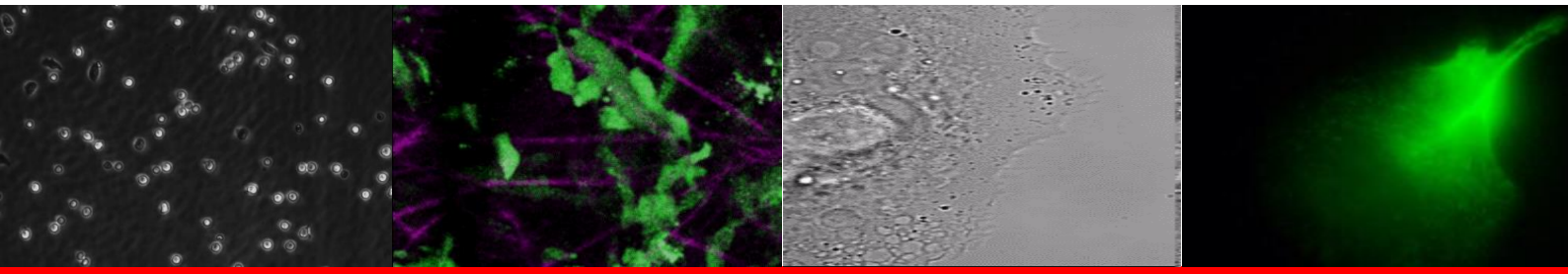

InCeM - RESEARCH TRAINING NETWORK ON INTEGRATED COMPONENT CYCLING IN EPITHELIAL CELL MOTILITY

Deliverable D6.4

PhD theses

Dissemination			
Lead	**Level**	**Type**	**Delivery Month**
UKA	☒ PU (Public) ☐ CO (Confidential) ☐ CI (Classified)	☒ R (Document, report) ☐ DEM (Demonstrator, pilot, prototype) ☐ DEC (Websites, patent fillings, videos etc.) ☐ OTHER	M48

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Version	Date	Author (Name, Institution)	Comments
1.0	18.12.2018	Lea Tomášová, Nikos Kavalopoulos, Nadieh Kuijpers, Anne Pora, Kritika Sahni, Galia Sakaeva, Laura Bornes, Tina Li, Rutuja Patwardhan, Katerina Lomanov, Roman Yakobenchuk, Saransh Vora, Victor Juma, Shore Salle Chota, Dmytro Kotsur, Davide Cusseddu	Final version

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1 PhD theses

All Early Stage Researchers have been involved in a PhD programme. Table 1 and institutions that will award PhDs are clearly identified.

Table 1: Overview of ESRs and their hosting resp. PhD awarding institution

No. ESR	Hosting institution / beneficiary	PhD awarding institution
ESR1	IBIDI	Rheinische Friedrich-Wilhelms-Universität Bonn (RFWU) • Germany
ESR2	GRAD	Uppsala University (UPU) • Sweden
ESR3	UKA	Rheinisch-Westfaelische Technische Hochschule Aachen (RWTH) • Germany
ESR4	UKA	Rheinisch-Westfaelische Technische Hochschule Aachen (RWTH) • Germany
ESR5	JUELICH	Rheinische Friedrich-Wilhelms-Universität Bonn (RFWU) • Germany
ESR6	JUELICH	Rheinisch-Westfaelische Technische Hochschule Aachen (RWTH) • Germany
ESR7	NKI (formerly KNAW)	Utrecht University (UUC) • The Netherlands
ESR8	WEIZMANN	Weizmann Institute of Science (WEIZMANN) • Israel
ESR9	UDE	Universitaet Duisburg-Essen (UDE) • Germany
ESR10	ANDOR	Queen's University Belfast (QUB) • United Kingdom
ESR11 (a)	SCCH	*Not applicable*
ESR11 (b)	SCCH	Universitat Linz • Austria
ESR12	UOS	University of Sussex (UOS) • United Kingdom
ESR13	TAU	*Not applicable*
ESR14	SCCH	Universitat Linz • Austria
ESR15	UOS	University of Sussex (UOS) • United Kingdom

1.1 ESR1 - Lea Tomášová

Table 2: Title and summary of submitted or prospective thesis for ESR1 - Lea Tomášová

Title	Advanced cell migration assays for chemotaxis of slow moving cells
Abstract	Chemotaxis of slow moving cells plays a crucial role in numerous physiological and pathophysiological processes; including tumour metastasis, development, and wound healing. Therefore, a comprehensive understanding of chemotactic behaviour, and the characterization of extracellular signals and cues that prompt directed cell migration is highly desirable. The state-of-the-art chemotaxis assays are in general designed either with respect to the migration characteristics of fast moving cells (such as cells of the immune system), or focus on an in-depth investigation of chemotactic behaviour, requiring a time-demanding and labour-intensive analysis of individual cell trajectories. Such approach poses a limitation for experiments challenged by an increased number of samples, e.g. screenings for chemoattractants, or molecules and genetic mutations with the potential to inhibit, or promote the chemotactic effect. Therefore, the first aim of this thesis was to develop a novel chemotaxis assay suitable for slow moving cells (e.g., keratinocytes, cancer cells), which would enable a fast and effortless evaluation of the chemotactic response. For that purpose, advanced stimuli-responsive materials and micropatterning methods were applied, and a chemotaxis chamber with a hydrogel migration arena was established. The assay employs a gradient generator that maintains a well-defined long-term stable chemical gradient, essential for the investigation of chemotaxis in slow moving cells. The design of the assay enables evaluation of the chemotactic effect from the end-point state of the experiment. This method substantially facilitates the analysis, providing for an increased experimental throughput. Characterization and quantification of the chemotactic behaviour of whole cell populations provide the fundamental information on chemotaxis-inducing stimuli and the relevant response, which is often cell-type specific. However, to get a complete picture of cell behaviour during chemotaxis, further detailed investigations of single cells in conditions close to their physiological environment is required. Therefore, in another part of the thesis, the focus was set on establishment of experimental procedures for studying the migratory behaviour of single chemotaxing cells in long-term stable gradients with advanced imaging techniques, such as the traction force microscopy, and the light-sheet fluorescence microscopy. Finally, the novel tool was employed to investigate the

