

Course: Optics, forces & development

In vivo 3D-microscopy for the analysis of cell behaviour in developing embryos

Santiago – Chile, January 14-29th, 2013

This practical and theoretical course is aimed at graduate students from Latin America interested in the use of optics and microscopic techniques for *in vivo* 3D visualisation and analysis of cell and tissue dynamics. Limited to 12 students.

Topics:

- . Development of zebrafish and annual fish
- . Confocal and spinning microscopy
- . Light-sheet microscopy
- . Super-resolution microscopy
- . Photoactivation and laser ablation
- . In vivo electroporation
- . Force estimation in cells & tissues
- . Optical tweezers
- . Particle tracking
- . Image processing and analysis

Teachers:

- . Roberto Bernal (U. Santiago, Chi)
- . Sebastián Brauchi (U. Austral, Chi)
- . Miguel Concha (U. Chile, Chi)
- . Mauricio Cerda (U. Chile, Chi)
- . Jorg Enderlein (Univ. Göttingen, Ger)
- . Nikta Fakhri (Univ. Göttingen, Ger)
- . Steffen Härtel (U. Chile, Chi)
- . Carl-Philipp Heisenberg (IST, Austria)
- . Jorge Jara (U. Chile, Chi)
- . Ulrich Kubitscheck (Univ. Bonn, Ger)
- . Omar Ramírez (U. Chile, Chi)
- . Florian Rehfeldt (Univ. Göttingen, Ger)
- . German Reig (U. Chile, Chi)
- . Felipe Santibañez (U. Chile, Chi)
- . Christoph Schmidt (Univ. Göttingen, Ger)
- . Jan Spille (Univ. Bonn, Ger)
- . Juan Pablo Staforelli (CEFOP, Chi)

Organisers:

- . Miguel Concha (U. Chile)
- . Steffen Härtel (U. Chile)

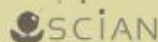
Travel Fellowships will be available. Indicate your interest in the application.

To apply send:

- . Curriculum Vitae
 - . Letter of Intention
 - . Reference from supervisor/mentor
- To: mconcha@med.uchile.cl

Deadline for application - December 26th 2012

Results - December 28th 2012



UNIVERSIDAD
DE CHILE

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FOFONDECYT 1120558, 1120579 & 3130590
FONDEF D111076



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2012 Curso PIB

> MSc Informatica Medica

Programa

> U-Chile

2006 Curso 3D-Microscopy I

2007 Curso 3D-Microscopy II

2007 Curso Colocalization

2007 Curso Microscopia

2008 Curso Fotografia

2008 Curso Colocalization

2008 Curso Microscopia

2009 Curso Colocalization

2011 Curso MMIMB

2011 Curso Bioinformatica

2012 Curso PIB

2012 Curso Bioinformática

2012 Curso Seminario MIM

> U-Chile / International

2005 Microscopy Uruguay

2007 3-D in vivo Imaging

2009 SCMI Conference

Optics, forces & development

| Santiago de Chile 14-29 de Enero 2013, Facultad de Medicina, U-Chile |

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Informaciones en:

-> www.scian.cl

Notas:

Prácticos (25 %)

Seminarios (25 %)

Examen Final (50%)

3 grupos de 2/4 para:

- el seminario:

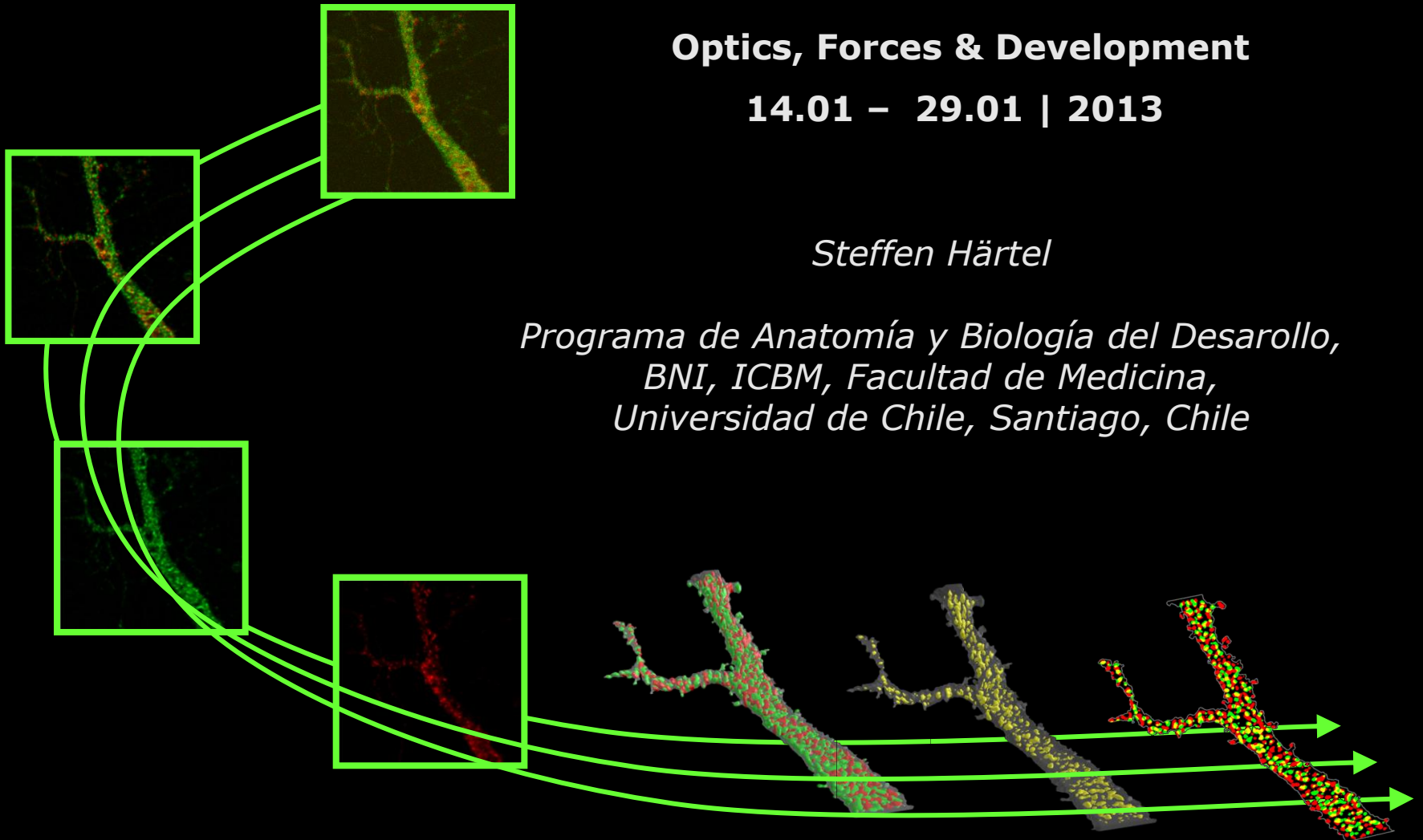
*90 min presentación,
preguntas y discusión*

Optics, Forces & Development

14.01 – 29.01 | 2013

Steffen Härtel

*Programa de Anatomía y Biología del Desarrollo,
BNI, ICBM, Facultad de Medicina,
Universidad de Chile, Santiago, Chile*

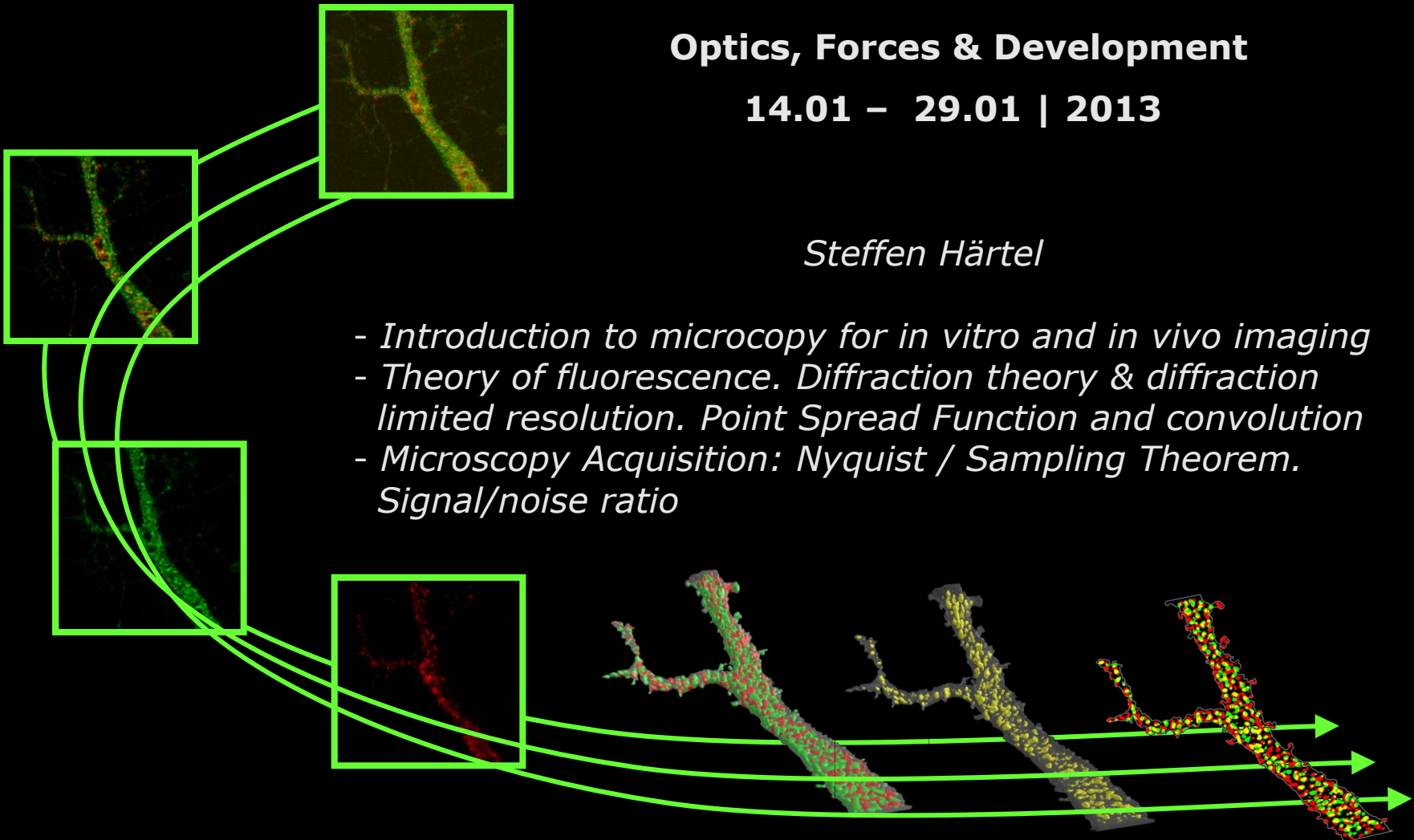


Optics, Forces & Development

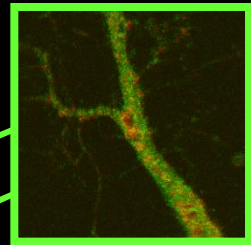
14.01 – 29.01 | 2013

Steffen Härtel

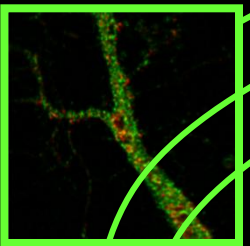
- Introduction to microscopy for *in vitro* and *in vivo* imaging
- Theory of fluorescence. Diffraction theory & diffraction limited resolution. Point Spread Function and convolution
- Microscopy Acquisition: Nyquist / Sampling Theorem. Signal/noise ratio



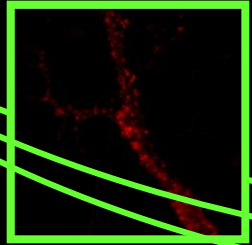
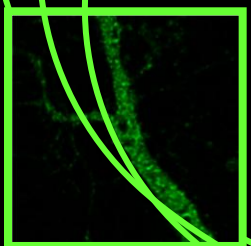
I. Image Adquisition



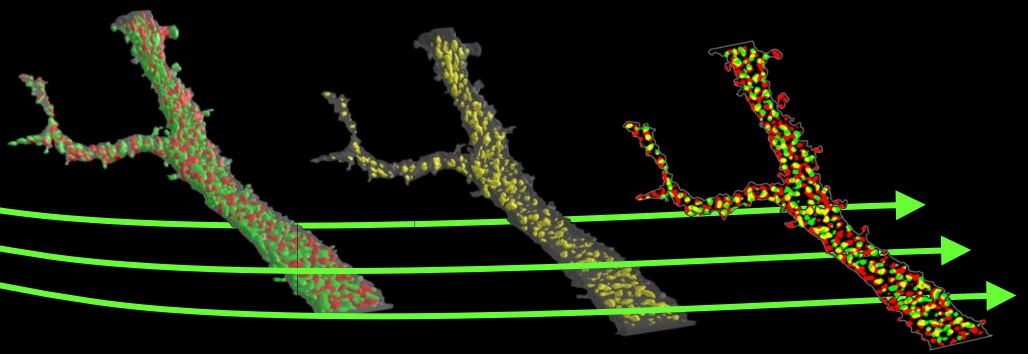
II. Deconvolution



III. Segmentation



IV. Analisis



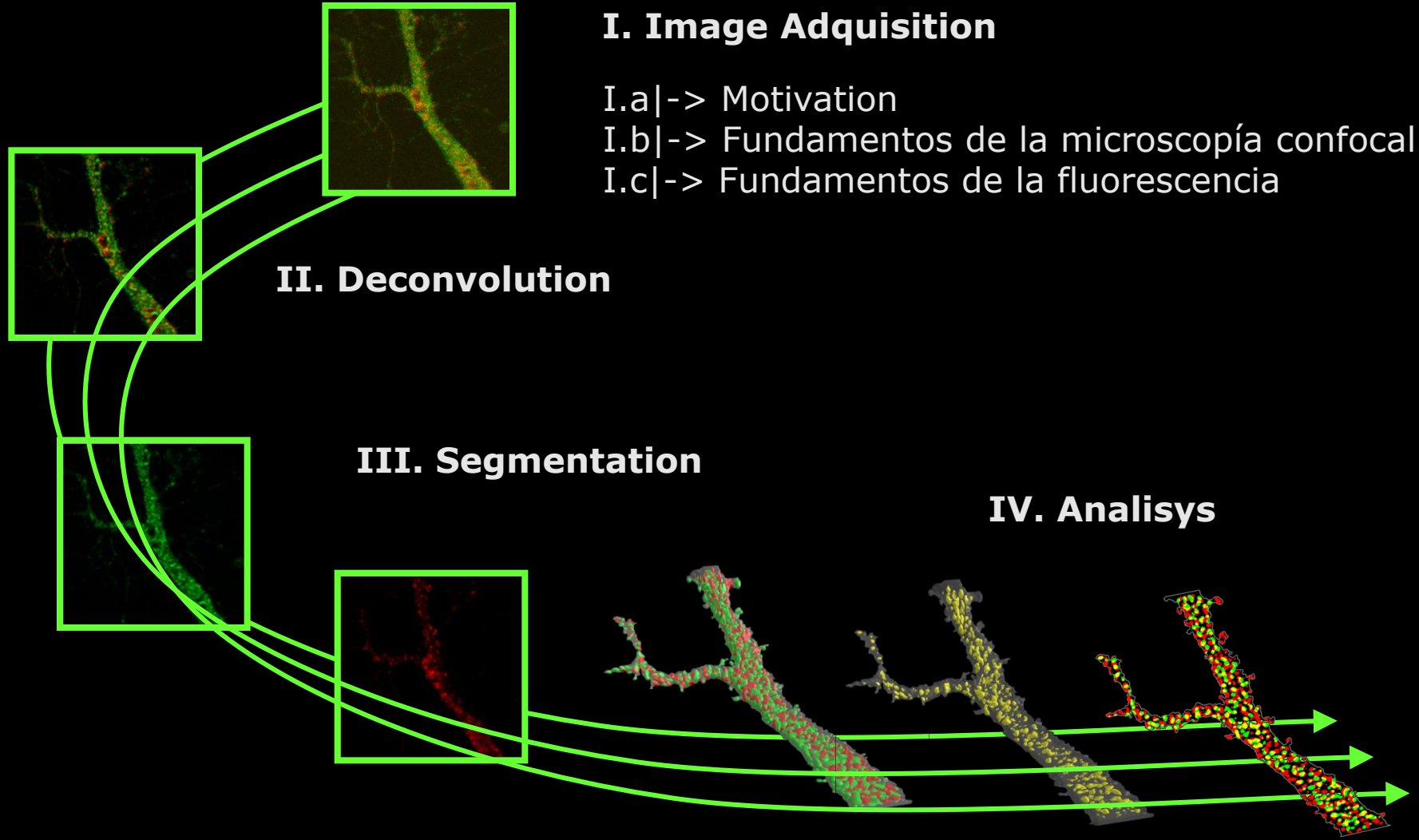
I. Image Adquisition

- I.a| -> Motivation
- I.b| -> Fundamentos de la microscopía confocal
- I.c| -> Fundamentos de la fluorescencia

II. Deconvolution

III. Segmentation

IV. Analisis





“It is very easy to answer many of these fundamental biological questions. You just look at the thing !

