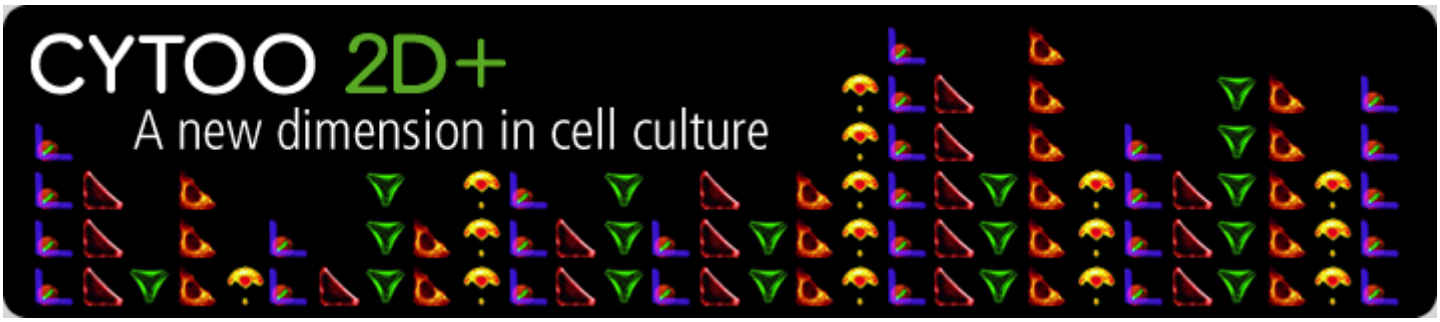


What is Micropatterns?

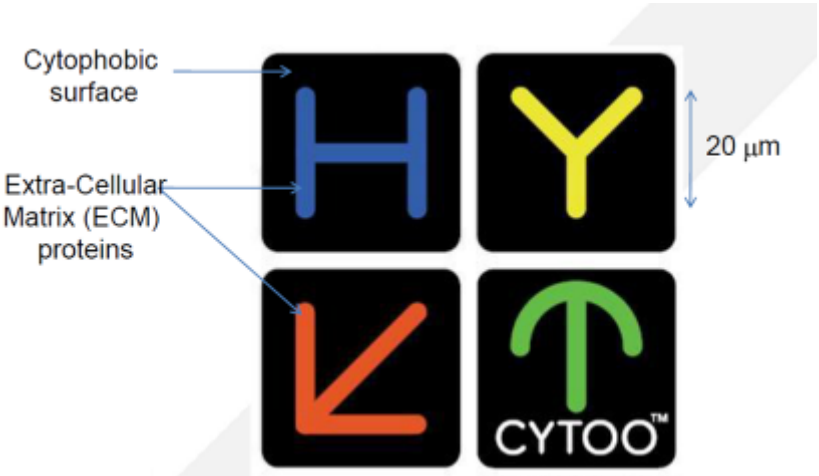
CTYOO 產品是在高階影像等級的玻璃上，先以使細胞無法貼附的 **Cytophobic** 塗料處理，再以光蝕刻的技術，精準地留下特定的幾何形狀，並在這樣的幾形狀上 coating 上特定的細胞間質蛋白 (extra cellular matrix , ECM, such as fibronecin, collgene, Laminin, ect…) 。這樣的細胞培養工具藉由精準的控制細胞貼附的型式，提供了體外培養細胞結構上的引導，使細胞表現出更接近體內生理的表型及框架，我們稱這樣的細胞培養技術為 2D + cell culture

這樣的工具可以：

☑ 提高試驗的再現性，靈敏性和歸納量化複雜的生物性狀

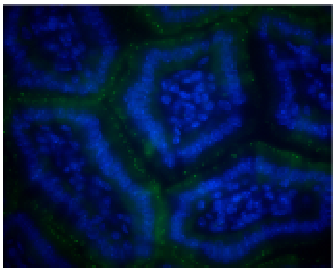
☑ 進一步分析細胞結構 、機械性刺激和細胞功能之間的關係

☑ 模擬重現細胞在生物體內的環境，並使性狀易於觀察分析



In conventional 2D cell culture the spatial information got by the cells in vivo is lost

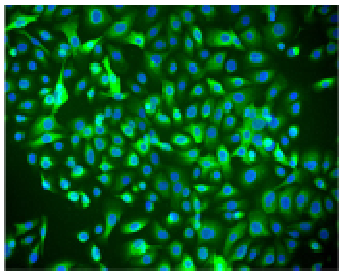
Cells in vivo



- organized
- uniform
- constrained
- polarized

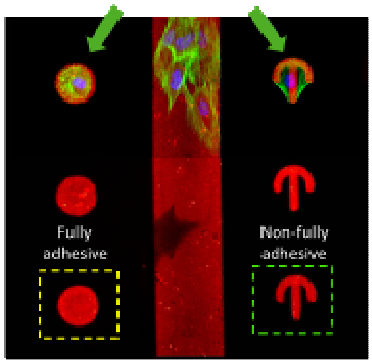
VS

Cells in 2D culture



- different shapes
- different sizes
- free to move
- non-polarized

Normalized cells

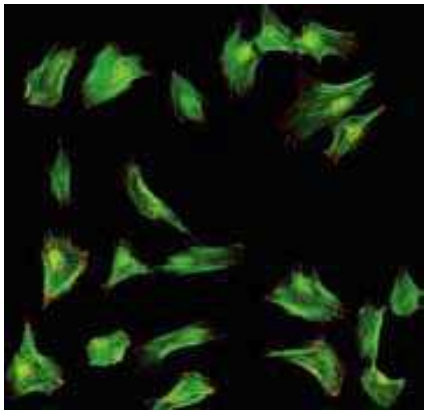


CYTOO adhesive micropatterns

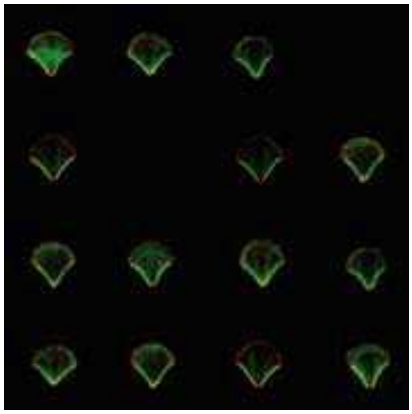
A breakthrough in quantitative cell analysis

精準的對照條件

在 CYTOO 陣列上，細胞呈現相同的結構



Cells in a standard culture dish



Cells cultured on CYTOO products

細胞貼附在塗有細胞間質蛋白的 micropattern 陣列上時，會隨著 micropattern 的引導在不能貼附的材質上張開成特定的幾何結構。這樣特定形狀的貼附接觸分布，促使細胞產生固定且再現性極高的極性機制。細胞位置、細胞形狀、細胞極性、以及細胞內部的結構都可標準化。

取得優勢

分析更直接，結果可信度更高，探索發現更快速

- Reduce cell to cell variability
- Control the location of cell compartments and protein networks
- Achieve simple and rapid image analysis
- Improve assay reproducibility
- Map a standard? averaged cell to be used as a Reference Cell™

引領潮流

Cell biology and high throughput screening

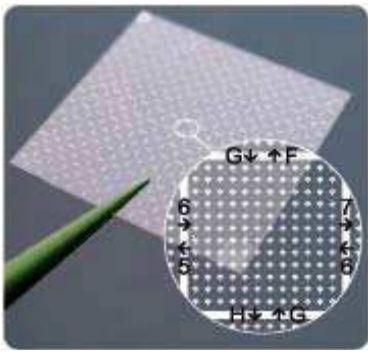
Application assays:

- Cell Shape and Actin Cytoskeleton
- Microtubule network
- Cell Polarity and Organelle Positioning
- Cell Division and Mitotic Spindle Orientation
- Quantitative Cell Phenotyping
- Cell signaling
- Toxicology
- ...

