.1233>
- Collier, J. R., Monk, N. A., Maini, P. K., & Lewis, J. H. (1996). Pattern formation by lateral inhibition with feedback: a mathematical model of delta-notch intercellular signalling. *Journal of theoretical biology*, 183(4), 429–446. <https://doi.org/10.1006/jtbi.1996.0233>

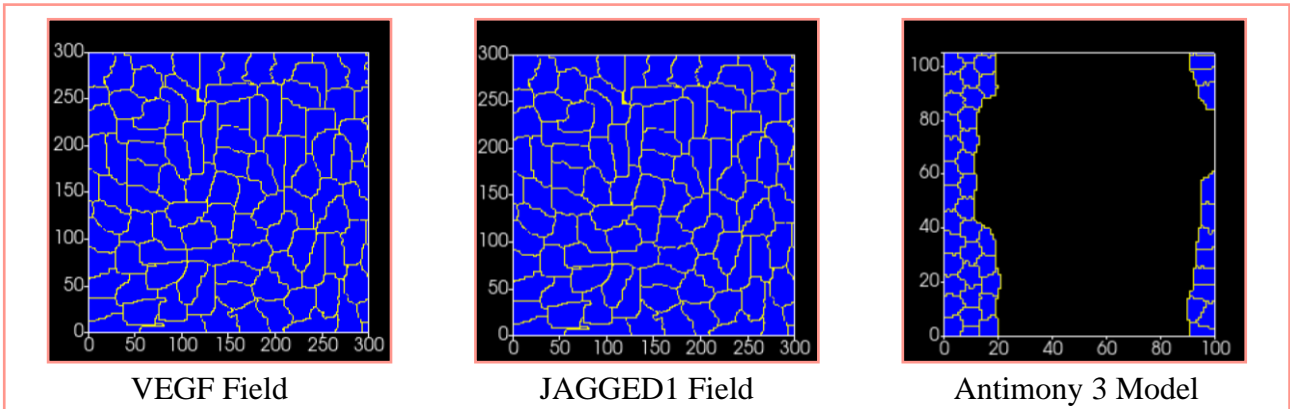
- Czerniak, J. M., Zarzycki, H., Apiecionek, L., Palczewski, W., & Kardasz, P. (2018). A Cellular Automata-Based Simulation Tool for Real Fire Accident Prevention. *Mathematical Problems in Engineering*, 2018, 1–12. <https://doi.org/10.1155/2018/3058241>
- De Smet, F., Segura, I., De Bock, K., Hohensinner, P. J., & Carmeliet, P. (2009). Mechanisms of vessel branching: filopodia on endothelial tip cells lead the way. *Arteriosclerosis, thrombosis, and vascular biology*, 29(5), 639–649. <https://doi.org/10.1161/ATVBAHA.109.185165>
- Ferrara, N., Hillan, K. J., & Novotny, W. (2005). Bevacizumab (Avastin), a humanized anti-VEGF monoclonal antibody for cancer therapy. *Biochemical and biophysical research communications*, 333(2), 328–335. <https://doi.org/10.1016/j.bbrc.2005.05.132>
- Ferreira, S. C., Jr, Martins, M. L., & Vilela, M. J. (2002). Reaction-diffusion model for the growth of avascular tumor. *Physical review. E, Statistical, nonlinear, and soft matter physics*, 65(2 Pt 1), 021907. <https://doi.org/10.1103/PhysRevE.65.021907>
- Fidler, I. J., & Ellis, L. M. (2004). Neoplastic angiogenesis--not all blood vessels are created equal. *The New England journal of medicine*, 351(3), 215–216. <https://doi.org/10.1056/NEJMp048080>
- Geudens, I., & Gerhardt, H. (2011). Coordinating cell behaviour during blood vessel formation. *Development (Cambridge, England)*, 138(21), 4569–4583. <https://doi.org/10.1242/dev.062323>
- Giese, A., & Westphal, M. (1996). Glioma invasion in the central nervous system. *Neurosurgery*, 39(2), 235–252. <https://doi.org/10.1097/00006123-199608000-00001>
- Grant, M. R., Mostov, K. E., Tlsty, T. D., & Hunt, C. A. (2006). Simulating properties of in vitro epithelial cell morphogenesis. *PLoS computational biology*, 2(10), e129. <https://doi.org/10.1371/journal.pcbi.0020129>