	chemotaxis of primary keratinocytes, i.e. slow moving cells that play a central role in re-epithelialization of wounds. For the first time, the normal human epithelial keratinocytes (nHEK) were exposed to chemical gradients of several growth factors over long time-periods. The chemotactic response to a range of varying chemical conditions was evaluated quantitatively with the end-point assay. From the tested substances, the epithelial growth factor (EGF) and transforming growth factor α (TGFα) were most potent in inducing the directed migration of nHEK cells. The identification and quantification of the parameters that prompt keratinocyte chemotaxis made it possible to establish a model for further investigations of gradient sensing and directed migration of epithelial cells, aiming for new therapeutic strategies to promote wound repair.
Expected date of thesis submission	March 2019
InCeM Supervisor(s)	Zeno von Guttenberg & Valentin Kahl (IBIDI)
InCeM Co-supervisor(s)	Bernd Hoffmann (JUELICH)
Academic PhD supervisor(s)	Rudolf Merkel & Ulrich Kubitscheck (RFWU)

Table 3: Information about current employment of ESR1 - Lea Tomášová

Employer	ibidi GmbH (IBIDI), Germany
Job title	Researcher
Short description of job position and main activities	Fulfilment of research and development projects

1.2 ESR2 - Nikos Kavalopoulos

Table 4: Title and summary of submitted or prospective thesis for ESR2 - Nikos Kavalopoulos

Title	3D printed tools and microfluidic systems for the development of methods to study cellular interactions
Abstract	Microfabrication and additive manufacturing has had an immense impact in the development and characterization of Biomedical Micro Electro Mechanical Systems (BioMEMS). In the work performed during this ITN the potential of these methods to create sustainable and easy to use systems to study cell interactions was investigated. Novel cell culturing components were produced using additive manufacturing technologies. These can easily replace equipment necessary for every life science lab worth thousands of euros with tailor made fabricated components that cost less than 100euros. That new capacity of everyone with access to a 3D printer to be able to produce affordably everything needed for basic cell culturing and live time lapse imaging of living cells, furthers the spread of life scientific research and enables

	labs of lesser means all over the world to perform complex imaging acquisition assays. Furthermore, traditional microfabrication technologies were combined with additive manufacturing assays to produce a novel microfluidic chip that can create clusters of cells of defined composition and number. That is a tool of importance in studies of cellular interactions, as it enables precise control of the environment and interacting cells. With this novel assay we studied the response of tumour growing cancer cells to paracrine stimulation from neighbouring pancreatic beta cells. The fine level of control and replicability of results inherent in the assay allowed for the detection of fine behaviour profiles previously unnoticed. All tools created during this ITN were designed so as not only to perform a single assay but to be able to act as method development interfaces for other interested parties to explore. It is our hope that with the work done here, novel applications in the field of cell interactions can be furthered explored.
Expected date of thesis submission	13.06.2019
InCeM Supervisor(s)	Sara Thorslund (GRAD)
InCeM Co-supervisor(s)	Johan Kreuger (UPU)
Academic PhD supervisor(s)	Johan Kreuger (UPU)

Table 5: Information about current employment of ESR2 - Nikos Kavalopoulos

Employer	Uppsala University, Sweden
Job title	PhD student
Short description of job position and main activities	PhD student candidate

1.3 ESR3 - Nadieh Kuijpers

Table 6: Title and summary of submitted or prospective thesis for ESR3 - Nadieh Kuijpers

Title	Analysis of keratin dynamics during migration
Abstract	Cytoskeleton dynamics are highly regulated and important for proper cell migration. The most abundant cytoskeletal components in epithelial cells are the keratin intermediate filaments. They have to interact with the other cytoskeletal components, microtubules and actin filaments, and probably with their motor proteins. However, the nature of these interactions during epithelial cell migration is mostly unknown. By combining cytoskeletal targeted drug treatments in primary human keratinocytes with several imaging techniques and micropatterning, we aim to dissect the interdependence the three major cytoskeleton components of epithelial cells during cell migration. Using

	image analysis tools, keratin dynamics can be compared to movement of the other cytoskeleton components. This has led to the observation that the actin-myosin system moves from the plasma membrane toward the cell interior together with newly-assembled keratin particles until it encounters the keratin filamentous network, whereupon its movement slows down and separates from the keratin system. Thus, a clear border between the actin and keratin cytoskeleton is delineated in the migrating keratinocytes. The keratin and microtubule networks do not co-localize in large parts of the cytoplasm. Nonetheless, newly forming keratin filaments seem to use microtubules as a template. Annihilation of both the microtubules and the actin filaments leads to a stepwise relaxation of the keratin network, confirming our observations for the dynamic interactions between keratin and these two systems separately.
Expected date of thesis submission	Summer 2019
InCeM Supervisor(s)	Rudolf Leube & Reinhard Windoffer (UKA)
InCeM Co-supervisor(s)	Alexander Bershadsky (MBI), Jacco van Rheenen (NKI, formerly KNAW)
Academic PhD supervisor(s)	Marc Spehr (Spehr Lab)

Table 7: Information about current employment of ESR3 - Nadieh Kuijpers

Employer	Ugentec, The Netherlands
Job title	Inside sales (first three months) / Outside sales
Short description of job position and main activities	Inside sales: customer approach via web or telephone, processing orders up to 25.000€ and forwarding (larger) orders to the right persons/departments. Outside sales: handling orders >25.000€ and visiting costumers in BeNeLux, Germany and Austria.