CYTOO 產品型式

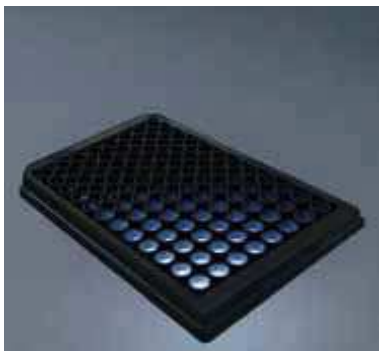
依您的需求分為

CYTOOchips™
for research



The glass coverslip format with an array of up to 20,000 micropatterns and a printed grid for easy localization.

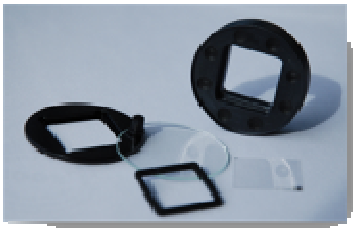
CYTOOplates™
for screening

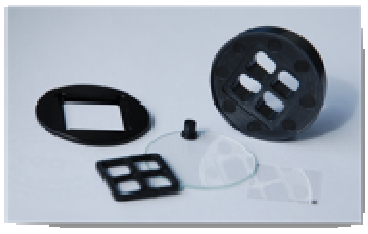


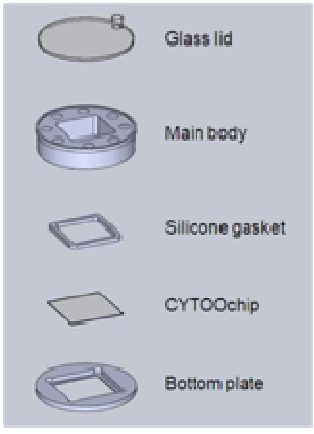
The standard glass bottom microplate format presenting over 1,000 micropatterns per well.

CYTOOchambers™ *1 and 4 wells*

另有專用規格的活體影像磁座可供長時間的活體影像擷取

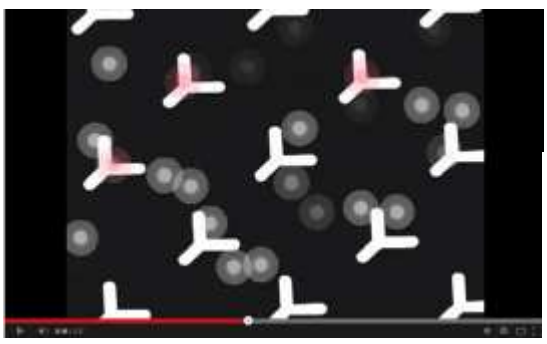






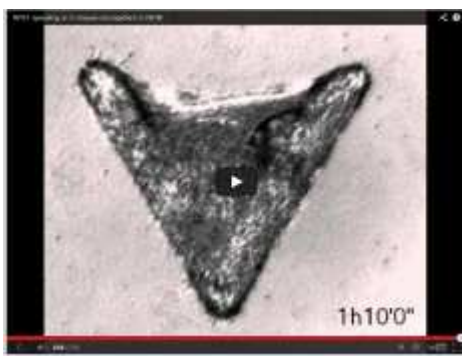
很抽象嗎？來看看影片吧！

細胞在 CYTOO micro pattern 陣列上形成一致的結構
可以平均多個細胞影像，得到多個樣品數的影像定量
http://www.youtube.com/watch?v=nKMDoHn8yFE



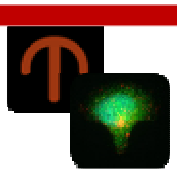
影片 QR code

RPE1 細胞在 V shape micropattern 張開的 RICM 影像 (credits : M Thery/ M Bornens)
http://www.youtube.com/watch?v=2ayd7H2c6fM



影片 QR code

2D+ Single cells



- Mitochondria assay
- Receptor internalization
- Actin Cytoskeleton
- Cell division
- Stem cell differentiation

選擇適合您應用的 micropattern

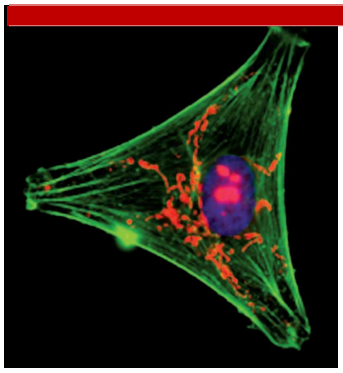
客製化特殊 micropattern 請洽創世紀生技

	Disc	Crossbow	H	Y	L
Micropatterns					
Stretched cells					
Application examples:					
Cell shape control and arraying	✓	✓	✓	✓	✓
Cell polarization		✓			
Cell division			✓	✓	✓
Cell contractility analysis				✓	✓
Noteworthy Applications	. Array cells . Control cell spreading and contraction . Control ciliogenesis . . .	. Polarize cells . Study internal organelle/endomembrane spatial organisation . Study microtubule dynamics . Control asymmetric cell division	. Control symmetric cell division . Quantify cell-cell adhesion/contacts . . .	. Study Multipolar divisions and supernumerary centrosomes . . .	. Measure contractility . Measure subtle alterations in spindle orientation during mitosis . . .
References:		PNAS 103(52):19771-6 Nature 447, 493-496.	Nature 447, 493-496. Genes Dev. 22(16): 2189-203.	CMC 63(6):341-55. Genes Dev. 22(16): 2189-203.	CMC 63(6):341-55. Nat. Cell Biol. 7(10): 947-53.