- Guarnaccia, L., Navone, S. E., Trombetta, E., Cordiglieri, C., Cherubini, A., Crisà, F. M., Rampini, P., Miozzo, M., Fontana, L., Caroli, M., Locatelli, M., Riboni, L., Campanella, R., & Marfia, G. (2018). Angiogenesis in human brain tumors: screening of drug response through a patient-specific cell platform for personalized therapy. *Scientific reports*, 8(1), 8748. <https://doi.org/10.1038/s41598-018-27116-7>
- Henkel, R., Endler, L., Peters, A., Le Novère, N., & Waltemath, D. (2010). Ranked retrieval of Computational Biology models. *BMC bioinformatics*, 11, 423. <https://doi.org/10.1186/1471-2105-11-423>
- Hoelzinger, D. B., Demuth, T., & Berens, M. E. (2007). Autocrine factors that sustain glioma invasion and paracrine biology in the brain microenvironment. *Journal of the National Cancer Institute*, 99(21), 1583–1593. <https://doi.org/10.1093/jnci/djm187>
- Hucka, M., Bergmann, F. T., Dräger, A., Hoops, S., Keating, S. M., Le Novère, N., Myers, C. J., Olivier, B. G., Sahle, S., Schaff, J. C., Smith, L. P., Waltemath, D., & Wilkinson, D. J. (2018). The Systems Biology Markup Language (SBML): Language Specification for Level 3 Version 2 Core. *Journal of integrative bioinformatics*, 15(1), 20170081. <https://doi.org/10.1515/jib-2017-0081>
- Hwang, M., Garbey, M., Berceles, S. A., & Tran-Son-Tay, R. (2009). Rule-Based Simulation of Multi-Cellular Biological Systems-A Review of Modeling Techniques. *Cellular and molecular bioengineering*, 2(3), 285–294. <https://doi.org/10.1007/s12195-009-0078-2>
- Guyon, J., Chapouly, C., Andrique, L., Bikfalvi, A., & Daubon, T. (2021). The Normal and Brain Tumor Vasculature: Morphological and Functional Characteristics and Therapeutic Targeting. *Frontiers in Physiology*, 12, 622615. <https://doi.org/10.3389/fphys.2021.622615>
- Karamysheva A. F. (2008). Mechanisms of angiogenesis. *Biochemistry. Biokhimiia*, 73(7), 751–762. <https://doi.org/10.1134/s0006297908070031>
- Kim, W. Y., & Lee, H. Y. (2009). Brain angiogenesis in developmental and pathological processes: mechanism and therapeutic intervention in brain tumors. *The FEBS journal*, 276(17), 4653–4664. <https://doi.org/10.1111/j.1742-4658.2009.07177.x>