Make microscopes a hundred times more powerful and many problems of biology would be made very much easier.”

Richard Feynman (1918-1988)

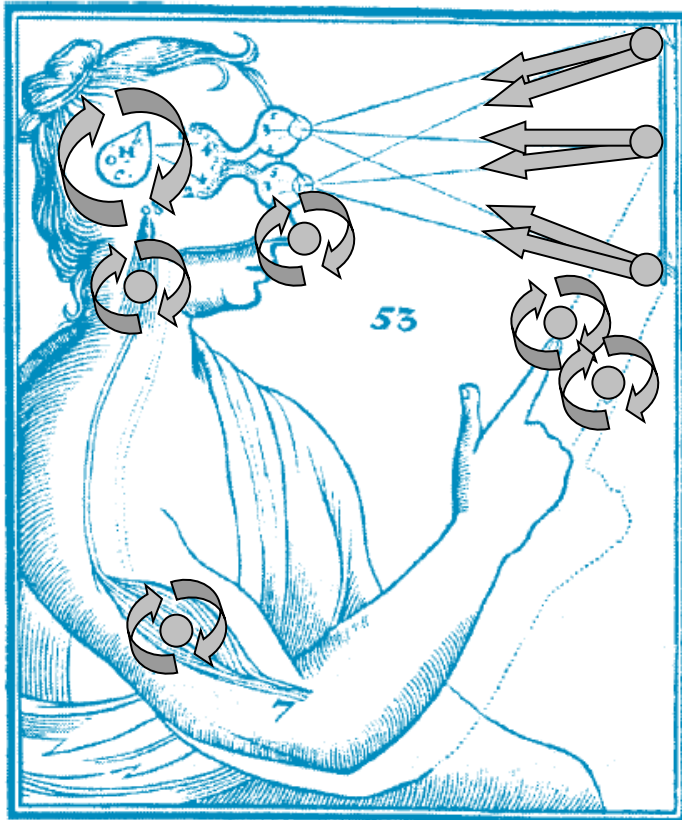


René Descartes (1596-1650)

**... just look at the thing ...
¿ Human visual perception ?**

Treatise of man (~ 1637)

Passions of the soul (~ 1649)



A combination of ...

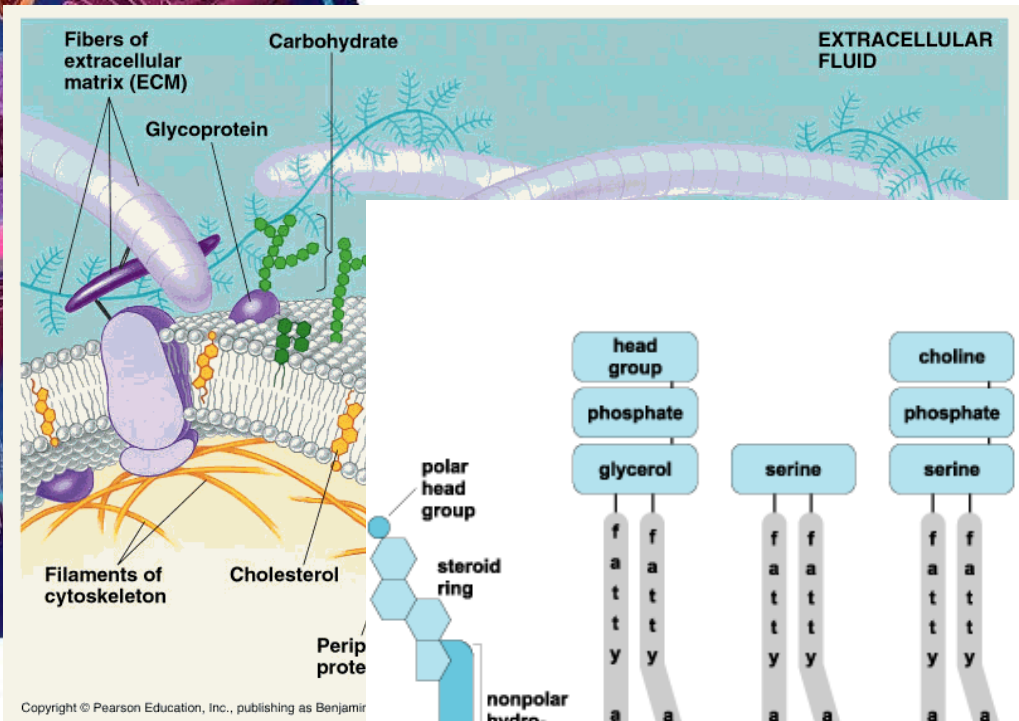
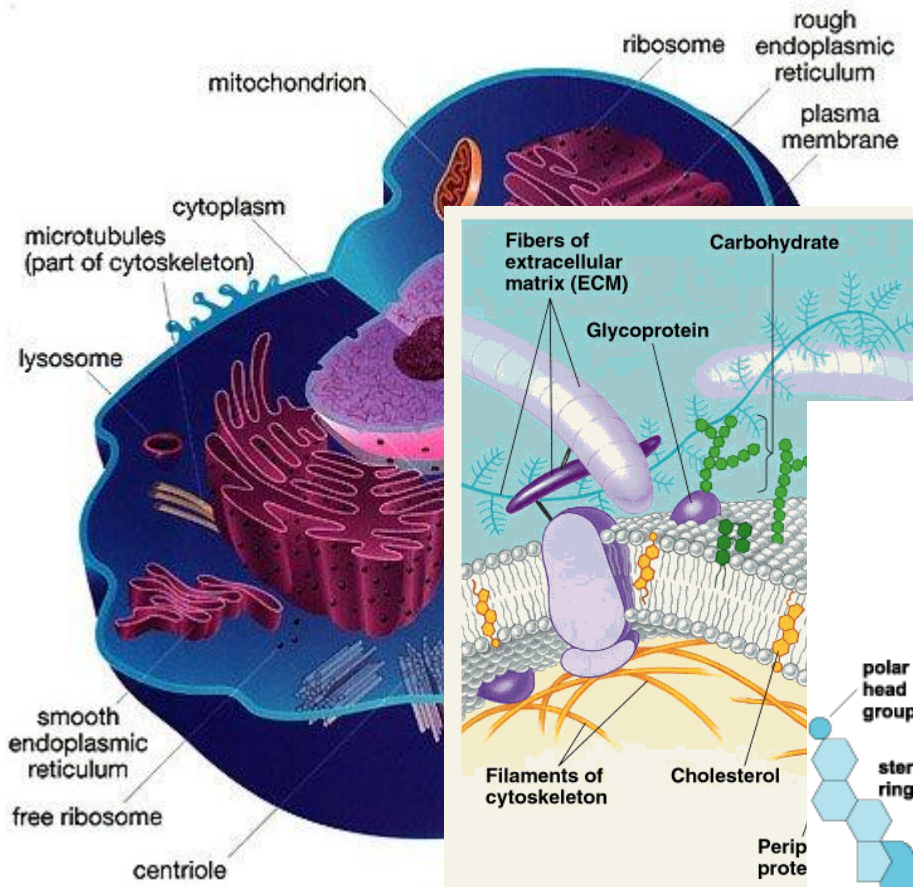
1| direct signals ...

2| signals from other senses ...

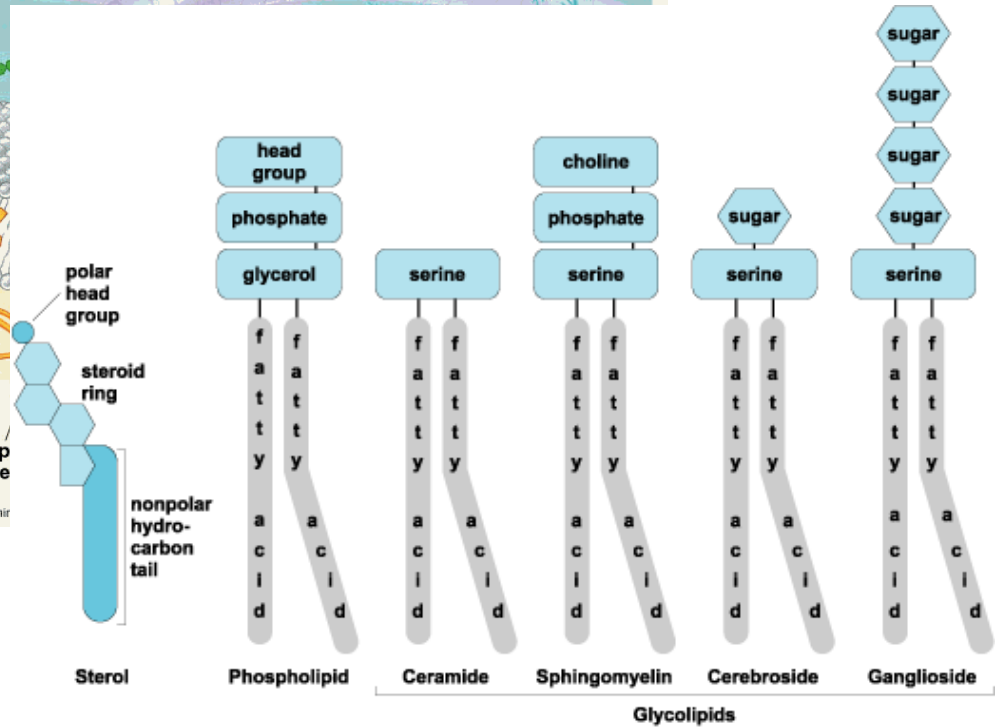
3| feedback loops ...

**... produce a symbolic
representation of an object.**

***glandula pinealis* / pineal organ**



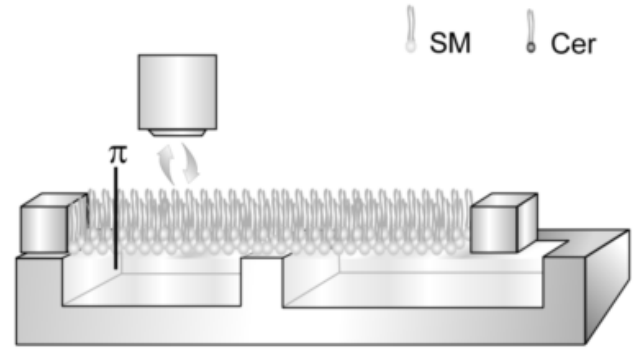
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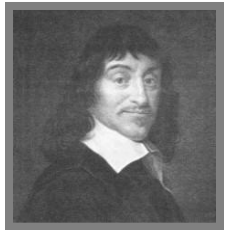


Glycolipids

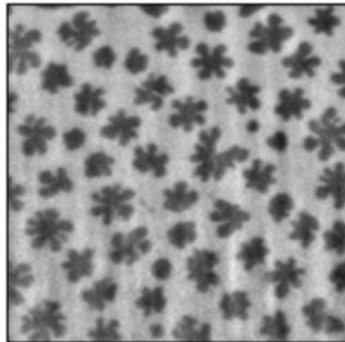


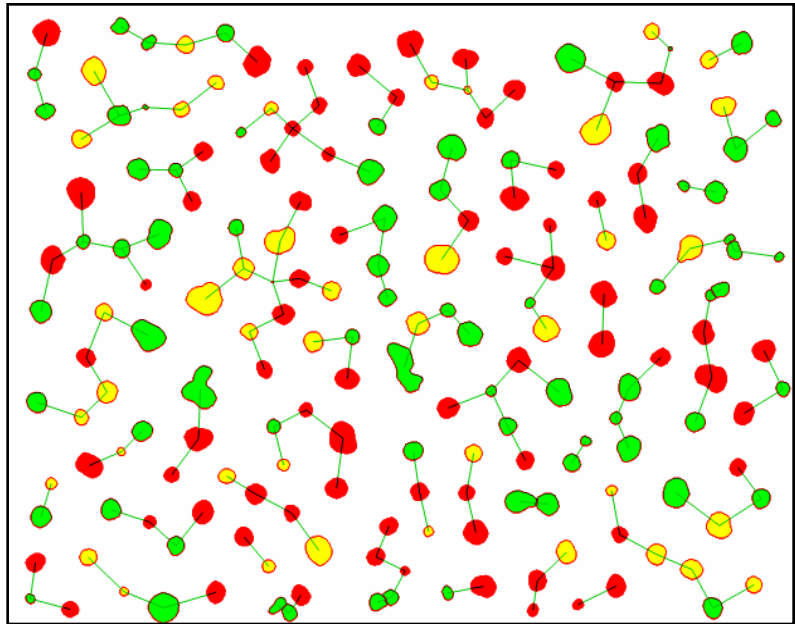
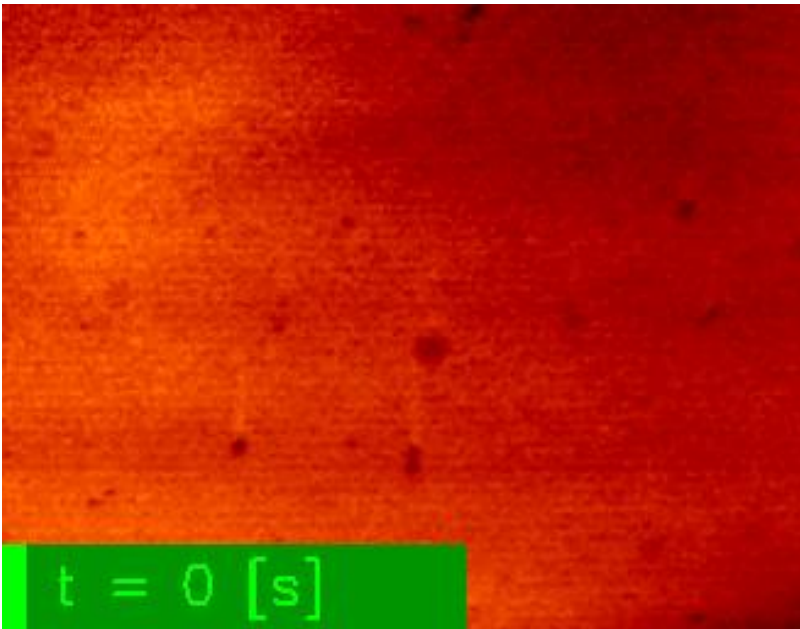
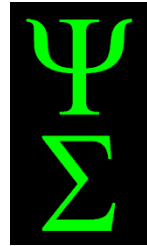
Langmuir trough coupled to time lapse microscopy