1.4 ESR4 - Anne Pora

Table 8: Title and summary of submitted or prospective thesis for ESR4 - Anne Pora

Title	Impact of keratin network regulation on migrating cells
Abstract	Cell migration is a highly complex process whereby different physical and chemical signals act on many different cell components, most notably the force-generating cytoskeleton. Intermediate filaments are one of the three major components of the cytoskeleton. Keratin intermediate filaments are the main type of cytoplasmic intermediate filaments of epithelial cells. They are anchored to hemidesmosomes, which are involved in the attachment of epithelial cells to the

extracellular matrix of the underlying basement membrane. Although keratin intermediate filaments are involved in the mechanical resilience of tissues, they are highly dynamic structures. They are subject to continuous turnover in sessile keratinocytes. This turnover is part of a spatially-defined cycle of assembly and disassembly. Similarly, hemidesmosomes contribute to the mechanical integrity of the epithelium, but their role in epithelial cell migration remains unclear.

I aimed at understanding how the dynamic behaviour of the epithelial keratin intermediate filament cytoskeleton and its associated hemidesmosomes are integrated in migrating cells. Most particularly, I investigated how the distribution and the kinetics of the keratin turnover cycle are affected by cell migration; and how this process is dependent on the mechanophysical environment of the cell. I determined how hemidesmosomes are organized and maintained during migration; and how this process depends on the adjacent actin-associated focal adhesions.

In migrating human primary keratinocytes, I show that keratin is highly dynamic presenting a well-defined spatial distribution with highest flow found at the cell periphery. Keratin dynamics are co-regulated with the speed of cell migration and the cell's trajectory. Upon changes in the mechanophysical environment, migration features and keratin flow are altered. The changes in keratin flow are most likely downstream of focal adhesion-dependent mechanosensation and flow. As a result, keratin dynamics correlate with migration speed. Keratin flow and actin flow show conspicuous similarities in their spatial distribution. The observed dynamic interplay suggests the existence of multiple feedbacks between the actin and keratin cytoskeleton. My observations further suggest that the associated cell-matrix adhesions play a key role in this feedback.

I then show that in migrating cells, hemidesmosomes cluster into highly ordered chevron-like patterns with intercalated focal adhesions in migrating primary human keratinocytes. This arrangement is maintained during migration by continuous assembly of adhesions at the cell front and disassembly of adhesions at the cell back. I further observed that the specialized hemidesmosome-focal adhesion distribution patterns emerge during substrate adhesion and cell spreading within three hours after seeding. During cell adhesion and migration, hemidesmosomes and focal adhesions affect each other's distribution.

Bidirectional cross-talk between focal adhesions and hemidesmosomes is the key for the coordination of the actin and keratin cytoskeleton during cell migration. Mechanical

	coupling at the cell-matrix adhesion level and at the cyto-skeletal network level together induce an increase in migratory persistence. Persistence in cell migration is crucial for efficient migration in a complex 3D environment.
Expected date of thesis submission	10.12.2018
InCeM Supervisor(s)	Rudolf Leube & Reinhard Windoffer (UKA)
InCeM Co-supervisor(s)	Bernd Hoffmann (JUELICH)
Academic PhD supervisor(s)	Björn Michael Kampa (MSN)

Table 9: Information about current employment of ESR4 - Anne Pora

Employer	NA
Job title	NA
Short description of job position and main activities	NA

1.5 ESR5 - Kritika Sahni

Table 10: Title and summary of submitted or prospective thesis for ESR5 - Kritika Sahni

Title	Correlation analysis of migration component dynamics in epithelial cell motility
Abstract	Cell migration is a fundamental process in development and maintenance of multicellular organisms. A coordinated assembly and release of focal adhesions has been reported to be crucial for harmonious cellular movement. Actin and myosin IIA are pivotal components of the cytoskeletal network, that work in accord with each other to facilitate directed cellular movement. In order to characterize the continuous flow of molecules as well as protein exchange behaviour in distinct structures during migration, we have used photo-convertible Dendra2 fusion proteins of actin, myosin IIA and vinculin in motility stimulated primary human epithelial keratinocytes. Following the photo-converted protein incorporation into non-converted structures, we have identified cytosolic diffusion of actin monomers and their subsequent exchange into different types of stress fibres, with very low exchange rates for transversal arcs but high incorporation rates for the rear-end stress fibre. In contrast, myosin IIA mobility was rarely measureable on the cytoplasmic level. Instead, myosin signal propagation took place exclusively along the existing actin stress fibre network. This myosin propagation speed was observed to correlate with the cell's mobility. For focal adhesions, our data confirm faster exchange kinetics for nascent compared to mature complexes. Interestingly, the mature rear end focal adhesions were observed to switch their vinculin

	exchange kinetics to those of nascent ones shortly before disassembly. Furthermore, high resolution analysis indicates different distal-proximal exchange kinetics for disassembling focal adhesions at the cell rear with higher dynamics at their peripheral tips.
Expected date of thesis submission	30.01.2019
InCeM Supervisor(s)	Bernd Hoffmann (JUELICH), Rudolf Merkel (RFWU)
InCeM Co-supervisor(s)	Leif Dehmelt (MPI), Christian Thirion (Sirion)
Academic PhD supervisor(s)	Rudolf Merkel (RFWU)