- Leon, S. P., Folkerth, R. D., & Black, P. M. (1996). Microvessel density is a prognostic indicator for patients with astroglial brain tumors. *Cancer*, 77(2), 362–372. [https://doi.org/10.1002/\(SICI\)1097-0142\(19960115\)77:2<362::AID-CNCR20>3.0.CO;2-Z](https://doi.org/10.1002/(SICI)1097-0142(19960115)77:2<362::AID-CNCR20>3.0.CO;2-Z)
- Jiang, Y., Pjesivac-Grbovic, J., Cantrell, C., & Freyer, J. P. (2005). A multiscale model for avascular tumor growth. *Biophysical journal*, 89(6), 3884–3894. <https://doi.org/10.1529/biophysj.105.060640>
- Kim, Y., Stolarska, M. A., & Othmer, H. G. (2011). The role of the microenvironment in tumor growth and invasion. *Progress in biophysics and molecular biology*, 106(2), 353–379. <https://doi.org/10.1016/j.pbiomolbio.2011.06.006>
- Koon, Y. L., Zhang, S., Rahmat, M. B., Koh, C. G., & Chiam, K. H. (2018). Enhanced Delta-Notch Lateral Inhibition Model Incorporating Intracellular Notch Heterogeneity and Tension-Dependent Rate of Delta-Notch Binding that Reproduces Sprouting Angiogenesis Patterns. *Scientific reports*, 8(1), 9519. <https://doi.org/10.1038/s41598-018-27645-1>
- Lakka, S. S., & Rao, J. S. (2008). Antiangiogenic therapy in brain tumors. *Expert review of neurotherapeutics*, 8(10), 1457–1473. <https://doi.org/10.1586/14737175.8.10.1457>
- Macklin, P., McDougall, S., Anderson, A. R., Chaplain, M. A., Cristini, V., & Lowengrub, J. (2009). Multiscale modelling and nonlinear simulation of vascular tumour growth. *Journal of mathematical biology*, 58(4-5), 765–798. <https://doi.org/10.1007/s00285-008-0216-9>
- Martinson, W. D., Ninomiya, H., Byrne, H. M., & Maini, P. K. (2021). Comparative analysis of continuum angiogenesis models. *Journal of Mathematical Biology*, 82(4), 21. <https://doi.org/10.1007/s00285-021-01570-w>
- Metzcar, J., Wang, Y., Heiland, R., & Macklin, P. (2019). A Review of Cell-Based Computational Modeling in Cancer Biology. *JCO clinical cancer informatics*, 3, 1–13. <https://doi.org/10.1200/CCI.18.00069>
- Michinaga, S., & Koyama, Y. (2015). Pathogenesis of brain edema and investigation into anti-edema drugs. *International journal of molecular sciences*, 16(5), 9949–9975. <https://doi.org/10.3390/ijms16059949>
- Milde, F., Bergdorf, M., & Koumoutsakos, P. (2008). A Hybrid Model for Three-Dimensional Simulations of Sprouting Angiogenesis. *Biophysical Journal*, 95(7), 3146–3160. <https://doi.org/10.1529/biophysj.107.124511>