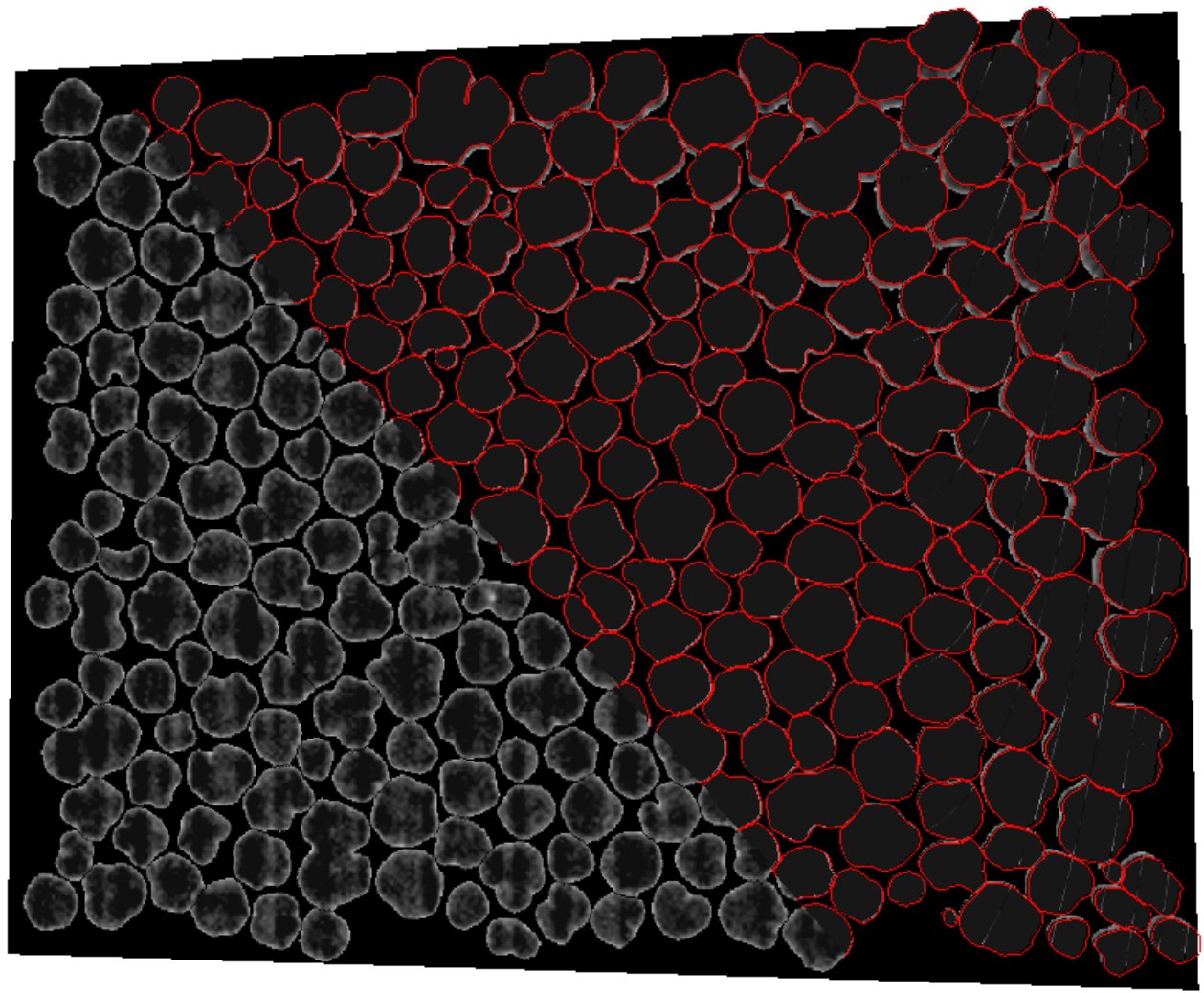


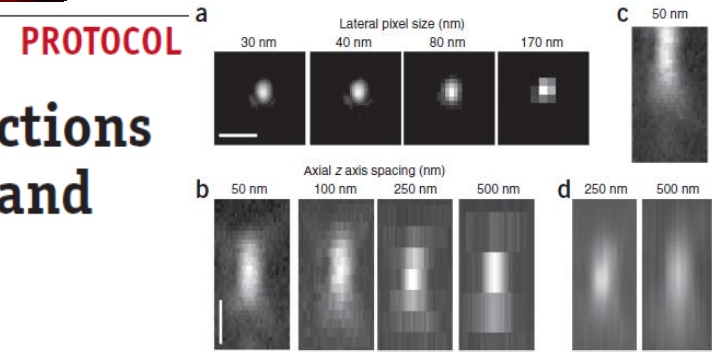
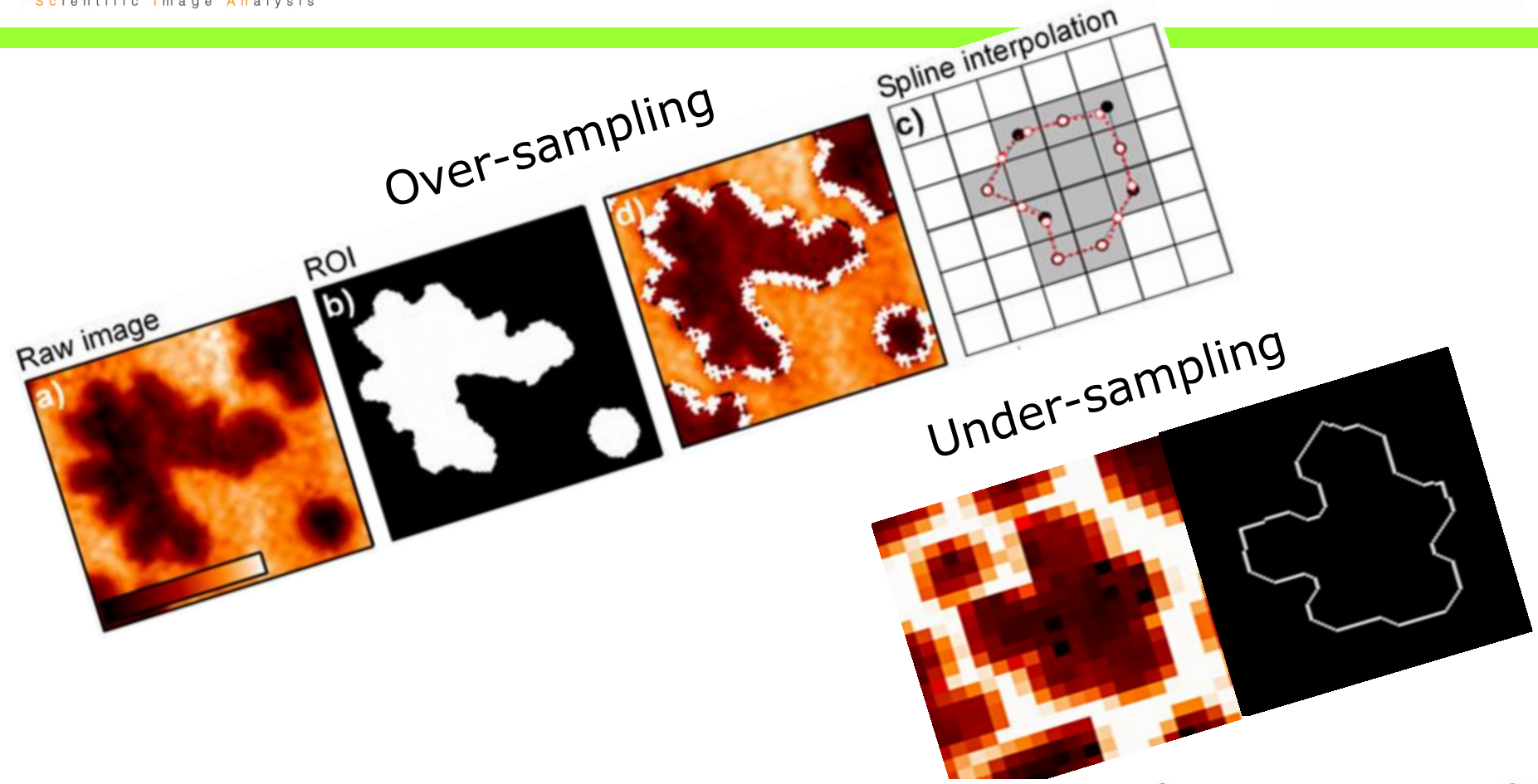


Fluorescence microscopy: visualization



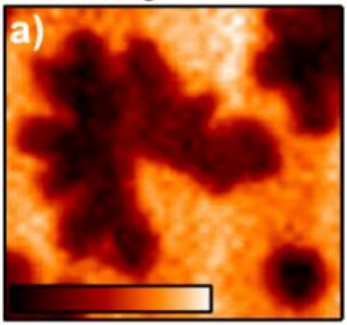




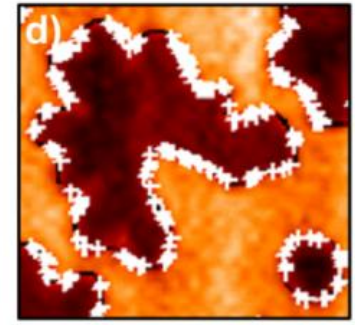
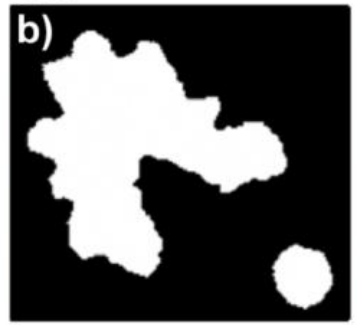


Measuring and interpreting point spread functions to determine confocal microscope resolution and ensure quality control

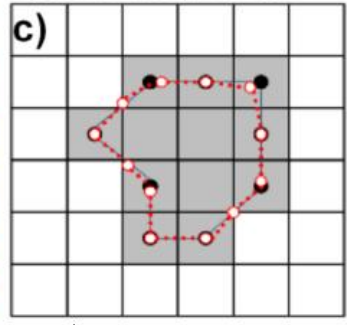
Raw image



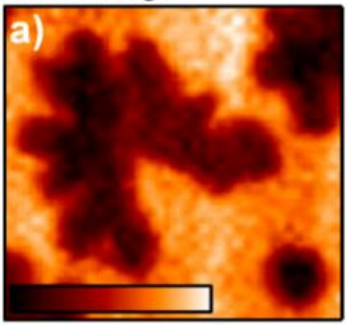
ROI



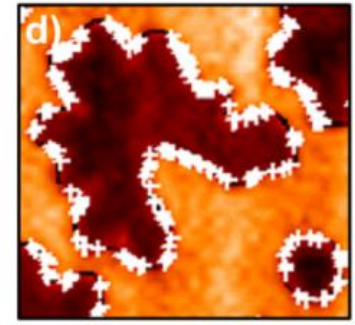
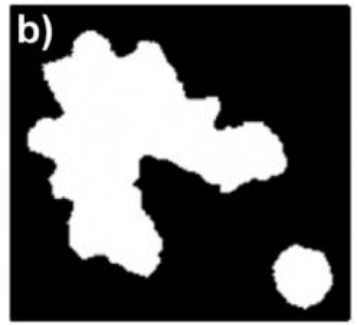
Spline interpolation



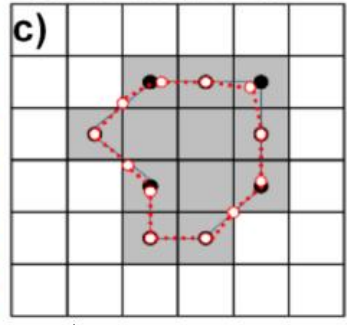
Raw image



ROI



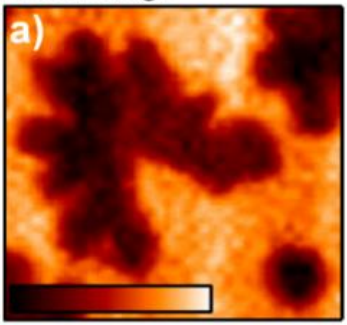
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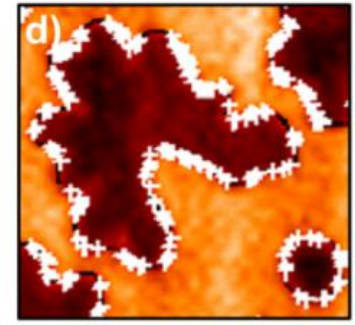
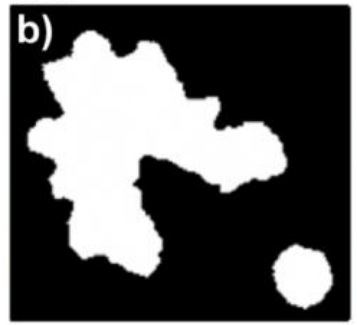
Parametric curve:

$$C=C(s,t) = [x(s,t),y(s,t)]$$

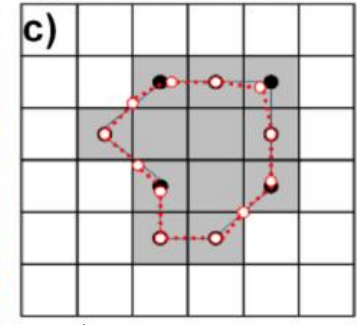
Raw image



ROI



Spline interpolation



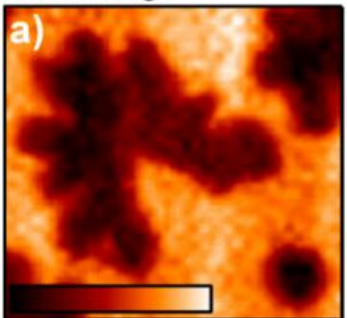
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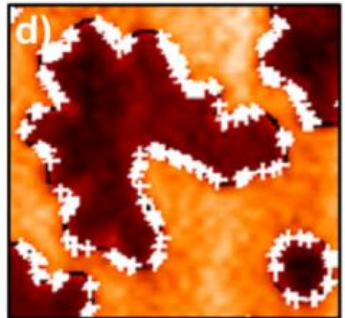
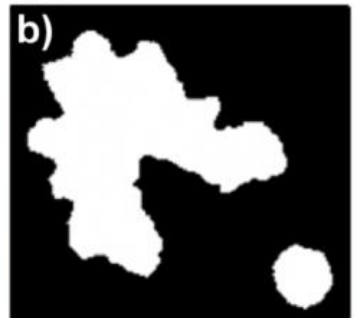
Force balance:

$$\gamma \frac{\partial C}{\partial t} = \underbrace{\beta \frac{\partial^4 C}{\partial s^4} - \alpha \frac{\partial^2 C}{\partial s^2}}_{\text{internal forces}} + \underbrace{f_{\text{img}}(C)}_{\text{image forces}}$$

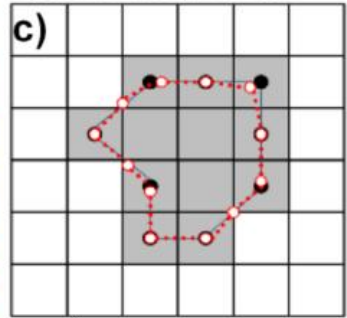
Raw image



ROI



Spline interpolation



Parametric curve:

$$C=C(s,t) = [x(s,t),y(s,t)]$$

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Force parameters:

- α : elasticity
- β : bending
- γ : viscosity

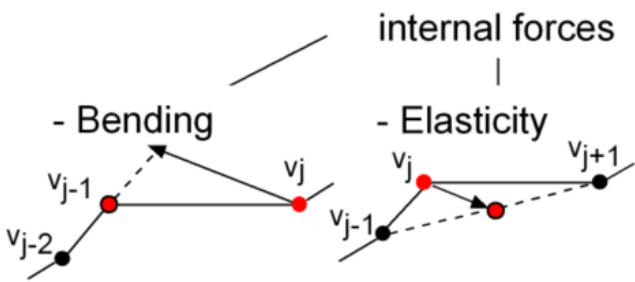
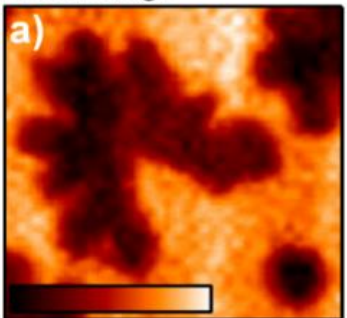


