3D cell culture & microtechnologies: new insights in carcinoma

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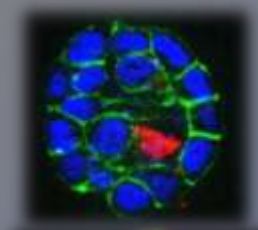
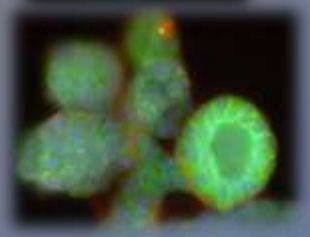
Biochips & Functional Genomics (Biomics) lab

in collaboration with LETI

Phenotypic screening in a physiologically-relevant context





3D cell models
Target discovery / validation
biologists

Emerging micro-technologies – 3D imaging
engineers

A multidisciplinary environment

CEA Grenoble

Hospital

Translational research
clinicians

CHU Inserm Université Joseph Fourier Cea tech Leti

Enter the Third Dimension !

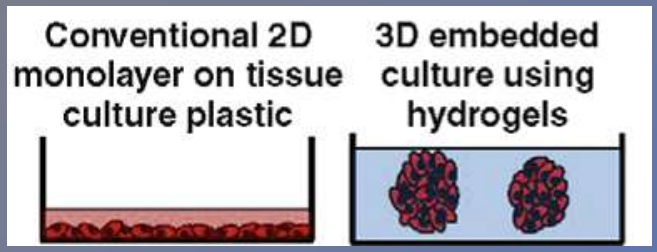
Why 3-D cell culture ?

« Because we're not flat !!!! »

(S. Takayama, Univ. Michigan, Ann Arbor)

=> Cell culture goes 3D with devices that better mimic *in vivo* conditions

The Scientist, *Lab Tools*, Sept. 2012

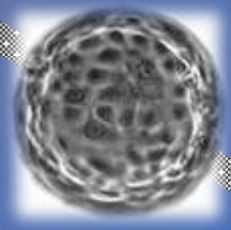


Justification for Using 3D Culture Models

- 3D culture models mimic tissue-like structures more effectively than monolayer cultures
- 3D cultures can exhibit differentiated cellular function absent in 2D cultures
- 3D cultures hold promise for being more predictive of *in vivo* responses to drug treatments

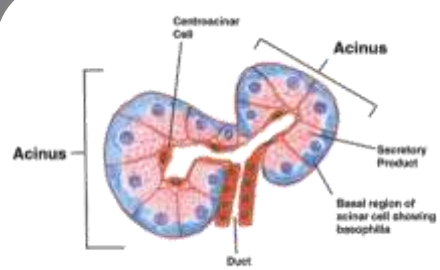
Traditional 2D cell culture

The 3D bridges the gap between cell culture and live tissue

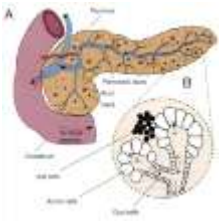


Tissue, animal

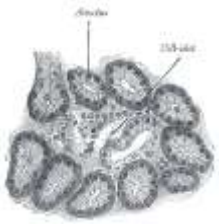
Secretory Epithelium



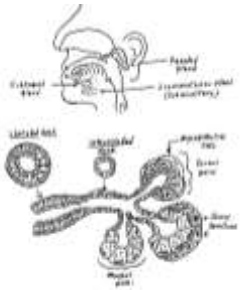
Prostate



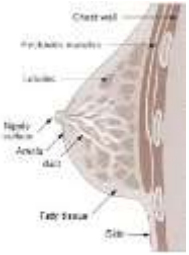
Pancreas



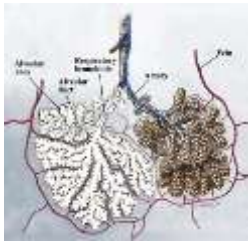
Lung



Salivary gland



Breast

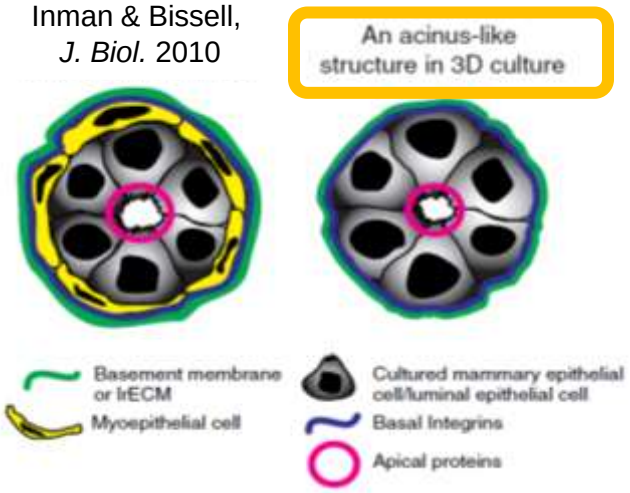


Sweat gland

Acinus

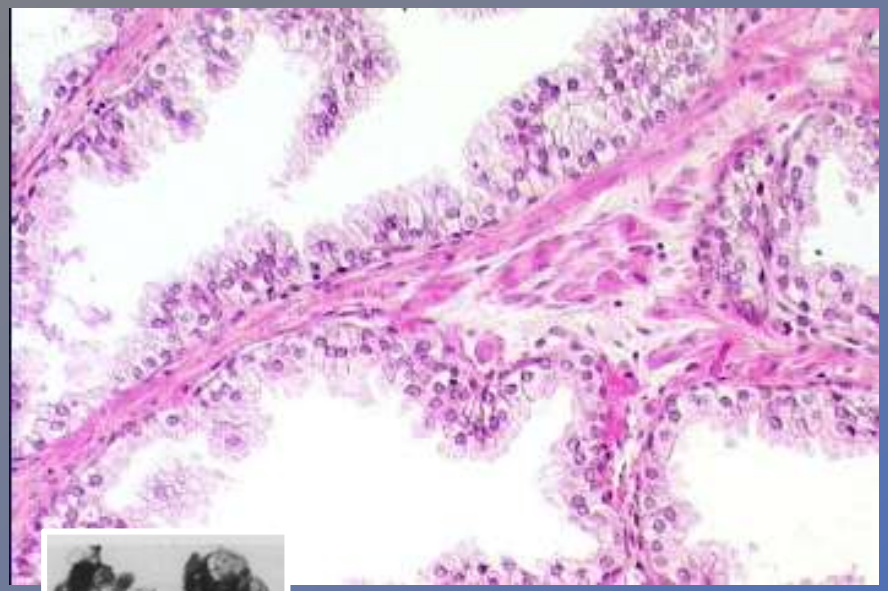
structural & functional unit of glandular tissues

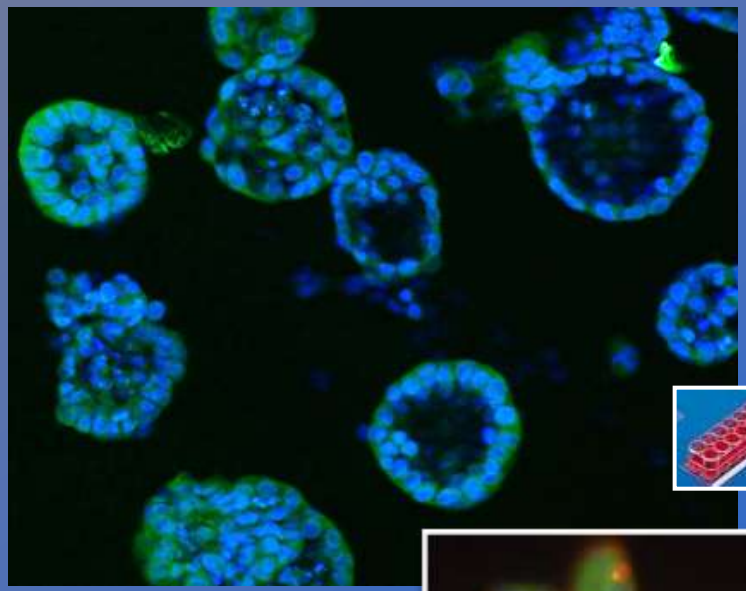
Inman & Bissell, *J. Biol.* 2010

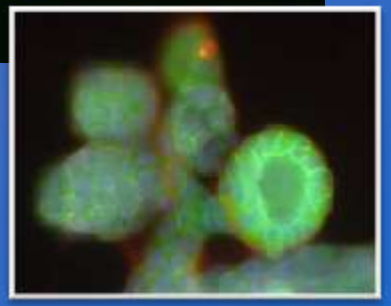


in vivo

in vitro : 3D model

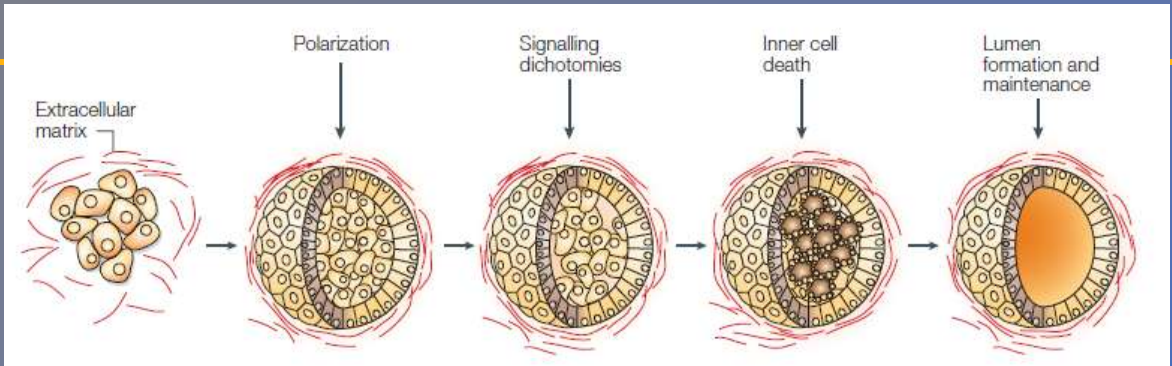






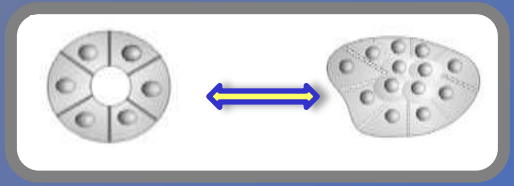
Tree-like structures
(acini + secretory ducts)

DEVELOPMENT



- Models of tumor initiation & cancer development

Differentiation



Proliferation

- Genes involved in 3D polarity (lumen clearance) ?
- Genes involved in the « filling » of lumen (model of carcinogenesis) ?

CANCER

How to reconstruct acini-like structures *in vitro* ?

Nathalie PICOLLET-D'HAHAN, CEA, FRANCE

General Classification of 3D Culture Methods

Scaffold

- Hydrogels
- Inert matrix

Scaffold-free

- Low adhesion plates
- Micropatterened surfaces
- Hanging drop
- Suspension using methyl cellulose, rolling vessel or magnetic levitation

Hydrogel Examples

Animal-derived

- Matrigel®
- Collagen

Alginate / Agarose (Plant-derived)

Synthetic

- QGel® Matrix
- 3-D Life Biomimetic
- Puramatrix

Standard 3D culture

Matrigel

The most widely used hydrogel.
A reconstituted basement membrane (laminin, collagen IV, entactin, GF)

Collagen

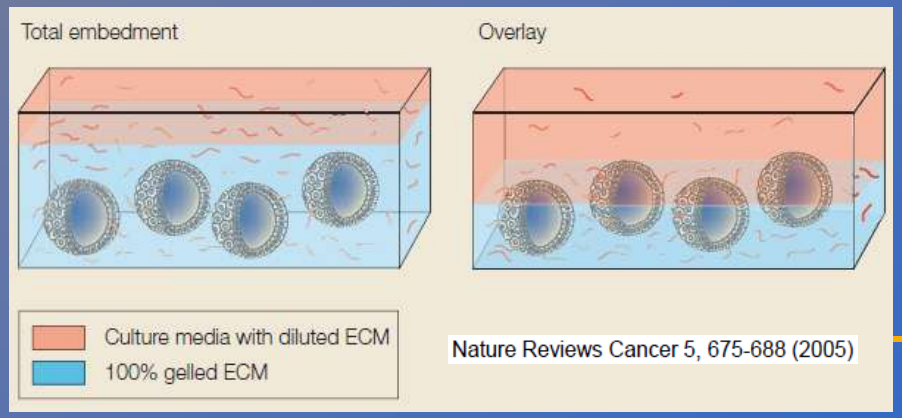
The most abundant class of ECM proteins.
Used to coat surfaces or to embed cells

Puramatrix

Synthetic- Peptide self-assembles into nanofiber structures that form hydrogel (native ECM)

☺ Often contains « magic potion » ingredients similar to serum
☹ Lot-to-lot variability. Challenging to handle at low temperature

Cells are supported with Extracellular Matrix proteins - a soft gel



Nature Reviews Cancer 5, 675-688 (2005)