Table 11: Information about current employment of ESR5 - Kritika Sahni

Employer	NA
Job title	NA
Short description of job position and main activities	NA

1.6 ESR6 - Galia Sakaeva

Table 12: Title and summary of submitted or prospective thesis for ESR6 - Galia Sakaeva

Title	Life cycle analysis of actin, focal adhesions and force measurements
Abstract	Actin stress fibres (SFs) play a central role in cell adhesion and migration. Recently laser ablation has become a popular tool to understand mechanical properties of actin SFs and to measure forces generated by a single or multiple actin SFs in non-locomoting or patterned cells, but still less is known about the role of individual actin SFs in migrating cells. In this study laser nanosurgery combined with live-cell microscopy and traction force microscopy (TFM) was applied to understand several aspects of actin SFs contribution cytoskeletal organization. First, the input of the actin ventral SF at the rear end of the polarized cell to cell polarity, migration and force transmission in human keratinocytes (NHEK) was analysed. In addition, a role of a substrate stiffness on mechanical properties if the rear end SF and were examined. The second part was devoted to investigation of a contribution of the SFs of different localization in cell, SFs in lamellae, in support of cell polarity and locomotion. And finally, mutant murine keratinless keratinocytes were used as a convenient cell model to better understand participation of intermediate keratin filaments in cell polarity. The main founding was that the ventral SF at the cell tail has an essential role in maintenance of cell polarity, migration and force generation. The substrate stiffness, however,

	proved to be not a major factor for the polarity formation. Moreover, laser destruction of the ventral SFs in lamellae demonstrated the importance of a structural integrity of these SFs for a preserved cell polarity and migration. Finally, keratin filaments in its turn were shown not to possess an inevitable role in cell polarity and migration. Overall, the results showed that myosin-derived tensile SF at the rear end of the polarized migrating cells as well as the structurally integral SFs in lamellae are of great importance for cell polarity and migration.
Expected date of thesis submission	February 2019
InCeM Supervisor(s)	Bernd Hoffmann (JUELICH), Rudolf Merkel (RFWU)
InCeM Co-supervisor(s)	Alexander Bershadsky (MBI, Christian Thirion (Sirion)
Academic PhD supervisor(s)	Rudolf Leube (UKA)

Table 13: Information about current employment of ESR6 - Galia Sakaeva

Employer	NA
Job title	NA
Short description of job position and main activities	NA

1.7 ESR7 - Laura Bornes

Table 14: Title and summary of submitted or prospective thesis for ESR7 - Laura Bornes

Title	Cell migration *in vivo*: From physiological necessity to pathological danger
Abstract	**Chapter 1: Dynamic migration of keratinocytes during wound closure** Epidermal wound healing is a crucial process to re-establish the protective barrier function of the skin. However, how epidermal keratinocytes dynamically migrate in order to close the wound has not been analysed *in vivo,* yet. Therefore, we established an *in vivo* scratch wound system within the back skin of a mouse to follow individual keratinocytes in the epidermal sheet during the course of wound healing using intravital microscopy. We discovered that single keratinocytes migrate independently within a collectively migrating epidermal sheet. Moreover, we show that keratinocytes change their direct neighbouring cells, indicating that they remodel their cell-cell-junctions during migration. Additionally, these dynamics of single cell migration within a migrating sheet enable keratinocytes to overcome obstacles such as hair follicles.

	Taken together using a newly developed *in vivo* system we can uncover the unexpected migratory behaviour of individual keratinocytes within the collectively migrating sheet. **<u>Chapter 2: Active vs. passive migration during tumor invasion and dissemination</u>** Metastatic growth is the major cause of cancer-associated mortality. However, whether cancer cell need to be able to migrate to leave the primary tumour and establish metastasis at the secondary side is not yet understood. To address this question we inhibited migration by knocking out the actin branching protein, ArpC3 without affecting proliferation. We could show that organoids are inhibited in their spreading capacity. Further we discovered that also ArpC3 knockout tumours, which were transplanted into the mammary fat pad decreased their migration and invasion compared to the WT. Additionally, these knockout tumours also demonstrated a lower metastasizing capacity. Taken together these findings suggest that actin branching is important for invasion and seeding to the distant site. **<u>Chapter 3: The role of epithelial to mesenchymal transition (EMT) during dissemination</u>** It is still debated whether epithelial to mesenchymal transition (EMT) plays a role in the metastatic cascade. Previous results indicate that EMT occurs naturally in mammary tumours by a small population of cells, which down regulate their E-cadherin. However, in an opposing study it has been shown EMT characterized by upregulation of fsp1 was not required to initiate lung metastasis. Here we combine these two markers and look at their overlay. We discovered that only a minority of cells expressed fsp1 at any time point during primary tumour growth. Further, we show that the majority of cells leaving the primary tumour, as well as the once establishing metastasis at the secondary side did not express fsp1 at any time. Moreover, we observed an E cadherin low population in the primary tumour, which differentially expressed typical EMT genes compared to the E-cadherin high population. Additionally, we found that this population increase when cells leave the primary tumour. Taken together these finding suggest that the markers of fsp1 and E-cadherin are not overlaying, while cells leaving the primary tumour have down regulated E-cadherin and typical EMT expression profile they do not express fsp1, which suggest an existence of an partial EMT.
Expected date of thesis submission	2020
InCeM Supervisor(s)	Jacco van Rheenen (NKI)