已經發表在文獻上 micropattern 應用的眾多細胞種類包括：

- Epithelial cells (HeLa, RPE-1, CHO, MDCK, BSC, MCF10A)
- Fibroblasts (murine NIH-3T3, BHK)
- Adenocarcinoma cell lines (MDA-231, A549)
- Hepatic cell lines (HepG2)
- Primary cells (Rat astrocytes, Rat ventricular myocytes, myoblasts)
- Neurons and neuron progenitors (SH-SY5Y; hippocampal and cortical neurons)
- Stem Cells (Human mesenchymal stem cells, mouse embryonic stem cells/mESCs)

Check here for an updated list of cell types:
www.cytoo.com/celltypes

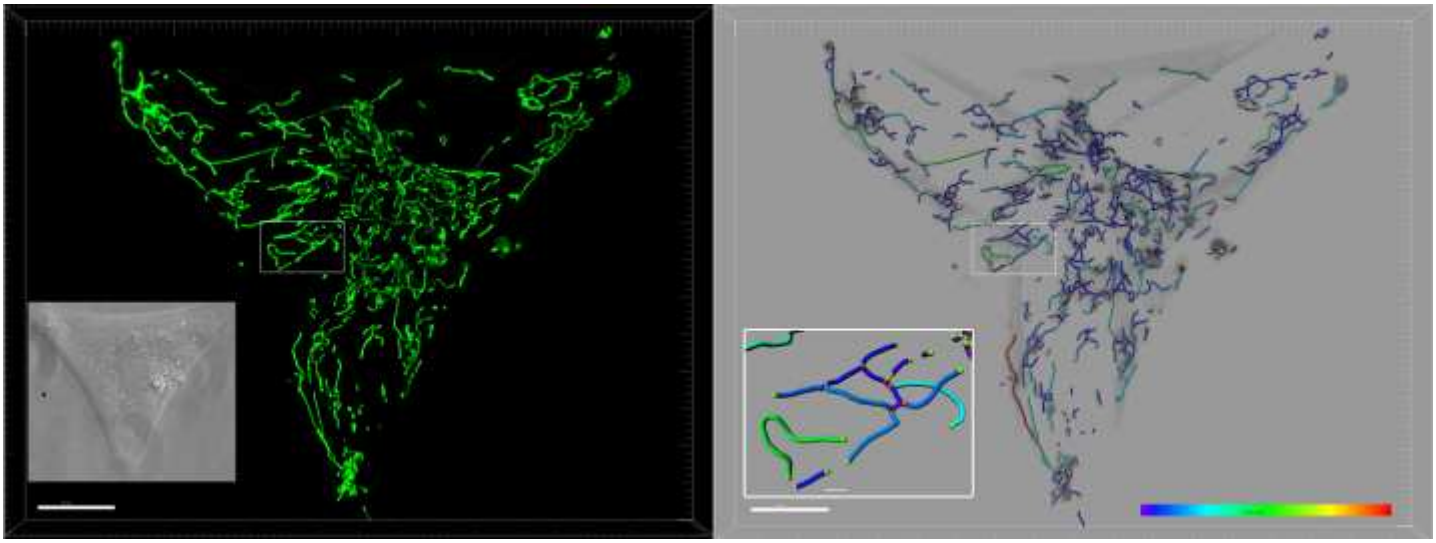


Efficient labeling of mitochondrial networks in micropatterned cells for toxicity studies

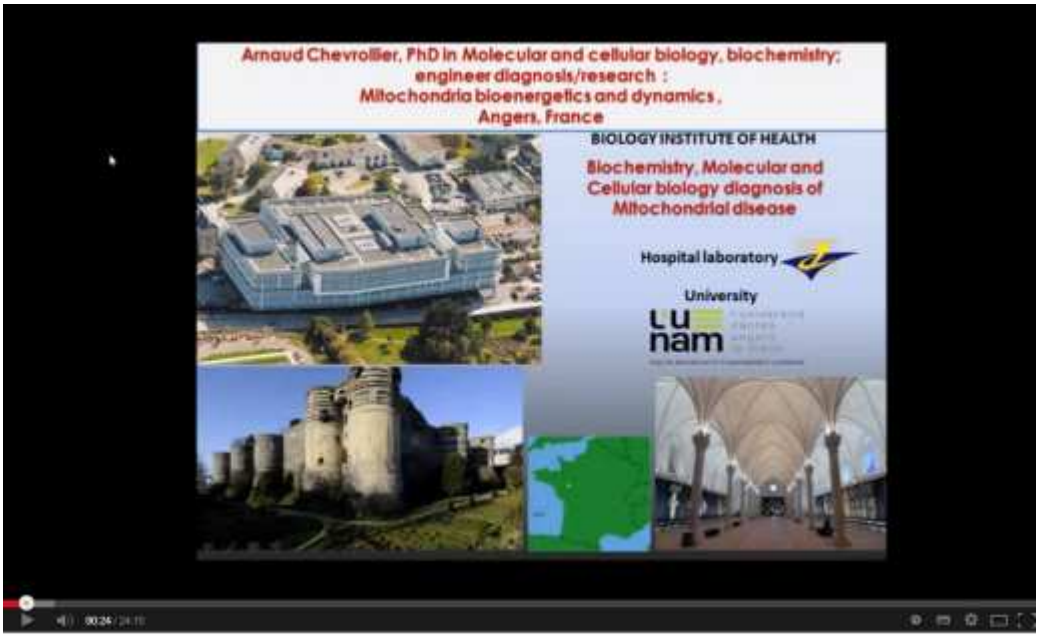
Yoran Margaron, Sebastien Degot, Alexandra Fuchs, Chloe Loiraud *

- Optimized protocol for mitochondrial network labeling in both live and fixed cells
- Cell individualization and normalization thanks to adhesive micropatterns
- Straightforward comparison between different experimental conditions

Standardization of mitochondrial network: diagnostic criteria of mitochondrial diseases – Primary fibroblasts



Cytoo micropattern array ; MitoTracker Green signal ; Mitochondrial network, branch point (red), The color codes show the tubules length between branch points. Control fibroblast



線上 seminar 請上
http://www.youtube.com/watch?v=AvwOnqSWqh4

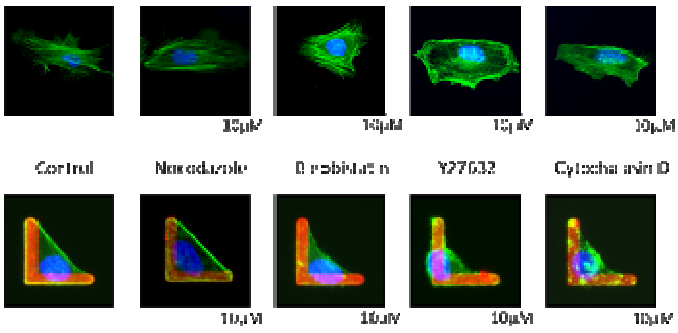
Build a Reference Cell™ for powerful cell phenotype quantification

Muriel Auzan*, Violaine Chapuis*, Joanne Young*, Anne Béghin**, Pauline Ménager*, Sébastien Degot*.