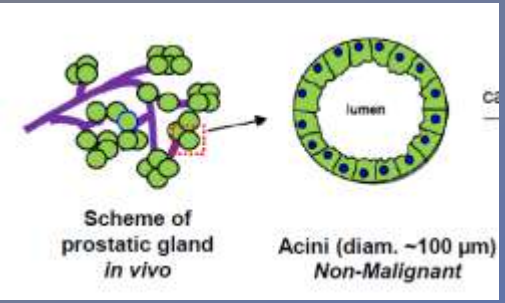
Matrigel properties

4° C → 37° C

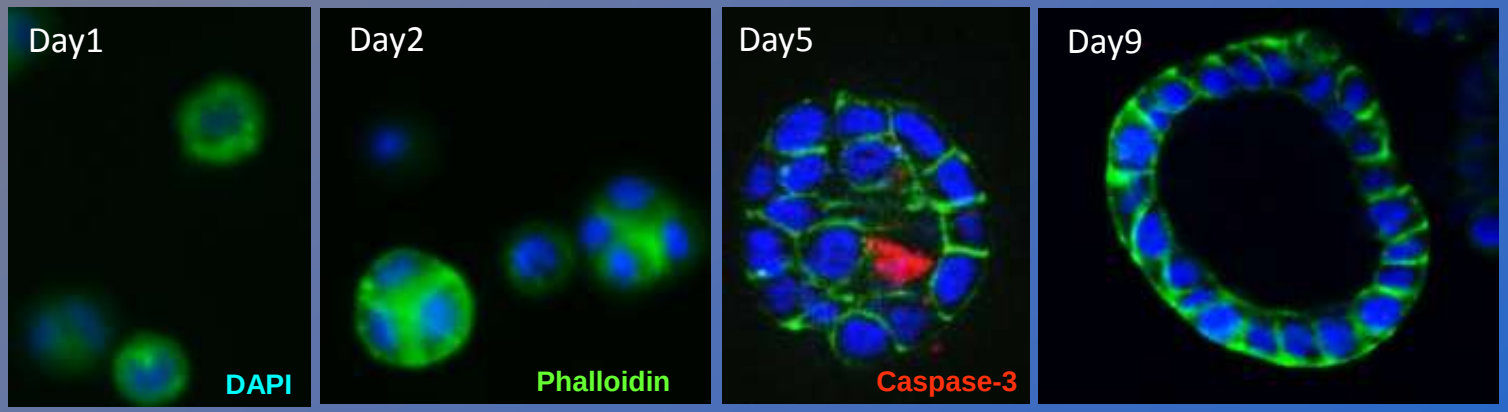
liquid **Gel formation**

Nathalie PICOLLET-D'HAHAN, CEA, FRANCE

Morphogenesis and polarity in 3D

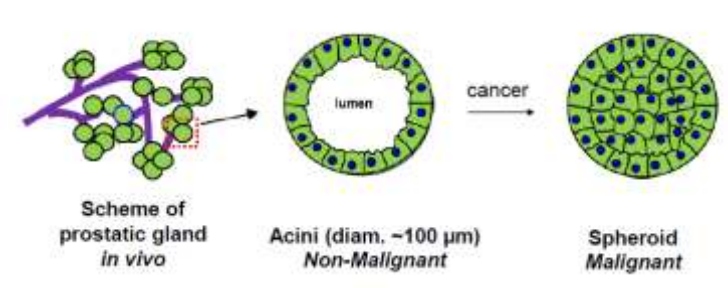


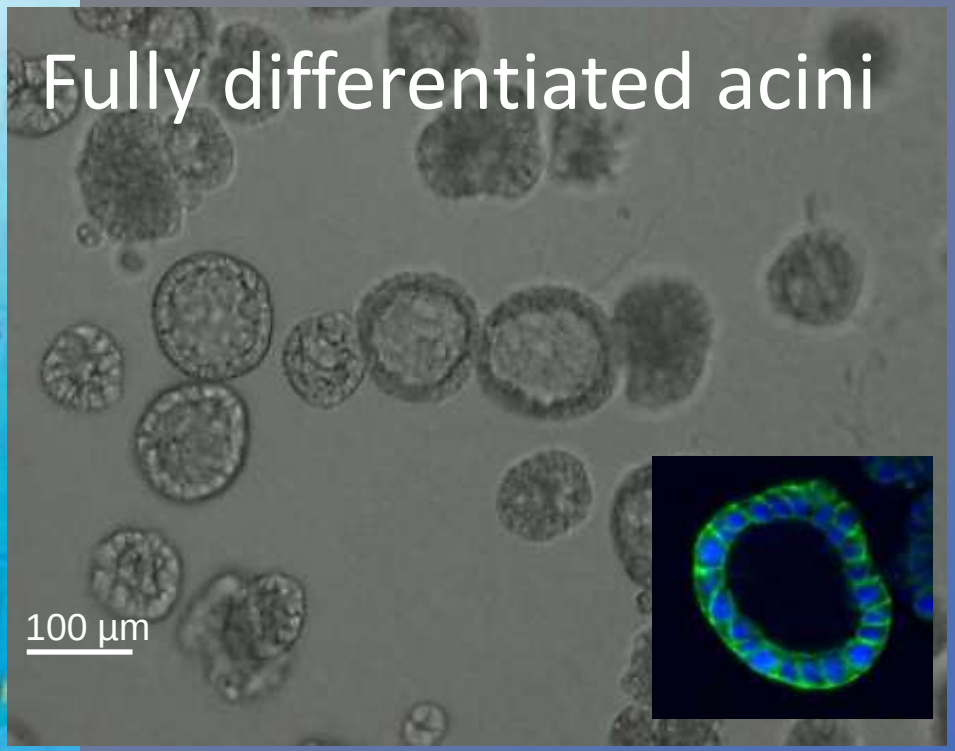
In 3D Matrigel culture, epithelial cells are able to differentiate into structures that highly resemble glandular tissue *in vivo*

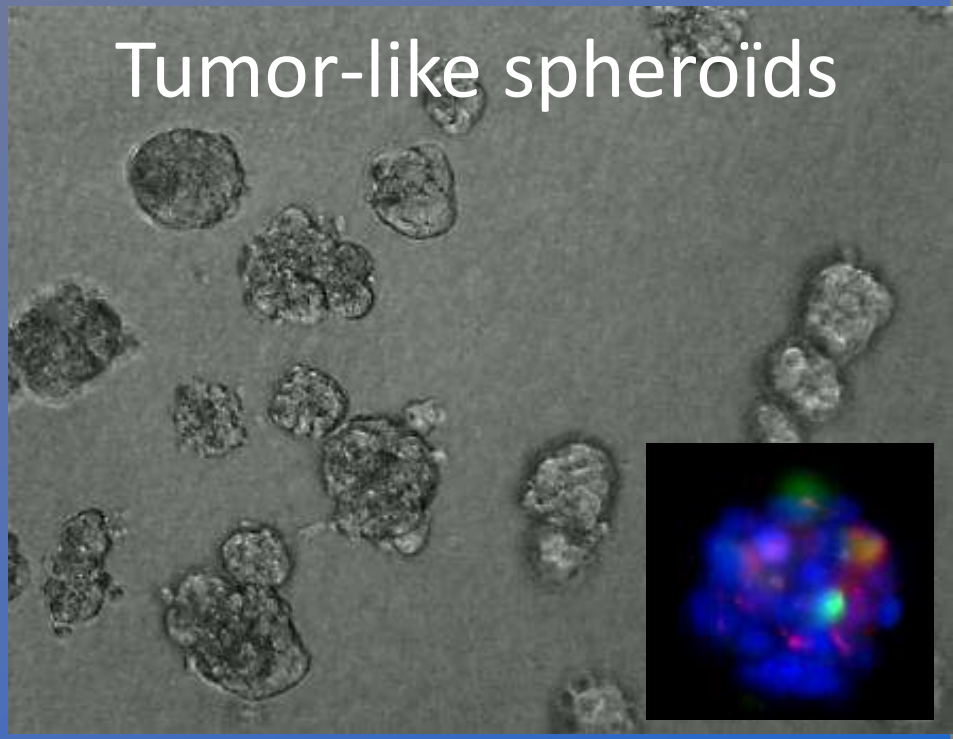


Features of acini

- clonal growth (not cell aggregation)
- differentiation (= polarity & death)
- integrin-ECM interactions







RWPE1 prostatic cell line

Immortalized cell line (by HPV)

WPE1-NB26 prostatic cell line

(transformed by N-methyl-n-nitrosurea (MNU))

HTS siRNA screen based on 3D structure polarity

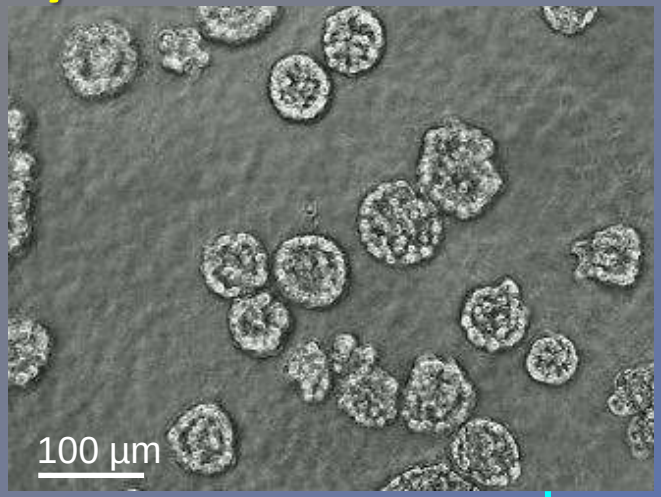
>> Genes involved in 3D polarity (lumen clearance) ?

- We used small interfering RNAs (siRNA), targeting kinases-related genes to identify 3D-specific effectors of prostate acini morphogenesis and polarity.

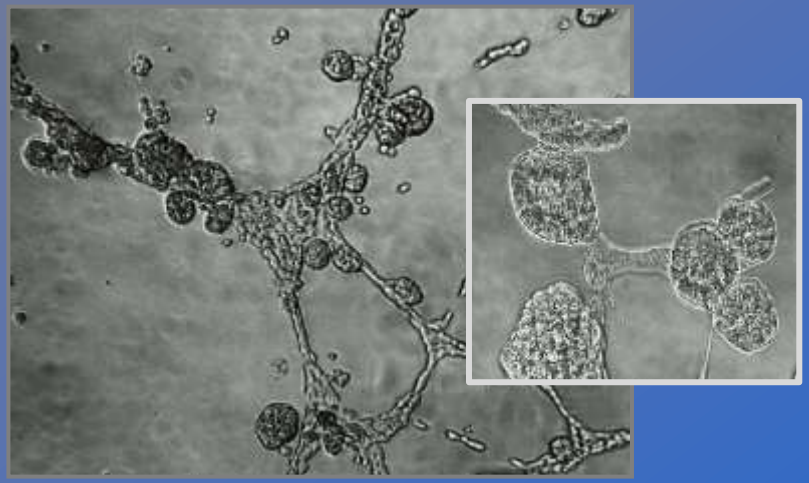
Limitations of current 3D approaches for HTS

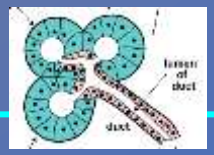
3D culture

Cyst-like structure



Duct-like structure



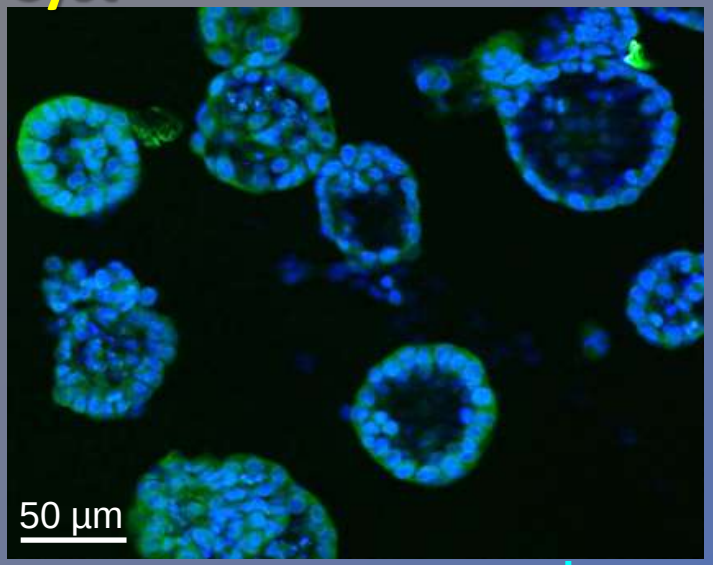


- Lack of control over acini size
- Overlapping acini
- Lack of dynamic response :
  - *Secreted biofluids ?*
  - *Tubulogenesis ?*
- Difficult to recover cells from the wells

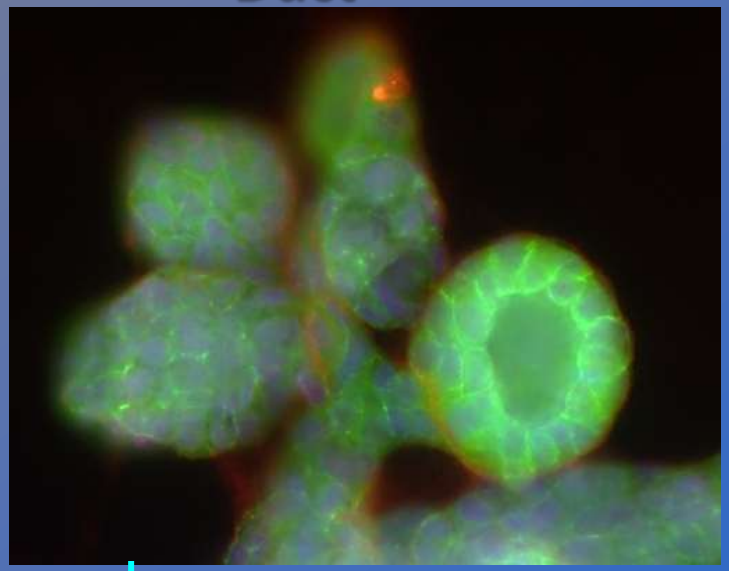
Limitations of current 3D approaches for HTS

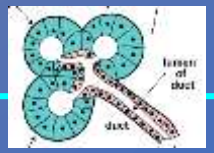
3D analysis

Cyst-like structure



Duct-like structure





Confocal

- Label dependent
- Labor intensive & time consuming
- Difficult to perform statistical analysis

Videomicroscopy

- Limited field of view
- Missed rare events
- Difficult to count a large number of « 3D objects »


SEM
©M-E. DOLEGA-PYZALSKA

How to meet the needs of HTS ?