InCeM Co-supervisor(s)	Rudolf Leube & Reinhard Windoffer (UKA)
Academic PhD supervisor(s)	Jacco van Rheenen (UUC)

Table 15: Information about current employment of ESR7 - Laura Bornes

Employer	Stichting het Nederlands Kanker Instituut-Antoni van Leeuwenhoek Ziekenhuis (Netherlands Cancer Institute - NKI), The Netherlands
Job title	PhD student
Short description of job position and main activities	Plan and execute the research plan in my research questions.

1.8 ESR8 - Tina Li

Table 16: Title and summary of submitted or prospective thesis for ESR8 - Tina Li

Title	The molecular basis for the structure and function of filopodia, induced by galectin-8
Abstract	Live cells were shown to be able to sense multiple features of the extracellular matrix (ECM) and respond to them. Typically, such studies were based on the plating of cells on one specific ECM component (e.g. fibronectin, vitronectin or laminin), and tested the effects of that particular matrix component on cell structure and function (e.g. cell migration, proliferation, gene expression etc.). While such experiments yielded interesting insights concerning the differential signalling capacity of specific ECM components, little attention has been devoted to the fact that physiologically, cells simultaneously encounter multiple ECM components and are responding, in fact, to the ensemble. Here, we investigated how cells respond to a combination of ECM components, each of which might have a distinct local and global effects on cell adhesion and fate. The pair of protein studied here included fibronectin and galectin-8, which drive very different spreading processes. Fibronectin induces alternating cycles of contraction and extension, leading to the formation of focal adhesions and stress fibres, while galectin-8 drives continuous protrusion with essentially no focal adhesions and stress fibres. Mixtures of the two proteins induced complex adhesive patterns, which depended on the concentration of galectin-8: increased galectin-8 addition to fibronectin induces decreased cell adhesion. In addition, galectin-8 doped with fibronectin increases cell adhesion and cells are more contractile. We then explored the mechanism of interactions between the two molecular signalling pathways. Cells on galectin-8 also form numerous adhesive filopodia, and these filopodia have a chiral bending tendency. We found the filopodia chiral

	bending is dependent on Myosin-X, and is Arp2/3 independent.
Expected date of thesis submission	30.05.2020
InCeM Supervisor(s)	Benjamin Geiger & Irit Sagi (WEIZMANN)
InCeM Co-supervisor(s)	Alexander Bershadsky (MBI)
Academic PhD supervisor(s)	Benjamin Geiger (WEIZMANN)

Table 17: Information about current employment of ESR8 - Tina Li

Employer	Weizmann Institute of Science (WEIZMANN), Israel
Job title	PhD student
Short description of job position and main activities	PhD student in understanding cytoskeleton structure, dynamics and function.

1.9 ESR9 - Rutuja Patwardhan

Table 18: Title and summary of submitted or prospective thesis for ESR9 - Rutuja Patwardhan

Title	Role of Rho GTPases in keratinocyte migration
Abstract	Keratinocytes are one of the major skin cell types and essential for the generation of the skin barrier throughout the lifespan of an organism. The epidermal growth factor (EGF) has been shown to be essential for orchestrating different Keratinocyte behaviours such as migration, differentiation and wound healing. However, molecular mechanisms that control the dynamic behaviour of Keratinocytes downstream of EGF receptor activation are still not fully understood. In our hands, EGF stimulation of primary human keratinocytes (NHEK) lead to one strong short cell contraction pulse at the cell periphery within the first minutes after treatment. Using a fluorescence sensor for Rho activity and TIRF (Total Internal Reflection Fluorescence) microscopy, we also found that EGF stimulation triggered a single major Rho activity peak in the cell periphery prior to the local cell contraction pulse. Recently, a signal network generating local pulses and propagating waves of Rho activity was identified in our lab (Graessl et al., 2017). These dynamic patterns are a result of positive feedback of Rho GTPase local activity by recruiting Rho-GEFs to the plasma membrane and a negative feedback on a slower time scale recruiting acto-myosin and associated RhoGAPs. Thus, growth factor signalling might trigger Rho excitability, presumably by raising basal Rho activity above the amplification threshold via activation of upstream GEFs. Indeed, we could show the presence of an excitable Rho signal network by stimulating Rho excitability by GEF-H1 and

	several other related Lbc-type Rho GEFs in primary human Keratinocytes (NHEKs). Furthermore, inhibition of MAPK signalling significantly delayed the peak onset suggesting crosstalk between growth factor mediated activation, MAPK signalling and Rho specific GEFs in the stimulation of the peripheral Rho activity peak. In addition to the transient pulse of Rho activation at the cell periphery upon EGF stimulation, we also measured persistently elevated local activity of another Rho GTPase, Cdc42 at the periphery of migrating Keratinocytes. Interestingly, this activity pattern was independent of growth factor stimulation suggesting that Cdc42 might be involved in basic migration dynamics in Keratinocytes. Correlation analyses with the direction of cell movement and acto-myosin dynamics further showed that Cdc42 activity was localized at the contractile trailing edge during migration. In addition, depletion of Cdc42 revealed significantly decreased migration efficiency presumably due to defects in directionality.
Expected date of thesis submission	September 2019
InCeM Supervisor(s)	Perihan Nalbant (UDE)
InCeM Co-supervisor(s)	Leif Dehmelt (MPI), Bernd Hoffmann (JUELICH), Rudolf Merkel (RFWU)
Academic PhD supervisor(s)	Perihan Nalbant (UDE)

