Computational reconstruction of Zebrafish early embryogenesis by mathematical methods of image processing

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Joint results with

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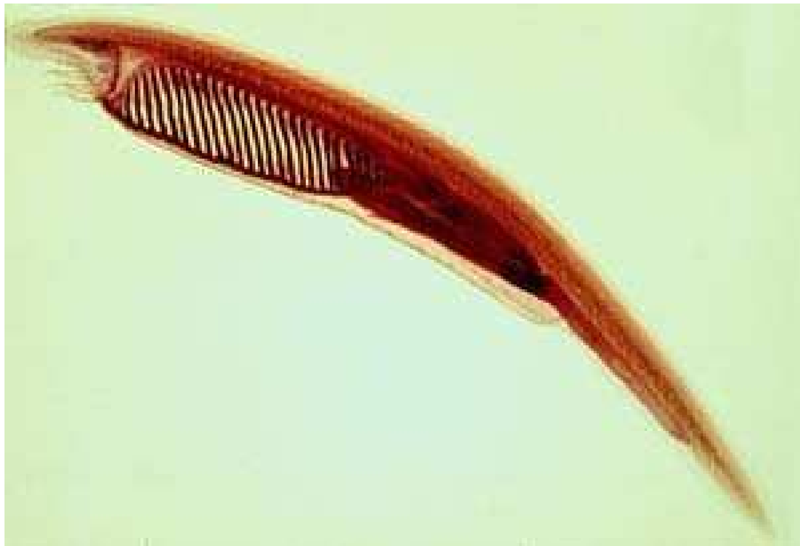
A.Sarti, M.Campana, C.Melani, B.Rizzi, C.Zanella (Bologna)
N.Peyrieras, P.Bourgine, E.Faure, T.Savy (Paris)

Motivation from biology and medicine

- cooperation with biologists (CNRS - Department of developmental biology, Institute Pasteur and Institute Curie, Paris), bioengineers (University of Bologna), computer scientists (Ecole Polytechnique, Paris, DENALI Brussels) and supercomputing center (IN2P3 Lyon) - European projects Embryomics and BioEmergences

- an automated reconstruction of the vertebrate early embryogenesis in space and time (zebrafish, sea urchin, phalusia mammilata, amphioxus - simple organisms which are relatively close to humans in many biological aspects - transparent for laser microscopes)

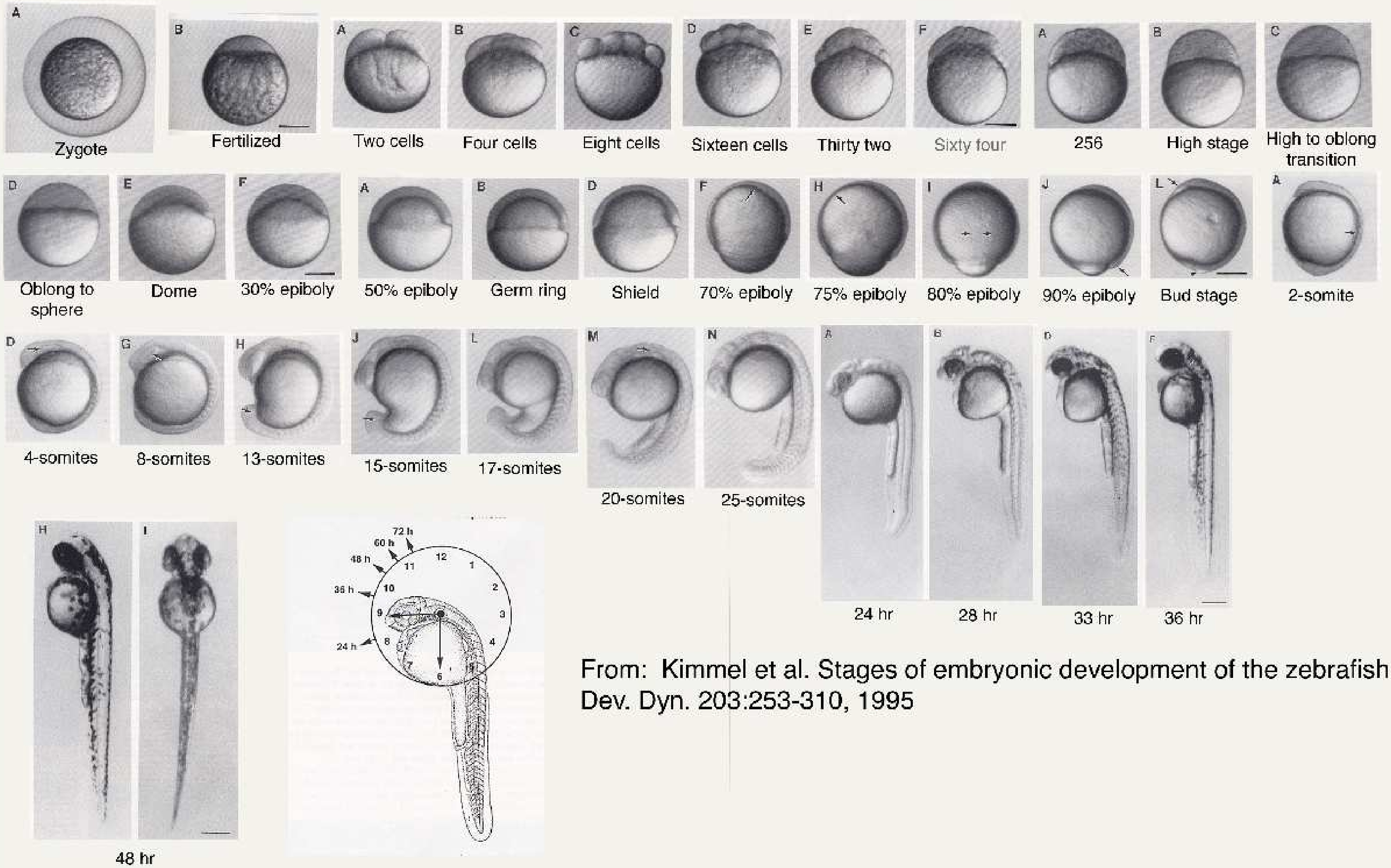


Using computational analysis of large-scale time sequences of 3D images taken by a two-photon microscope, the goals are