3D models	▪ Reproducible ▪ Scalable ▪ Easy accessibility	▪ Relevance:	❖ Differentiation ❖ Vascularization ❖ Co-culture ❖ Patients samples
3D analysis	▪ Direct ▪ Rapid	▪ Statistics	▪ Functional assays

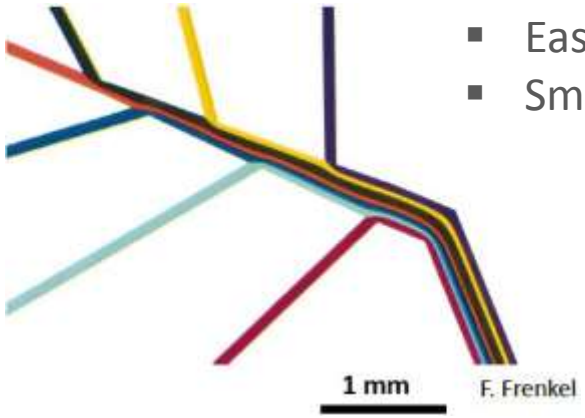
> Emerging technologies for 3D cell cultures suited for HTS

① Handling acini in flux with microfluidics

② 3D direct lens-free imaging

Droplet Microfluidics for 3D cell culture

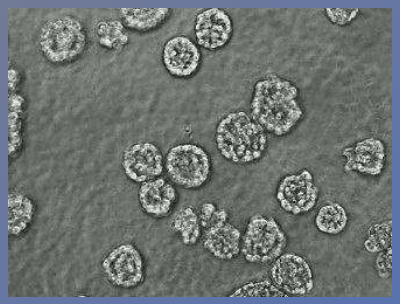
The microscale offers perfect control over flow conditions



- **Laminar flow**
- Statistics via repeated reactions
- Easy control of reaction conditions with millisecond resolution
- Small volumes of solvents and reagents

- High controllable volume of droplets
- One droplet = one chemical/biological reactor

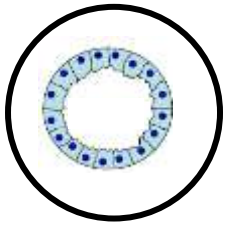
Microfluidic encapsulation brings the third dimension into epithelial cell culture

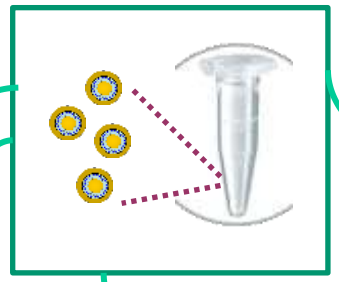


Standard 3D culture
in a well

3D culture
in beads

Microfluidic encapsulation





Handling in flux

Sorting

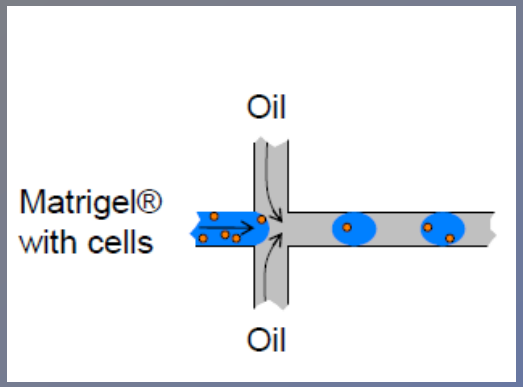
Analysis

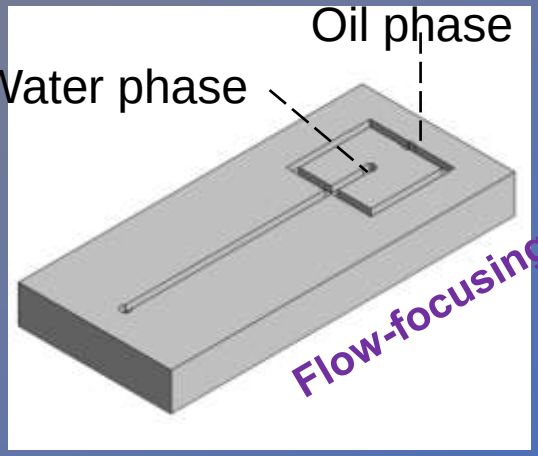
Storage

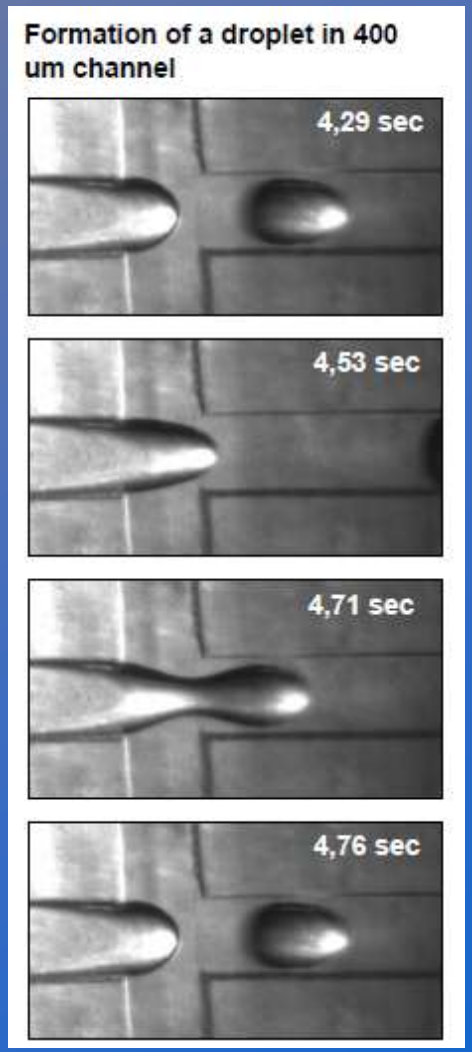
(…)

How does it work?

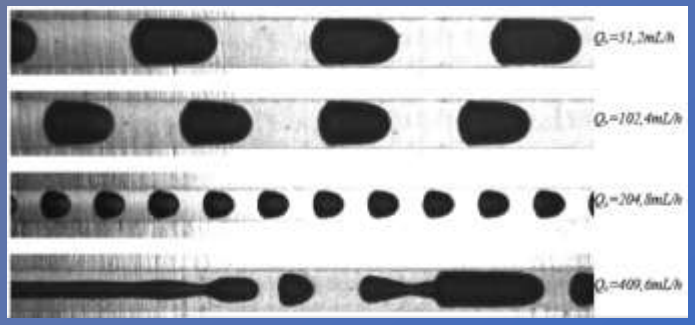
Formation of water-in-oil emulsions







Flow-focusing formation of droplets – control of flow-rate

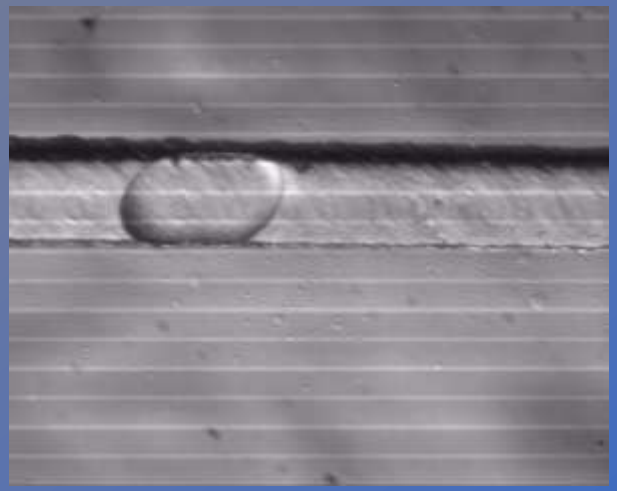


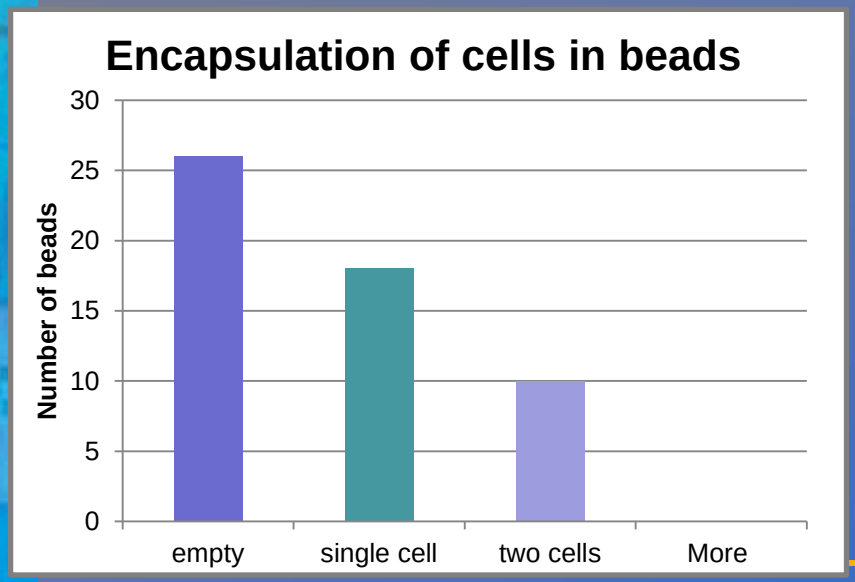
Encapsulation of controlled number of cells

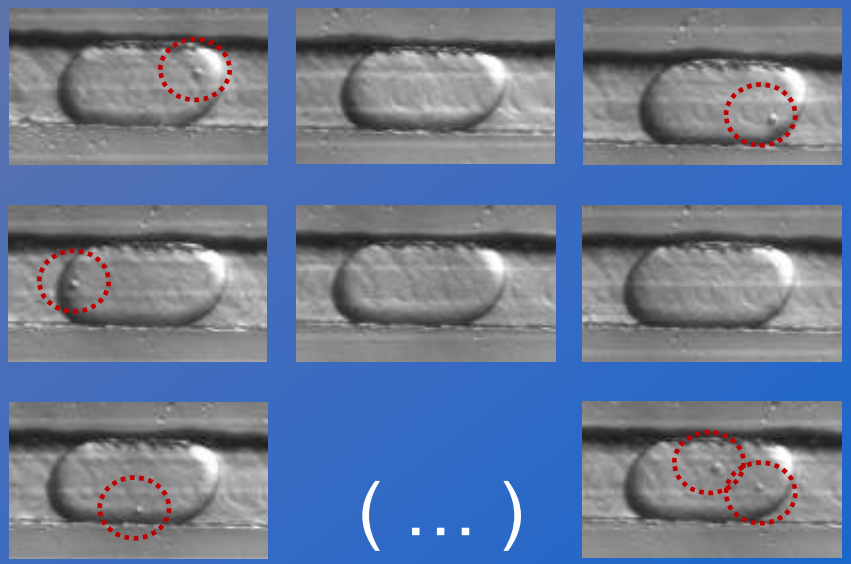
$$P(n,\overline{n}) = \frac{\overline{n}^{n} e^{-\overline{n}}}{n!}$$

$$\overline{n} = \rho V_{microparticle}$$

Relies on Poisson distribution



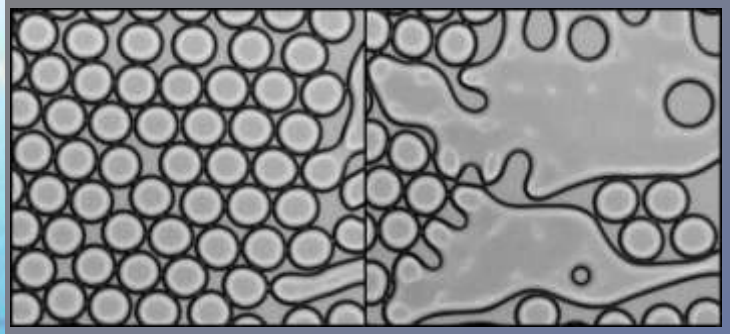




Observation of number of cells in flowing droplets

Formation of droplets of Matrigel - Coalescence

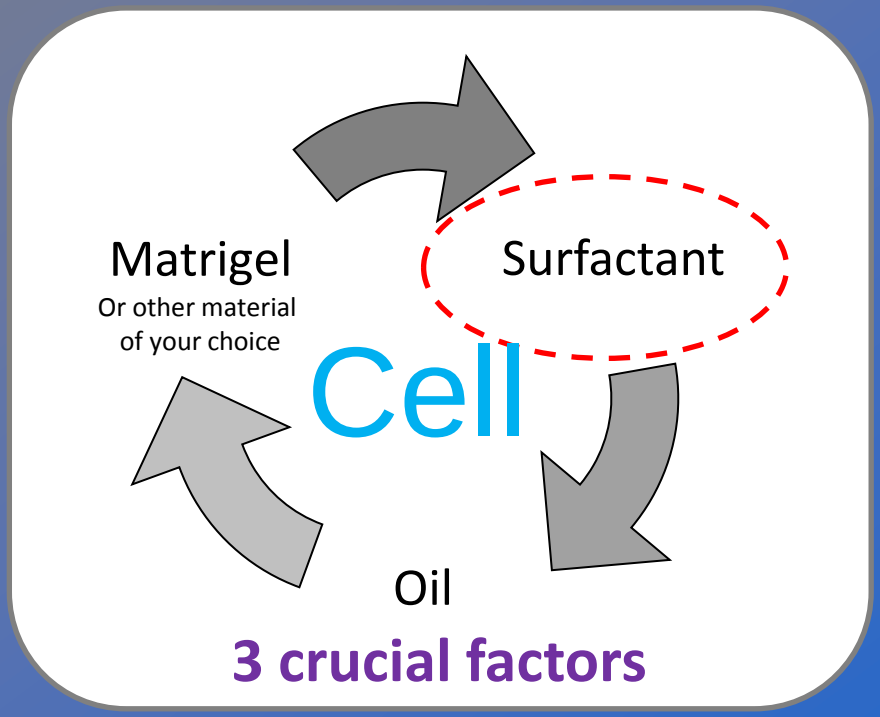
Coalescence



Coalescence of droplets (N.Bremond; *PRL*,2011)

With surfactant

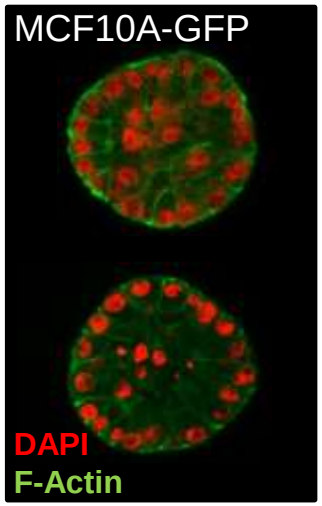
Flow of Matrigel droplets (~100 µm) in tubing



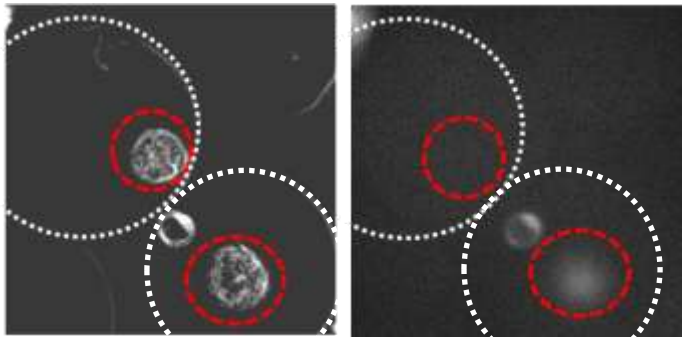
Not universal !