Table 19: Information about current employment of ESR9 - Rutuja Patwardhan

Employer	Universitaet Duisburg-Essen (UDE), Germany
Job title	PhD Student
Short description of job position and main activities	Biochemistry, Cell culture, Phase contrast microscopy, TIRF microscopy, microscopy image analysis and interpretation

1.10 ESR10 - Katerina Lomanov

Table 20: Title and summary of submitted or prospective thesis for ESR10 - Katerina Lomanov

Title	Cell detection and tracking
Abstract	Interacting Multiple Model algorithm combined with the double use of the linear assignment problem for data association. Performance has been evaluated using a real confocal microscopy image sequence of a migrating cell stained for focal adhesions. The tracker has been compared against several state-of-the-art, commercially available tracking algorithms, and outperforms each of these other methods in terms of multiple object tracking accuracy. Although we have tailored the tracking algorithm to focal adhesions, it can be used for tracking cells as well.

	To ensure robust tracking of cells, reliable detections are necessary. Given the recent successes of Deep Learning approaches for cell segmentation and the availability of partially labelled data, we made use of a U-Net architecture. In particular, we propose strategies for training convolutional neural for cell localization and segmentation in microscopy images with both little training data and in presence of significant label noise. Insufficient availability of ground truth is a common issue in the field of microscopy image analysis, hence the usefulness of such approaches. Performance evaluation is done using phase contrast microscopy human fibrosarcoma (HT1080) cells and comparing the resulting F-scores. Some of our methods outperform standard cell detectors in terms of F-score, while requiring little effort to collect training data.
Expected date of thesis submission	May 2019
InCeM Supervisor(s)	Hugh Gribben (ANDOR), Peter Majer (Bitplane)
InCeM Co-supervisor(s)	Bernd Hoffmann (JUELICH), Rudolf Merkel (RFWU)
Academic PhD supervisor(s)	Jesus Martinez del Rincon & Paul Miller (QUB)

Table 21: Information about current employment of ESR10 - Katerina Lomanov

Employer	ANDOR, United Kingdom
Job title	PhD Fellow
Short description of job position and main activities	Research in computer vision

1.11 ESR11a - Roman Yakobenchuk

Table 22: Title and summary of submitted or prospective thesis for ESR11a - Roman Yakobenchuk

Title	Image analysis of cytoskeletal network dynamics
Abstract	Actin stress fibers play an essential role for cell migration which is fundamental for phenomena as wound healing or tissue invasion during carcinogenesis. Thus, extraction of a single actin stress fiber allows measuring its geometric and dynamic properties. These measurements can serve as a basis for modeling the behavior of stress fibers and the extracted fibers themselves can be used for further tracking. The structure of the cytoskeleton is very complex and has numerous intersections as well as filaments with low intensity. The results of segmentation based on a graph representation, obtained by state-of-the-art methods (such

	as BFS or A* for example) are not satisfying as these methods don't take into account the curvature and bending of filaments. We present a method for identifying a single actin stress fibre in the actin network appearing in live cell images. In order to remove noise from actin tagged fluorescence microscopy images and to enhance the brightness and the contrast of the actin network, we propose an image pre-processing routine, which improves the results of further segmentation. A graph representation of the actin cytoskeleton is used in order to transfer the segmentation problem into the domain of graph processing. Therefore, the task of single stress fiber extraction can be solved by means of a specially adapted shortest path search algorithm. Thereby, the assessment of paths is based on geometrical properties of the corresponding actin fibers. The proposed method is efficient and almost fully automatic, only requiring user interaction in a form of two given end-points in the network. The fiber between these two end-points is detected and segmented. We give an overview of the proposed methodology and report on initial evaluation results obtained for videos of NHEK cells with a complex actin cytoskeleton structure.
Expected date of thesis submission	*PhD thesis will not be submitted*
InCeM Supervisor(s)	Bernhard Moser (SCCH), Julian Mattes (MATTES)
InCeM Co-supervisor(s)	Rudolf Leube & Reinhard Windoffer (UKA)
Academic PhD supervisor(s)	Sergivy Pereverzyev (RICAM)

Table 23: Information about current employment of ESR11a - Roman Yakobenchuk

Employer	Thermo Fisher Scientific, Czech Republic
Job title	Software Engineer
Short description of job position and main activities	Software development in computer vision

1.12 ESR11b - Saransh Vora

Table 24: Title and summary of submitted or prospective thesis for ESR11b - Saransh Vora

Title	A computational framework for simulating the dynamics of keratin filament networks
Abstract	In this work, we propose a biomechanical computational framework, which allows us to perform numerical simulations in order to mimic the behaviour of keratin filament networks as observed in microscopy data. The developed automated computational methods allow us to:

	(1) generate, by a randomized process, a filament network topology with predefined properties; (2) simulate the motile behaviour of the filament network; (3) synthesize image data based on the produced simulations. The synthetic image sequences can then be used to evaluate filament extraction and tracking algorithms as the underlying ground truth is available. The core of our simulation module consists of a spring-mass-damper discrete mechanical system, which allows simulating elastic behaviour of the filaments. Additional stochastic external forces are also incorporated, which allow exhibiting the effect of Brownian motion and the influence of the actin network resulting in an inward-directed filament flow. Based on the produced simulations we are able to synthesise artificial image data mimicking the behaviour of the filament network. Artificial images can be exposed to additional microscopy-related imaging artefacts such as Poisson/Gaussian noise, convolution with a predefined point spread function.
Expected date of thesis submission	April 2019
InCeM Supervisor(s)	Bernhard Moser (SCCH), Julian Mattes (MATTES)
InCeM Co-supervisor(s)	Rudolf Leube & Reinhard Windoffer (UKA)
Academic PhD supervisor(s)	Sergivy Pereverzyev (RICAM)

Table 25: Information about current employment of ESR11b - Saransh Vora

Employer	Software Competence Center Hagenberg
Job title	Researcher, Knowledge-based Vision Systems (KVS)
Short description of job position and main activities	The main responsibilities are: to do high-quality work to reach the individual object tasks and the whole project goals; to develop the individual research topic; to coordinate own work with other researchers in the project(s); to support others within specific research topic; to perform the administrative tasks according to the defined procedure to enable project (company) managers to organizationally and financially control the project and the whole company.

1.13 ESR12 - Victor Juma

Table 26: Title and summary of submitted or prospective thesis for ESR12 - Victor Juma

Title	Data-driven mathematical modelling and simulation of Rho-Myosin temporal dynamics
Abstract	In this thesis, a full repertoire of model formulation, mod-el analysis, numerical analysis, sensitivity analysis and Bayesian method to parameter identification are presented, that seek to describe faithfully the temporal dynamics of GEF-Rho-

	Myosin signalling pathway as observed experimentally. The thesis is based on rigorous mathematical and numerical analysis to provide robust models and numerical results that exhibit the temporal dynamics as observed in experiments. The modelling is based on the experimental observations, and therefore three different mathematical models are formulated from first principles depending on the constitutive laws for the interaction between the chemical species, entailing that new mathematical models are obtained. Detailed mathematical analysis of the stability of uniform steady states using nullcline theory, linear stability theory and sign pattern analysis is carried out, to characterise mathematically the key temporal dynamics of stability, oscillations, excitability and bistability as observed in experiments. Numerical bifurcation analysis using Matcont and numerical simulations illustrates the theoretical analytical results through parameter variations for the key temporal dynamics. Rigorous sensitivity analysis provides a powerful tool for investigating the effect of parameter variations through local and global sensitivity. In particular, we use local sensitivity theory to characterise the limit cycle behaviour of an oscillatory dynamical system in terms of parameter variations and therefore the thesis provides premises to characterise or study amplitude and period sensitivity to parameter variations. Finally a full Bayesian approach is applied to the model for the identification of parameters that best-fits the model to experimental results. The theis provides a new framework for incorporating prior knowledge about parameters, which results in obtaining full probability distribution for parameters.
Expected date of thesis submission	06.12.2018
InCeM Supervisor(s)	Anotida Madzvamuse (UOS)
InCeM Co-supervisor(s)	Stephanie Portet (UMA), Leif Dehmelt (MPI)
Academic PhD supervisor(s)	Anotida Madzvamuse (UOS)

Table 27: Information about current employment of ESR12

Employer	NA
Job title	NA
Short description of job position and main activities	NA

1.14 ESR13 - Shore Salle Chota

Table 28: Title and summary of submitted or prospective thesis for ESR13 - Shore Salle Chota

Title	Modelling cell shape changes by actin filaments
Abstract	Cell shape and migration are controlled by dynamic actin cytoskeleton. In mathematical and physical modelling, representation of the interaction of actin filament with plasma membrane and the movement of plasma membrane remains a challenge. Here we developed a simplified computational model of dynamic behaviour of the plasma membrane at the cell leading edge driven by polymerizing actin filaments. Our approach is based on Helfrich theory of membrane elasticity and the study of actin filament by Brownian dynamics. At first we developed a biophysically motivated computational model of single actin filament which takes care of monomer interactions, and hence formation of actin filament. This result is then extended to two parallel actin filaments, which is followed by networks of actin filaments. In presence of an elastic obstacle (membrane), the growth of actin filaments exerts a force on the membrane and deforms it. Taking advantage of the time scale separation of actin polymerization and plasma membrane equilibration, we minimize the membrane elastic energy. In general, analytic solution for minimizing membrane energy is non-existent, and hence we developed a numerical optimization software based on Quasi newton algorithm to get the minimum energy configuration of the cell leading edge, which in turn provide as the shape of the cell. We report the computational result for different elastic parameters of the cell membrane. The general theoretical result is being compared and tuned according to experimental observation of specific actin architecture; lamelipodia and filopodia.
Expected date of thesis submission	*PhD thesis will not be submitted*
InCeM Supervisor(s)	Michael Kozlov (TAU)
InCeM Co-supervisor(s)	Benny Geiger (WEIZMANN)
Academic PhD supervisor(s)	Michael Kozlov (TAU)

Table 29: Information about current employment of ESR13 - Shore Salle Chota

Employer	NA
Job title	NA
Short description of job position and main activities	NA

1.15 ESR14 - Dmytro Kotsur

Table 30: Title and summary of submitted or prospective thesis for ESR14 - Dmytro Kotsur