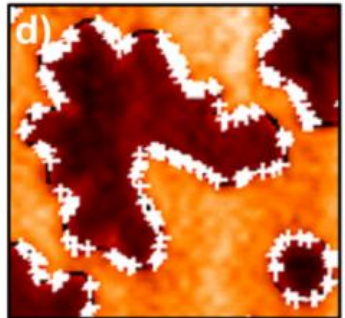
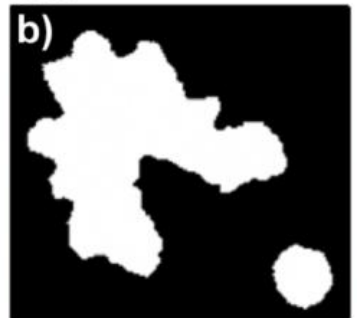
image forces

- $k \cdot V(C)$
GVF field $V(C)$
- $q \cdot B(C)$
Balloon force $B(C)$

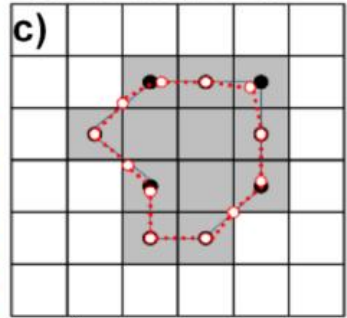
Raw image



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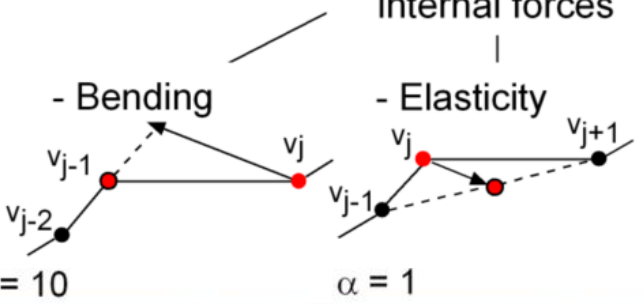


image forces

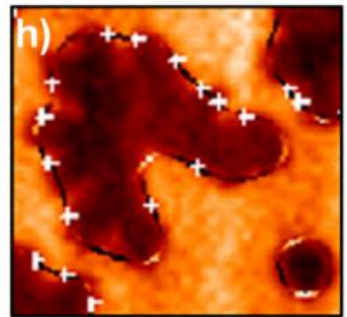
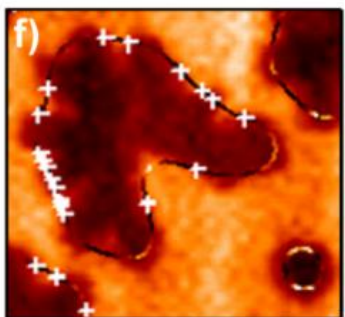
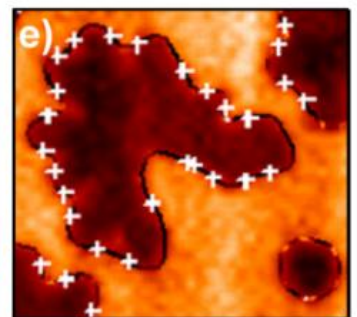
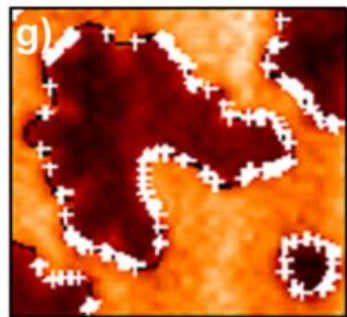
- $k \cdot V(C)$
GVF field $V(C)$
- $q \cdot B(C)$
Balloon force $B(C)$

$\beta = 0$

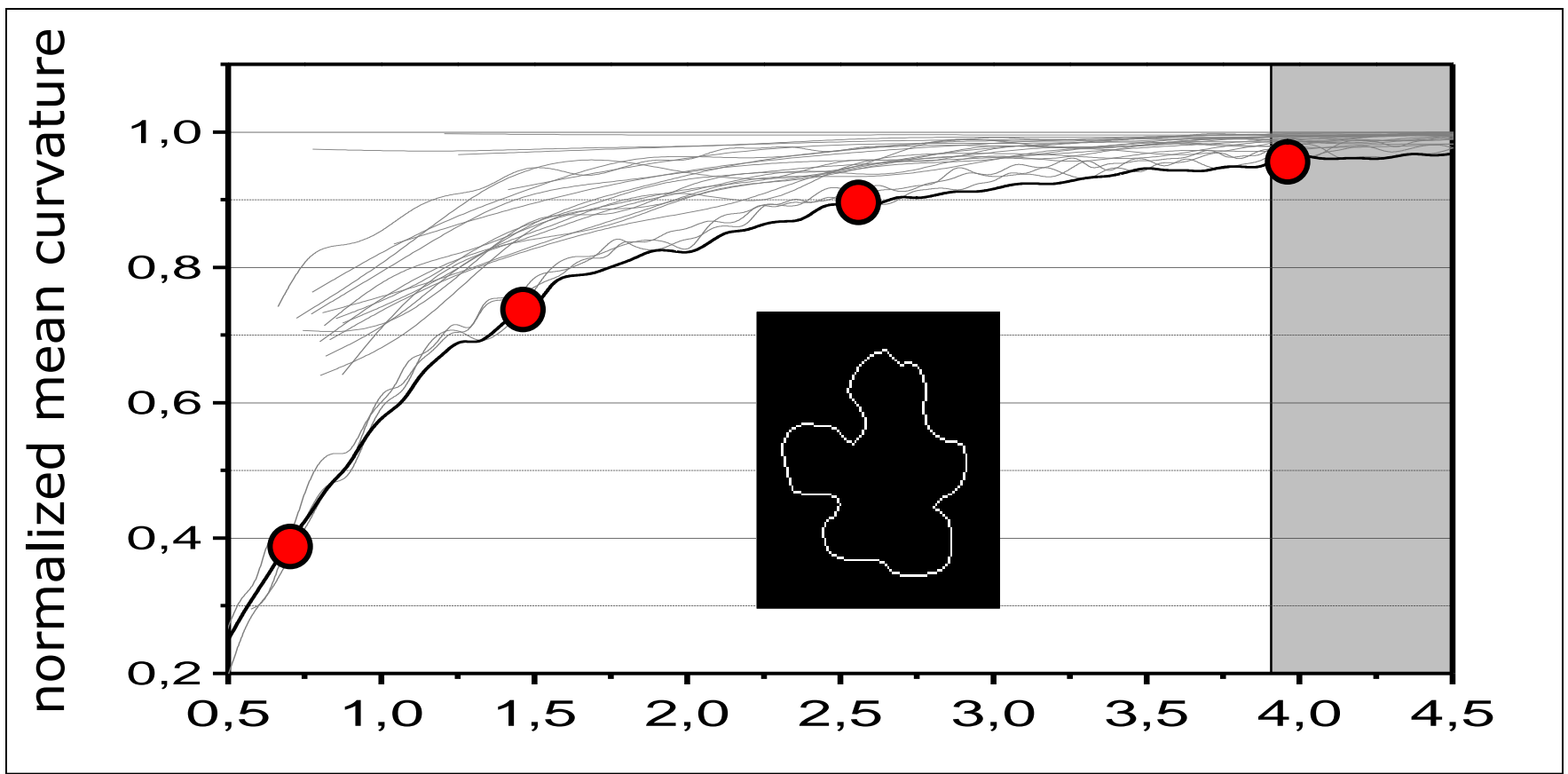
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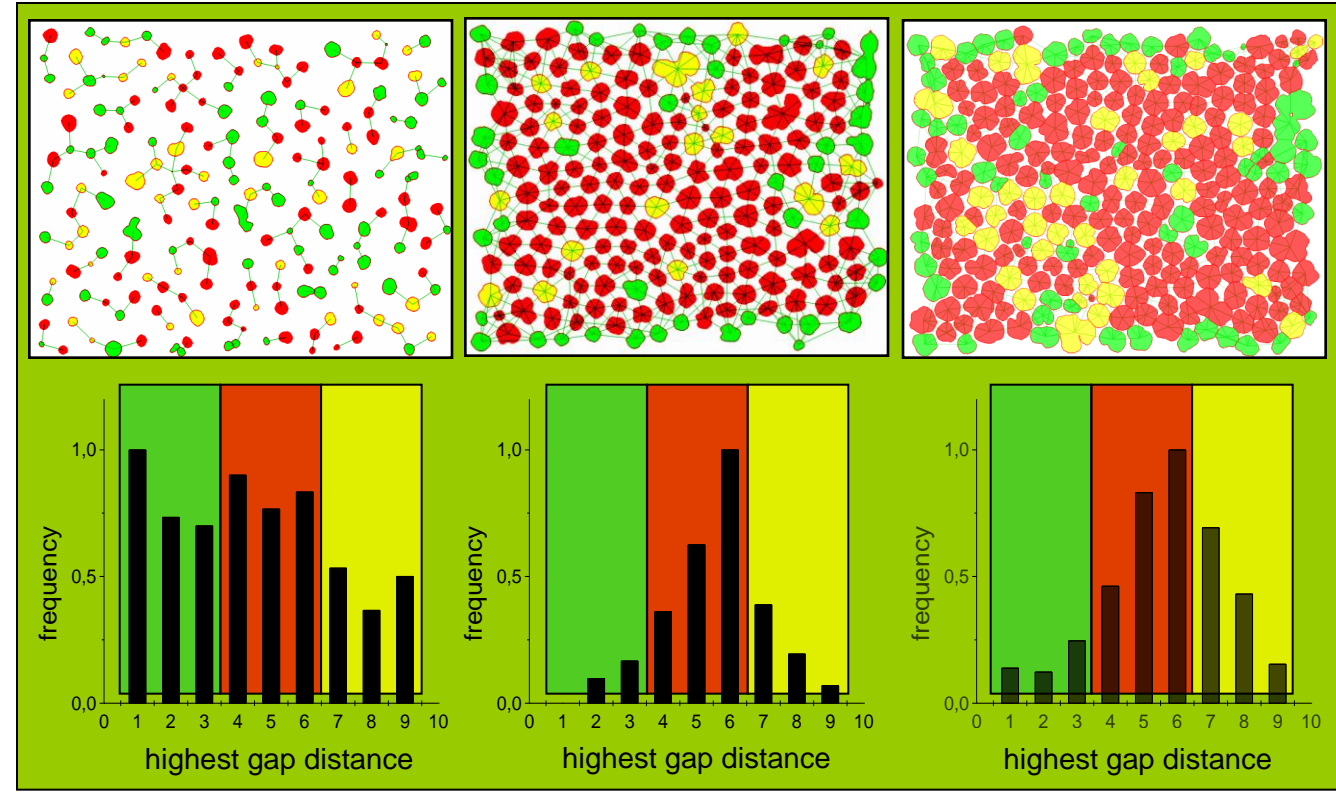
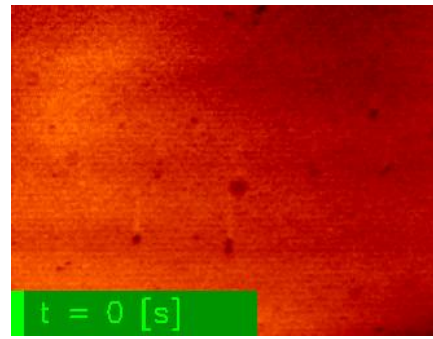
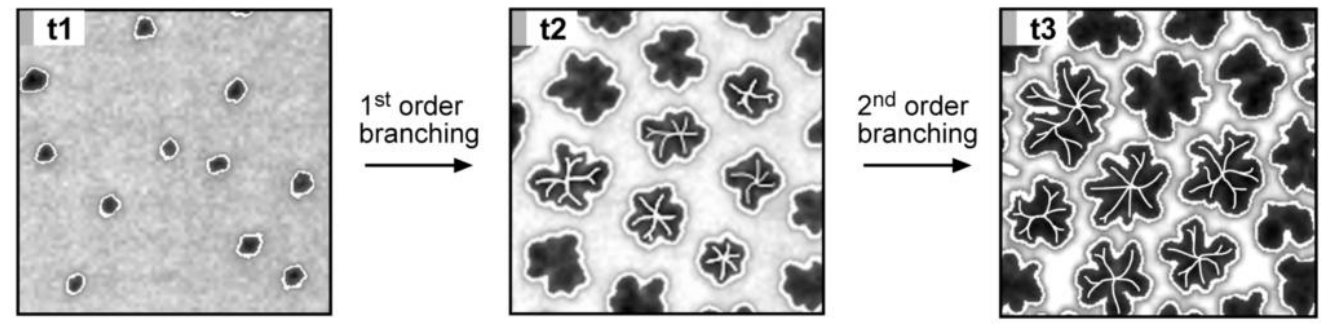
$\alpha = 1$

it = 10

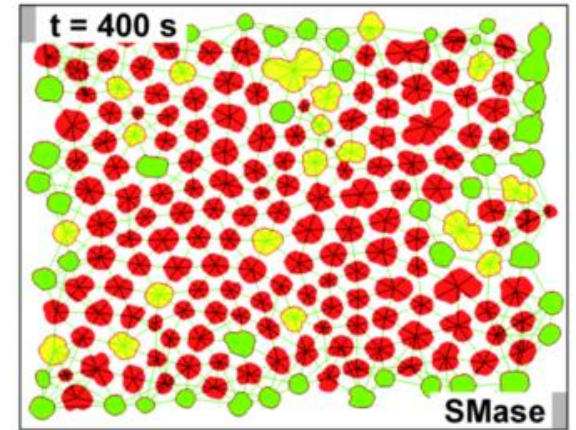
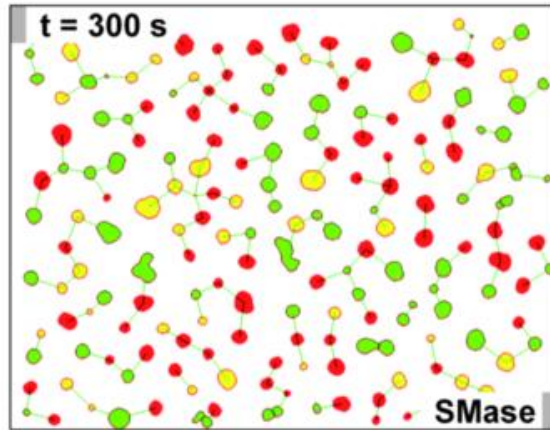
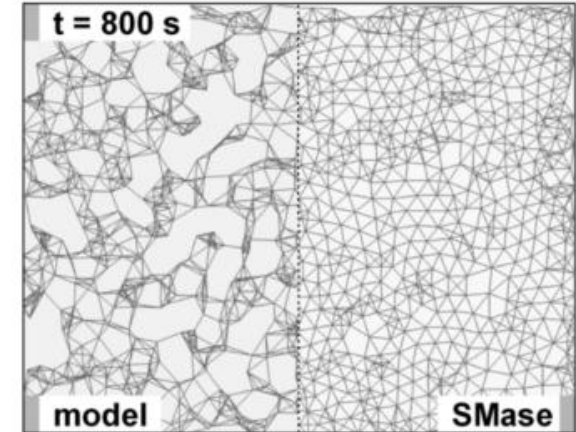
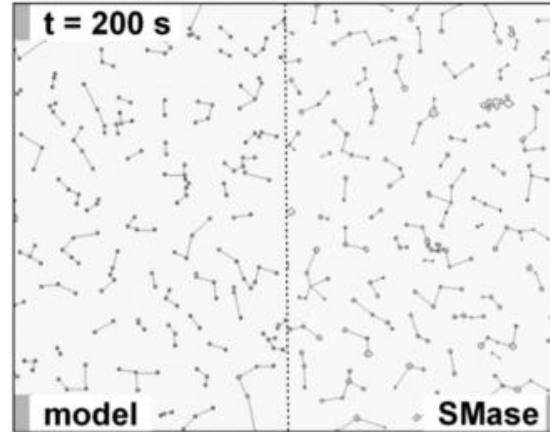
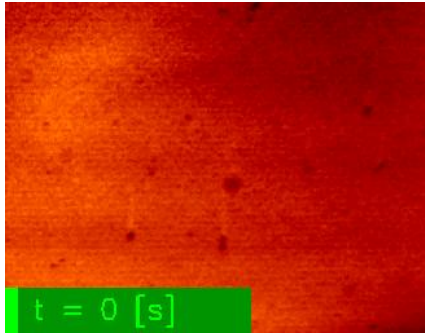