- extraction of the cell lineage tree - fundamental question in developmental biology - when, from which cell and by which mechanism a development of biologically meaningful structures (like vertebra, eyes, neural system) arises during embryogenesis

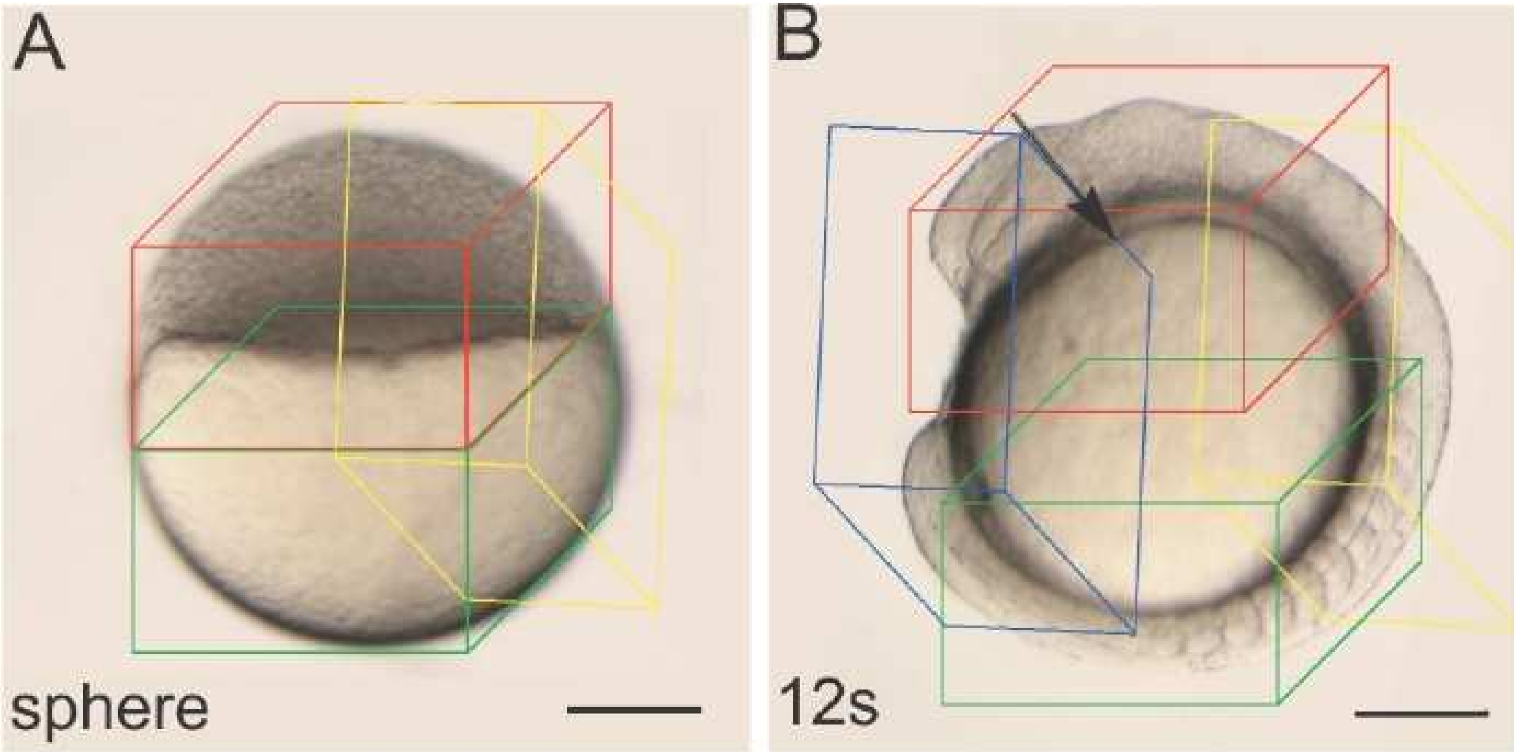
- computational reconstruction of morphogenetic fields

- comparison of untreated and treated cell populations development - anticancer drug testing

