



CellArchitects Symposium

Using Micropatterns for **Quantitative Cell Analysis**
18 May 2010 - EMBL Heidelberg, Germany

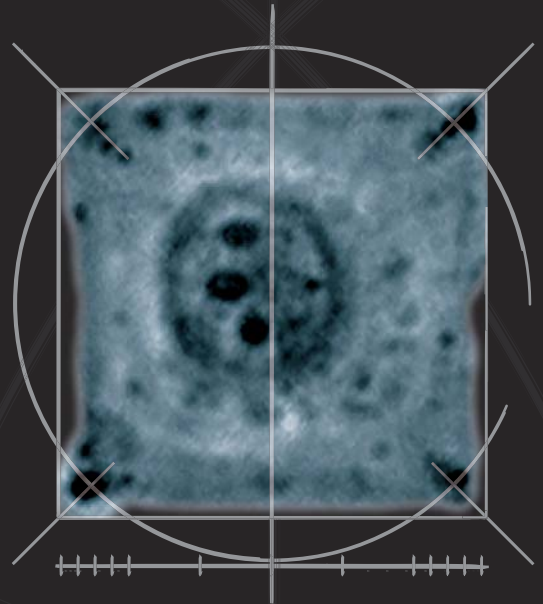
PROGRAM

Scientific Organizer:

Michel Bornens - Institut Curie, France

Symposium Organizers:

EMBL





Welcome to the participants of this first Cell Architects Symposium, hopefully the first of a long series.

It is known from the early days of modern cell biology that cell shape in metazoa can change extensively during migration or division, as well as during differentiation. But it is only recently that it has been realized that conversely, modifying cell shape can be a very efficient way to modify cell functions, including cell fate and differentiation. For most cells, the best way to control cell shape is to control cell adhesion. In response to the pattern and compliance of adhesion, the cell is setting a specific and dynamic

architecture of the actin network whose activity largely defines its shape. The progressive maturation of micro-engineering techniques to control cell adhesion has led to the possibility of defining conditions in which global and local actin contractility can be precisely controlled, providing invaluable tools for quite novel and promising developments in cell biology. A Cell Architects symposium appears thus timely to help spreading these techniques and methods into the community of cell biologists. For the first year, we have given a European format to the symposium. The invited speakers are representative of the best state of the art, both on theoretical and experimental grounds. They will cover major aspects of the current use of micro-patterns.

This year the Cell Architects Symposium is a satellite meeting of the ELMI International Meeting. Participants attending ELMI are invited to register for one of the micropattern technology workshops organized by CYTOO. These practical sessions will provide participants with a hands-on experience of micropattern technology as well as guidelines for easy automated image analysis and cell morphology quantification.*

Michel Bornens, Institut Curie, Paris

*We thank Leica Microsystems for kindly providing a microscope for the technology workshops.

Tuesday, 18 May 2010

- 11:00 - 13:00 Arrival and Registration
- 12:30 - 13:00 Welcome Coffee
- 13:00 - 13:15 Opening / Welcome remarks by **Michel Bornens**
- 13:15 - 14:00 **Keynote lecture - Buzz Baum** (UK). Extrinsic and intrinsic control of cell shape and polarity.
- 14:00 - 14:30 **Invited lecture - Ulrich Schwarz** (Germany). Modeling cell shape and mechanics as studied with micropatterned substrates and laser cutting.
- 14:30 - 14:45 **Presentation Abstract - Alexa Kiss** (Switzerland). Investigating nuclear migration with the help of micropatterned surfaces.
- 14:45 - 15:00 **Presentation Abstract - Arnaud Chevrollier** (France). Micropatterned cells give new insights into the organization of the mitochondrial network.
- 15:00 - 15:15 **Technology showcase - Alexandra Fuchs** (France). CYTOO's ready-to-use adhesive micropatterns: from Starter's to custom-made.
- 15:15 - 15:45 Coffee Break and Poster Session
- 15:45 - 16:15 **Invited lecture - Manuel Théry** (France), Mechanical Equilibrium in Multicellula Arrangements.
- 16:15 - 16:30 **Presentation Abstract - Paolo Ronchi** (Germany). A novel laser nanosurgery approach allows the characterization of Golgi de novo biogenesis
- 16:30 - 16:45 **Presentation Abstract - Kristine Schauer** (France). A computational imaging approach to systematically study the global organization of endomembranes with probabilistic density maps.
- 16:45 - 17:00 **Presentation Abstract - Dencho Gugutkov** (Spain). Fibronectin patterning on substrates of electrospun Poly-(Ethyl Acrylate) – an Approach for Guiding the Cellular Behaviour.
- 17:00 - 17:15 **Presentation Abstract - Sébastien Degot** (France). Quantification of Blebbistatin effects at very low doses using Adhesive Micropatterns.
- 17:15 - 17:40 **Round table** (Q/A) with representatives of industry and academia.
- 17:40 Concluding remarks by **Michel Bornens**