- Moya, I. M., Umans, L., Maas, E., Pereira, P. N., Beets, K., Francis, A., Sents, W., Robertson, E. J., Mummery, C. L., Huylebroeck, D., & Zwijsen, A. (2012). Stalk cell phenotype depends on integration of Notch and Smad1/5 signaling cascades. *Developmental cell*, 22(3), 501–514. <https://doi.org/10.1016/j.devcel.2012.01.007>
- Nakamura, K., Martin, K. C., Jackson, J. K., Beppu, K., Woo, C. W., & Thiele, C. J. (2006). Brain-derived neurotrophic factor activation of TrkB induces vascular endothelial growth factor expression via hypoxia-inducible factor-1alpha in neuroblastoma cells. *Cancer research*, 66(8), 4249–4255. <https://doi.org/10.1158/0008-5472.CAN-05-2789>
- Nishida, N., Yano, H., Nishida, T., Kamura, T., & Kojiro, M. (2006). Angiogenesis in cancer. *Vascular health and risk management*, 2(3), 213–219. <https://doi.org/10.2147/vhrm.2006.2.3.213>
- Peng, L., Trucu, D., Lin, P., Thompson, A., & Chaplain, M. A. (2017). A Multiscale Mathematical Model of Tumour Invasive Growth. *Bulletin of mathematical biology*, 79(3), 389–429. <https://doi.org/10.1007/s11538-016-0237-2>
- Peirce S. M. (2008). Computational and mathematical modeling of angiogenesis. *Microcirculation (New York, N.Y. : 1994)*, 15(8), 739–751. <https://doi.org/10.1080/10739680802220331>
- Qutub, A. A., Mac Gabhann, F., Karagiannis, E. D., Vempati, P., & Popel, A. S. (2009). Multiscale models of angiogenesis. *IEEE engineering in medicine and biology magazine : the quarterly magazine of the Engineering in Medicine & Biology Society*, 28(2), 14–31. <https://doi.org/10.1109/EMEB.2009.931791>

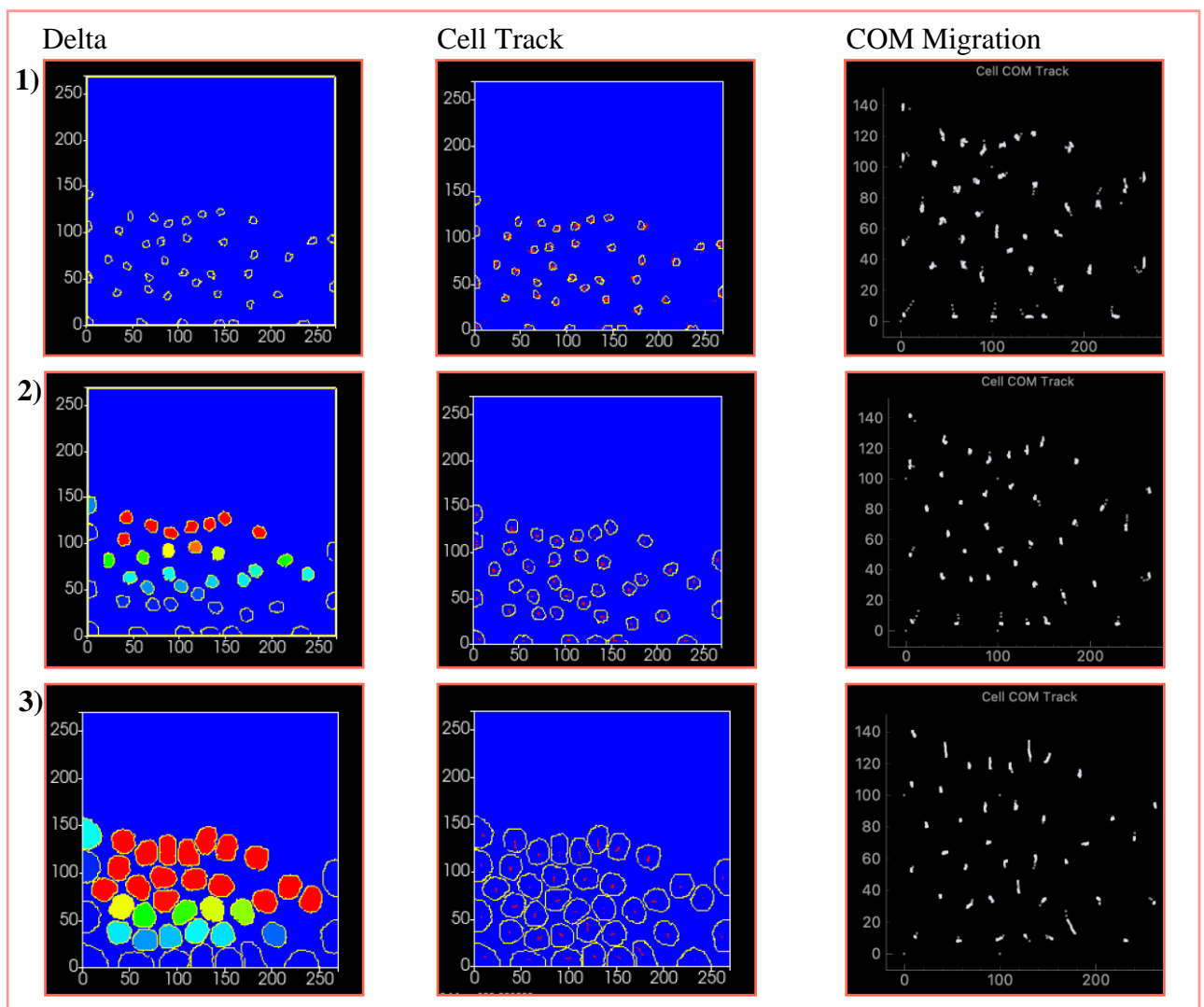
- Rundhaug J. E. (2005). Matrix metalloproteinases and angiogenesis. *Journal of cellular and molecular medicine*, 9(2), 267–285. <https://doi.org/10.1111/j.1582-4934.2005.tb00355.x>
- Santé, I., García, A. M., Miranda, D., & Crecente, R. (2010). Cellular automata models for the simulation of real-world urban processes: A review and analysis. *Landscape and Urban Planning*, 96(2), 108–122. <https://doi.org/10.1016/j.landurbplan.2010.03.001>
- Shirinifard, A., Gens, J. S., Zaitlen, B. L., Popławski, N. J., Swat, M., & Glazier, J. A. (2009). 3D multi-cell simulation of tumor growth and angiogenesis. *PLoS one*, 4(10), e7190. <https://doi.org/10.1371/journal.pone.0007190>