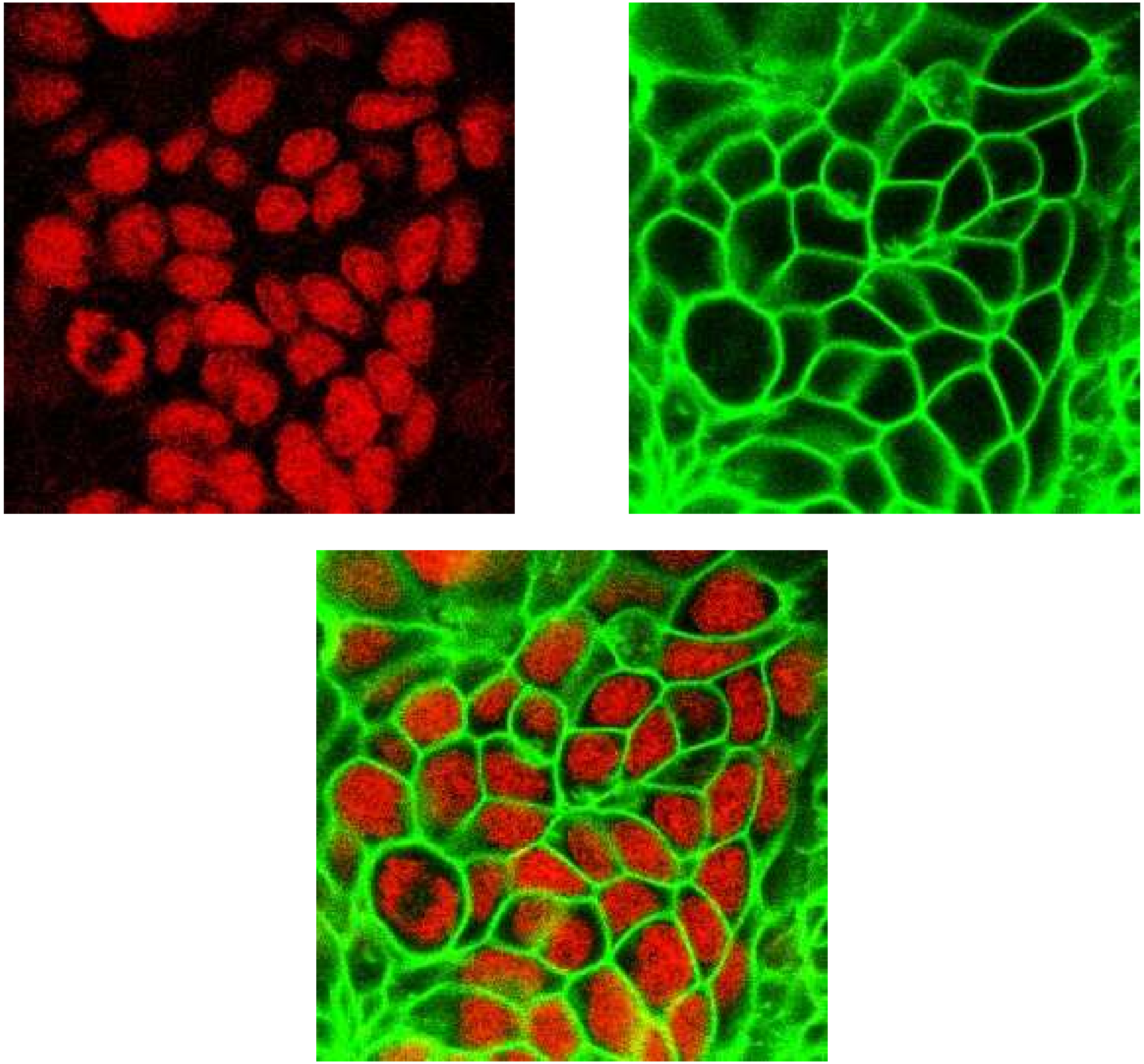


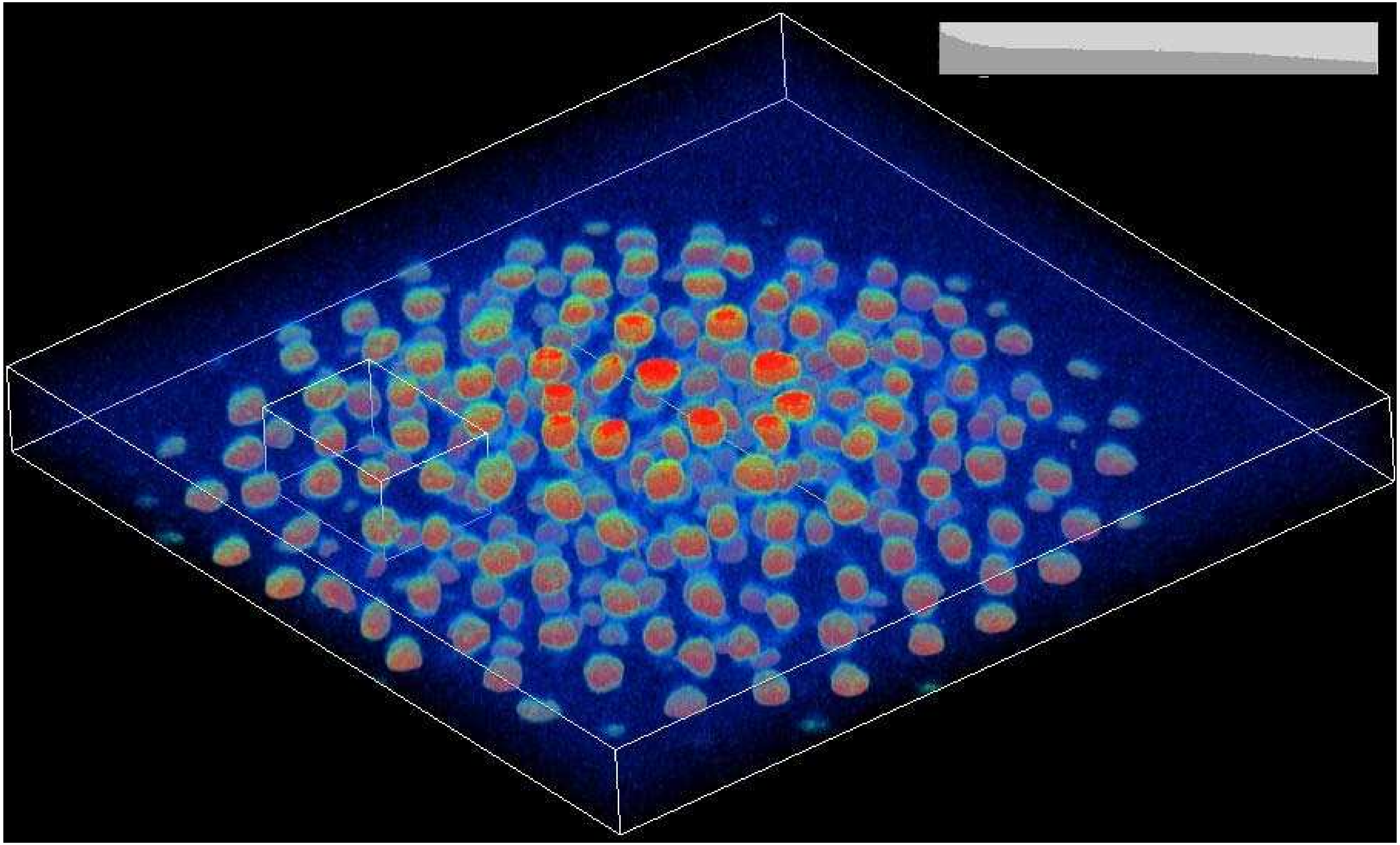
From: Kimmel et al. Stages of embryonic development of the zebrafish Dev. Dyn. 203:253-310, 1995

• two-photon laser scanning microscopy - several hundreds (100-300) of 2D image slices (512 x 512 pixels) of cell nuclei and cell membranes are taken subsequently - 3D image volume is constructed (in 50 seconds)