Interpolación de contornos

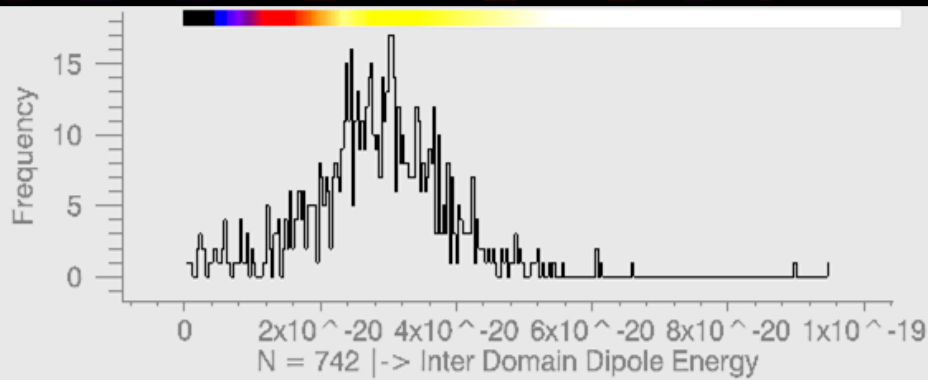


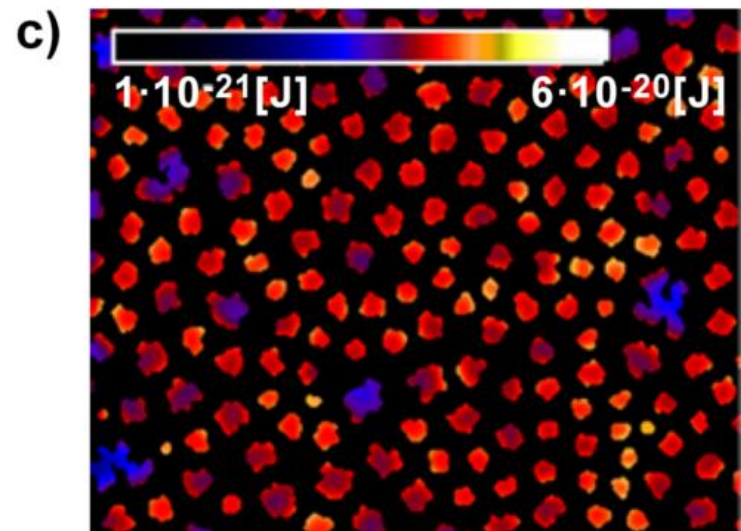
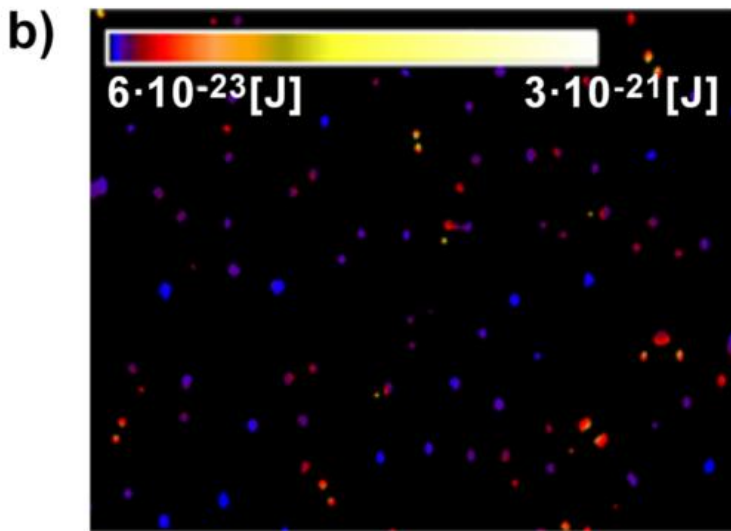
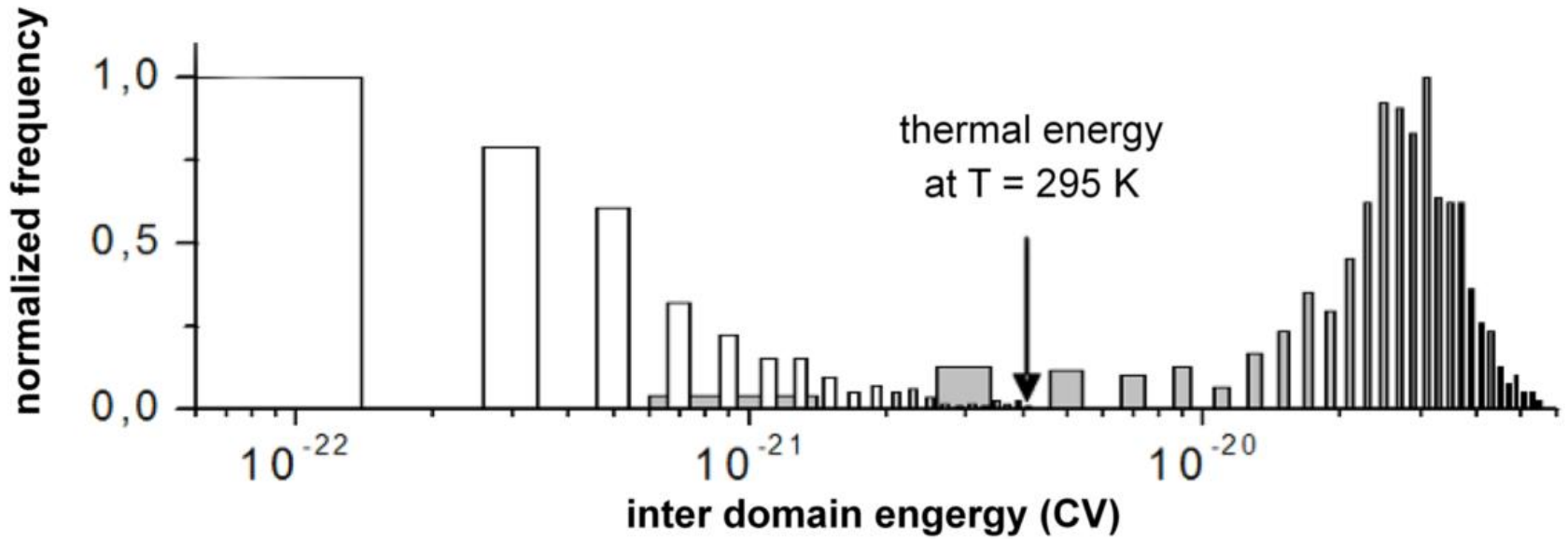


Parametrización

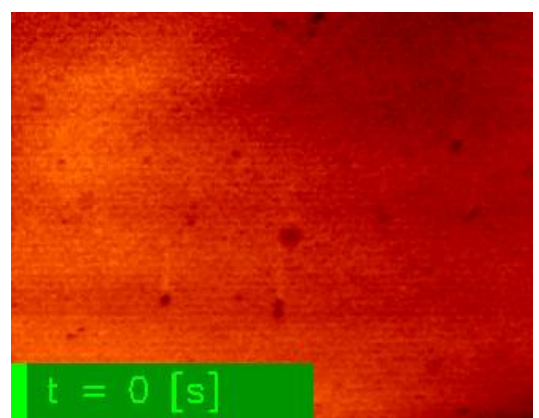
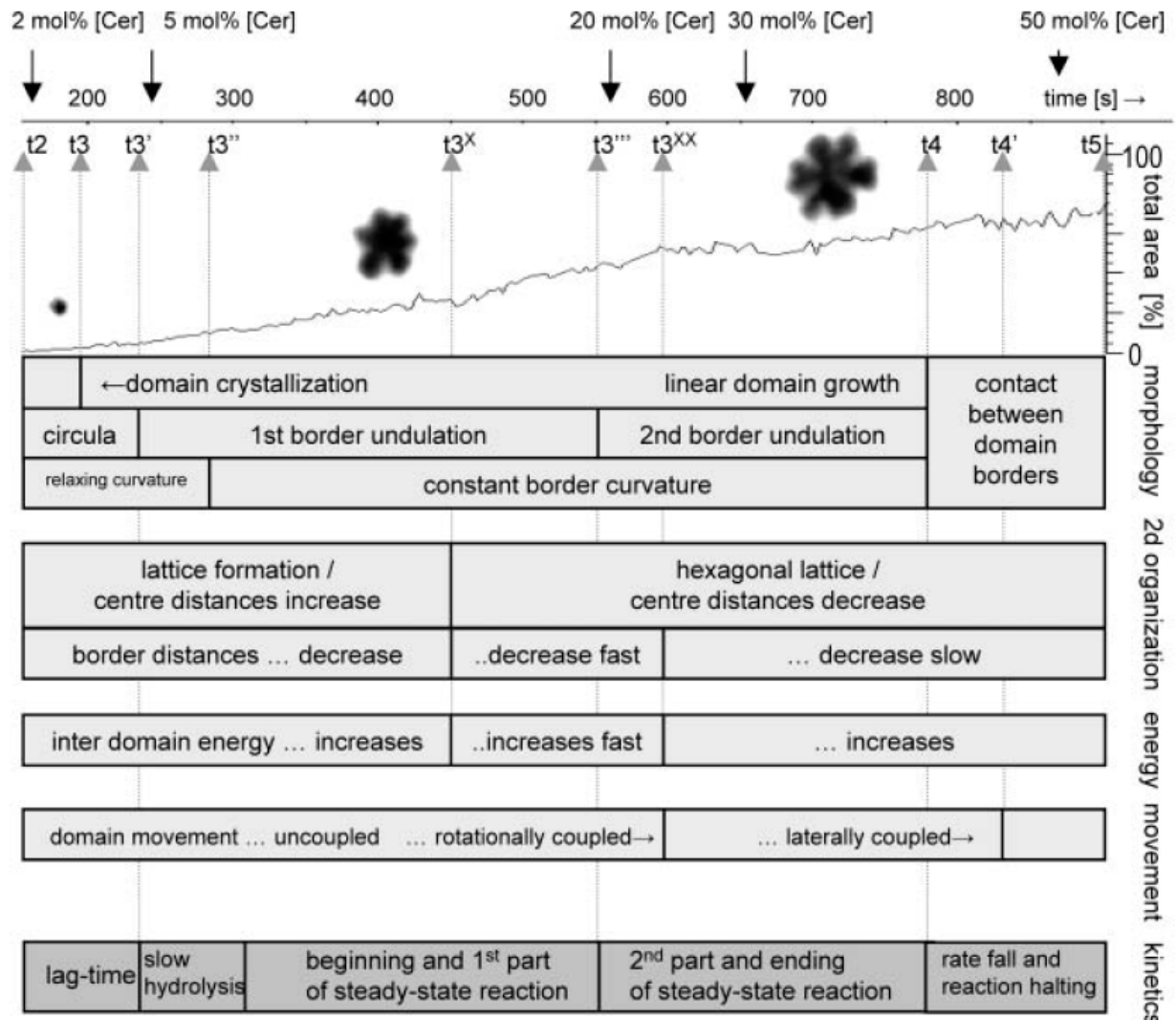


dipoles -> dipole moment density -> inter domain energy

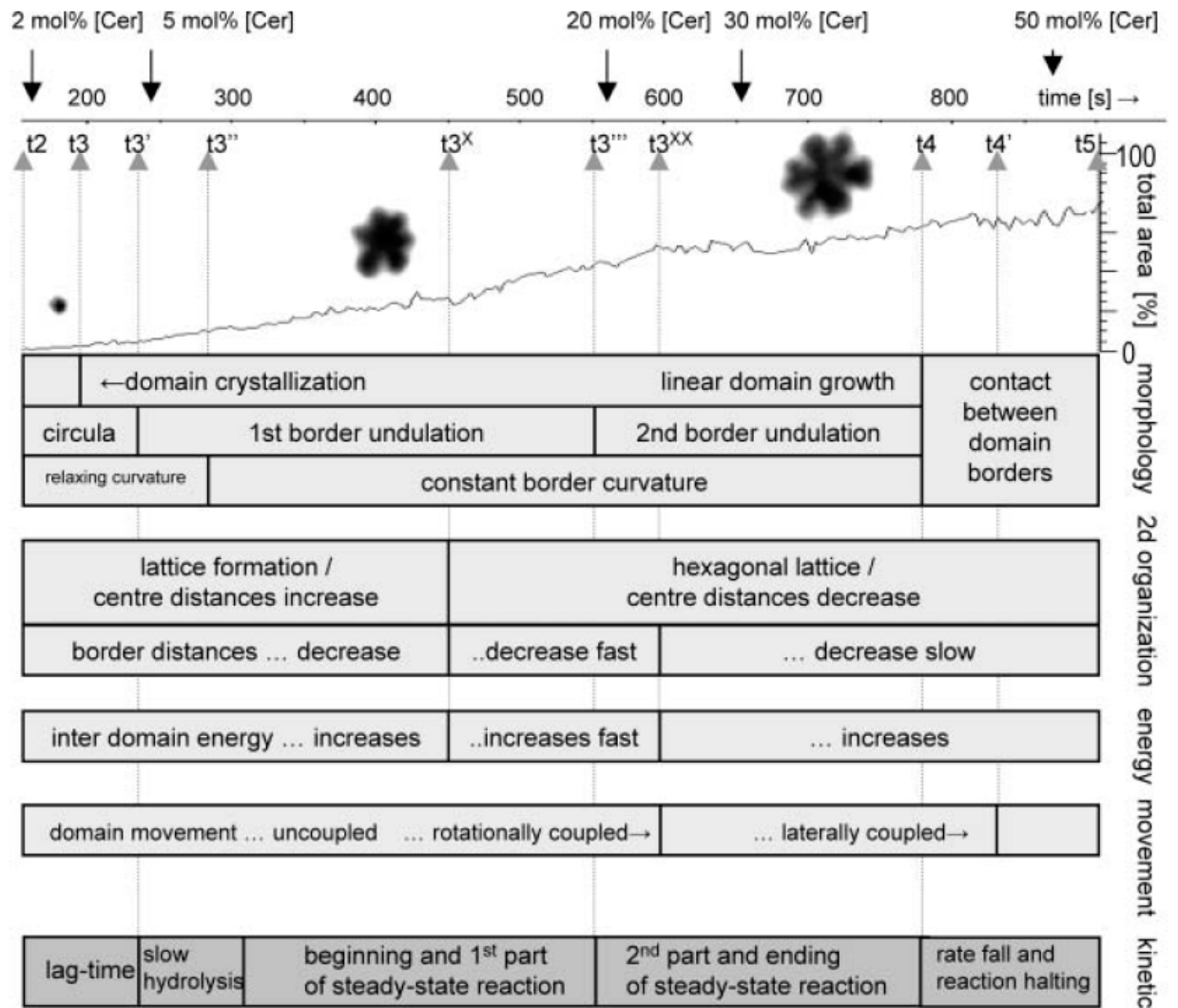
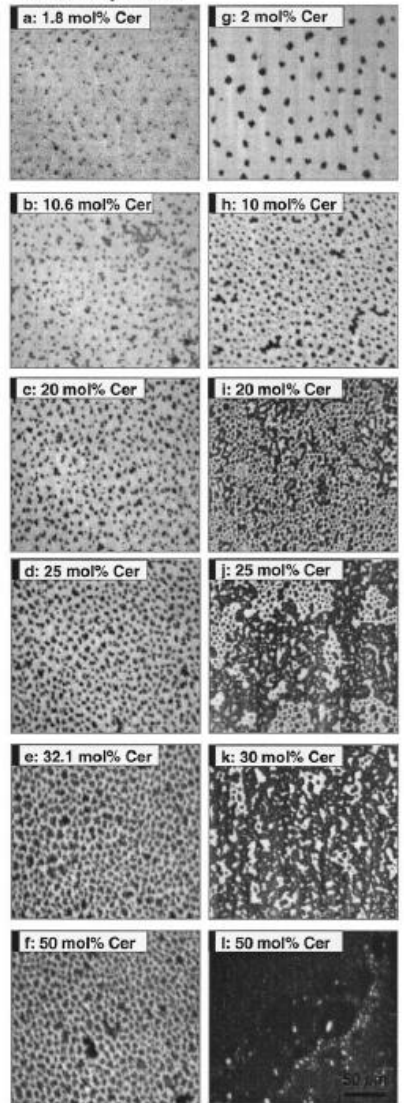