KEYNOTE LECTURE

13:15 - 14:00

Extrinsic and Intrinsic Control of Cell Shape and Polarity

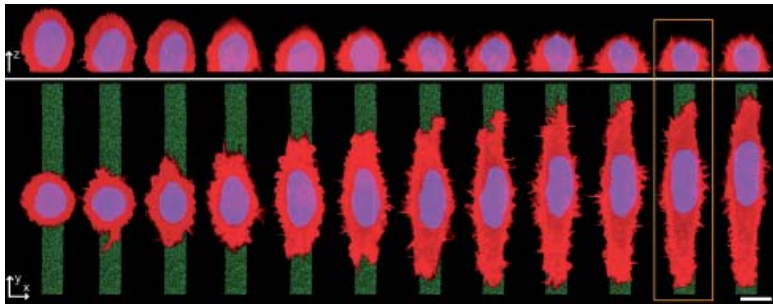
Buzz Baum

MRC Laboratory of Molecular Cell Biology, University College London, London, United Kingdom.

Because physical form and function are intimately linked, mechanisms that maintain cell shape and size within in tight limits are likely to be important for a wide variety of biological processes. However, the extent to which individual cells from a multicellular animal are able to regulate their plastic form remains unclear. For many years we have been using RNAi to identify and characterise the genetic controls that regulate cytoskeletal organisation, cell size and cell shape.

In this work, we have taken this analysis further by using micro-contact printing to limit the spreading of cells to one dimension on micro-patterned protein lines. This analysis revealed that animal cells on lines spread to a characteristic steady-state length that is independent of their mass, pattern-width and lamellipodial actin. Instead, homeostatic length control depends on a population of dynamic oriented microtubules. Moreover, a similar type of microtubule-dependent cell length homeostasis was observed in a tissue and developmental context in the zebrafish neural tube.

These data show the strengths of using micro-patterning to ask specific questions in cell biology, and point the importance of studying the dialogue between a cell and its environment for a proper understanding of cell morphogenesis.



INVITED LECTURE

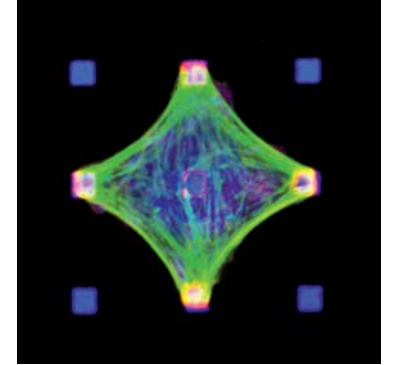
14:00 - 14:30

Modeling Cell Shape and Mechanics as Studied with Micropatterned Substrates and Laser Cutting

Ulrich Schwarz

University of Heidelberg, BioQuant, Heidelberg, Germany

Ulrich.Schwarz@bioquant.uni-heidelberg.de



Cell adhesion to discrete sites on a micropatterned substrates leads to cell shape characterized by inward-curved circular arcs.

Mechanical force has emerged as a major regulator of cellular function, but experimentally it is very difficult to locally measure forces in the cytoskeleton and at sites of adhesion. However, for especially suited experimental setups, theoretical models can be used to calculate force.

By performing laser cutting experiments, we showed that the retraction of actin stress fibers is restricted to their cut ends and that there new adhesions form^[1]. A mechanical model was defined and parametrized that allowed us to quantitatively describe fiber retraction and to calculate the force between fiber and substrate. By following fluorescent redistribution of the focal adhesion protein zyxin after laser cutting, we found that this protein co-localized with the calculated mechanical stress, suggesting a direct force-sensing mechanism. For adhesion to discrete sites,

the mechanics of actin bundles is also a main determinant of cell shape. Using cell adhesion to small adhesive islands on a micropatterned substrate, we showed that shape typically resembles a sequence of inward-curved circular arcs reinforced by contractile actin bundles^[2].