• several hundreds of 3D volumes are aquized during a time and represent imaged early embryogenesis during first (24) hours of development





Videos of embryogenesis

Steps in our computational embryogenesis reconstruction

- **data acquisition** - large-scale 3D image data sets of cell nuclei and cell membranes

- **image filtering** - by nonlinear (geometrical) diffusion equations

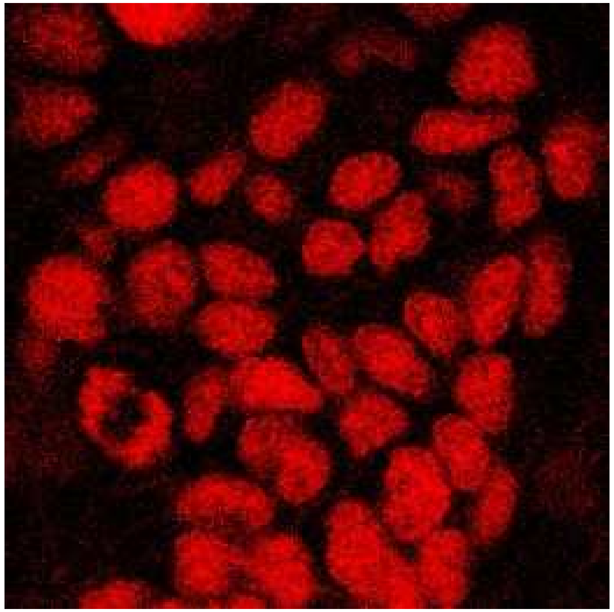
- **nuclei center detection** - by convection-diffusion level set equation $\longrightarrow$ approximate number of cells (proliferation rate), detected nuclei centers are starting points for the image segmentation

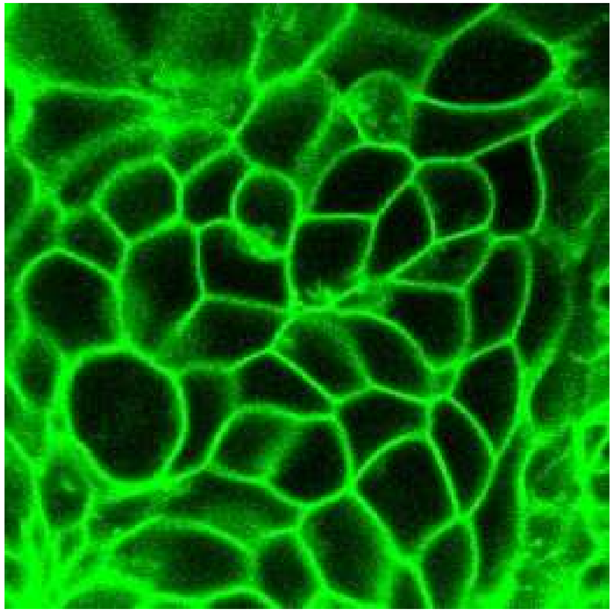
- **nuclei segmentation** - by the generalized subjective surface method (geometrical PDE) $\rightarrow$ 3D nuclei shapes during development, correction of number of cells and positions of the nuclei centers - basis for lineage tree construction

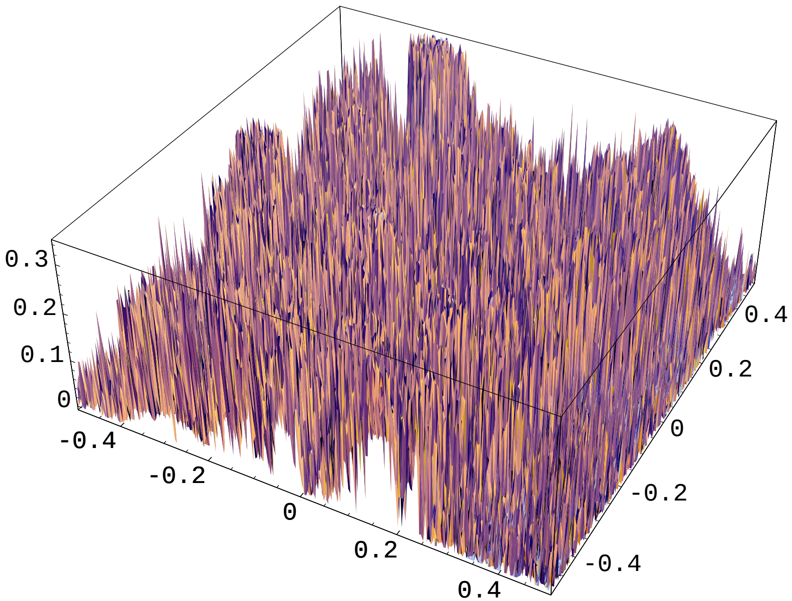
- **whole embryo segmentation** $\longrightarrow$ cell density evolving in time

- **membranes segmentation** $\longrightarrow$ 3D cell shapes during development, detection of cell divisions (mitosis) - helps in cell lineage tree construction

- **cell tracking** - by minimization of a heuristic functional by the simulated annealing algorithm $\longrightarrow$ cell trajectories and binary cell lineage tree