Beads in solution = analysis in flow

> HT RNAi screens on acini with flow-based detection

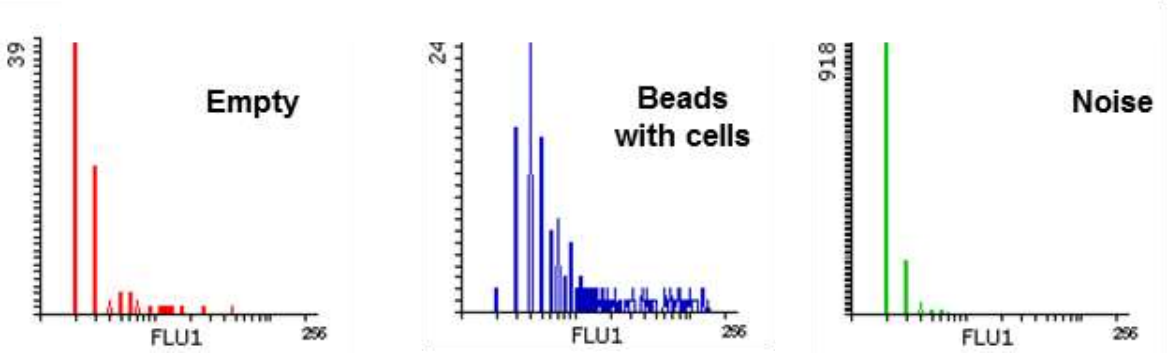


+ siRNA_GFP



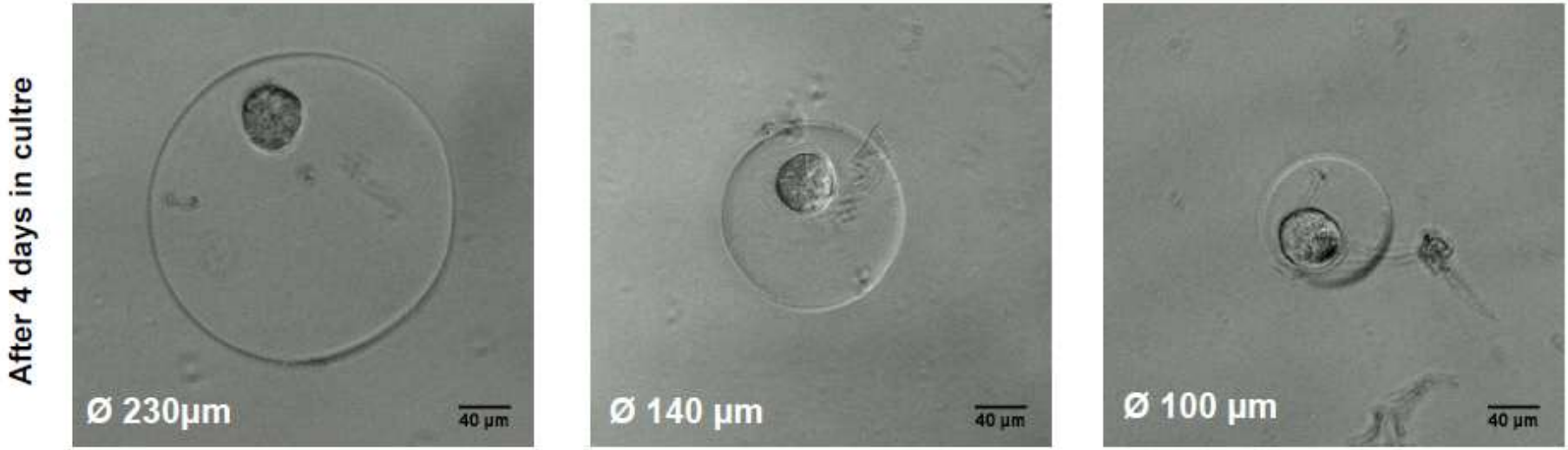
Phase contrast GFP 50ms

Method validation with Large Particle FACS



Control over constraint

> How does the constraint of µenvironment influence the growth and polarity of acini ?

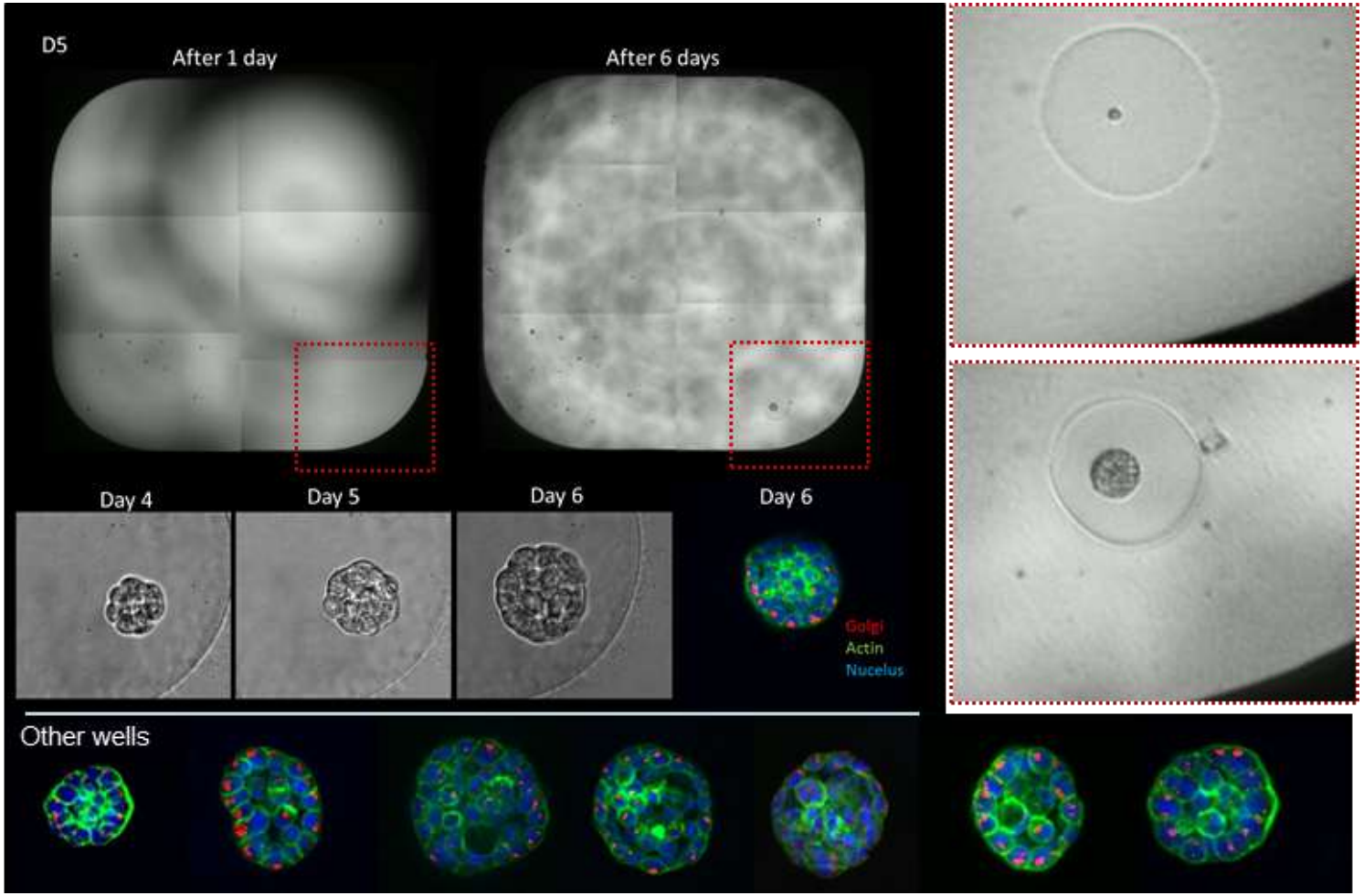


The size of droplets changes according to the adjusted flow rates

Control over number of cells

> Is a single cell able to develop into an acinus?

Encapsulation of a single cell and its isolation in well of 384 well plate



Day1

Day6

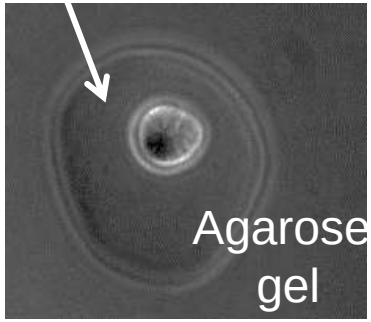
Control over rigidity

> How does environmental rigidity influence acini polarity?

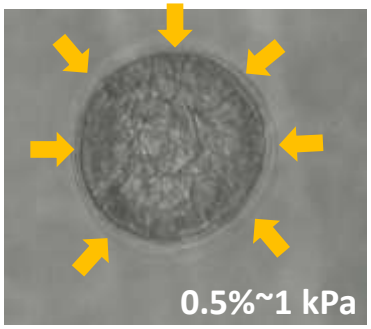
Encapsulation of cells in Matrigel (~400 Pa) and further immobilization of capsules in hydrogel of controlled rigidity

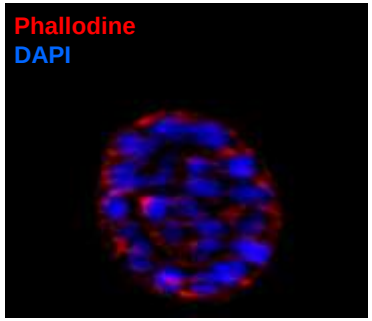
Agarose controls the stiffness & Matrigel gives the chemical signals

Polymerized matrigel

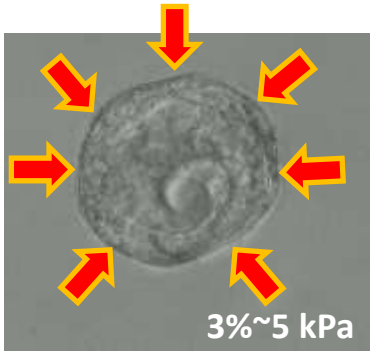


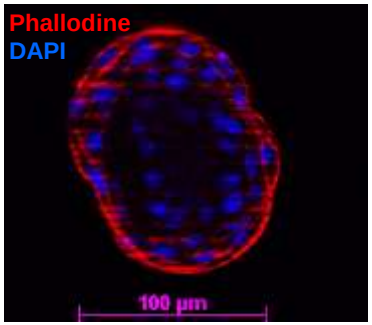
Capsule of 100µm





1kPa



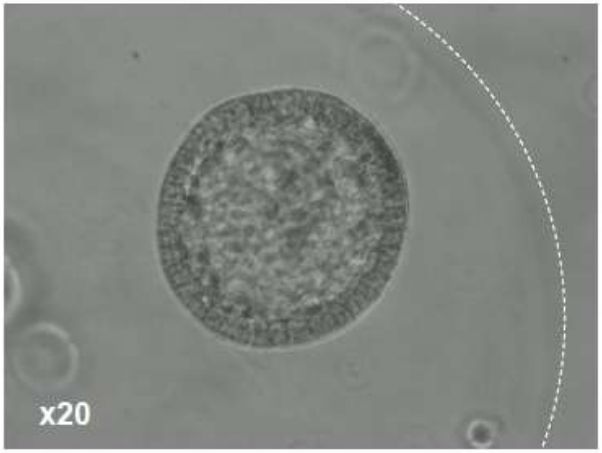


5kPa

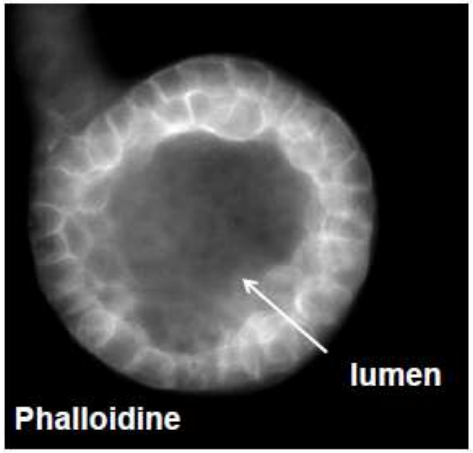
Tension on acini generated in 3D

- Elongated cells/nucleus at the basal layer
- Occurrence of actin stress fibers

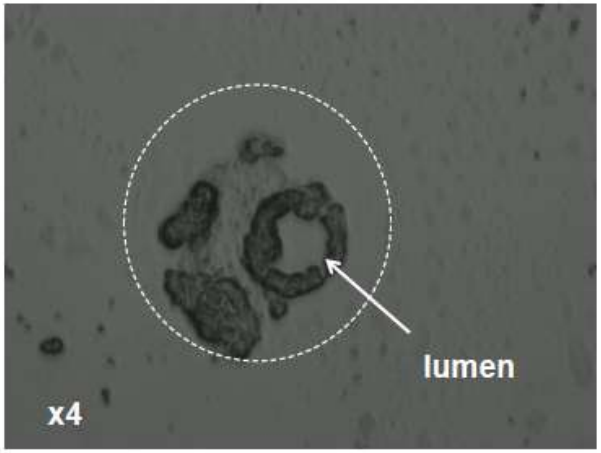
Are morphology & polarity maintained in beads ?