Surprisingly, a quantitative shape analysis revealed that arc radius increases with spanning distance, suggesting a theoretical model that combines the elasticity of filamentous networks with active contractility. Our mechanical model can be used to predict cellular forces from shape, without the need of explicit force measurements^[3].

[1] J. Colombelli et al., Mechanosensing in actin stress fibers revealed by a close correlation between force and protein localization. *J. Cell Sci.*, 122:1665-79, 2009.

[2] I.B. Bischofs et al., Filamentous network mechanics and active contractility determine cell and tissue shape. *Biophys. J.*, 95:3488-3496, 2008.

[3] I.B. Bischofs et al., Effect of adhesion geometry and rigidity on cellular force distributions. *Phys. Rev. Lett.*, 103:048101, 2009.

PRESENTATION ABSTRACT

14:30 - 14:45 - Poster P01

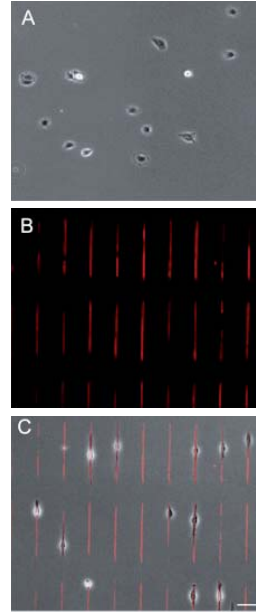
Investigating nuclear migration with the help of micropatterned surfaces

Alexa Kiss, Peter Horvath, Ulrike Kutay, Gabor Csucs

ETH Zürich Light Microscopy Centre, Zürich, Switzerland

Nuclear migration is a general term for a non-random movement of the nucleus toward specific sites in the cell. This phenomenon has been described throughout the eukaryotes from yeast to mammals. However, compared to other organisms (*S. cerevisiae*, *C. elegans*) the process is still poorly understood in mammalian cells. By using micro-contact printing we are able to regulate the geometry and spreading of cultured cells. Adhesive micropatterns of fibronectin provide an attachment surface for the cells whereas the passivation of the surface by poly-L-lysine-poly-ethylen-glycol (PLL-PEG) prevents protein, thus cell attachment. Live cell imaging by time-lapse microscopy showed that under these conditions cells gain a bipolar shape, and more interestingly, the nuclei of the cells showed auto-reserved motion. We have used a self-developed software, which enables automated tracking of the nuclei. Our research tries to understand the molecular cues and mechanisms behind the observed cellular and nuclear movement. We have already shown that the cellular cytoskeleton plays an important role in the observed phenomena but the exact players and the detailed mechanism remain to be clarified. We plan to apply drug treatments and siRNA experiments to identify the most important components and their relationship. Although our research concentrates mainly on the motility of the nucleus, it may also help to get a better understanding of the general theme of cell migration.

C6 rat glioma cells were plated on glass coverslips (A) or on 5x200 µm rectangles of fluorescently labeled fibronectin (B, C: overlay) Scale bar: 50 µm.



D: kymograph of a single C6 glioma cell showing oscillation of the nucleus (cells were plated on 5x200 µm rectangles and imaged in every 5 minutes) Scale bar: 20 µm.



PRESENTATION ABSTRACT

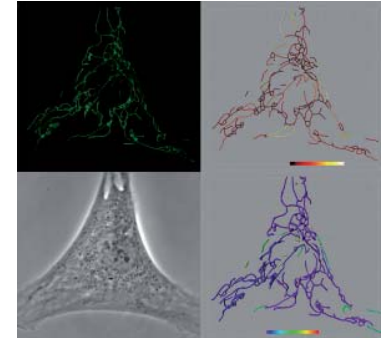
14:45 - 15:00 - Poster P02

Micropatterned cells give new insights into the organization of the mitochondrial network

**Arnaud Chevrollier^{1,2}, Julien Cassereau^{2,3},
Patrizia Amati-Bonneau^{1,2}, Vincent Procaccio^{2,3},
Dominique Bonneau^{1,2}, Pascal Reynier^{1,2}**

¹INSERM U694, Angers, France; ²CHU d'Angers, Angers, France; ³CNRS UMR6214 and INSERM U771, Angers, France.