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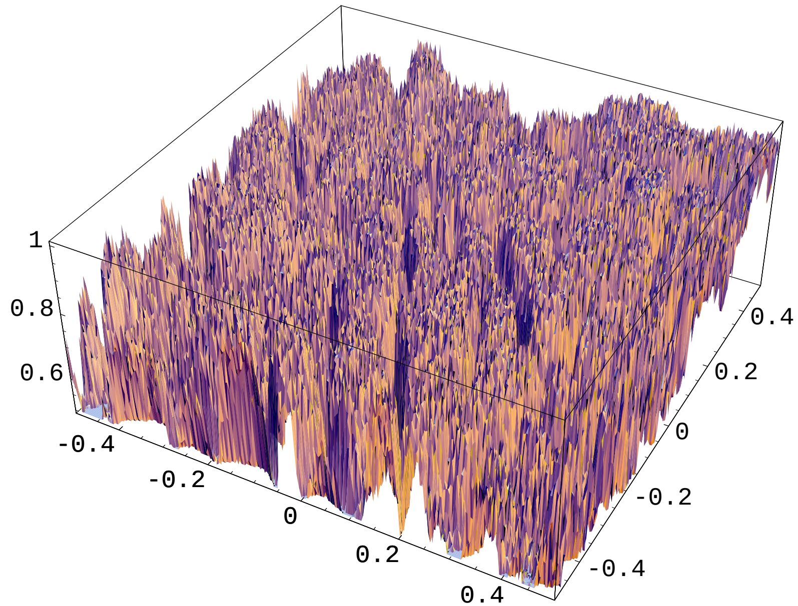
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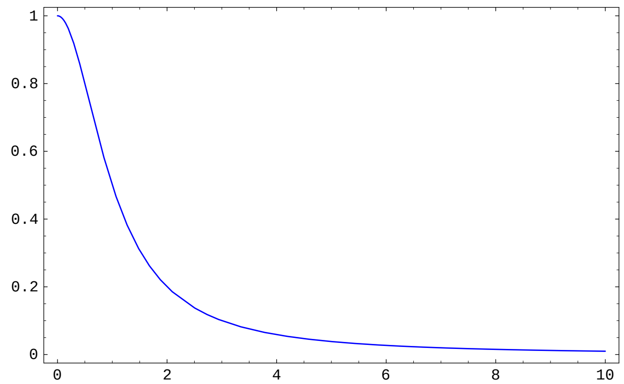
Image filtering

- **Geodesic mean curvature flow equation**

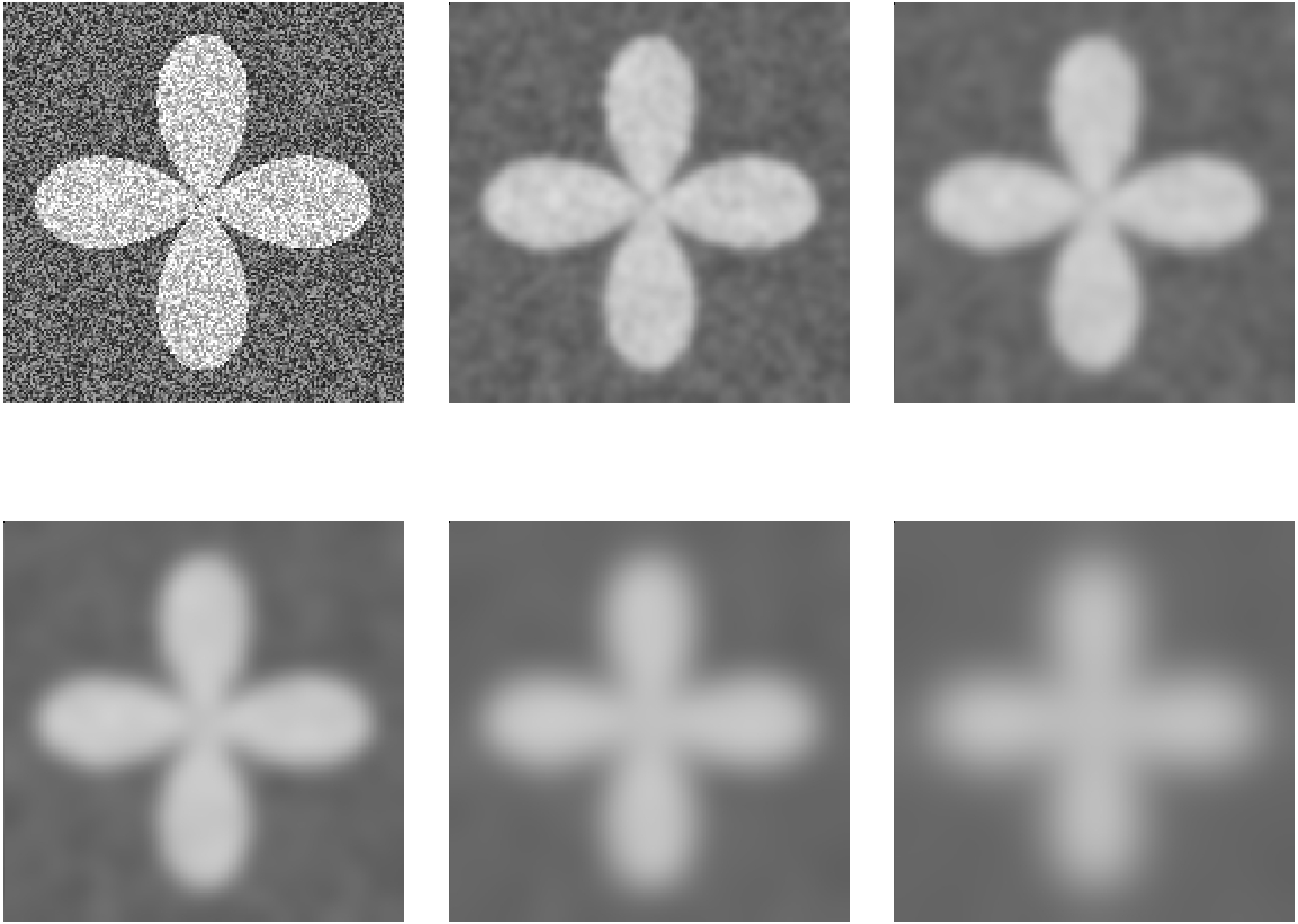
(Caselles, Kimmel, Sapiro and Chen, Vemuri, Wang)