Phase contrast



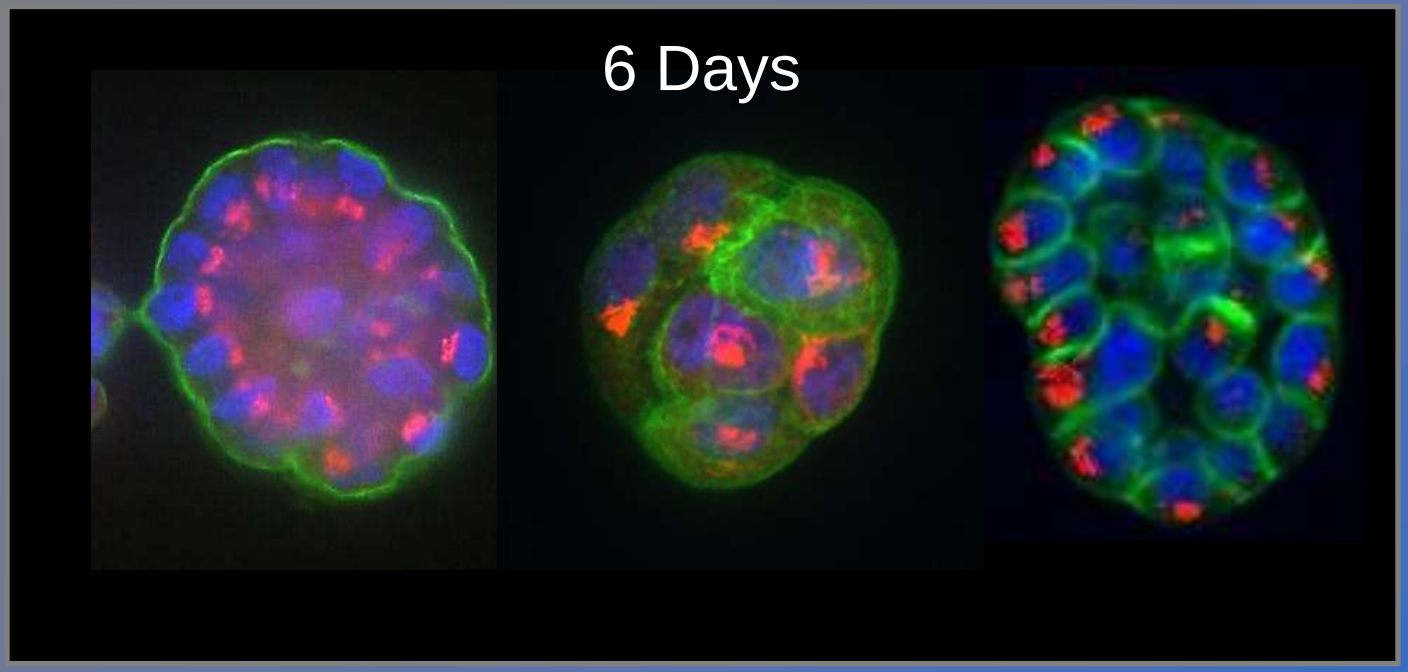
Fluorescent staining

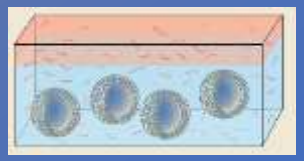


Cryosection (~10 µm) of acini in a bead

Encapsulated cells have grown into spherical organized structures with lumen as observed by fluorescent labeling with phalloidin (actin staining) and cryosectioning of beads. **There is no negative influence of microfluidics on growth of acini**

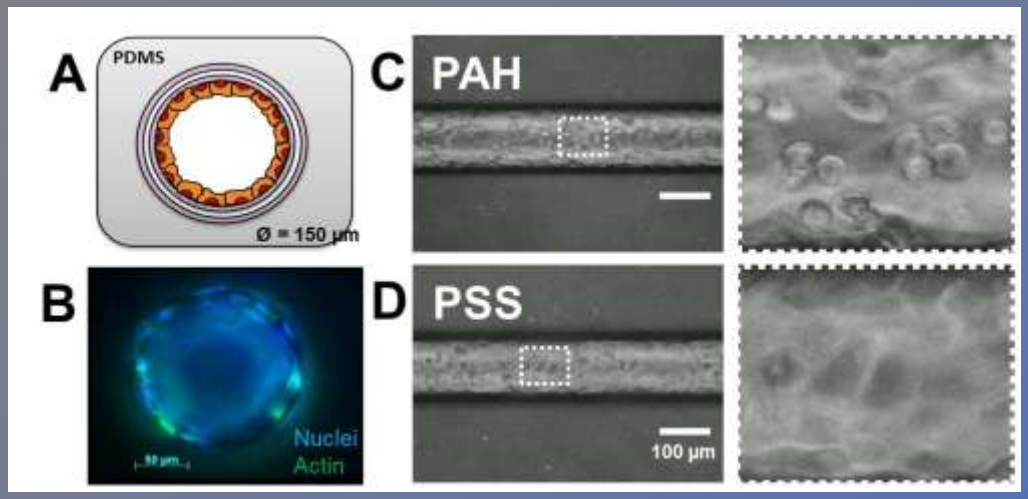
Top-coat Embedded Capsules



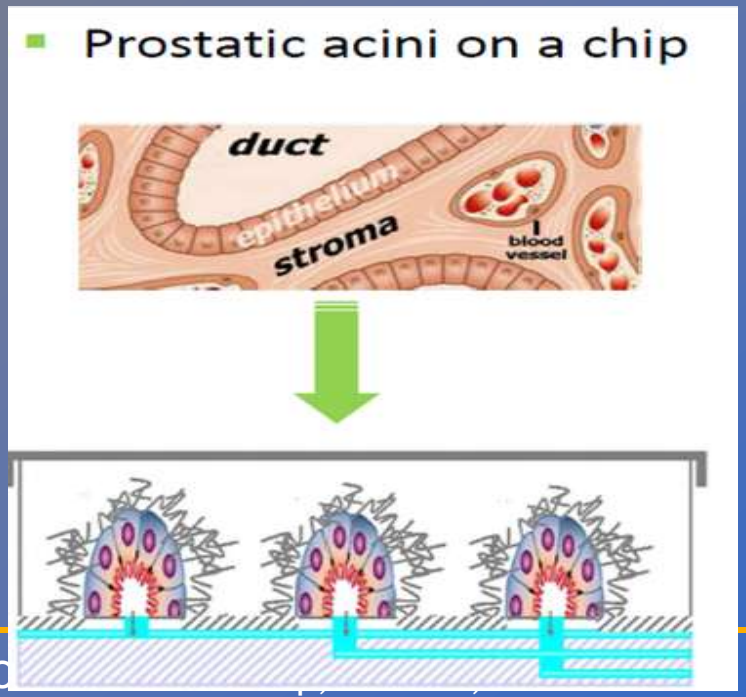


✓ Proper polarity ✓ No polarity ✓ Inverted orientation of Golgi

From acini to 3D ductal structure



- Picollet-D'hahan et al, *Biomaterials* **34**, 2013.
- Dolega M et al. *PloSOne* **9** (6), e99416, 2014.



Polymeric microtubes to mimic prostatic secretory ducts and identify predictive biomarkers in biofluids

How to meet the needs of HTS ?

3D models	▪ Reproducible ▪ Scalable ▪ Easy accessibility	▪ Relevance:	❖ Differentiation ❖ Vascularization ❖ Co-culture ❖ Patients samples
3D analysis	▪ Direct ▪ Rapid	▪ Statistics	▪ Functional assays

> Emerging technologies for 3D cell cultures suited for HTS

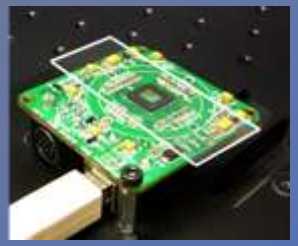
① Handling acini in flux with microfluidics

② 3D direct lens-free imaging

Direct Read-out for HTS

Microscope

CMOS sensor



Lensfree system

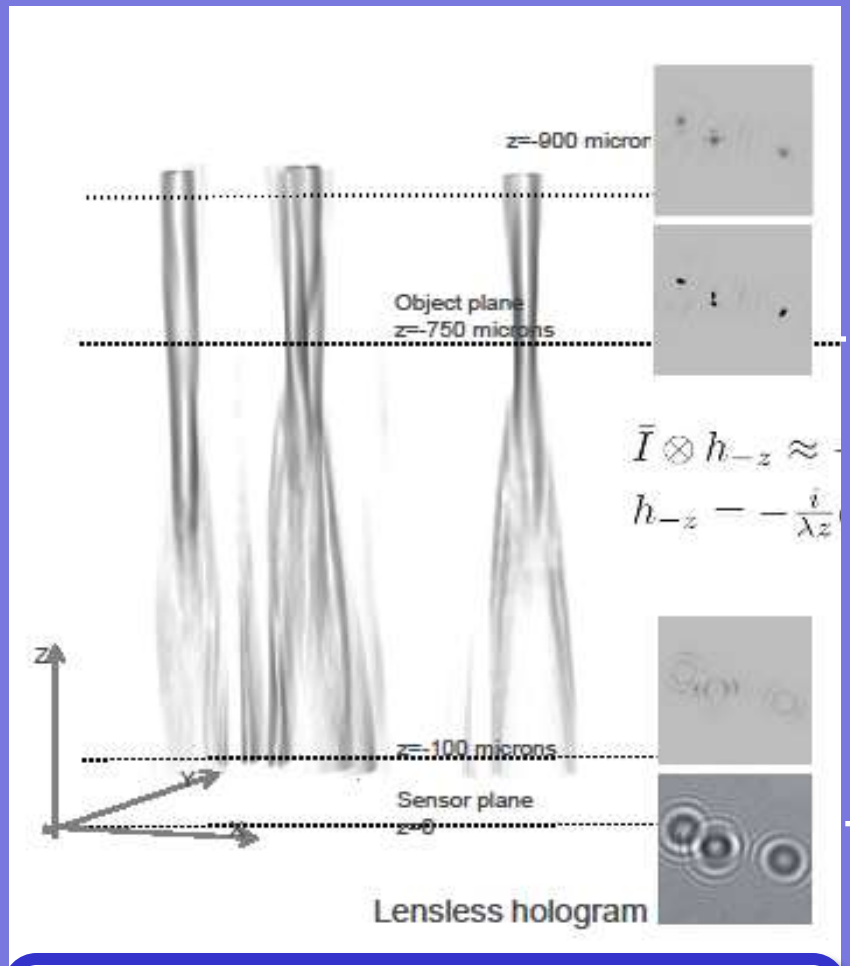
- Large field of view (> 20 mm²)
- 10,000 cells at the same time
- Large dynamic scale from virus to acini
- Miniaturized
- Label-free
- Simple, small & cheap (< 300 $)
- Video-microscopy (live)

Lensfree Imaging

CEA / LETI (C. Allier), FR

Holographic reconstruction of cells

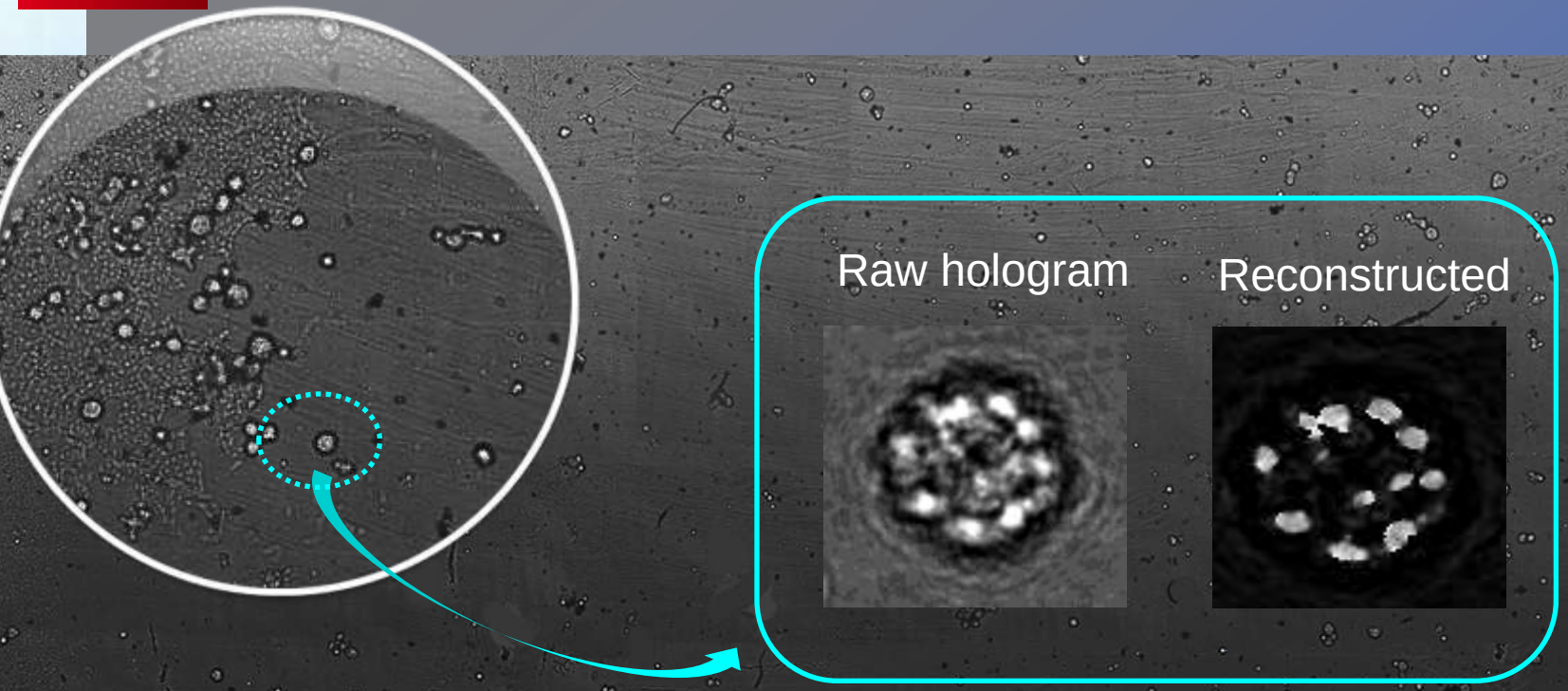
Holograms or patterns of interference



Metrics / algorithms for image reconstructions

Dolega *et al*, *Biosens Bioelec*, 2013

Lensfree imaging of 3D prostatic cells

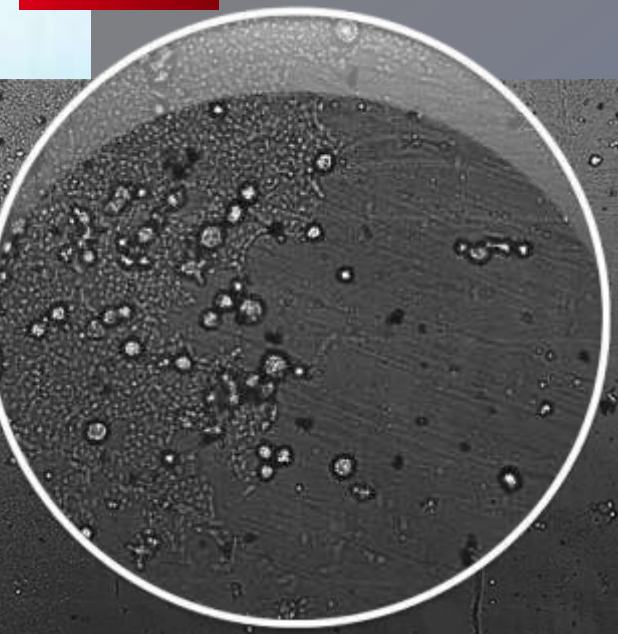


1.8 cm² (~12 images)