Title	Model based quantification of cytoskeletal motion
Abstract	The cytoskeleton (CS) plays a vital role in the motion and migration of living cells. CS is essential for biological processes as tissue formation, wound healing and it drives tissue invasion during carcinogenesis. The main constituents of the CS are: actin filaments, which play a major role in force generation, intermediate filaments (e.g., keratin), which are responsible for cell stabilization against external forces, and microtubules participating in cell division and intercellular transport. Each of these three classes of constituents forms a sophisticated and highly dynamic interconnected network inside the cell. The study of the network's properties and the interplay of such networks is currently an active topic of research in the field of microbiology. However, manual analysis of such networks could be extremely time consuming. In order to facilitate the above analysis, one requires automated instruments, which would allow a quantification of the network's motion. In this dissertation, we present a framework, which automatically extracts the geometry of the filamentous network and quantifies the motion of individual cytoskeletal filaments (or bundles of filaments) within such a network based on the time-sequences of confocal fluorescent microscopy images. The position of each individual filament is represented by a parametric curve, which we fit to the image data using a further development of the stretching open active contour model which we call "endpoint-controlled active contours". We also tackle the problem of automated tuning of the model's parameters based on recently developed parameter choice strategies (e.g., linear functional strategy, quasi-optimality principle). In order to evaluate the proposed framework, we have developed a simulation tool which mimics the behaviour of the filamentous network and produces artificial image data based on numerical simulations. Finally, we demonstrate an application of our framework for the problem of parameter estimation in a model of interconnected actin stress bundles (spring mass-based model) coupled with an elastic substrate model.
Expected date of thesis submission	April 2019
InCeM Supervisor(s)	Bernhard Moser (SCCH) Julian Mattes (MATTES)
InCeM Co-supervisor(s)	Bernd Hoffmann (JUELICH)
Academic PhD supervisor(s)	Sergej Pereverzyev (RICAM)

Table 31: Information about current employment of ESR14 - Dmytro Kotsur

Employer	Software Competence Center Hagenberg GmbH (SCCH)
Job title	Researcher, Knowledge-based Vision Systems (KVS)
Short description of job position and main activities	The main responsibilities are: to do high-quality work to reach the individual project tasks and the whole project goals; to develop the individual research topic; to coordinate own work with other researchers in the project(s); to support others within the specific research topic; to perform the administrative tasks according to the defined procedures to enable project (company) managers to organizationally and financially control the project and the whole company.

1.16 ESR15 - Davide Cusseddu

Table 32: Title and summary of submitted or prospective thesis for ESR15 - Davide Cusseddu

Title	Mathematical modelling in cellular biology through compartmentalisation and conservation laws
Abstract	The thesis is centered around main chapters: **1. chapter** The first key chapter studies a minimal model for cell polarisation driven by Rho GTPases. We present a three-dimensional generalisation of the well-known wave pinning model (Mori et al., *Biophys. J.*, 2008) through the maturing theory of coupled bulk-surface semilinear partial differential equations in which protein compartmentalisation in the cytosol and the cell membrane becomes natural. We show how a local perturbation over the surface can trigger propagating reactions, eventually stopped in a stable profile by the interplay with the bulk component. We describe the behaviour of the model through rigorous asymptotic and local perturbation analysis, in which the role of the geometry is investigated. The bulk-surface finite element method is used to generate numerical simulations over simple and complex geometries, which confirm our analytical results, showing pattern formation due to the propagation and pinning dynamics of the wave. The generality of our mathematical and computational framework allows studying more complex biochemical reactions and biomechanical properties associated with cell polarisation in multi-dimensions. **2. chapter** The second key chapter derives, analyses and simulates an experimentally-driven predictive mathematical model describing the spatio-temporal dynamics of the keratin network in epithelial cells. This network spans the whole cell interior, providing mechanical support to the cell, and protects the nucleus through a dense filamentous cage. Its

	dynamical behavior shows a very interesting balance between assembly and disassembly kinetics and a permanent inward flow towards the nuclear cage. Our model substantially extends the ideas of a simpler model previously proposed by Portet et al., *Plos One* (2015). A research visit at the Institute of Molecular and Cellular Anatomy of the Uniklinik RWTH Aachen has been a fundamental step towards the development of the new model, since in this occasion we had the opportunity to reinterpret the biological data previously used in the work by Portet et al. With new data-based modelling assumptions, plus some relaxation of the hypothesis, our new approach is able to better describe the experimental measurements, predicting the spatio-temporal assembly and disassembly rates as well as regions of sources and sinks, supporting the biological model proposed by Windoffer et al., *Journal of Cell Biology* (2011). **3. chapter** The last key chapter extends the previous one-dimensional study to a multi-dimensional spatiotemporal model for keratin network remodelling. The chapter details the derivation of the model equations from first principles and states some of its important properties. Given the intrinsic nature of the interaction and balance between transport and reaction kinetics, a robust and stable SUPG numerical method based on finite elements is employed. Preliminary results seem to reveal new spatiotemporal dynamics of the keratin material in multidimensions.
Expected date of thesis submission	01.02.2019
InCeM Supervisor(s)	Anotida Madzvamuse (UOS)
InCeM Co-supervisor(s)	Stephanie Portet (UMA)
Academic PhD supervisor(s)	Anotida Madzvamuse (UOS)

Table 33: Information about current employment of ESR15 - Davide Cusseddu

Employer	NA
Job title	NA
Short description of job position and main activities	NA