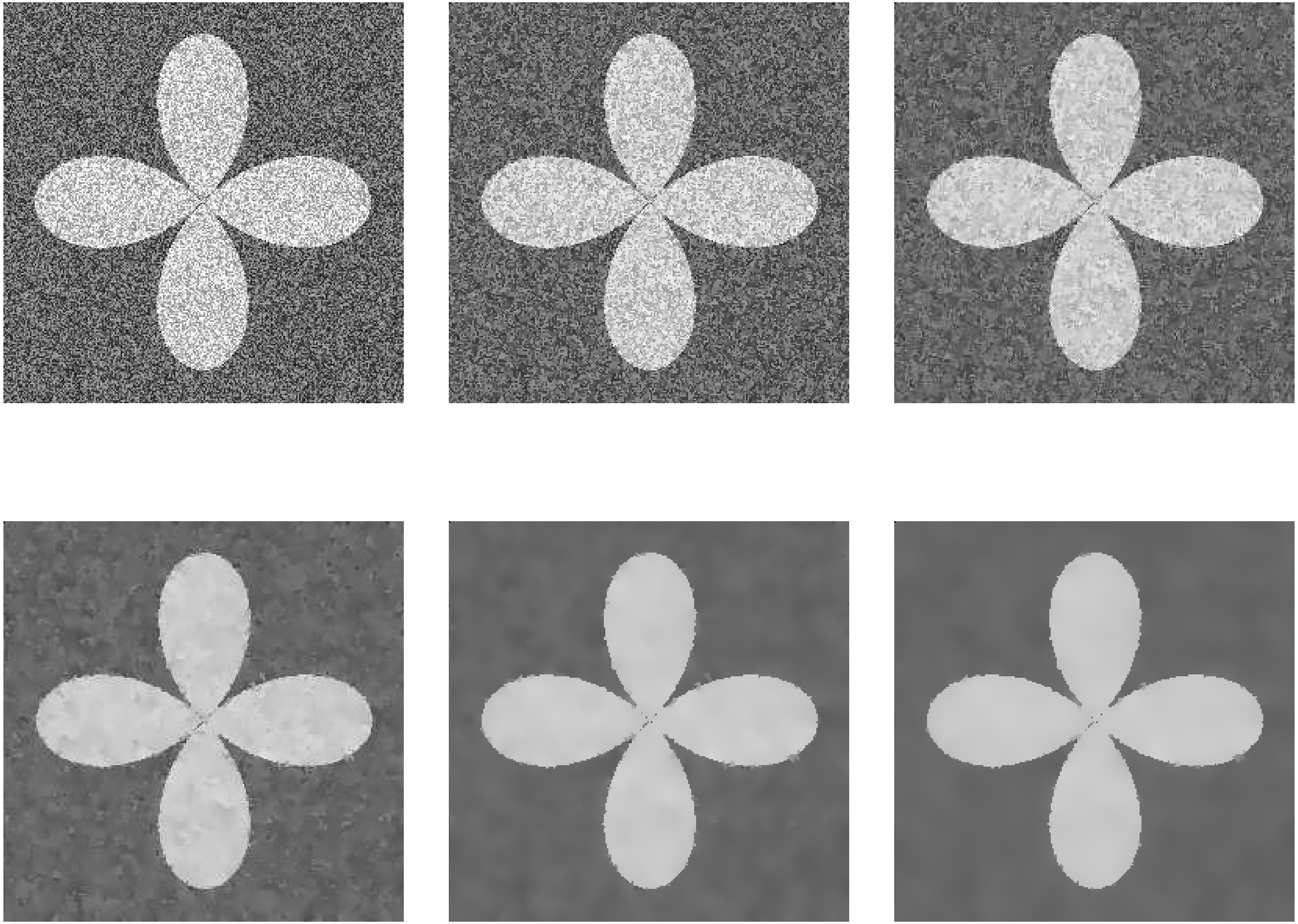
$$u_t = |\nabla u| \nabla . \left(g(|\nabla G_\sigma * u|) \frac{\nabla u}{|\nabla u|} \right) \quad u(0,x) = I^0(x), \quad \text{h.N.b.c} \qquad (1)$$



- $g(s) = 1/(1 + Ks^2), K > 0$ - small values for large gradients (edges)