0.5 cm

Nunc® Lab-Tek® Chamber Slide™ system. 4 wells slide, 1.8 cm²/well

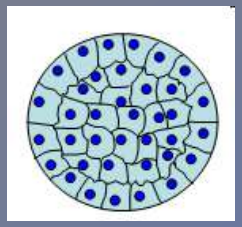
Lensfree imaging of 3D prostatic cells

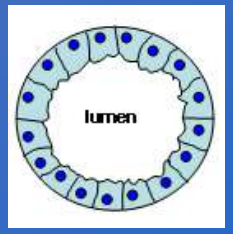


96-well plate (1 well=33 mm²)	Lensfree [2592 x 1944 px]	Spinning-Disk [512x512 px]
Field Area	**24 mm²**	0.6 mm²
% of well covered	**70 %**	2 %
Nb images to cover a well	**4**	100
Magnification	X 7	X 10

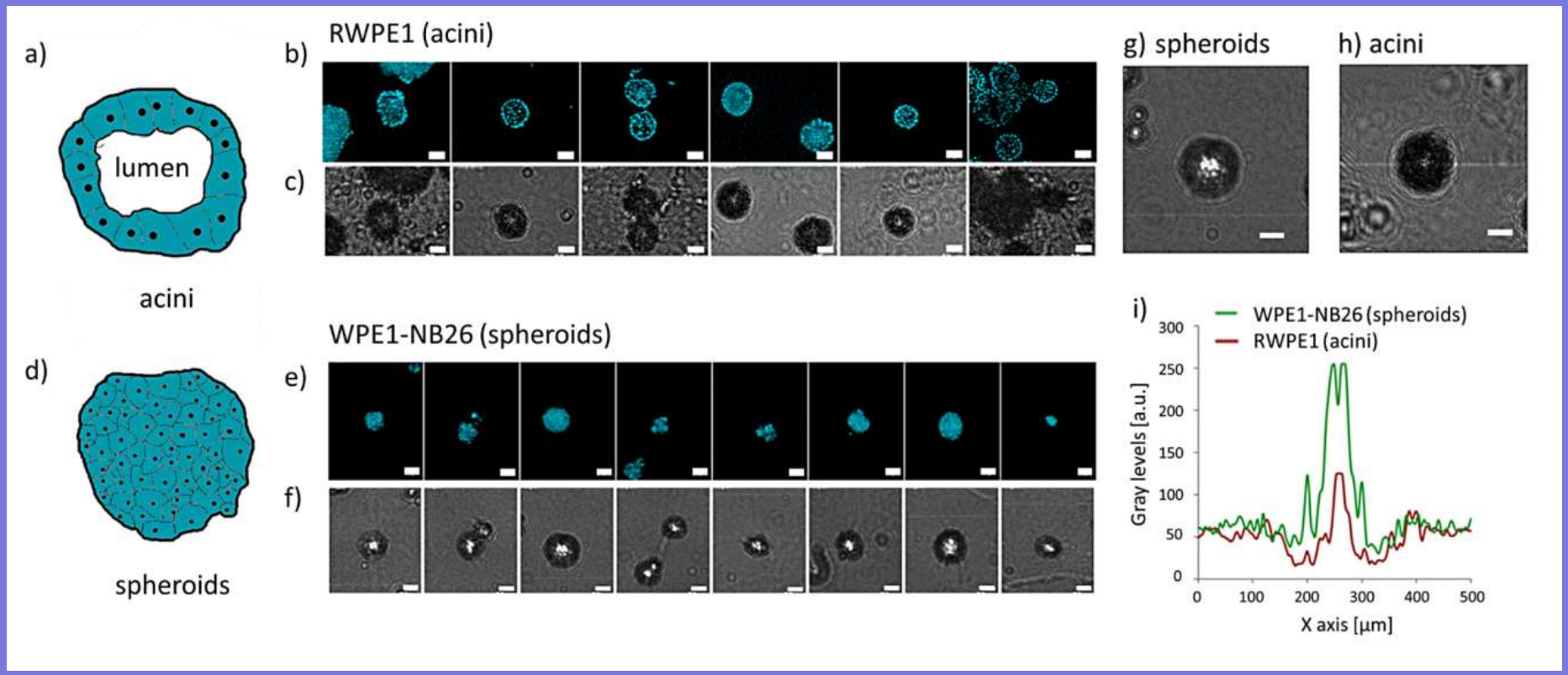
0.5 cm

With lensfree ?





Qualitative analysis of 3D structures : a specific holographic signature



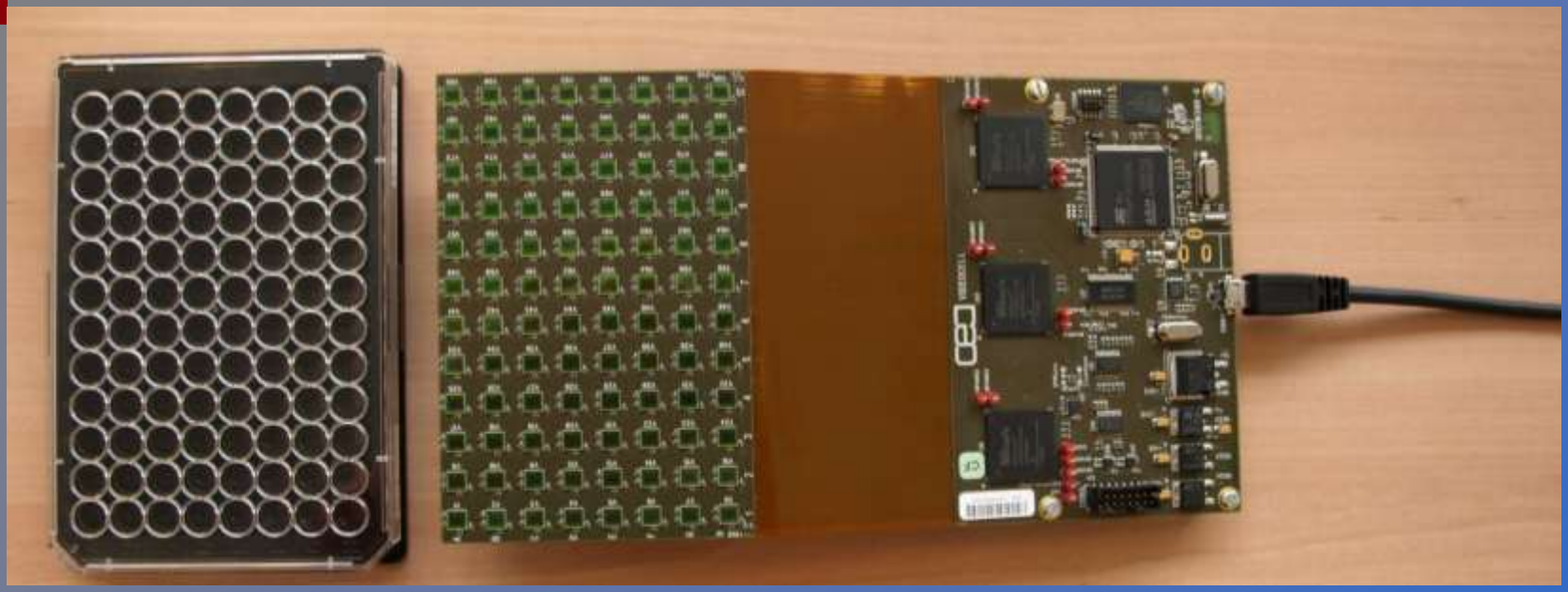
Dolega ME *et al. (2013).* Label-free analysis of prostate acini-like 3D structures by lensless imaging. *Biosensors & Bioelectronics* 49: 176-183.

- Genes involved in the development of prostatic acini (lumen formation) ?

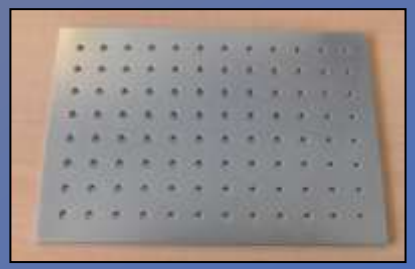
➢ evaluate the role of the RNAi-mediated down regulation of kinases-related genes

➢ effect of siRNA on « acini / spheroids » ratio ?