Multiples parametros



SM→Cer conversion Mixture of SM / Cer induced by SMase



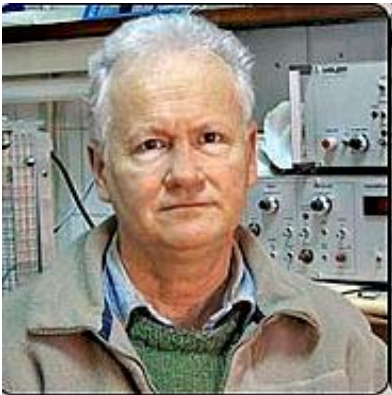
Laura Fanani



2D · Morpho-topology in lipid monolayers

2010 BBA
2009 Biophysical Journal
2007 Cell Biochemistry and Biophysics
2005 Biophysical Journal
2003 Chemistry and Physics of Lipids
2002 Biophysical Journal

Bruno Maggio

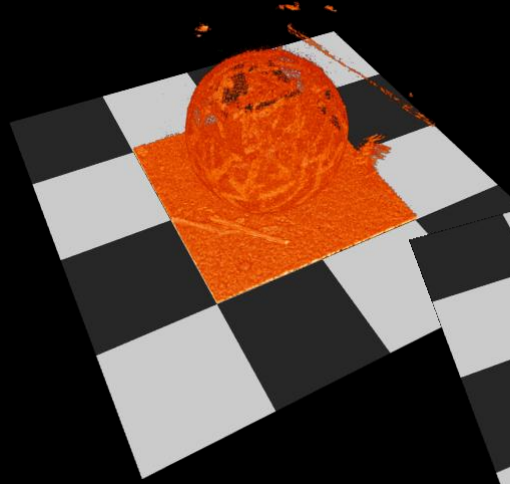


For enzyme-free SM/Cer monolayers, the LC domains formed cover an area larger than expected. Cer-enriched domains at equilibrium conditions contains about 50% of SM.

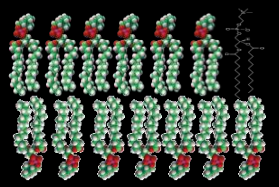
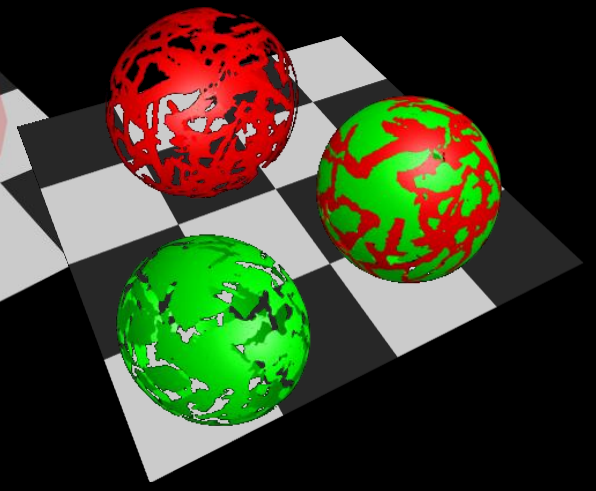
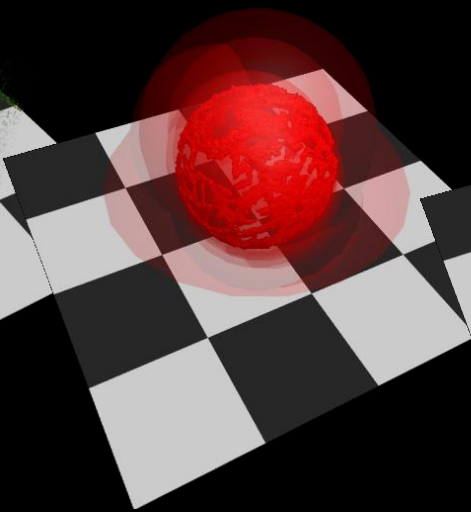
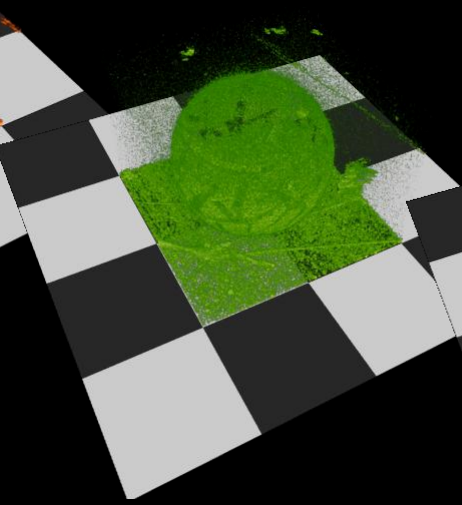
In SMase-domains present a high content of Cer, **higher intradomain repulsion** and, as a consequence, star-like shaped domains, while condensed rounded domains formed in the enzyme-free films.

The self-organization into highly ordered hexagonal lattice patterns is a consequence of a enhanced interdomain repulsion. **Repulsion is lower in enzyme-free monolayers.**

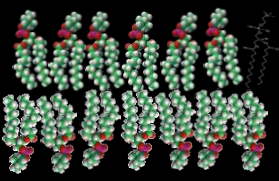
DPPC



DLPC

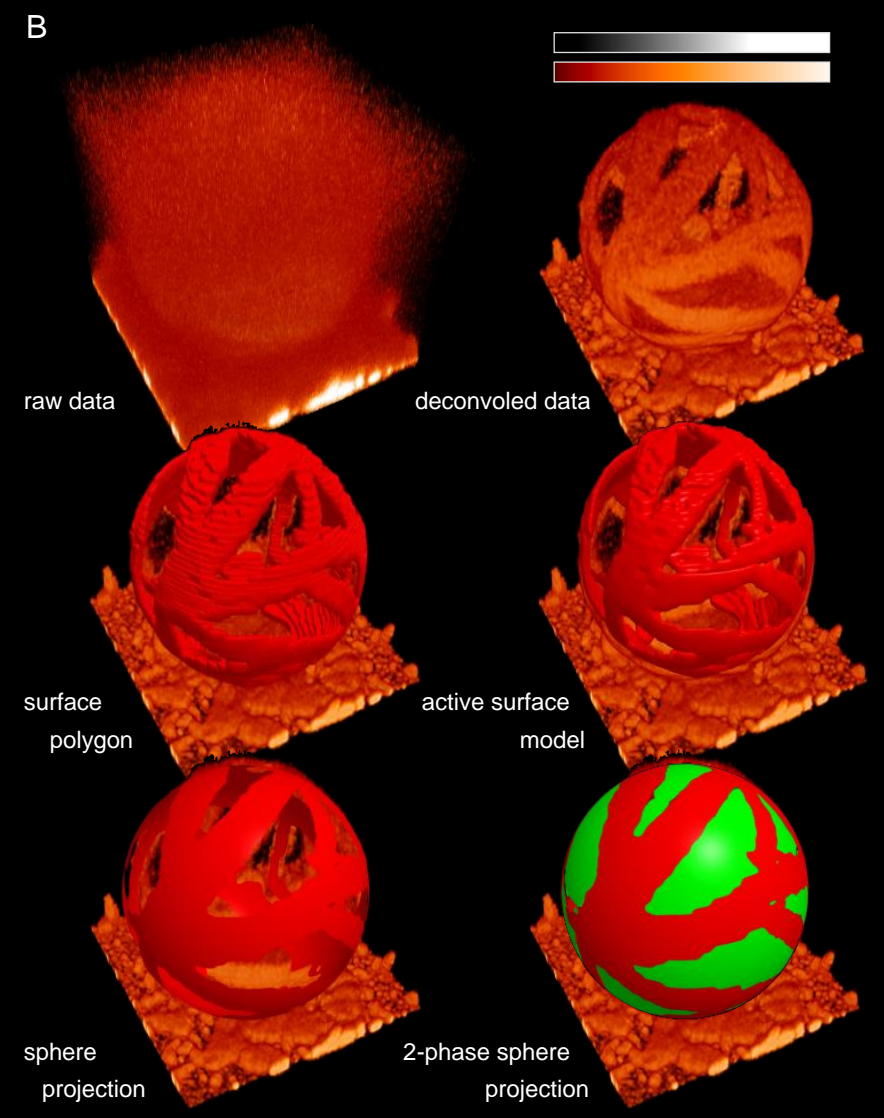
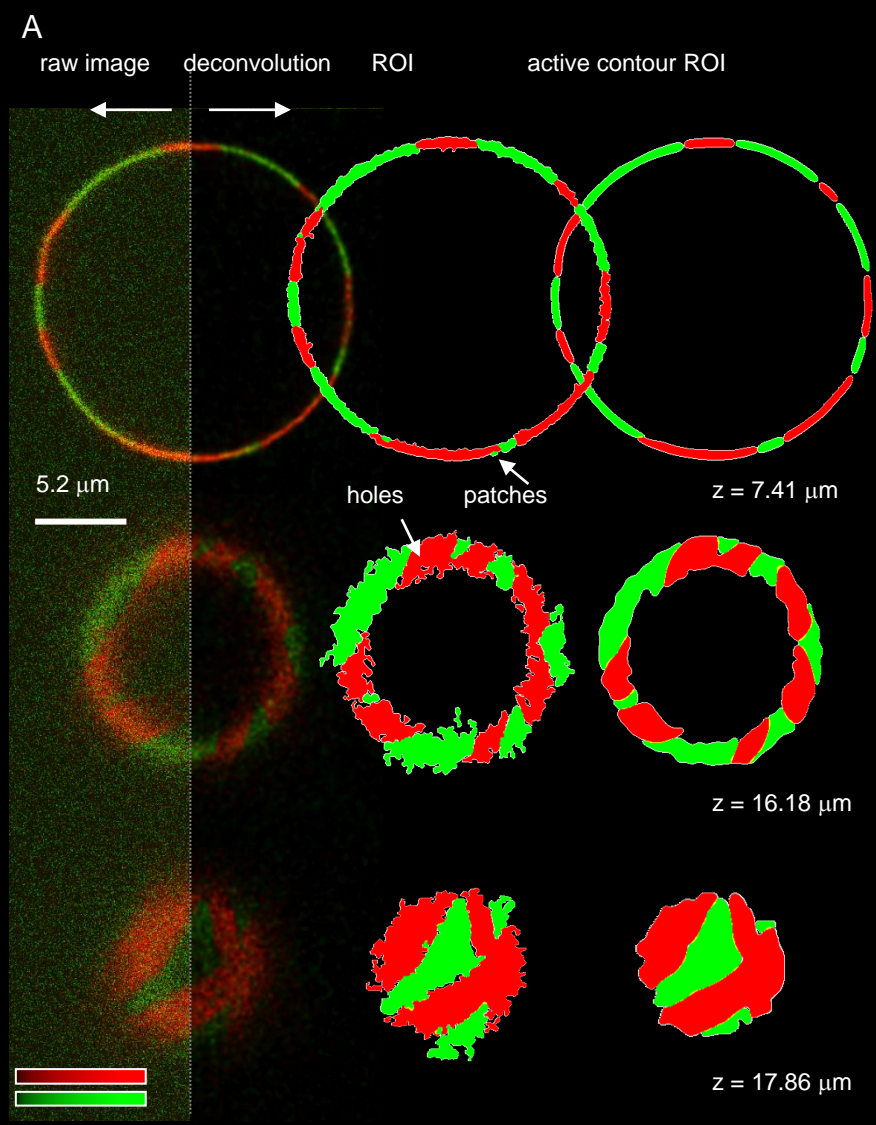


At $T = 20\text{ }^{\circ}\text{C}$: **Solid Gel or S_0 phase is formed by DPPC 16:0** (1,2-Dipalmitoyl-*sn*-Glycero-3-Phosphocholine) + 0.5 mol% DiIC₁₈.

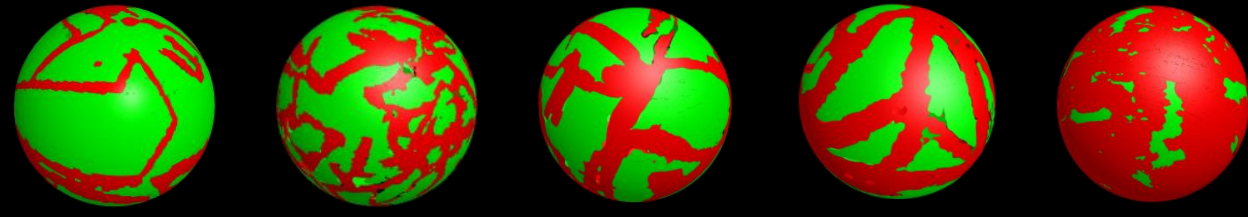


At $T = 20\text{ }^{\circ}\text{C}$: **Fluid phase is formed by DLPC 12:0 PC** (1,2-Dilauroyl-*sn*-Glycero-3-Phosphocholine) + 0.5 mol% BODIPY-PC.