Defective mitochondrial metabolism is associated with a wide range of human diseases involving almost all medical subspecialties. Mitochondria are dynamic organelles that form a tubular networks adapt to cellular requirements by changing their shape through fission and fusion of the organelles. These dynamic processes are essential to mammalian development and their defects lead to neurodegenerative diseases. Currently, fluorescence microscopy is used to examine the overall morphology of mitochondria in skin fibroblasts obtained from human biopsies. However, the precise analysis of the mitochondrial network in cultured cells is complicated by variations due to the cell cycle, the organisation of the cytoskeleton, and the metabolic environment. We propose a novel approach to the investigation of mitochondrial structure by means of coverslips with micropatterns designed to impose normalized size and shape of fibroblasts. An elaborate system of 3D imaging and computational analysis is then used to determine mitochondrial length and volume per cell, and the number of network connection points. Results show that the volume of the mitochondrial network adapts rapidly to the volume of the cell. Quantification indicates that smaller cells have a higher probability of creating connections within the network than larger cells. The exploration of cells with mutations in fusion genes OPA1 and MFN2 shows that OPA1 _S545R leads to a fragmented network associated with a reduced mitochondrial volume, and that Mfn2R94Q increases the mitochondrial mass and the number of branch points within the network. The sequential analysis presented here thus constitutes a useful tool for studying the relationship between the organization of the mitochondrial network and bioenergetics.



TECHNOLOGY SHOWCASE

15:00 - 15:15

CYTOO's ready-to-use adhesive micropatterns: from Starter's to custom-made.

Alexandra Fuchs

CYTOO S.A., Grenoble, France.

Alexandra Fuchs (co-CEO) will present CYTOO's offer of micropatterned products in both coverslip and microplate formats.



take a
break!

INVITED LECTURE

15:45 - 16:15 - Poster P08

Mechanical Equilibrium in Multicellular Arrangements

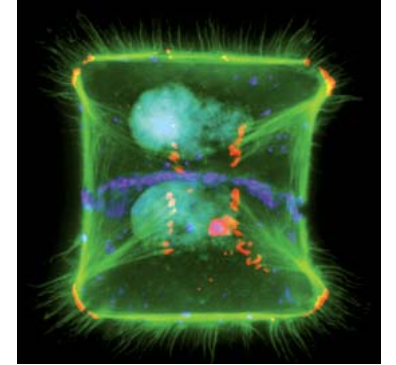
**Qingzong Tseng¹, Eve Duchemin-Pelletier¹,
Hervé Guillou², Odile Filhol-Cochet¹
and Manuel Théry¹**

¹Institut de Recherches en Technologie et Sciences pour le Vivant, CEA, Grenoble, France. ²Institut Néel, CNRS, Grenoble, France.

We investigated the physical laws governing the mechanical equilibrium of multicellular arrangements. Breaking and maintaining this equilibrium are fundamental for embryonic morphogenesis and tissue homeostasis.

Since multicellular equilibrium relies on a spatial regulation of the balance between cell-cell and cell-extra cellular matrix (ECM) adhesions, we studied human epithelial cell pairs confined on defined ECM micropatterns geometries. We found that cells ability to migrate or adopt a mechanical equilibrium was highly sensitive to ECM geometry.

Using new technique of soft substrate micropatterning, we measured the forces cell pairs exert on their microenvironment. Tensions along cell edges were highly dependent on local adhesion. Cell-cell junctions appeared to play a relaxing effect on these tensions. The incorporation of this effect in simple physical modeling of force distribution in cell doublets account for the observed mechanical equilibria.



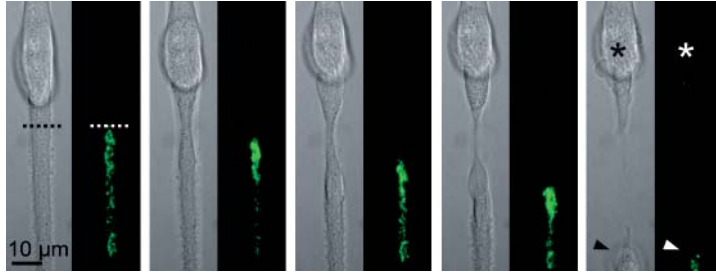
A cell doublet in a mechanical equilibrium on micropatterned ECM. Green: Actin, Red: Paxillin, Purple: β -catenin, Light blue: Nuclei