細胞種在常規培養皿時細胞呈現不同的形狀,細胞內的結構, 變異性更是大。創新的 CYTOO micropattern , 可標準化細胞的形狀和形態, 使細胞表現出重複性極高的內部架構,克服細胞圖像定量分析中精確度的挑戰。

Unveiling drug-induced phenotypes on micropatterned cells

HeLa cells were seeded in parallel on full fibronectin or on fibronectin L micropatterns, then treated with drugs at 10µM for 1h (except for Nocodazole at 5µM) or left untreated. Nucleus; Actin; Fibronectin micropattern



Robust quantification of drug effects with only 50 cells

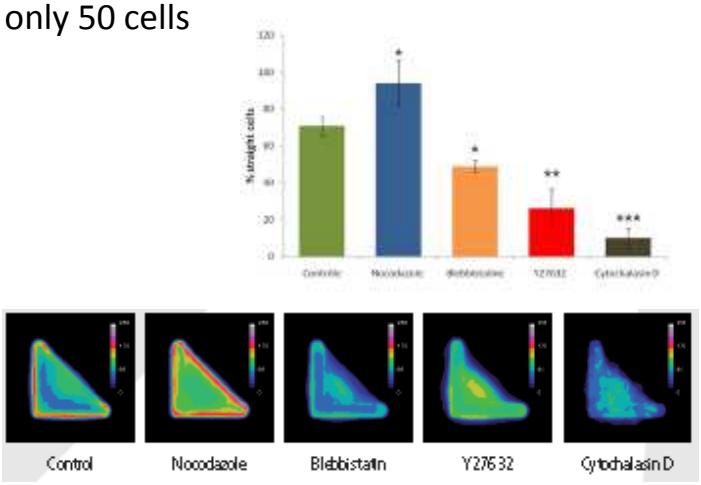


Figure 3: Reference Cell gallery depicting drug effects on the actin distribution in cells on L micropatterns and performed in a 96-well CYTOOplate™. Nocodazole (5µM), Blebbistatin (10µM), Y27632 (10µM) and Cytochalasin D (10µM), n=50 cells for all conditions.

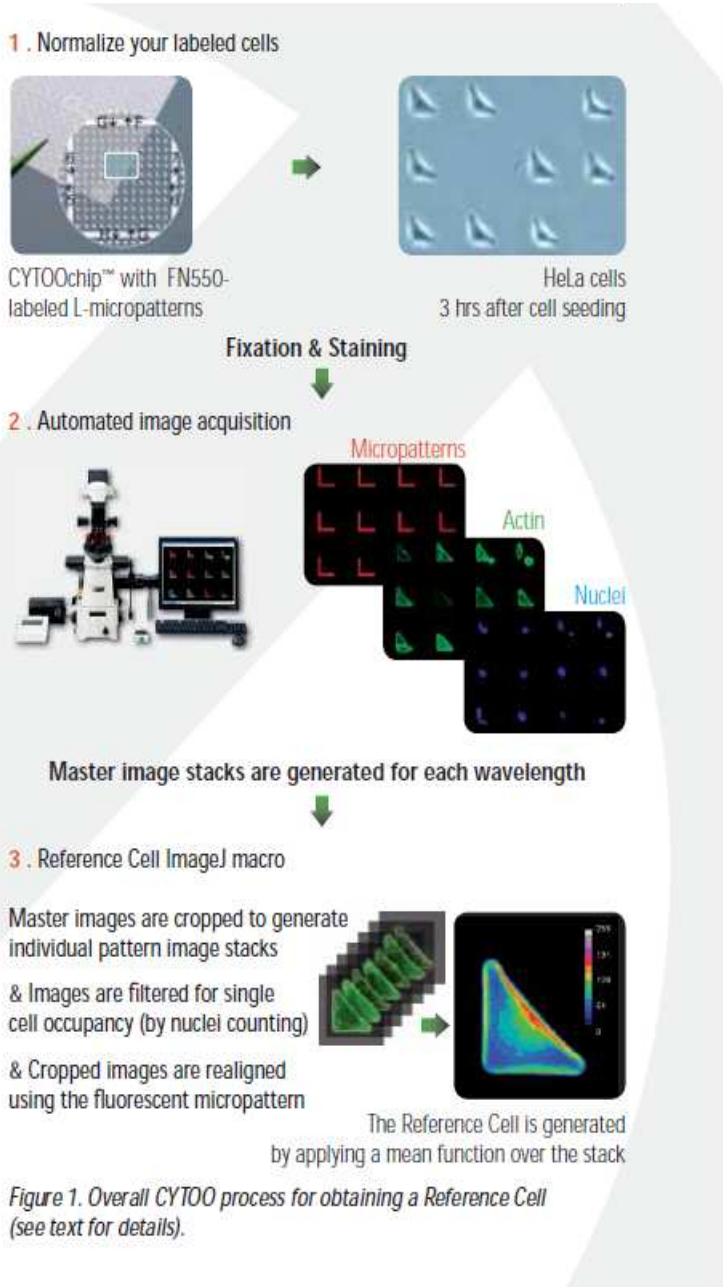
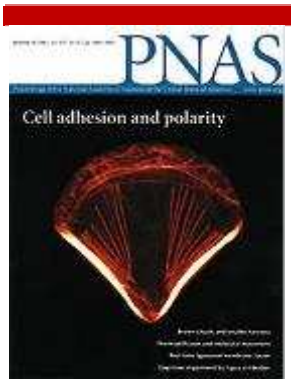


Figure 1. Overall CYTOO process for obtaining a Reference Cell (see text for details).

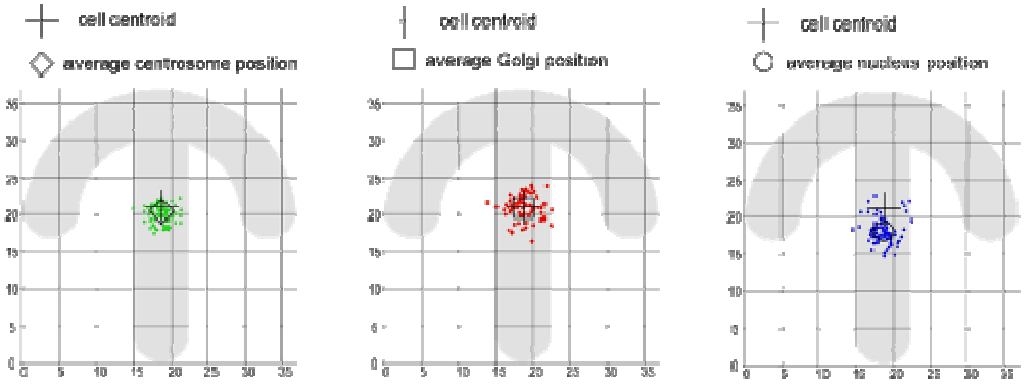
詳細實驗步驟方法請見 JoVE website :
JoVE 46: http://www.jove.com/index/Details.stp?ID=2514

* CYTOO Cell Architects, 7 parvis Louis Néel, Grenoble, France - www.cytoo.com
** Centre Commun de Quantimétrie, Faculté de Médecine Rockefeller, 8 av. Rockefeller Lyon, France
*** 最新的 Reference Cell 分析程式巨集 和 完整的使用說明請洽創世紀生技 tech@biogenesis.com.tw