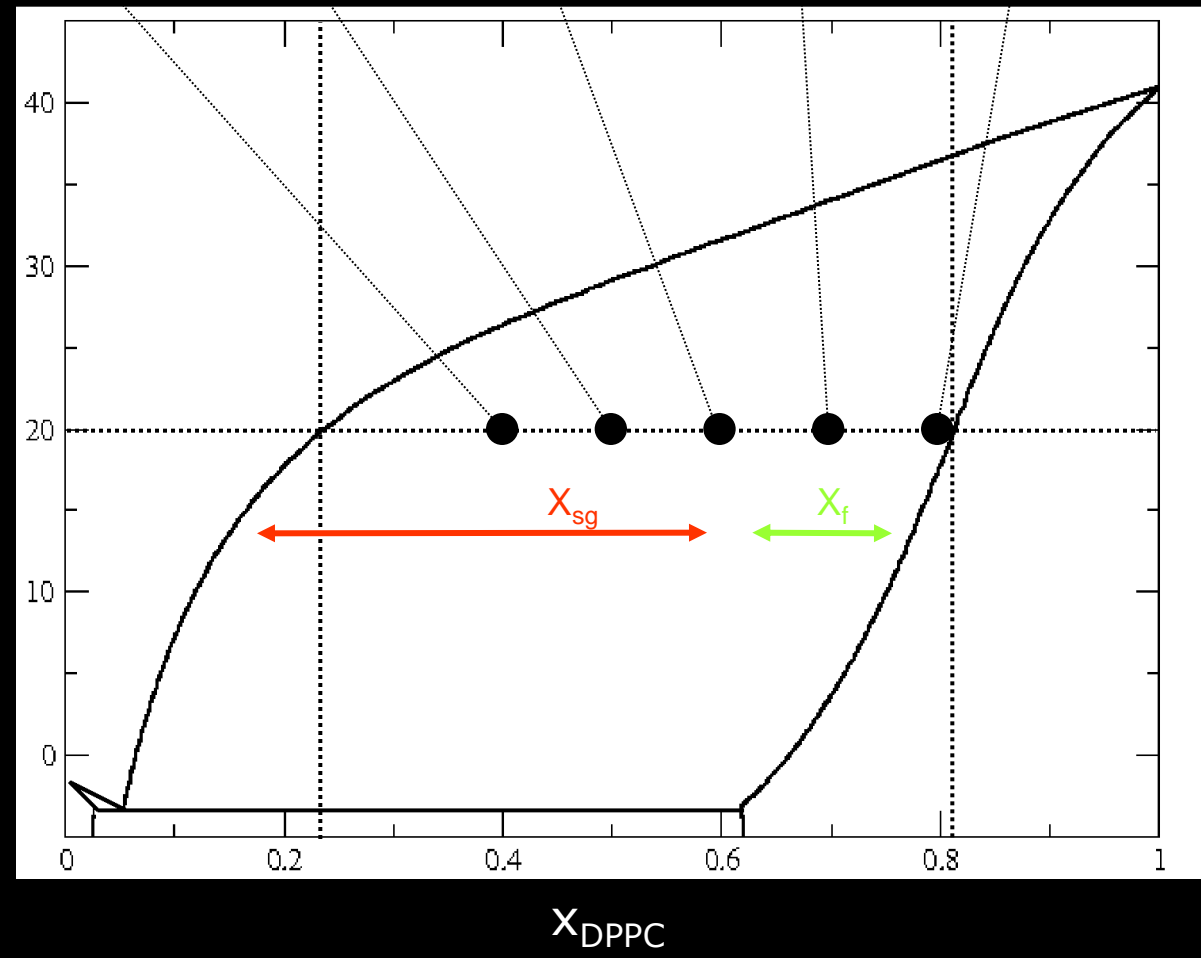
Interpolación de contornos



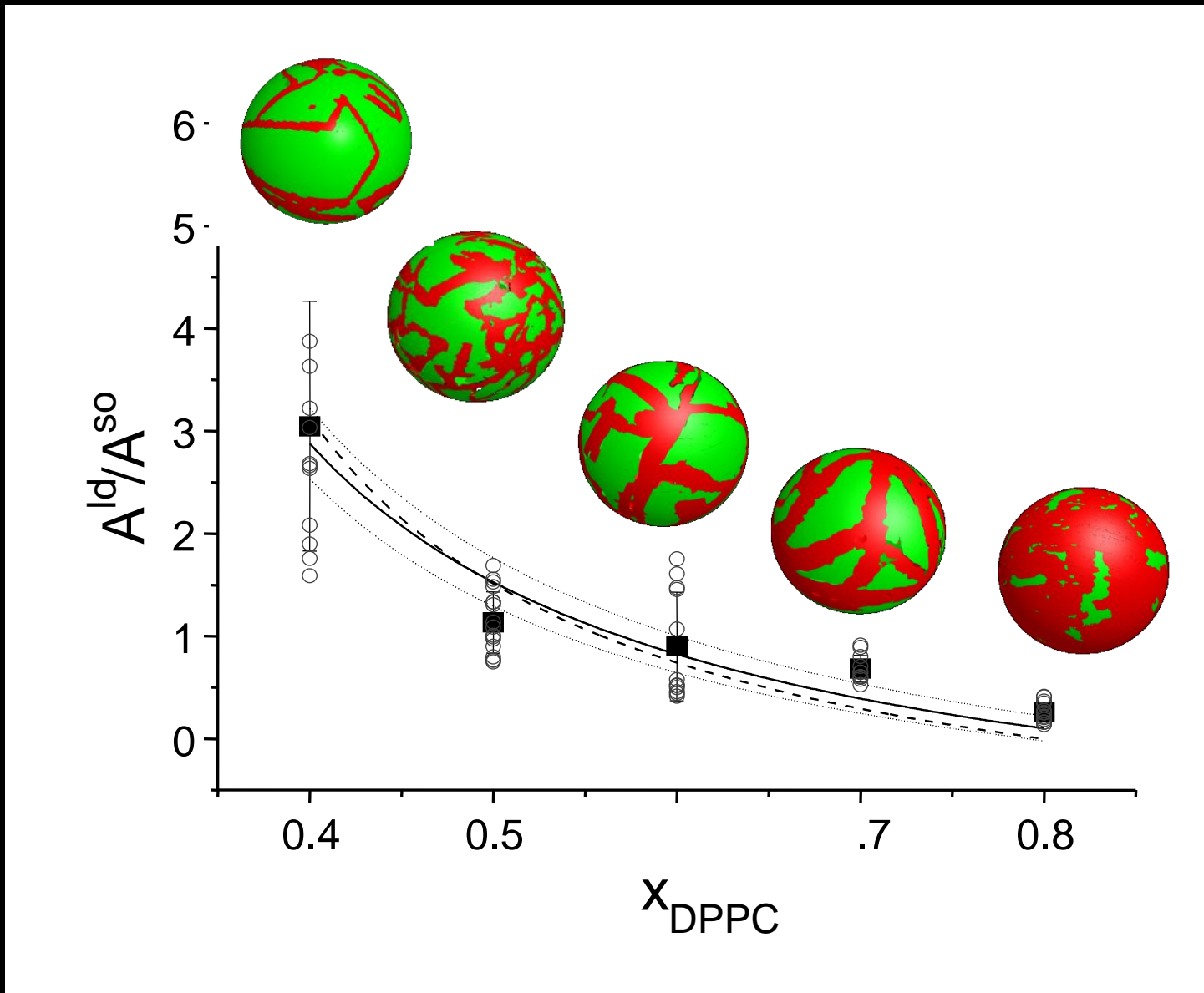
Interpolación de contornos



T [°C]



Interpolación de contornos

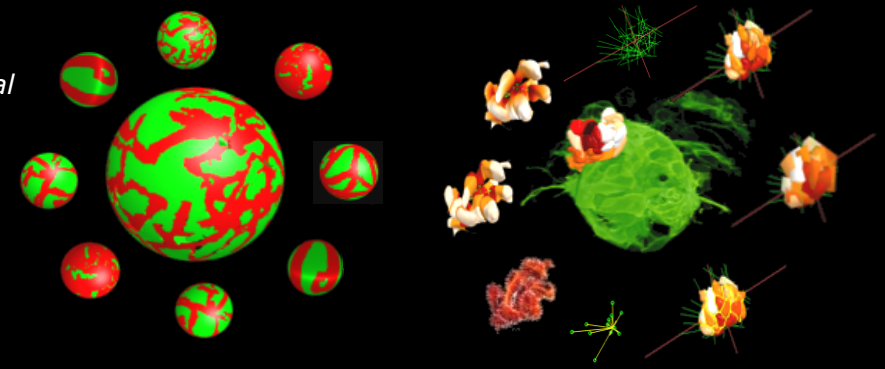


Luis Bagatolli (MEMPHYS, DK)



3D · Morpho-topology

- 2012 *European Biophysics Journal*
- 2010 *J of Struct Biol*
- 2010 *Development*
- 2010 *Biological Research*
- 2009 BBA**
- 2007 *Computational Modeling*



Miguel Concha



GUV populations can be prepared with excellent reproducibility.

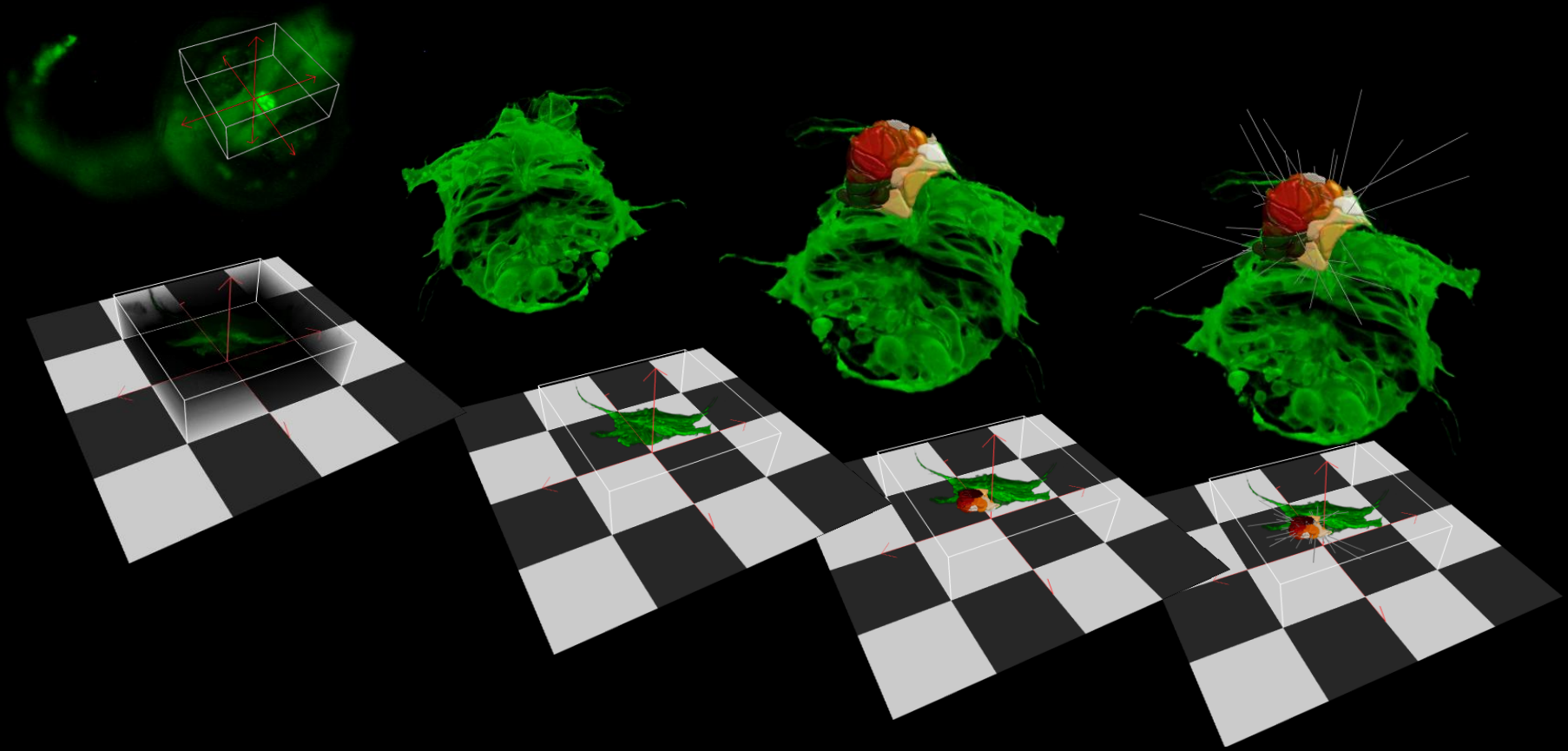
The theoretical lever was approved experimentally in GUVs .

2D-lipid domain surfaces can be determined reliably from 3D image stacks.

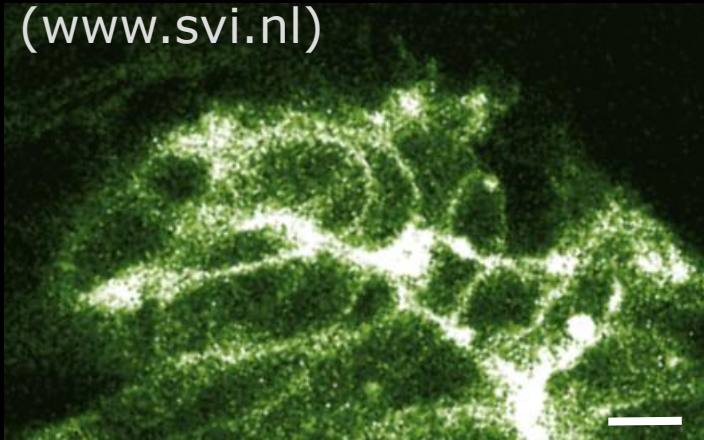
Facultad de Medicina, U-Chile

Morpho-genetic mechanisms, form, function in developmental biology

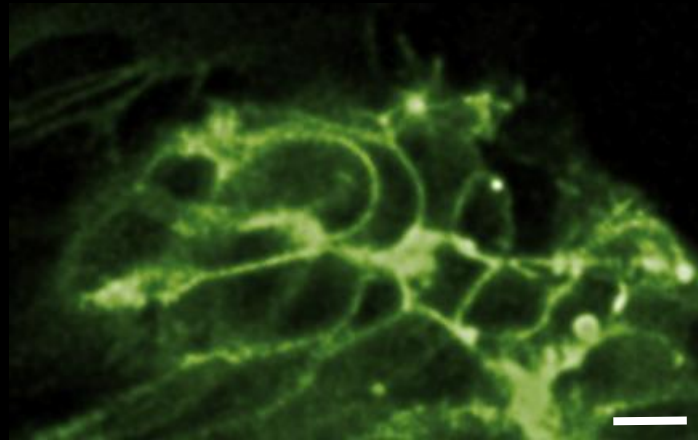
Transgenic *flh::GFP*



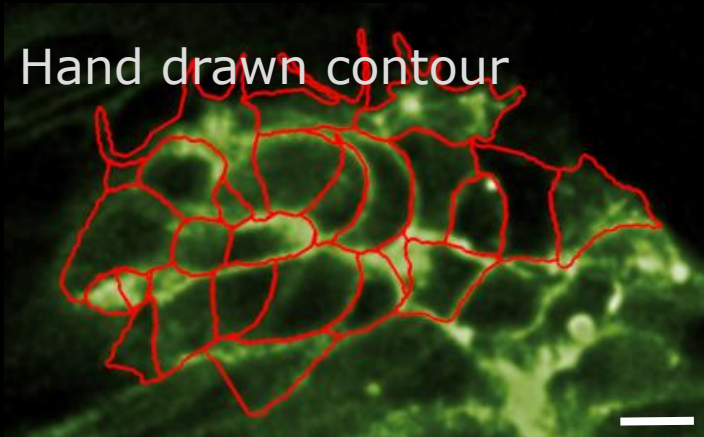
Original Data
(www.svi.nl)



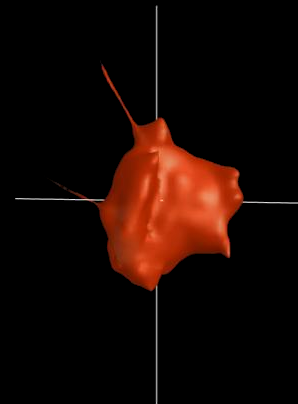
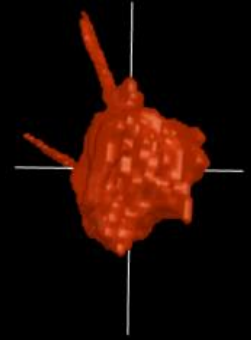
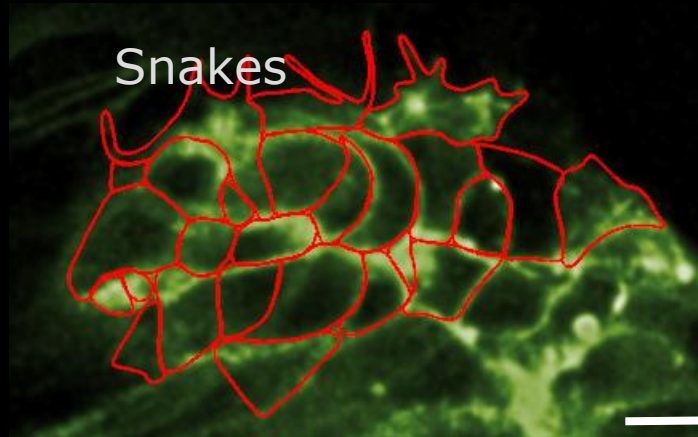
Deconvolved Data



Hand drawn contour



Snakes



Kass M. et al (1988) Int. J. Comp. Vis. 1:321-331.
Xu & Prince (1998) Signal Processing 71: 131-139.