PRESENTATION ABSTRACT

16:15 - 16:30 - Poster P03

A novel laser nanosurgery approach allows the characterization of Golgi de novo biogenesis

Paolo Ronchi, Carolina Taengemo, Rainer Pepperkok
EMBL, Heidelberg, Germany
ronchi@embl.de



A BSC1 cell, expressing the Golgi marker GalT-GFP and grown on patterned coverslips (Gum thick lines), undergoes nanosurgery to generate a living Golgi-free karyoplast. (image by C. Taengemo)

The Golgi complex is a key organelle of the secretory pathway that receives and distributes material from the endoplasmic reticulum. Live cell studies monitoring the dynamics of GFP-tagged Golgi proteins have demonstrated that this organelle is highly dynamic, raising the fundamental question of how it acquires and maintains its steady state architecture and function. Two major models for Golgi biogenesis have been proposed. One suggests that the Golgi is synthesized de novo from the endoplasmic reticulum, the second postulates a pre-existing Golgi template that serves as a scaffold for Golgi biogenesis. To test these hypotheses directly, we have developed an approach, in which we remove the entire Golgi complex from living cells with an approach based on microcontact printing and laser nanosurgery and subsequently we analyse the “Golgi-less” karyoplast by time-lapse and electron microscopy (EM). We show that biosynthetic transport is blocked after Golgi removal but restores 12 hours after laser nanosurgery coincident with an ordered assembly of stacked Golgi structures with Golgi matrix proteins preceding Golgi enzymes. Functional analyses by siRNA mediated GM130 knockdown further demonstrate the importance of the Golgi matrix protein during this de novo Golgi biogenesis. With high resolution time-lapse microscopy and EM we are now trying to understand the mechanisms and kinetics of this process in more details.

PRESENTATION ABSTRACT

16:30 - 16:45 - Poster P04

A computational imaging approach to systematically study the global organization of endomembranes with probabilistic density maps.

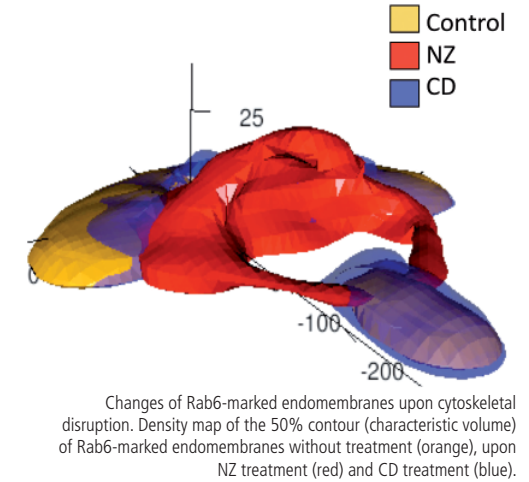
Kristine Schauer, Tarn Duong, Kevin Bleakley, Sabine Bardin, Michel Bornens and Bruno Goud.

Institut Curie, CNRS UMR 144, Paris, France

Despite a good knowledge of the molecular mechanisms involved in membrane trafficking and membrane homeostasis, the global organization of membranous compartments is not known. Taking advantage of adhesive micropatterning technology, which enforces cells to take a certain reproducible shape and impairs migration, we have developed a computational imaging approach that quantifies the 3D steady-state organization of several membranous compartments.

Applied to several well-known marker proteins, this revealed the average steady-state organization of early endosomes, multivesicular bodies/lysosomes, endoplasmic reticulum exit sites, the Golgi apparatus and Golgi-derived transport carriers in crossbow-shaped cells. The steady-state organization of each tested endomembranous population was well-defined, unique and in some cases depended on the cellular adhesion geometry. Density maps of all endomembrane populations became stable when pooling several tens of cells only, and were reproducible in independent experiments, allowing construction of a standardized cell model. A statistical test was developed and applied to reveal changes in 3D organization. Subtle changes in steady-state distribution of endomembranes induced by disruption of the cellular cytoskeleton were detected with strong statistical significance requiring only twenty cells.

Conclusions: Combining micropatterning with construction of endomembrane density maps provides a first and powerful tool to systematically study intracellular trafficking determinants. Beyond implications in the field of cellular biology, this interdisciplinary methodology is potentially applicable to the automatic detection of abnormal phenotypes in diverse applications including high-content screening.