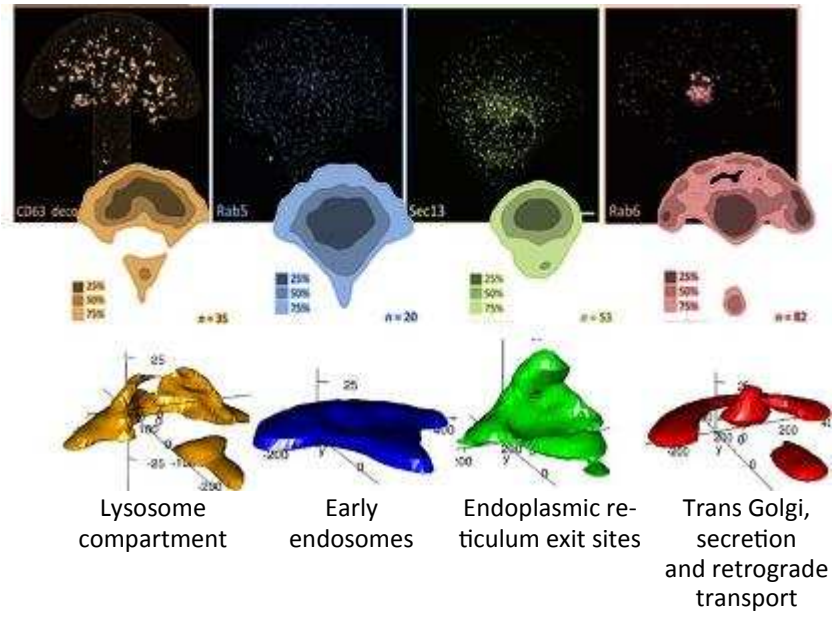
Reproducible internal cell organization in response to the geometry of the micropattern

看看標準化極性的細胞胞器的分布！！



Thiery et al. PNAS (2006)

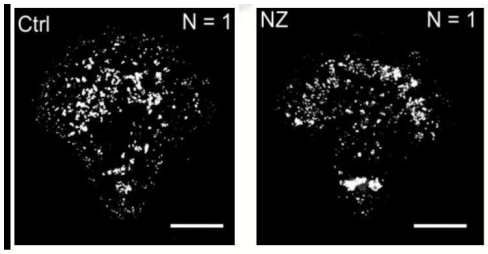
Quantifying the undetectable: Probabilistic density maps on normalized cells



Lysosome compartment | Early endosomes | Endoplasmic reticulum exit sites | Trans Golgi, secretion and retrograde transport

http://www.cytoo.com/CYTOO-applications-endomembrane-network.php

Figure 1: Stable density maps that represent the organization of different endomembrane compartments were obtained from the indicated number of cells (n=35 to 82). 2D and 3D density maps of multivesicular bodies (CD63), early endosomes (Rab5), ER exit sites (Sec13) and the secretory compartment (Rab6) are shown.



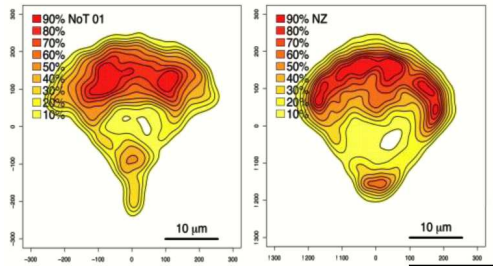
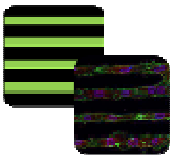


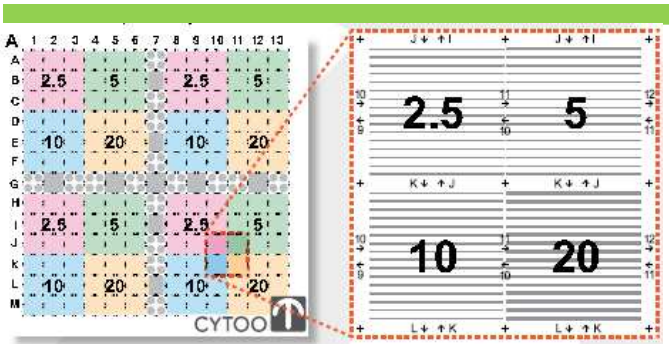
Figure 2:
Top: representative images of individual cells labeled with anti CD63 (left: control, right: nocodazole) yield little insight into the effect of this drug on the multivesicular body (MVB) network.
Bottom: 2D Density maps calculated for the MVB compartment show significant differences between the control and the Nocodazole (NZ) treated cells. Automatically calculated P value < 10-4 with only 20 cells.

Further reading:
1. Duong T, Goud 3, Schauer K. Closed-form density-based framework for automatic detection of cellular morphology changes. Proc. Natl. Acad. Sci. U.S.A. 2012;109(22):8382–8387.
2. Schauer K, Duong T, Bleakley K, Bardin S, Bornens M, Goud B. Probabilistic density maps to study global endomembrane organization. Nat. Methods. 2010;7(7):560–566.

2D+ Cell groups



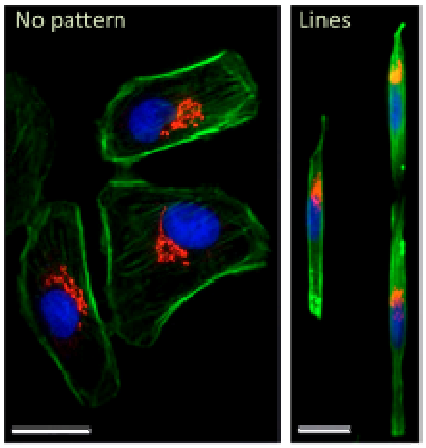
- Angiogenesis
- Mature cardiomyocytes
- Neurite outgrowth
- Myotube hypertrophy
- Hepatocyte BS
- EMT



CYTOO chips™ & CYTOO plates™ Motility

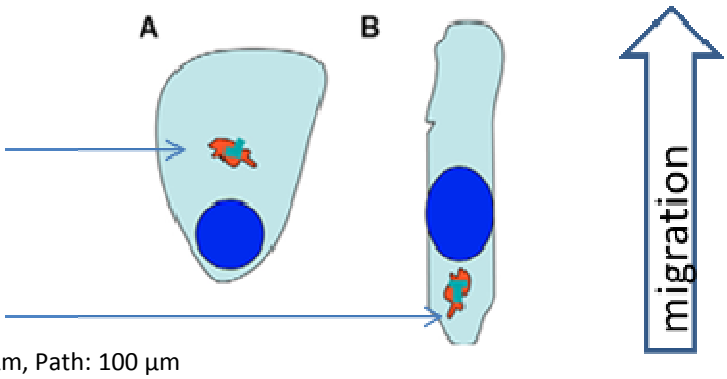
Cell Migration

Cells on fibronectin-patterned lines are polarized



Pouthas F, J Cell Science 2008

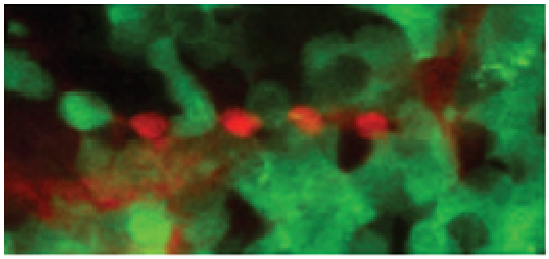
Distinctive mode of migration

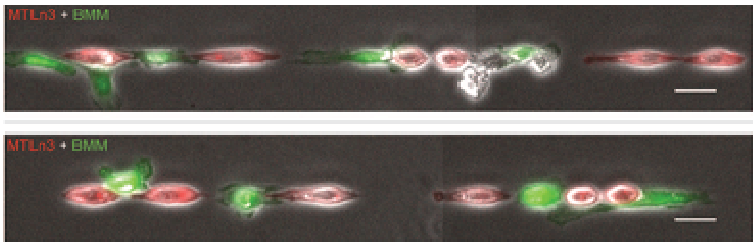


Nodediameter: 70 µm, Path: 100 µm
Structure optimizedfor SH-SY5Y neuroblastomacellline
Protein: PDL; Cytophobigcoating: PLL-g-PEG
Formation of neuriteconnection between adjacent groups of cells; 6 to 10 cell bodies per pattern in average