- Starruß, J., de Back, W., Brusch, L., & Deutsch, A. (2014). Morpheus: a user-friendly modeling environment for multiscale and multicellular systems biology. *Bioinformatics (Oxford, England)*, 30(9), 1331–1332. <https://doi.org/10.1093/bioinformatics/btt772>
- Tandle, A., Blazer, D. G., 3rd, & Libutti, S. K. (2004). Antiangiogenic gene therapy of cancer: recent developments. *Journal of translational medicine*, 2(1), 22. <https://doi.org/10.1186/1479-5876-2-22>
- Tang, L., van de Ven, A. L., Guo, D., Andasari, V., Cristini, V., Li, K. C., & Zhou, X. (2014). Computational modeling of 3D tumor growth and angiogenesis for chemotherapy evaluation. *PLoS one*, 9(1), e83962. <https://doi.org/10.1371/journal.pone.0083962>
- Toomey, D. P., Murphy, J. F., & Conlon, K. C. (2009). COX-2, VEGF and tumour angiogenesis. *The surgeon : journal of the Royal Colleges of Surgeons of Edinburgh and Ireland*, 7(3), 174–180. [https://doi.org/10.1016/s1479-666x\(09\)80042-5](https://doi.org/10.1016/s1479-666x(09)80042-5)
- Uwamori, H., Ono, Y., Yamashita, T., Arai, K., & Sudo, R. (2019). Comparison of organ-specific endothelial cells in terms of microvascular formation and endothelial barrier functions. *Microvascular research*, 122, 60–70. <https://doi.org/10.1016/j.mvr.2018.11.007>
- Vasudev, N. S., & Reynolds, A. R. (2014). Anti-angiogenic therapy for cancer: current progress, unresolved questions and future directions. *Angiogenesis*, 17(3), 471–494. <https://doi.org/10.1007/s10456-014-9420-y>
- Verhoeff, J. J., van Tellingen, O., Claes, A., Stalpers, L. J., van Linde, M. E., Richel, D. J., Leenders, W. P., & van Furth, W. R. (2009). Concerns about anti-angiogenic treatment in patients with glioblastoma multiforme. *BMC Cancer*, 9(1), 444. <https://doi.org/10.1186/1471-2407-9-444>
- Weavers, H., & Skaer, H. (2014). Tip cells: master regulators of tubulogenesis?. *Seminars in cell & developmental biology*, 31(100), 91–99. <https://doi.org/10.1016/j.semcdb.2014.04.009>
- Yadav, L., Puri, N., Rastogi, V., Satpute, P., & Sharma, V. (2015). Tumour Angiogenesis and Angiogenic Inhibitors: A Review. *Journal of clinical and diagnostic research : JCDR*, 9(6), XE01–XE05.