PRESENTATION ABSTRACT

16:45 - 17:00 - Poster P05

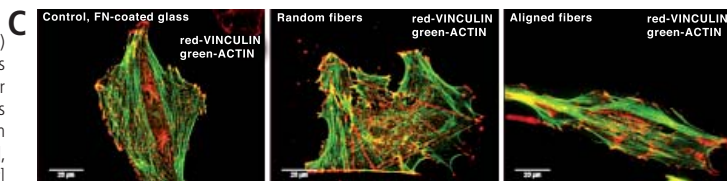
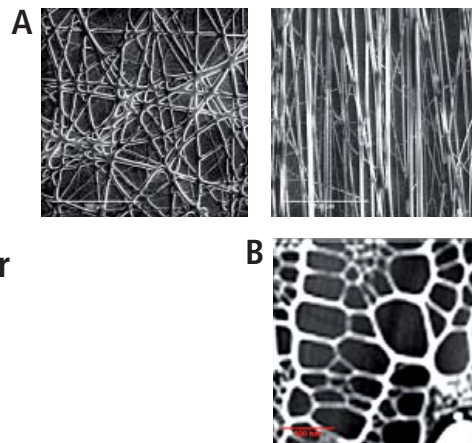
Fibronectin patterning on substrates of electrospun Poly-(Ethyl Acrylate) – an Approach for Guiding the Cellular Behaviour

Dencho M. Gugutkov, Manuel Sánchez, George Altankov

Institute for Bioengineering of Catalonia (IBEC), Barcelona, Spain

Previous research show that the process of fibronectin (FN) fibrillogenesis can be induced on the surface of poly(ethyl acrylate) (PEA) in the absence of cells. It enhances FN-FN interactions leading to the formation of a biologically active protein network. In this work we further explore this phenomena through deposition of this FN network on a spatially organized electrospun PEA microfibers to mimic the organization of ECM. Randomly oriented and aligned PEA-microfibers with average diameter of $\sim 3\mu\text{m}$ were successfully electrospun and deposited on glass plates. AFM reveal successful deposition of FN-network on fibres but not on the glass support. The overall morphology of fibroblasts adhering for 2 hours on randomly oriented PEA-microfibers showed irregular cell spreading with multiple projections, resembling early stellate morphology. Conversely, an extended cell shape, parallel to the fibres axis was observed on aligned ones. The cells respond to these micropatterned surfaces developing linearly organized focal adhesion complexes and actin stress fibres. It is widely accepted that FN fibrillogenesis occurs only on the surface of living cells, but here we show that FN is able to self-assemble on the surface of some biomaterials. That is to say, as a consequence of the protein-material interaction, leading to a substrate-induced FN fibrillogenesis. More importantly however is that this FN network is biologically active and may be used for guiding the cellular behavior at biomaterials interface.

Fibronectin patterning on substrates of electrospun Poly-(Ethyl Acrylate)
A: SEM micrographs of randomly deposited and aligned PEA-fibers
B: AFM micrograph of FN absorbed in network-like manner over the PEA-fibers e. g. substrate-induced fibrillogenesis
C: Indirect immunofluorescence of focal adhesions and actin cytoskeleton staining of fibroblasts cultured on FN-coated glass [left], randomly deposited [middle] and aligned PEA-fibers [right]



PRESENTATION ABSTRACT

17:00 - 17:15 - Poster P06

Quantification of Blebbistatin effects at very low doses using Adhesive Micropatterns

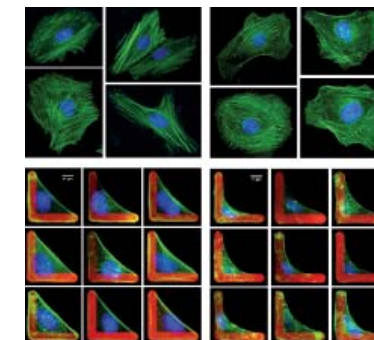
Sébastien Degot, Muriel Auzan, Violaine Chapuis, Alexandra Fuchs and Michel Bornens

CYTOO Cell Architects, 7 parvis Louis Néel, Grenoble, France
www.cytoo.com

HCS is a powerful tool whereby complex cellular pathways and processes can be studied in individual cells. To date, most HCSs are carried out with adherent cell lines grown on homogeneously adhesive surfaces, resulting in a large variability of cell shape, morphology and behavior, which in turn complicate data analysis and limit the potential of HCS.