Model for macrophage- tumor cell pairing and streaming :

robust & physiological





Courtesy of Landes Bioscience, reproduced from IntraVital 2012;1(1):77-85. (Left: in vivo imaging and Right: in vitro imaging on micropatterned 1D tracks)

Model similarities between in-vivo and 1D micropatterns:

- MorphologyBehaviors
- Motility rates

Specificities:

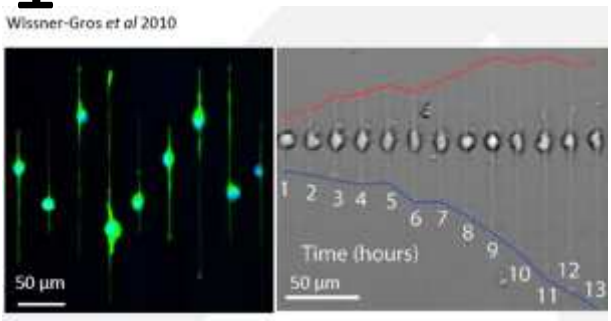
- Tumor cell velocity on 1D reproduce high velocity in vivo (2D model: velocity 10 folder lower)
- Assembly of alternating tumor cells and macrophages identified as steams in vivo were reproduced on 1D lines

CYTOOplate Neuro plate

http://www.youtube.com/watch?v=0rglGMAP78c

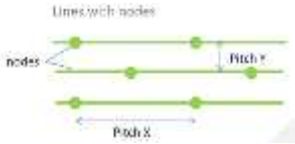
1

Bipolar neuriteoutgrowth & Migration

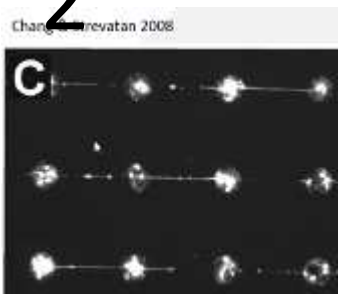


- Neurite outgrowth is highly bi-directional; no branching observed
- Easy single cell neurite length quantification possible
- But the position of cell bodies is not controlled

2

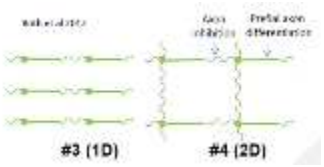


Controlling neuriteoutgrowth & Cell body position

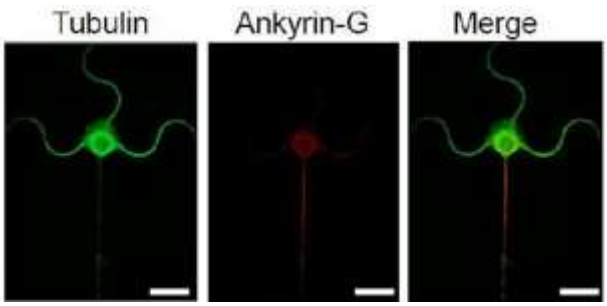


- Cell bodies and outgrowing processes are clearly separated
- The neuronal cell bodies adhere preferentially to the nodes where local adhesiveness is greater and are stabilized there

3 & 4

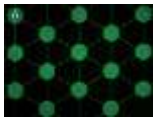


Single neuron network with axon Guidance.
Neuron polarization

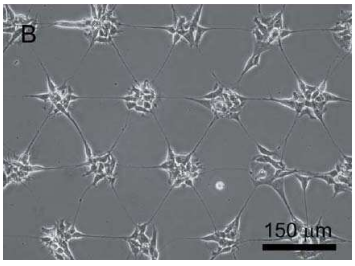


- The curved path geometry provides a strong inhibitory effect on axon specificationand prevents multiple axon formation. Axon rafficking studies are facilitated.
- Curved lines for neuriteoutgrowth are supposed to mimic in vivo neuron path-finding in a crowded environment

5

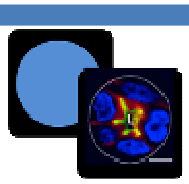


Network Formation Assay



- Connections are progressively created between nodes within 72 h
- Quantification of network formation= Number of connections per node
- Kinetics of neuriteoutgrowth can be measured in time lapse

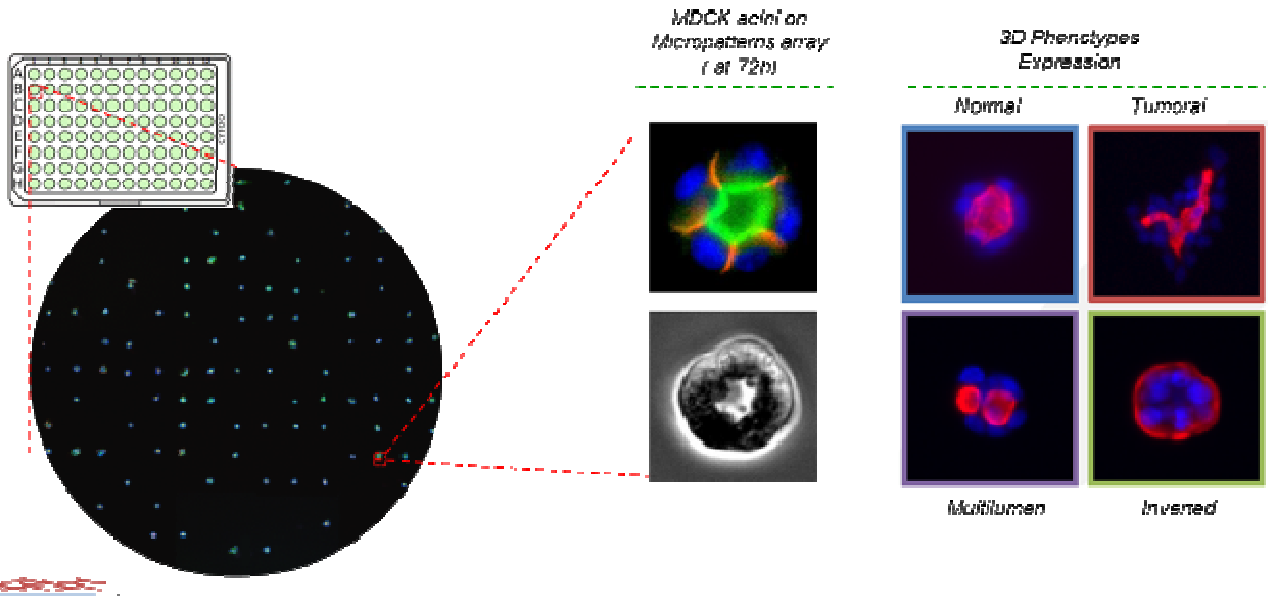
3D+
Cell structures

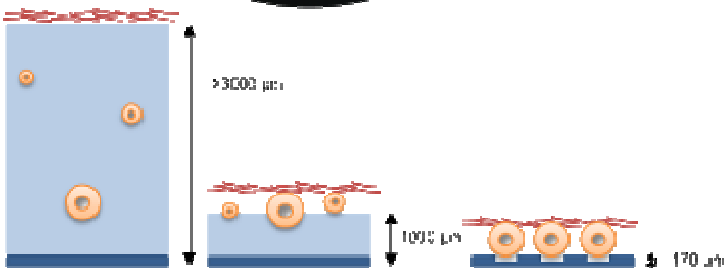


- Acini formation
- Spheroids
- Kidney tubes

3D+ Technologies :