Parallelized (96) lens-free imaging system



96-pinholes

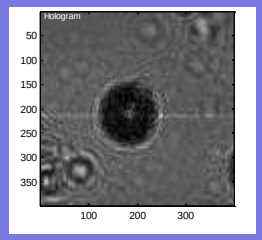


96-LEDs

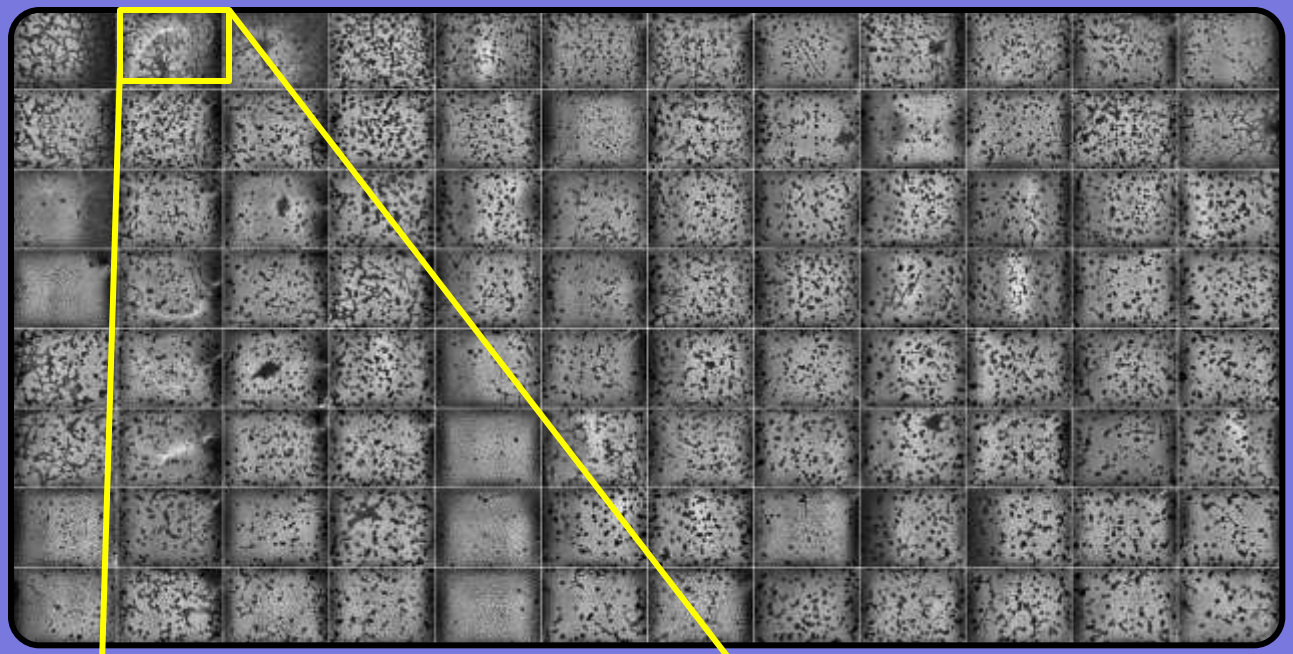
One 96-well plate = 10 min

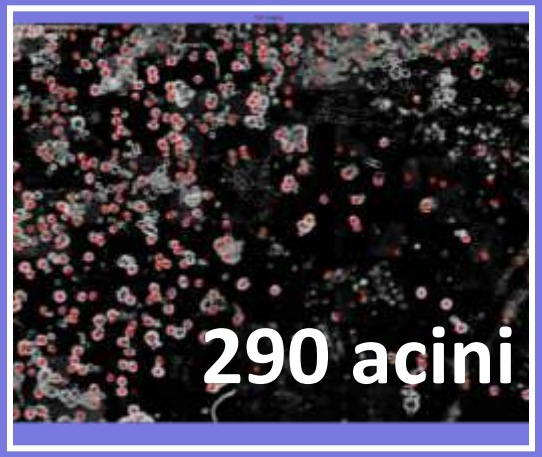
3D RNAi-based HTS

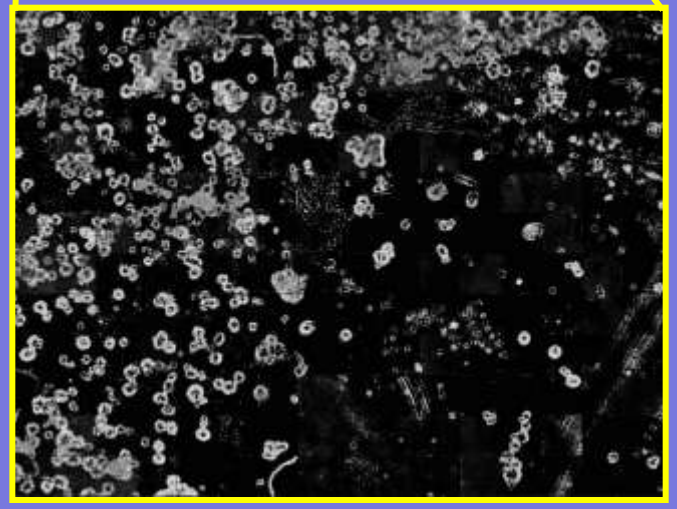
Pattern recognition

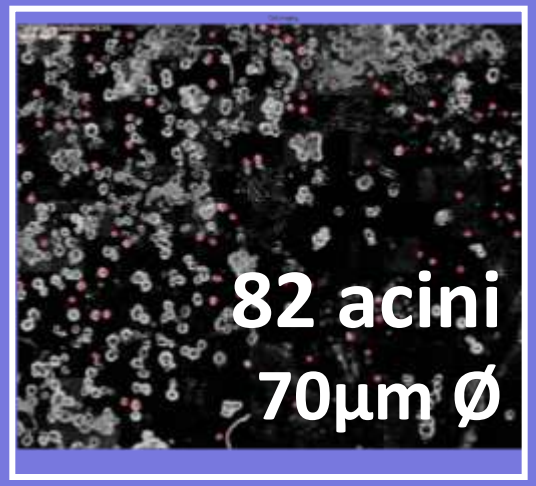


>1000 'objects' / well







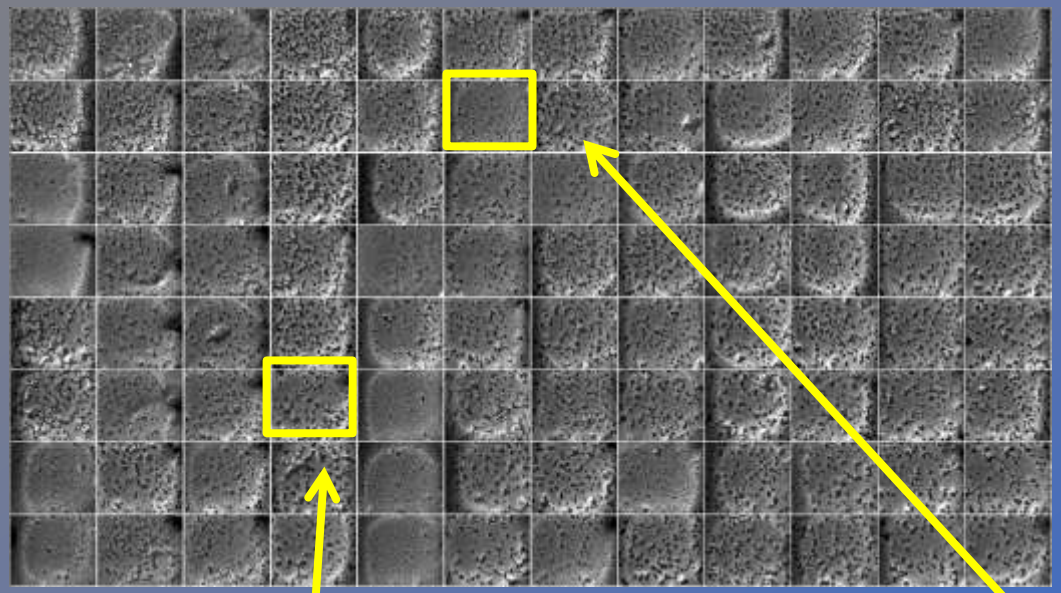


One field = 24 mm²

Quantitative analysis of 3D structures

Fast tracking of siRNA screening

Effect of siRNA on the « acini / spheroids » ratio

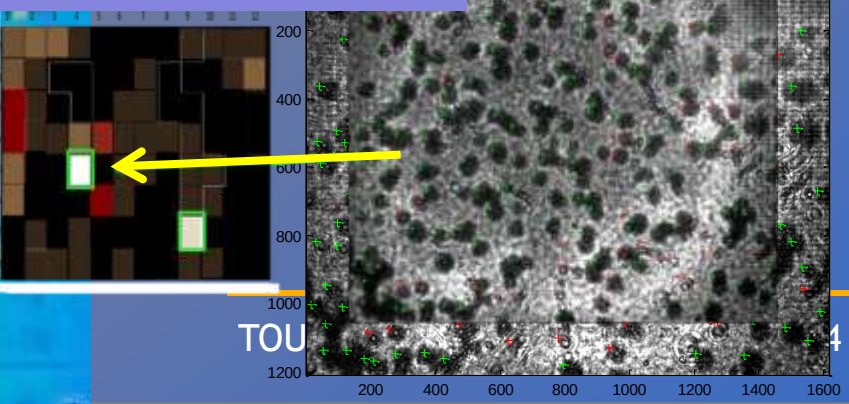


Extensive statistics :

- 14.000 3D objects
- 145+/-15 / well

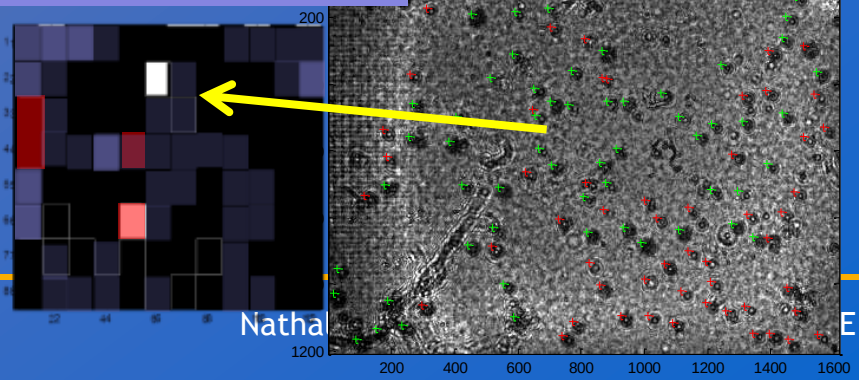
Hit 1
(acini)

More acini (> 5σ)
Large diameter (> 2σ)



Hit 2
(tumor-like spheroids)

More spheroids (> 5σ)
Small diameter (> 2σ)

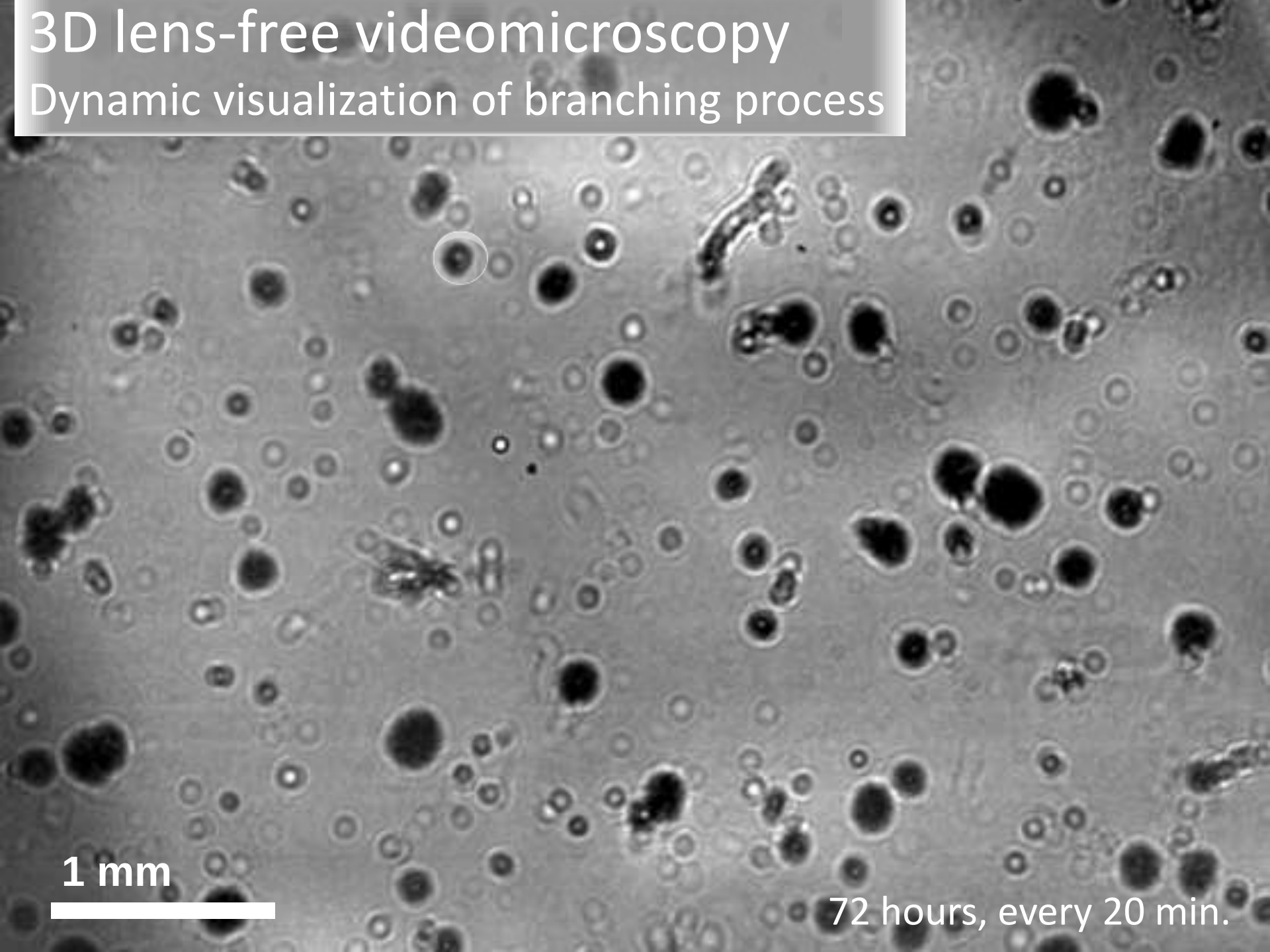


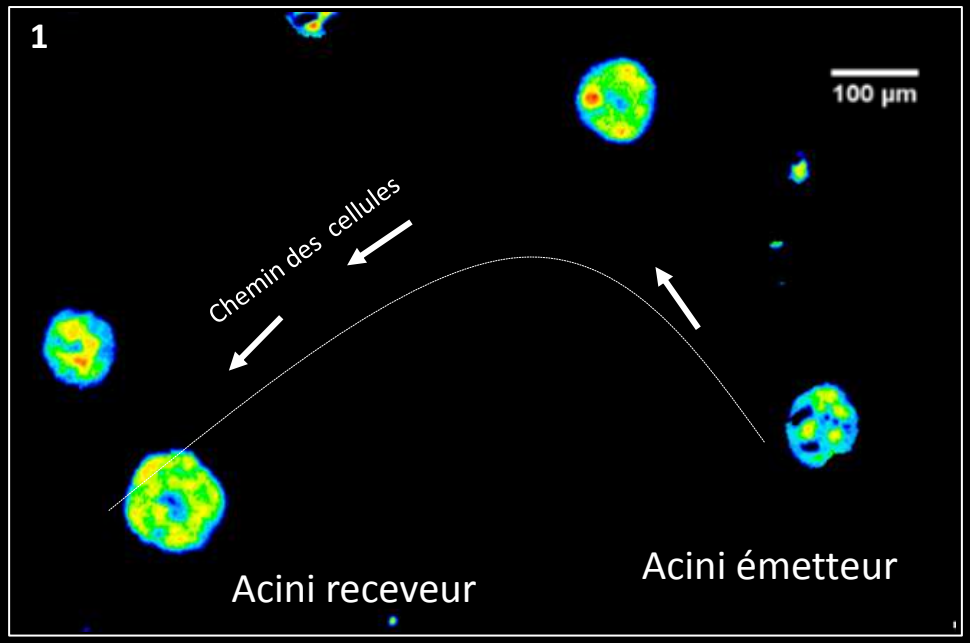
Can we use lensfree imaging to observe dynamic cellular 3D assemblies ?

Nathalie PICOLLET-D'HAHAN, CEA, FRANCE

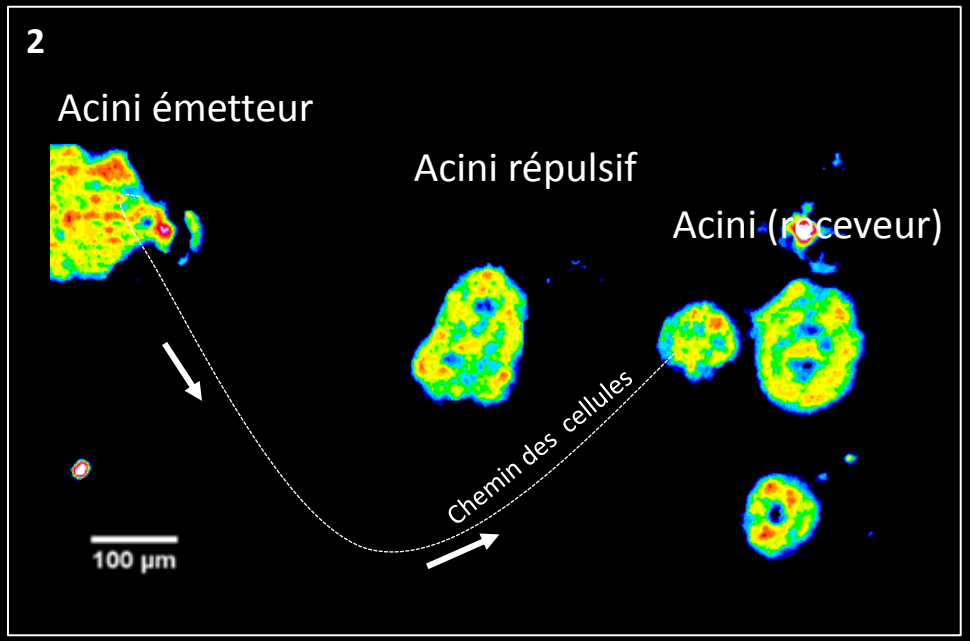
3D lens-free videomicroscopy

Dynamic visualization of branching process





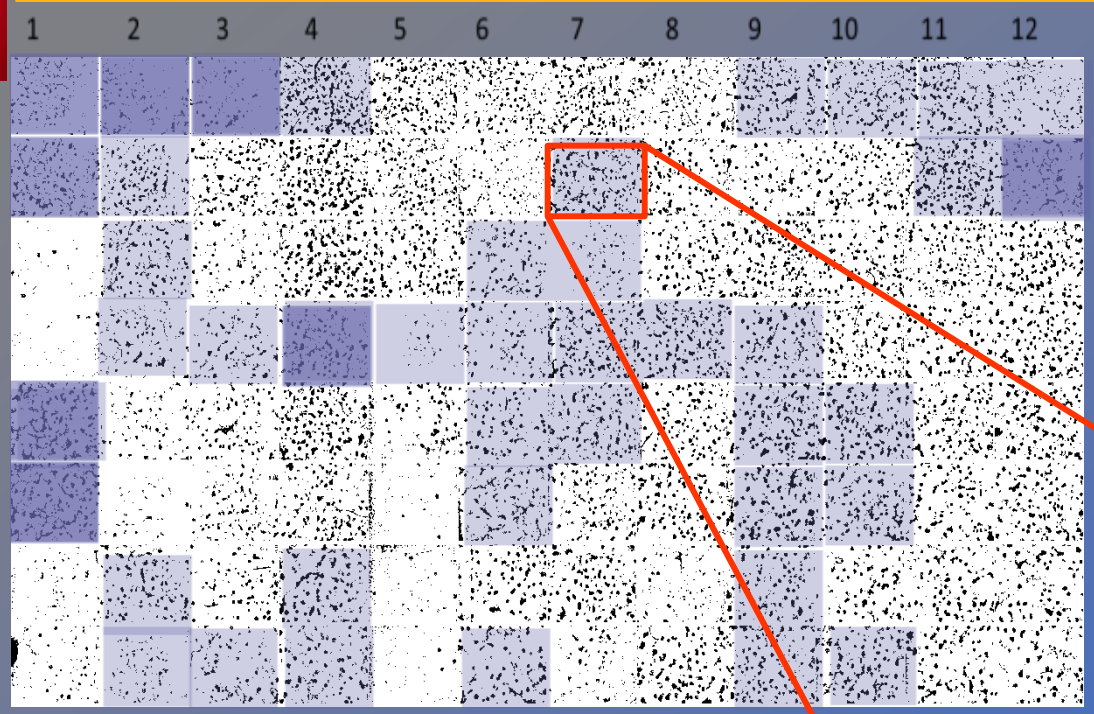
« Path finders »
&
« Path generators »



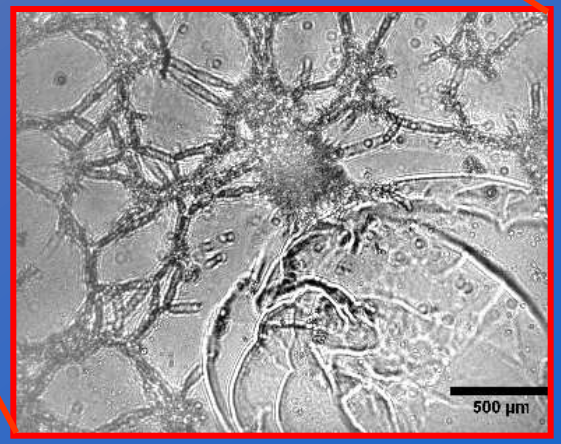
Read-out based on dynamic networking (branching) ?