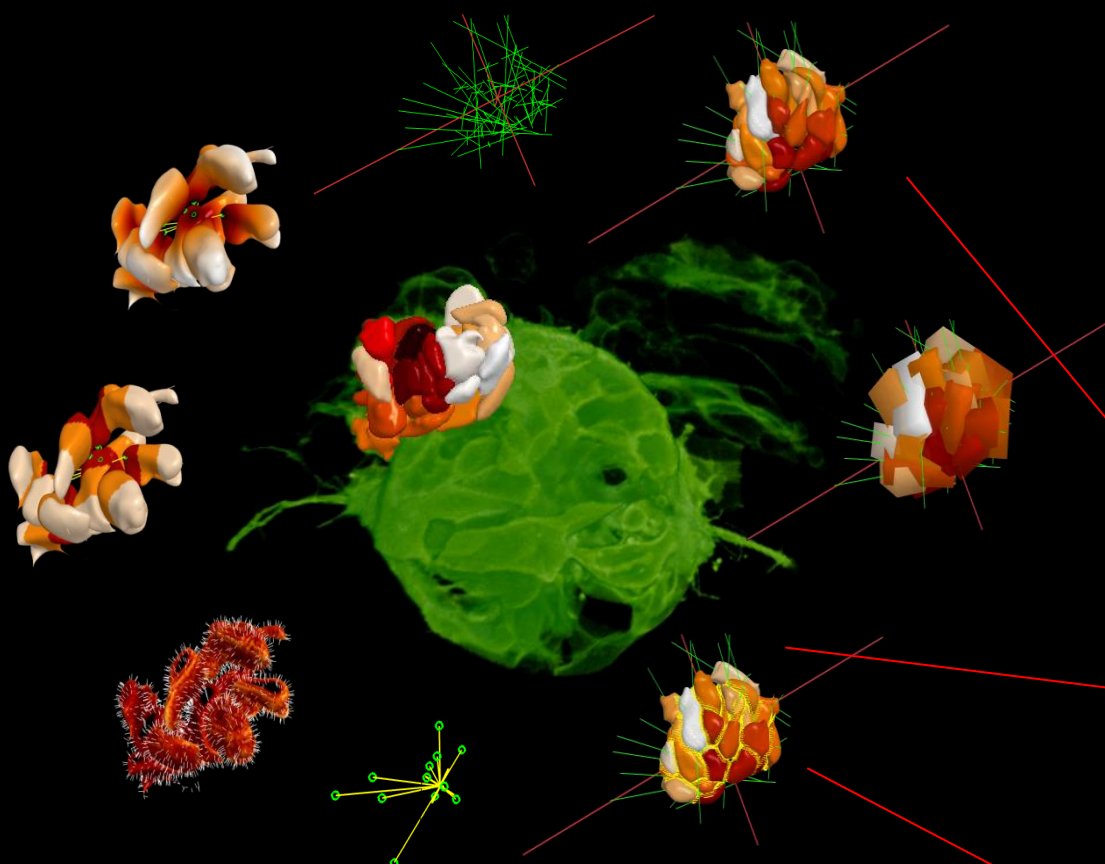
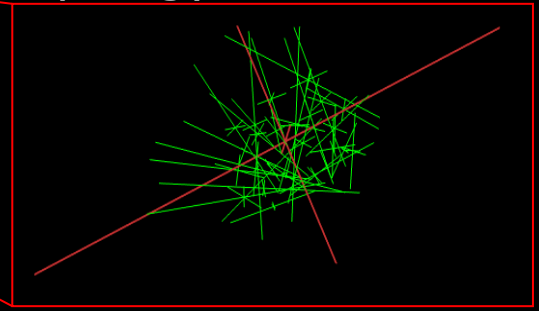
2D

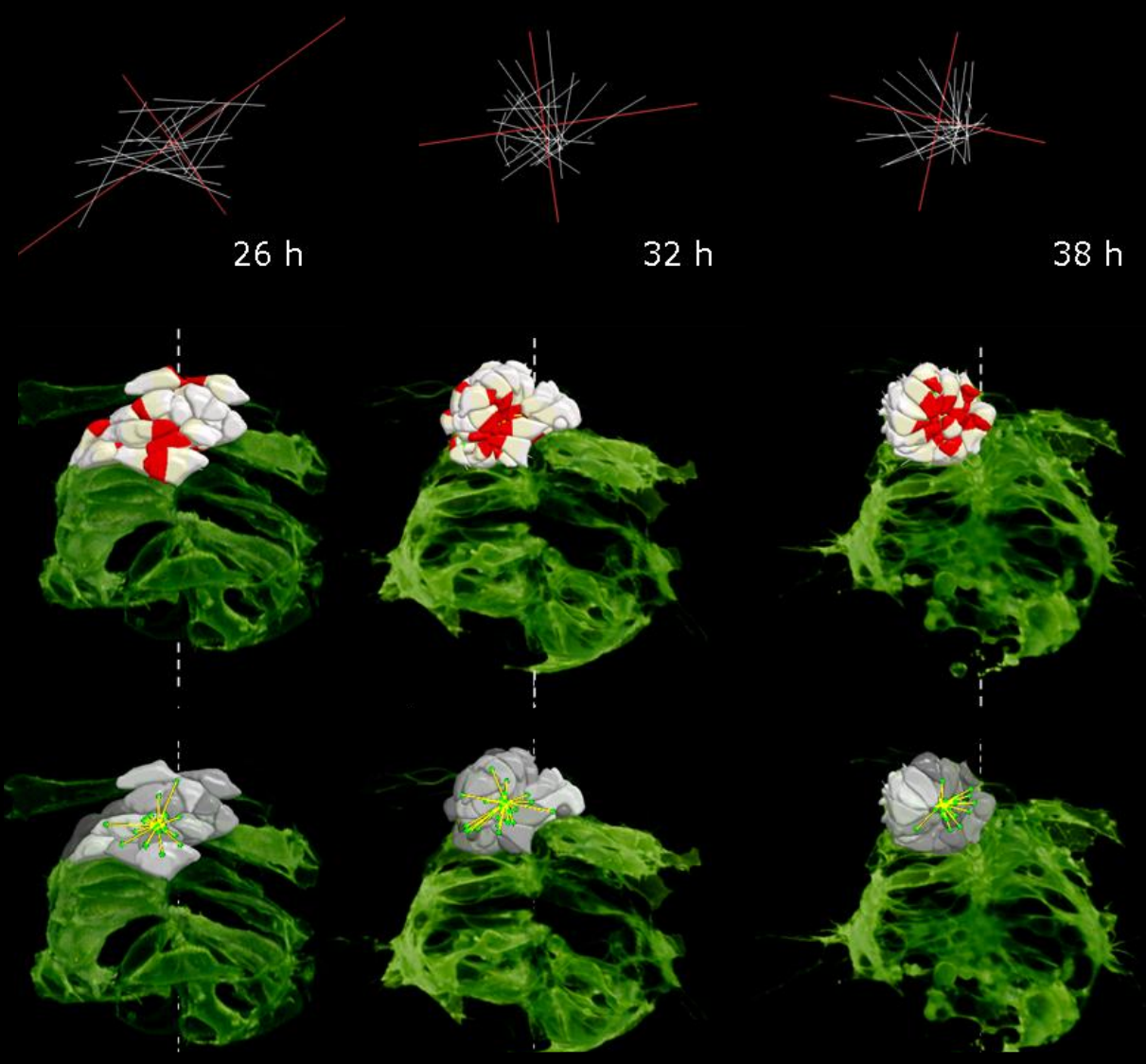
3D

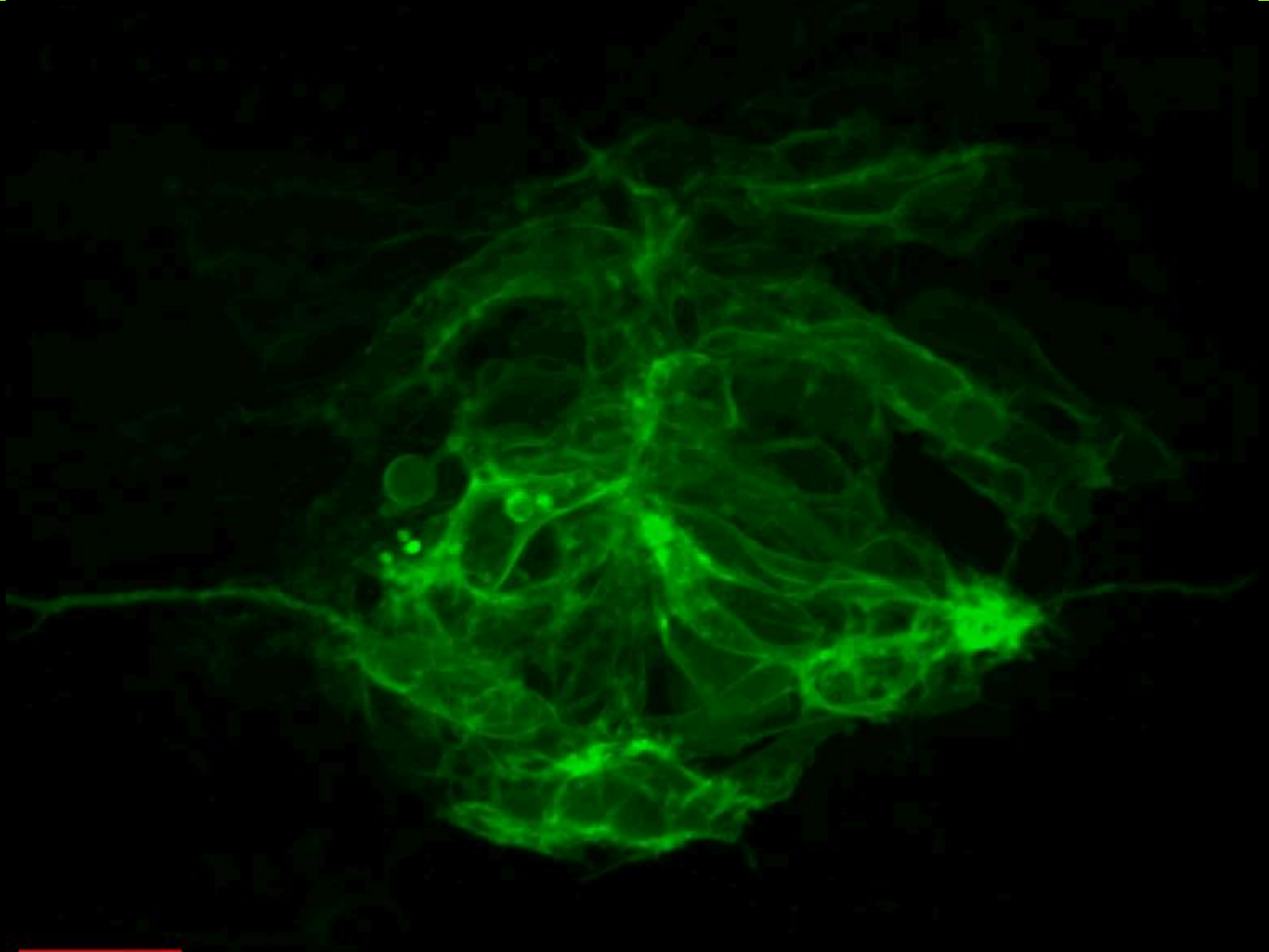
morphology



topology





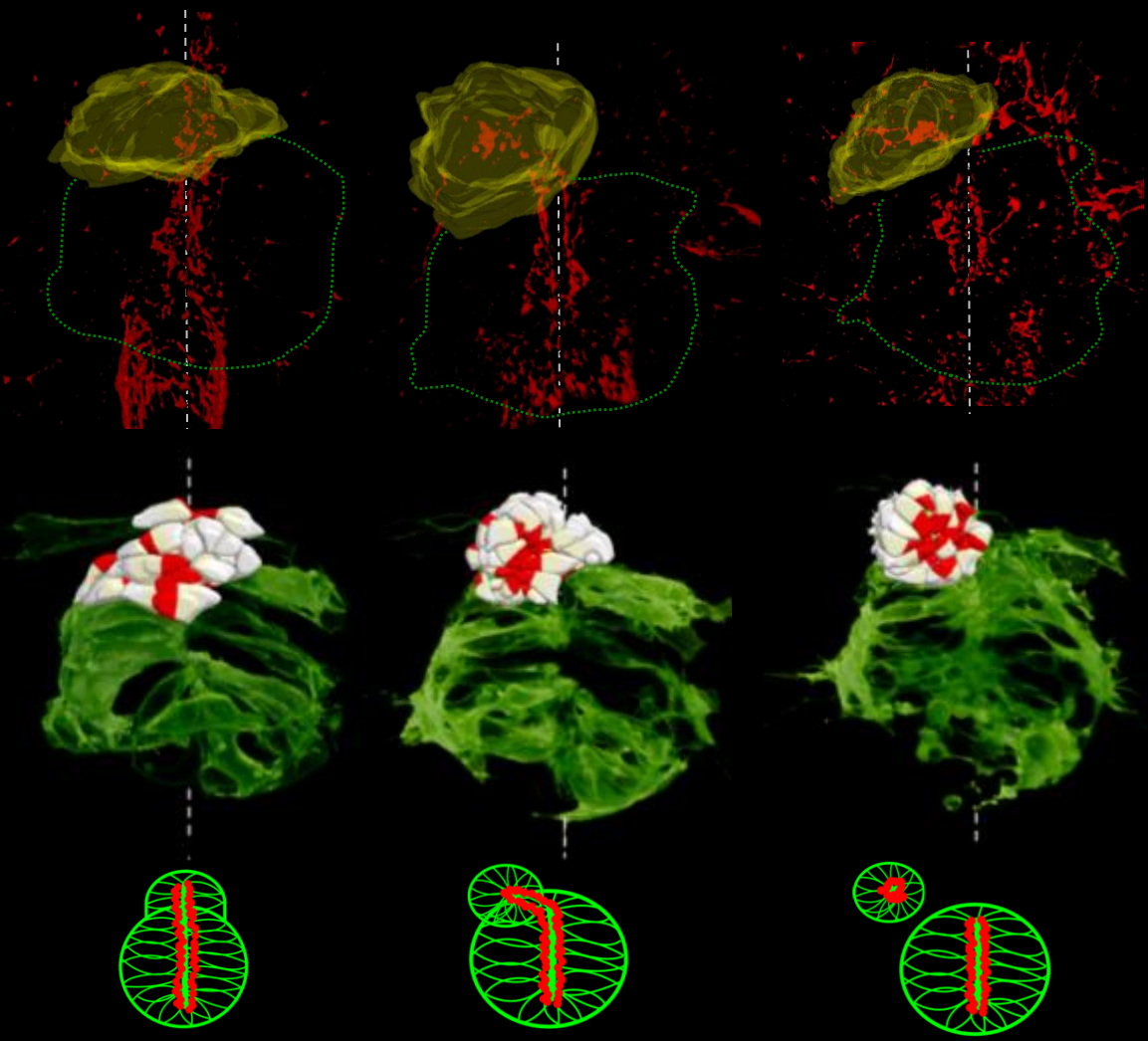



100,00 μm

26 hpf

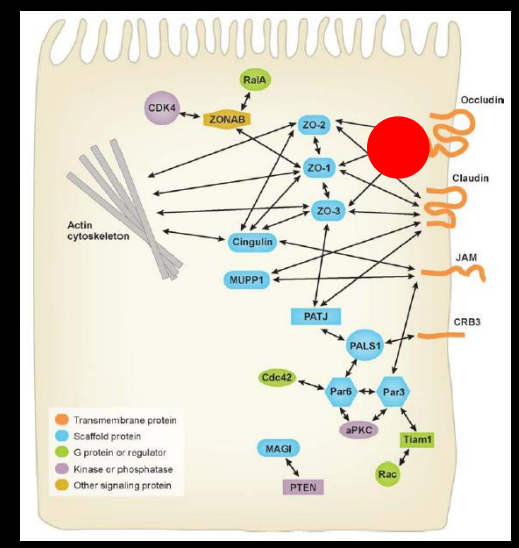
32 hpf

38 hpf



ZO-1 

apical



basolateral

Similar for α -tubulin,
 γ -tubulin,
 Phalloidin,

$$E = \int_0^1 \frac{1}{2} \left[\alpha \left| \frac{\delta C(s)}{\delta s} \right|^2 + \beta \left| \frac{\delta^2 C(s)}{\delta s^2} \right|^2 \right]$$

$$+ E_{ext}[C(s)] ds$$

$$+ E_n[C(s), C_i(s)] ds$$

$$+ E_f[C(s), C(s, t+1), C(s, t-1)] ds$$

internal energy
contour dependent

(Kass et al 1988)

external energy
image dependent

(GGVF Xu&Price 1988)

distance energy
neighbour
dependent

(us ...)

**CLG-3D optical flow
energy**

time dependent

++ multi-grid

(Bruhn et al. 2003/2005)

++ 'subjective surfaces'

(Sarti et al 2000)