Normalized Acini Structures





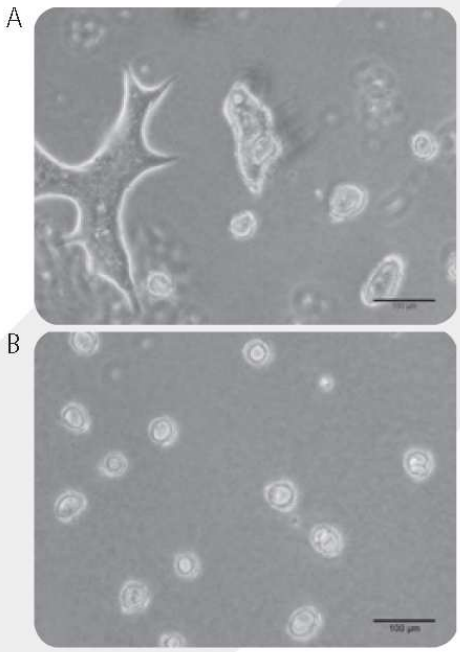
- Screening of drugs in a 3D and automated context
- Validate Drug Targets and Candidates in a polarized 3D context
- Model the effect of Oncogenes, tumor suppressors

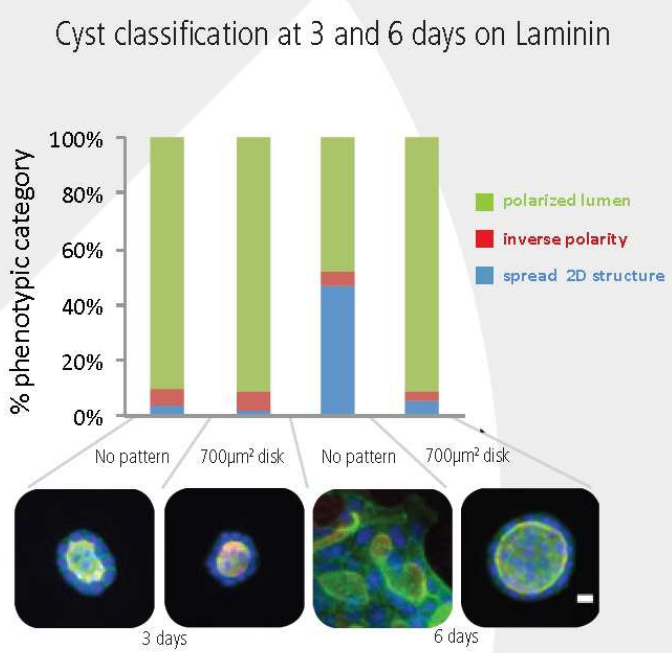
Further reading

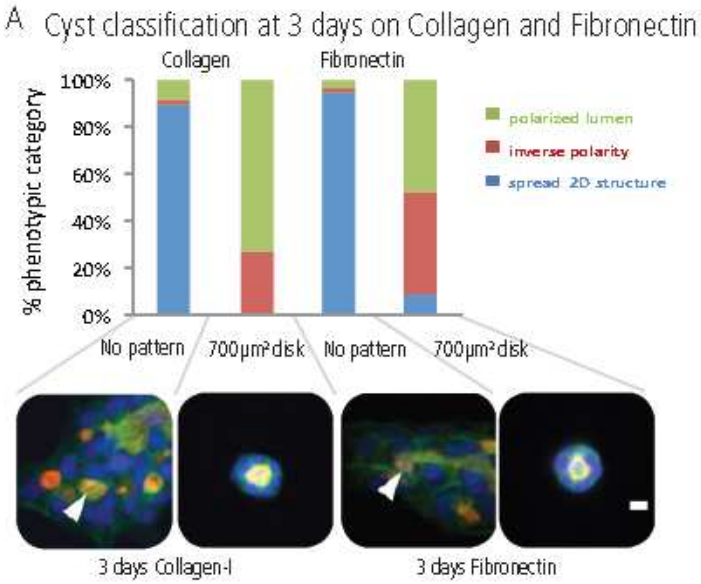
1. Debnath J, Muthuswamy SK, Brugge JS. Morphogenesis and oncogenesis of MCF-10A mammary epithelial acini grown in three-dimensional basement membrane cultures. Methods. 2003;30(3):256–268.
2. Wendt MK, Smith JA, Schiemann WP. Transforming growth factor-ß-induced epithelial-mesenchymal transition facilitates epidermal growth factor-dependent breast cancer progression. Oncogene. 2010;29(49):6485–6498.
3. Kumar A, Xu J, Brady S, et al. Tissue transglutaminase promotes drug resistance and invasion by inducing mesenchymal transition in mammary epithelial cells. PLoS ONE. 2010;5(10):e13390.
4. Sang L, Miller JJ, Corbit KC, et al. Mapping the NPHP-JBTS-MKS protein network reveals ciliopathy disease genes and pathways. Cell. 2011;145(4):513–528.
5. Li H, Yang W, Mendes F, Amaral MD, Sheppard DN. Impact of the cystic fibrosis mutation F508del-CFTR on renal cyst formation and growth. Am. J. Physiol. Renal Physiol. 2012;303(8):F1176–1186.
6. Qin X-Y, Fukuda T, Yang L, et al. Effects of bisphenol A exposure on the proliferation and senescence of normal human mammary epithelial cells. Cancer Biol. Ther. 2012;13(5):296–306.
7. Wang H, Lacoche S, Huang L, Xue B, Muthuswamy SK. Rotational motion during three-dimensional morphogenesis of mammary epithelial acini relates to laminin matrix assembly. Proc. Natl. Acad. Sci. U.S.A. 2013;110(1):163–168.
8. Härmä V, Virtanen J, Mäkelä R, et al. A comprehensive panel of three-dimensional models for studies of prostate cancer growth, invasion and drug responses. PLoS ONE. 2010;5(5):e10431.