- advective vector field $-\nabla g(|\nabla G_\sigma * u|)$ points towards edges





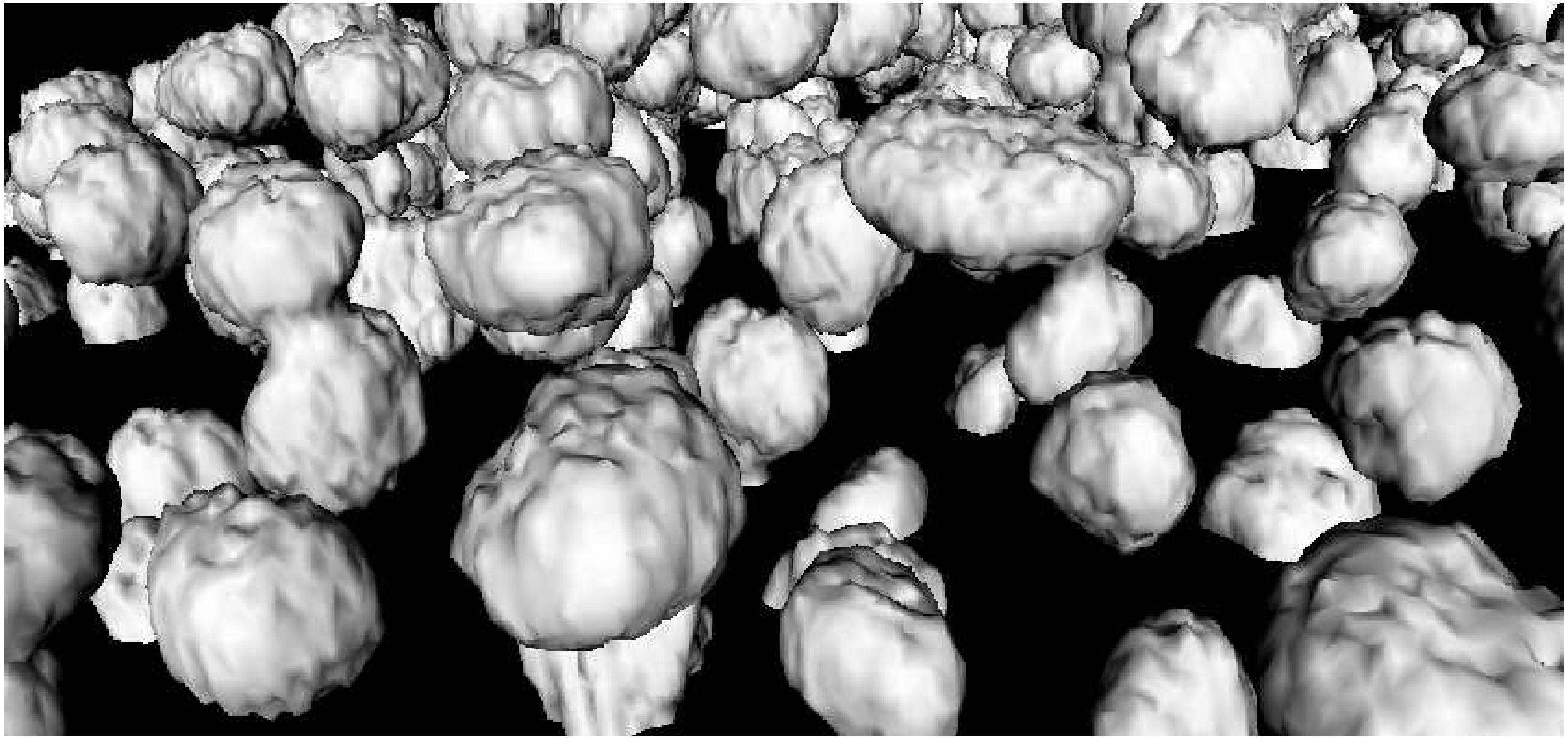


Image segmentation

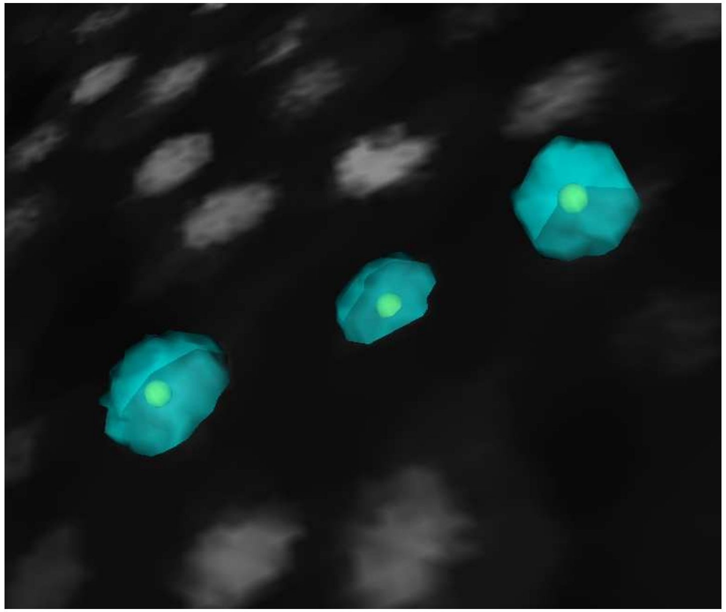
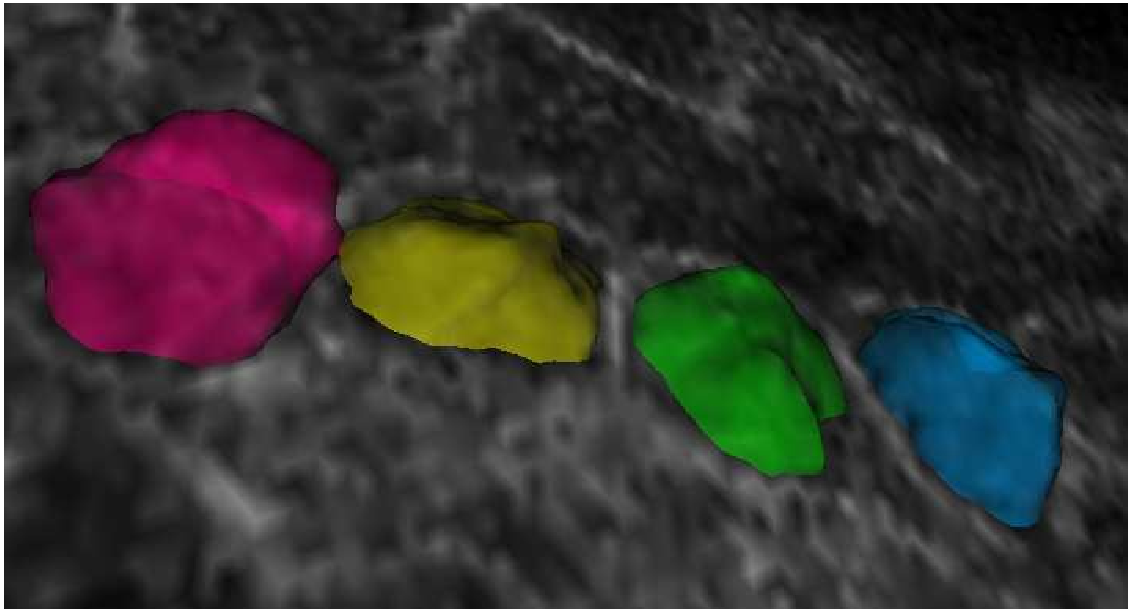
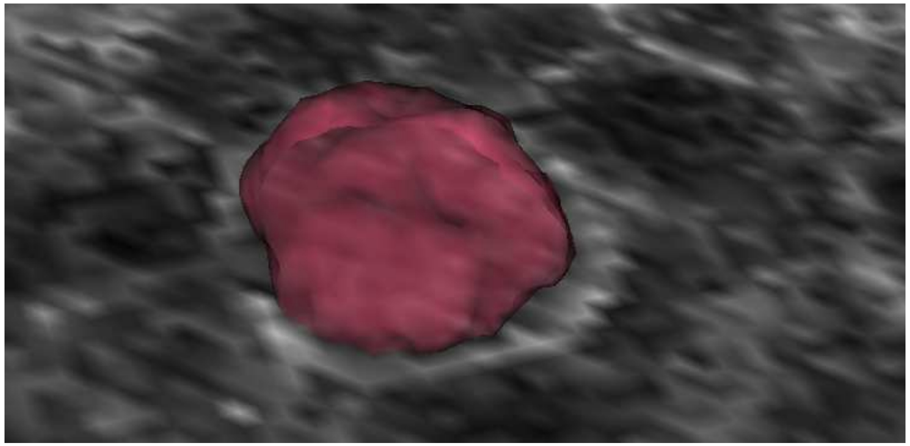
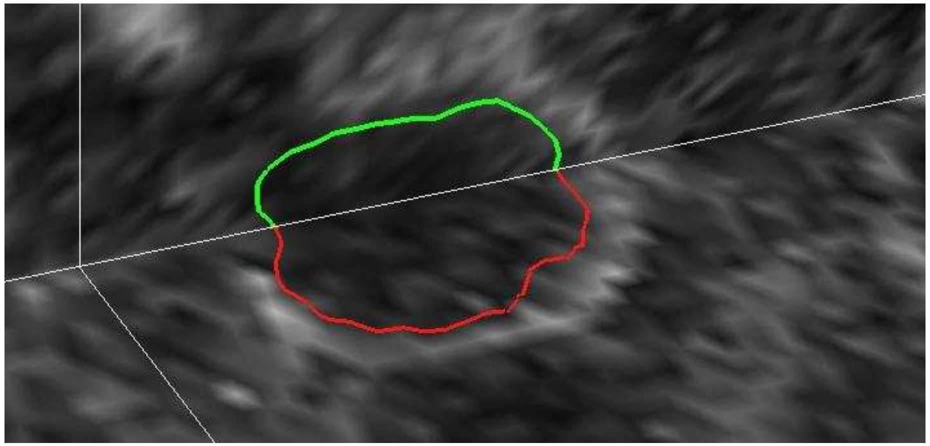
- subjective surface method due to Sarti, Malladi, Sethian (2000) - ε-regularization of the geodesic mean curvature flow equation