- Yanagisawa, H., Sugimoto, M., & Miyashita, T. (2021). Mathematical simulation of tumour angiogenesis: angiopoietin balance is a key factor in vessel growth and regression. *Scientific reports*, 11(1), 419. <https://doi.org/10.1038/s41598-020-79824-8>
- Yoo, S. Y., & Kwon, S. M. (2013). Angiogenesis and its therapeutic opportunities. *Mediators of inflammation*, 2013, 127170. <https://doi.org/10.1155/2013/127170>

Appendices

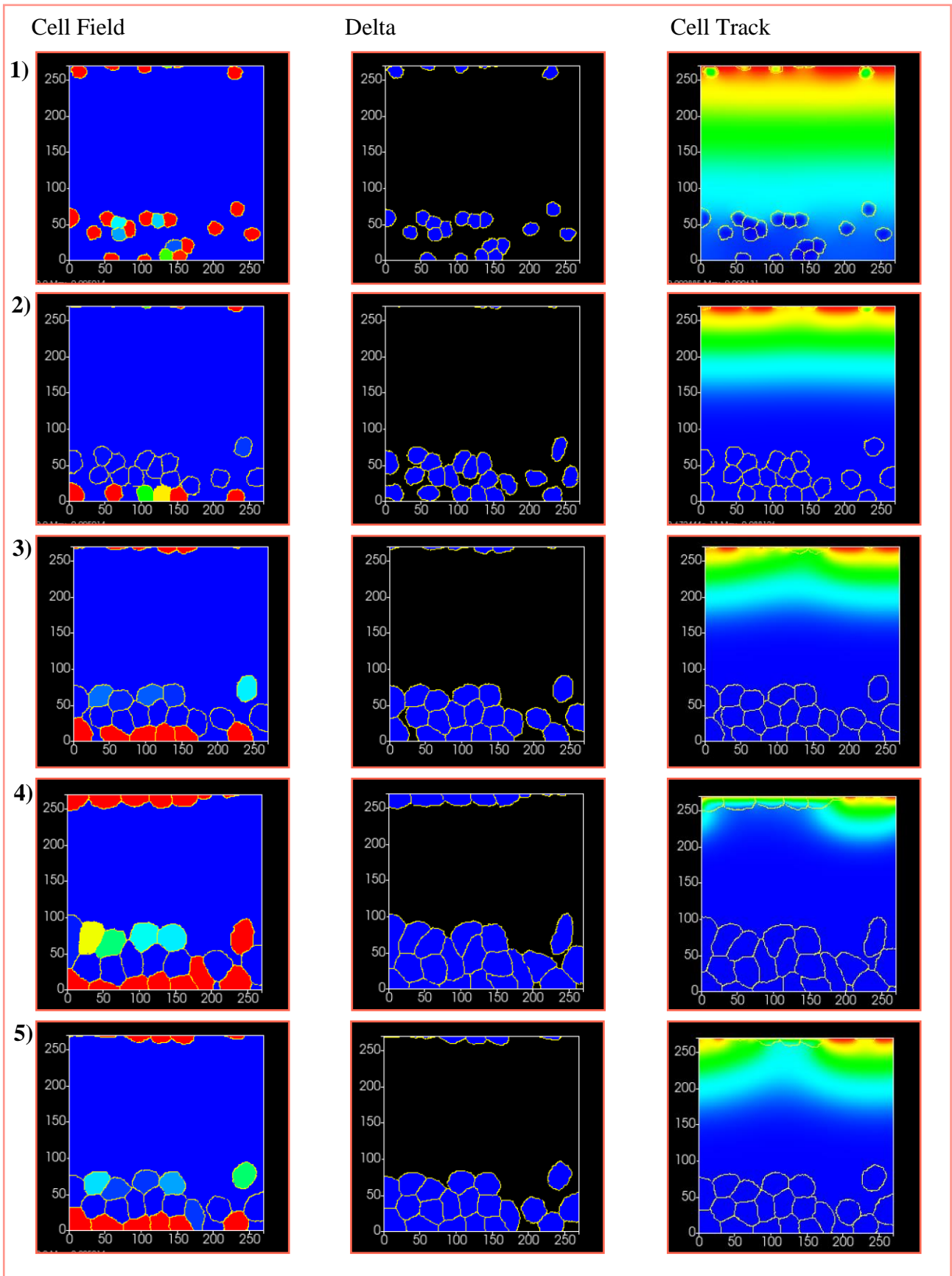
Appendix 1. VEGF and JAGGED1 Field Simulation, Implementation of Antimony Model with experimental data.



Appendix 2. Tracking Tip Cell Formation Through Delta-NOTCH Signalling In COM.



Appendix 3. Tracking Tip Cell Formation Through Delta-NOTCH Signalling In COM.



Appendix 4. Building Model 3 Without Volume Constraints.