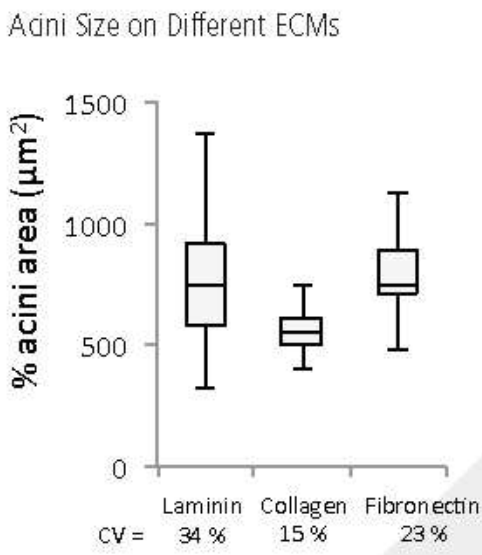
取得以下操作技術資料請洽聯創世紀生技:

- Protocol for seeding MDCK cells on CYTOOchips (MO-EXT-19)
- Hints for using Matrigel (MO-EXT-20).









配合 micropattern, ECM coating, 取代膠體的 3D 細胞培養, 使 MDCK 細胞的 Acini 大小及形成比例再現性更高,結構可維持更久, 並且在 Collagen-I 及 Fibronectin 的基質也能形成 Acini。

ECM	**Laminin-111**		**Collagen-I**		**Fibronectin**	
2D versus 2D+	***No pattern***	***µpattern***	***No pattern***	***µpattern***	***No pattern***	***µpattern***
Lumen formation efficiency (%) at 3 days						
Chip format	70-90	70-90	0	70	0	50
96-well plate	75	60	N.A	40	N.A	N.T
Acini size distribution (CV%)						
Chip format	34	34	N.A	15	N.A	23
Conclusion	Without micropatterns acini collapse and degenerate after 3 days	Laminin coated micropatterns sustain long-term 3D culture. Best conditions for high levels of lumen formation.	N.A	On collagen-I acini formation is dependent on micropatterns. Best conditions for creating a uniform population.	N.A	On fibronectin acini formation is dependent on micropatterns.

Table 3: Performance of conventional 2D flat surfaces compared to 2D+ micropatterns. N.A = Not Applicable. N.T = Not tested

Contents

更多 CYTOO 應用 在

http://www.cytoo.com/CYTOO-adhesive-micropatterns-applications.php

Cell migration
Reproduce *in vivo* conditions to study cell motility

YAP/TAZ
Role of YAP/TAZ in mechanotransduction

Ciliogenesis
Primary cilia growth on micropatterns

Cell Shape and Actin Cytoskeleton
Discover how micropatterns can help you study the cytoskeleton organization

Microtubule Network
Visualizing microtubule dynamics in a predetermined actin cytoskeleton architecture

Cell Polarity and Organelle Positioning
Control, quantify and predict organelle positioning

Cell Division and Mitotic Spindle Orientation
Control mitotic spindle orientation and analyze symmetric, asymmetric and multipolar cell divisions

Quantitative Cell Phenotyping of Cell Behavior
Example of WT and TTL null Mouse Embryonic Fibroblasts

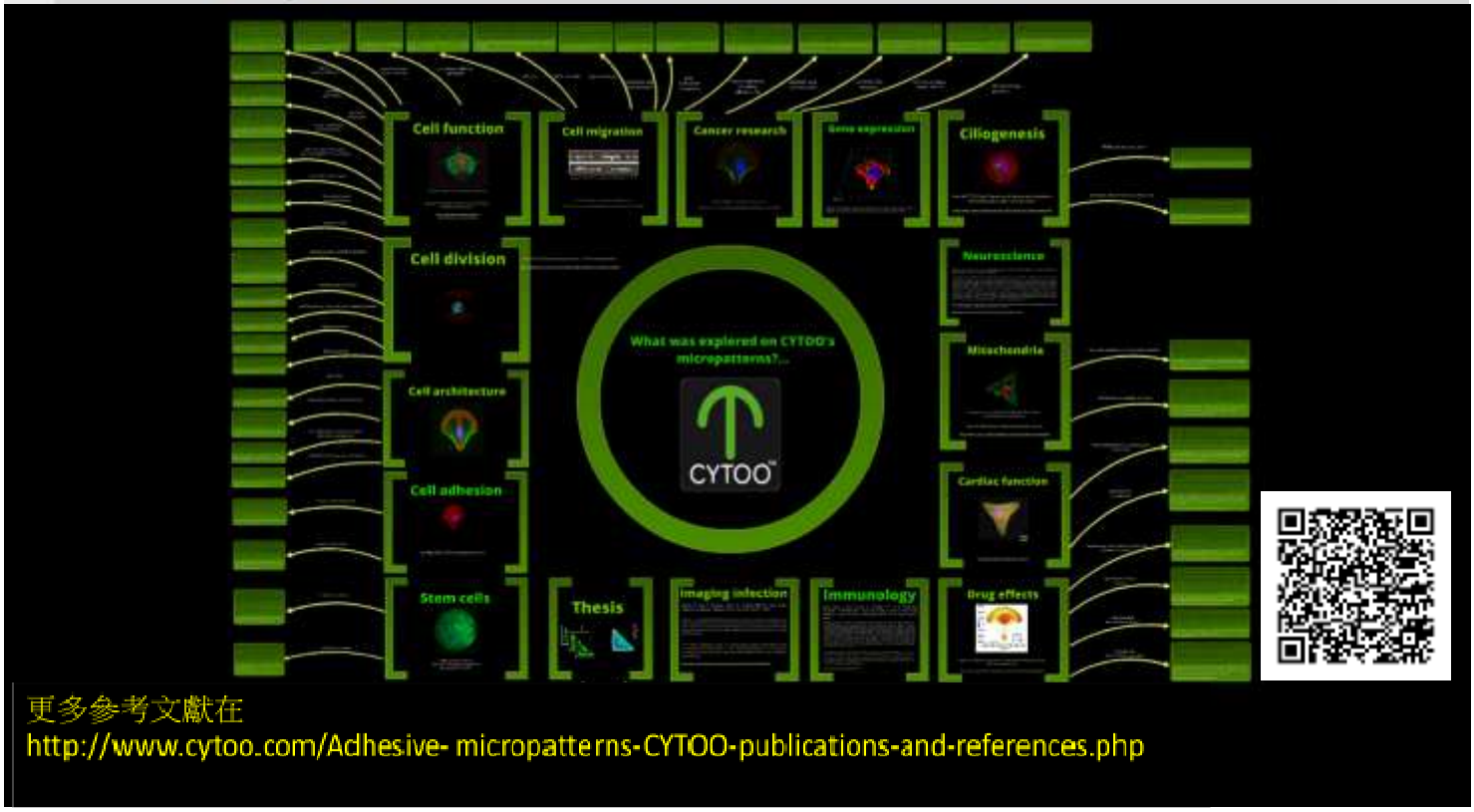
The Endomembrane Network
Quantifying the 3D organization of the various endomembrane compartments under various drug treatments

Cardiomyocyte Sarcomer Organization
Normalized sarcomeric organization of primary cardiomyocytes on micropatterns

Myotube Fusion Assay
Normalizing Myoblast Differentiation into myotubes

Neuron networks
Primary neurons growing neurites on patterned networks

Mitochondrial networks
Watch Arnaud Chevrollier's webinar on the quatification of fusion/fission and mitochondrial dynamics on micropatterns



更多參考文獻在
http://www.cytoo.com/Adhesive- micropatterns-CYTOO-publications-and-references.php

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