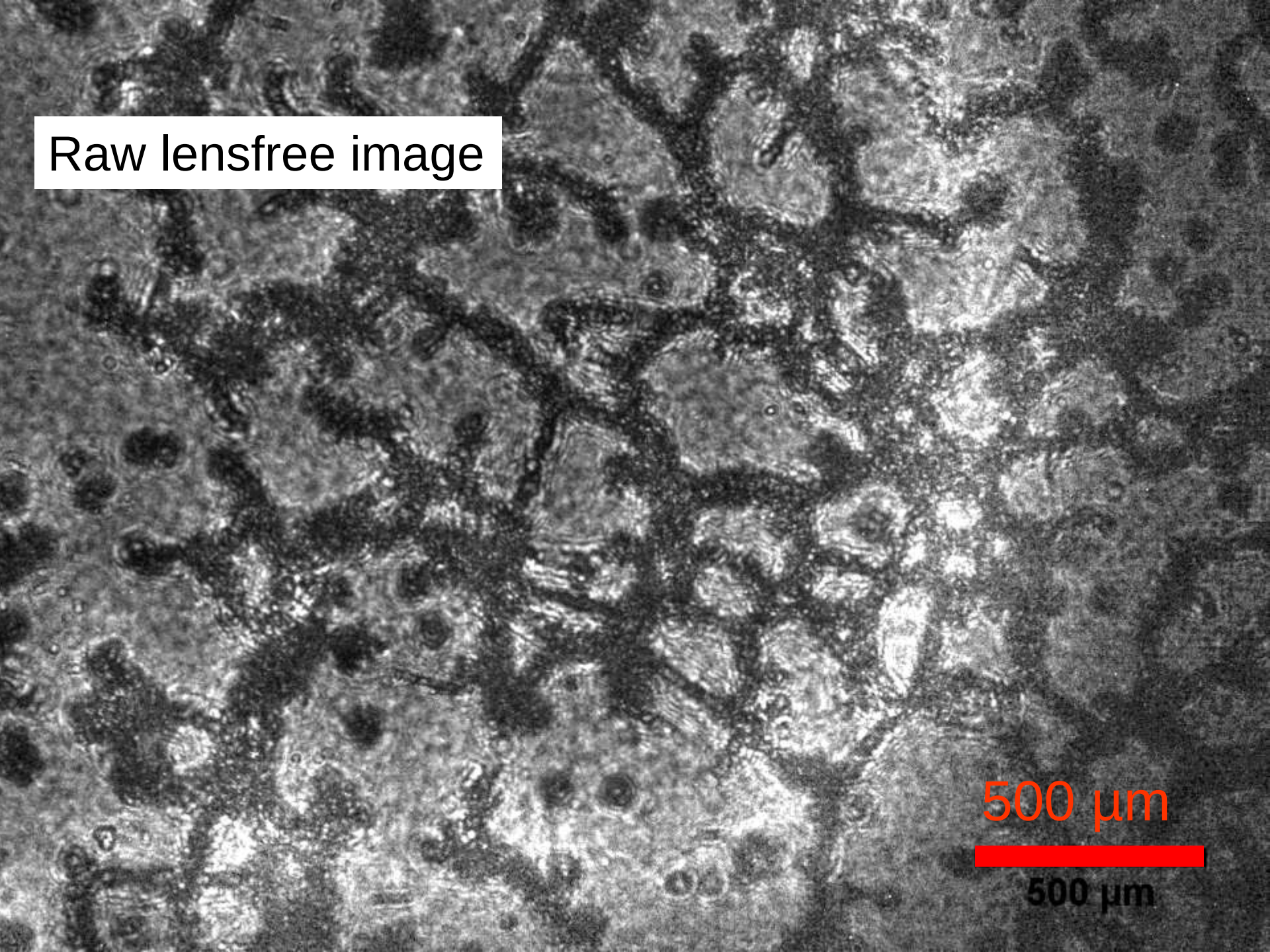
>> Useful to study the invasive properties and metastatic potential of tumor cells and for conducting screening assays for cell migration

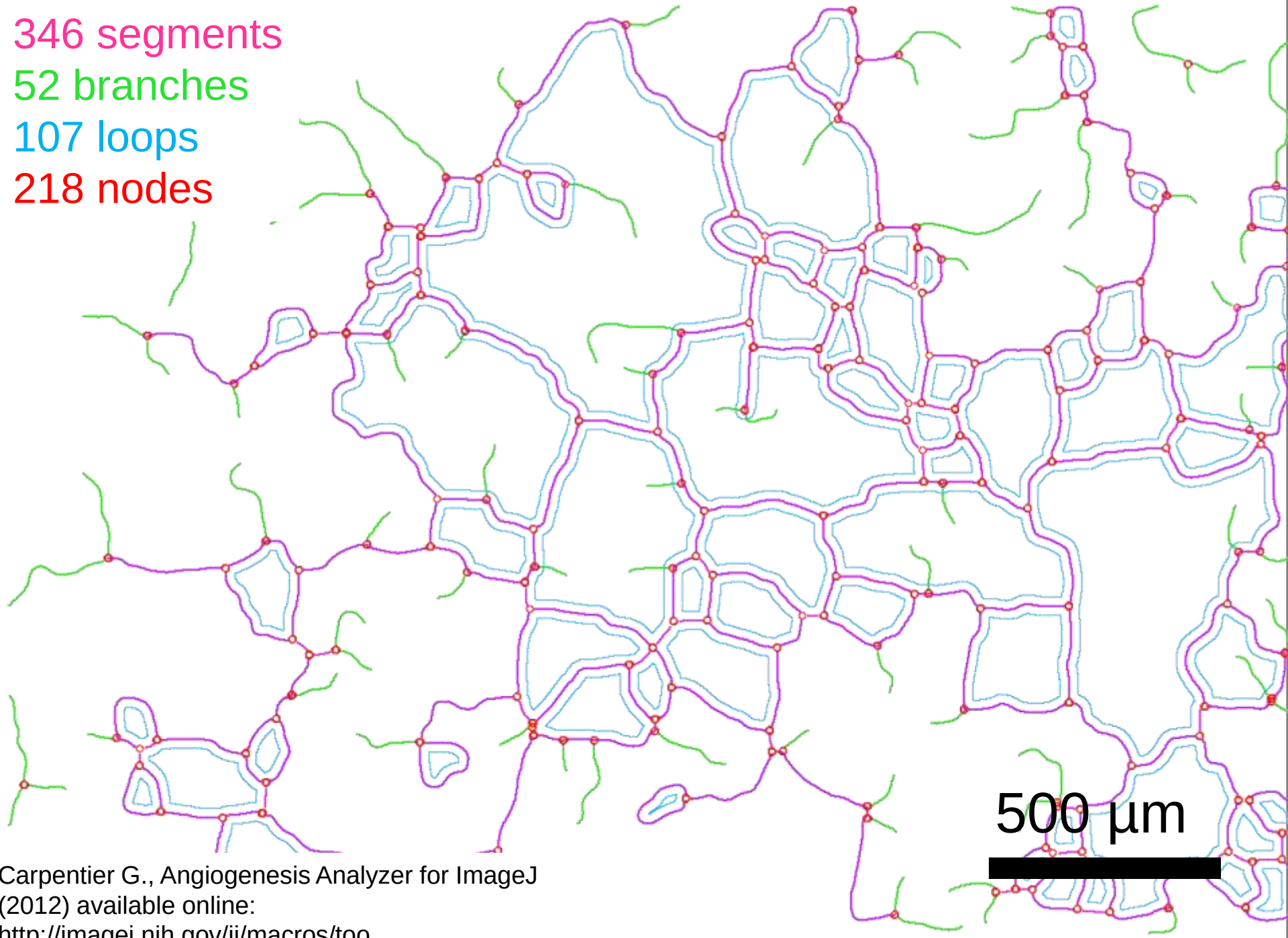
Branching



Holographic reconstruction

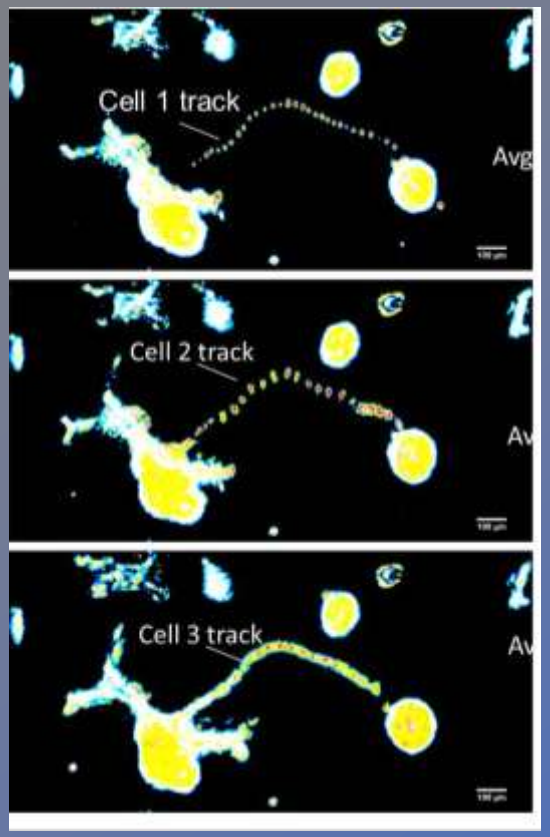



Raw lensfree image
500 µm
500 µm



Carpentier G., Angiogenesis Analyzer for ImageJ (2012) available online: http://imagej.nih.gov/ij/macros/too...

Cell velocities



>> Migration assays

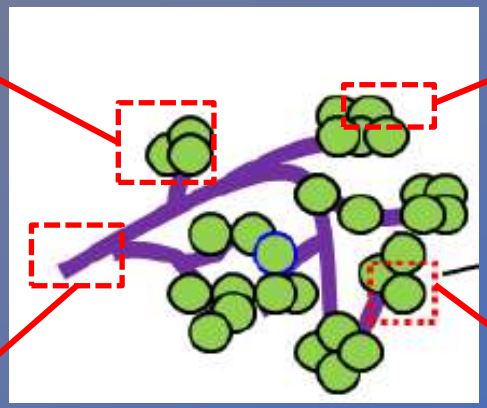
>> Drug screening

>> Toxicology studies

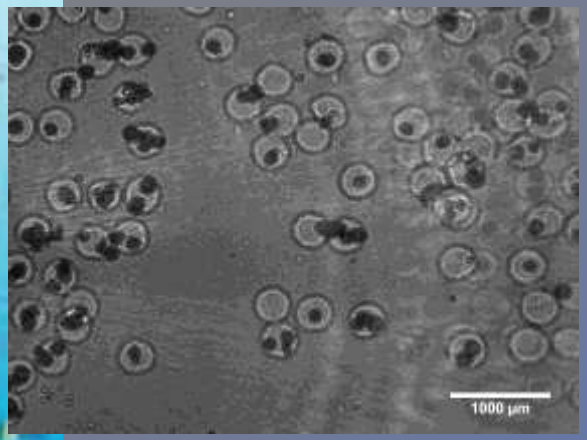
in organ-like models

Conclusion: Innovating 3D constructs using µfluidics & µtechnologies

« 3D Tool-Box »

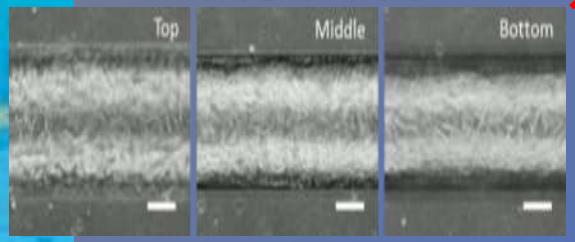


Capsules



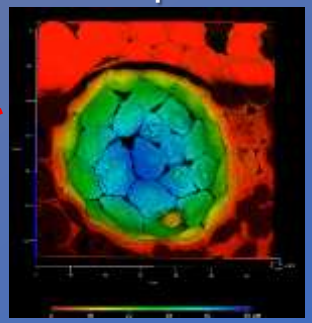
- Dolega *(submitted)*

Microchannels



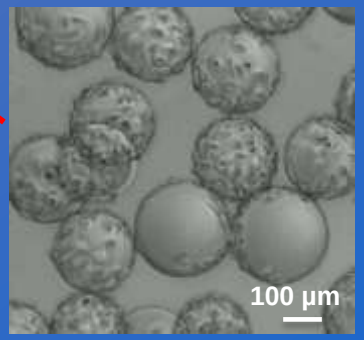
- Dolega, *PlOS One* **2014**

Hemispheres



- Picollet, *Biomaterials* **2014**

Microcareers



- Abeille, *L.O.C (in press)*

Xavier GIDROL

3D cell culture

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Nathalie BERTACCHI
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Stéphanie PORTE

Microfluidics

Monika DOLEGA

Lensfree imaging

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RNAi screening

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- 3D cell culture

Odile FILHOL-COCHET (CEA)

- Biomimetism - µfabrication

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