CYTOO has developed an innovative technology based on AMP that opens new avenues for HCS. By controlling the location of cellular adhesive and non-adhesive areas AMP allow a highly reproducible and polarized cell internal organization similar to that found in tissue, which translates into a dramatic reduction in cell-to-cell variability. By introducing cell normalization, AMP combines reliable quantification with improved cell phenotyping when screening drugs or siRNAs. In addition to being compatible with adherent cell lines, current assays and existing equipment, the major advantages of AMP are: straightforward image capture, simplified analysis, improved assay reproducibility and higher sensitivity.

To illustrate the potential of AMP, we conducted a series of assays with blebbistatin, a model drug whose effects on the actin cytoskeleton are almost impossible to quantify using conventional 2D cultures. We first determined cellular parameters for studying blebbistatin effects and show that one can efficiently quantify drug impact on cells at very low doses, a thousand times less than what is commonly used. We also demonstrate that such analyses on AMP can be performed using very few cells (<50) in contrast to the hundreds of cells usually necessary to obtain statistically valid results. Finally, we introduce the concept of the "Reference Cell™", which can be used as a universal standard for comparability between platforms in dose response assays or screens.



HeLa cells on plain fibronectin and on fibronectin L micropatterns treated with 5 μM Blebbistatin for 1h or left untreated (Control).
Blue: Nucleus, Green: Actin, Red: Fibronectin micropattern

POSTER

Poster P07

Collective cell migration during morphogenesis: role of atypical cadherins Dachsous (Ds) and Fat (Ft)

Amit Kumar, Priya Srivastava, Satish Sasikumar, Pradip Sinha

Indian Institute of Technology, Kanpur, India

Collective cell migration involves movement of interconnected cells and is important for animal morphogenesis. Dorsal closure during *Drosophila* embryogenesis or healing of epithelial wounds are examples of collective cell migration. While reorganization of cytoskeleton is a necessary prelude for collective cell migration, mechanisms regulating such cytoskeletal reorganization en masse are yet to be understood. It is also believed that the triggers for directed and collective cell migration are generated at the interface of cells with differential adhesive properties and/or tension. Here we show that the atypical cadherin Dachsous (Ds) and its binding partner Fat (Ft) regulate collective cell migration during epithelial morphogenesis. Using Laser Scanning Confocal Microscopy, we have observed that both Ds and Ft localize at the Leading Edges (LEs) formed at the interface of differentially adhesive cells in *Drosophila* imaginal epithelia or at embryonic lateral epithelium during dorsal closure or healing epithelial wounds. We further show that Ft/Ds serve as global regulators of collective cell migration, a role reminiscent of their function during planar cell polarity (PCP). Our results provide a novel role of the PCP pathway in the regulation of collective cell migration during animal morphogenesis.

POSTER

Poster P09

Micropatterned fibronectin-gradients induce polarization and haptotaxis in primary fibroblasts

Tatjana Autenrieth, Martin Bastmeyer

Zoology Institute, University Karlsruhe, Germany

Cell polarization and migration are essential for the function of multicellular organisms and can be evoked and/or directed by gradients of guidance molecules. Cell movement induced by gradients of soluble factors is a well-studied phenomenon and referred to as chemotaxis. In contrast, much less is known about haptotaxis. A possible candidate for haptotaxis is fibronectin, a molecule of the ECM mediating cell adhesion through integrin-receptor signaling.

For studying polarization and specific cell migration on adhesive, microstructured fibronectin gradients (Haptotaxis) we use μ CP. Centrosome position is a marker for cell polarization as it is orientated towards the leading edge of a polarized cell. We use this fact to study cell polarization in primary fibroblasts on graded, striped fibronectin patterns. Quantitative analysis reveal, that upward polarization (>70%) is provoked by the gradient in high fibronectin-coated areas. Treatment with Blebbistatin, a myosin II inhibitor, further shows that upward polarization depends on intracellular forces.

In addition we perform time-lapse studies to investigate cellular migration in micropatterned fibronectin dot gradients. These gradients we use induce specific upward migration in primary fibroblasts (80%). Furthermore we display, that in 80% of the upward migrating cells the Golgi complex is located behind the nucleus. After treatment with Nocodazole and Taxol only half the cells migrate upward with a 30% rear localization of the Golgi. These results reveal that the reorientation of the Golgi by microtubules plays a fundamental role in haptotaxis.



High Content Analysis. Precisely.