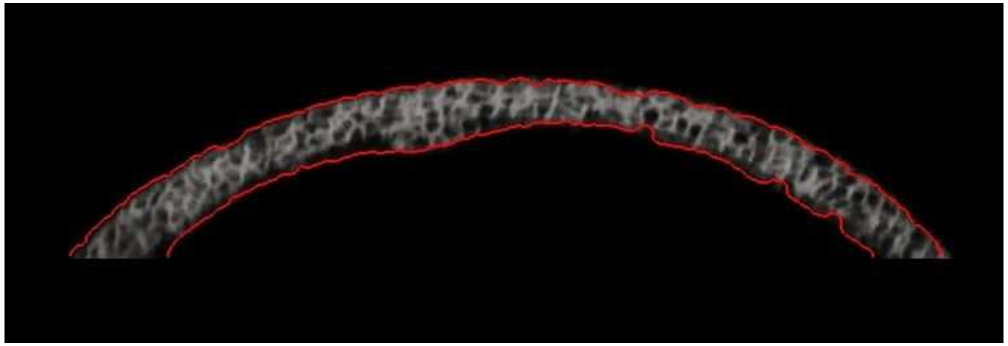
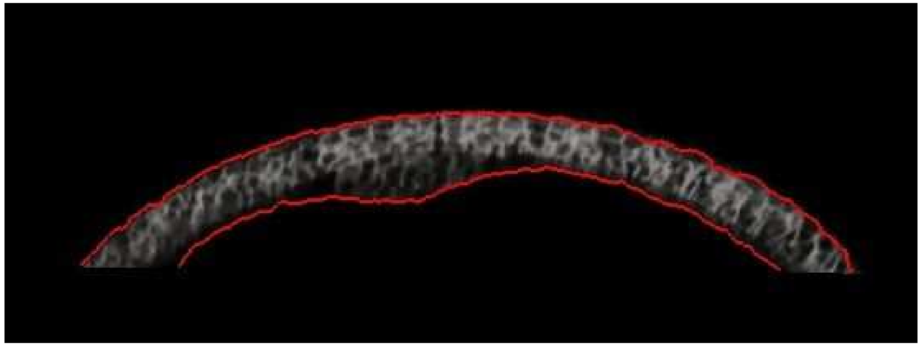
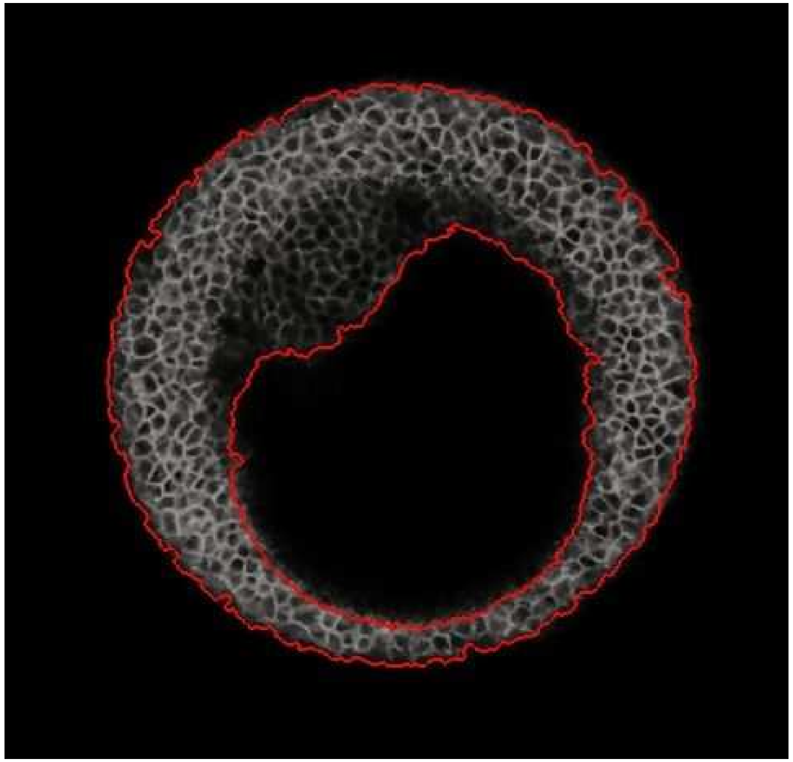
$$u_t = \sqrt{\varepsilon^2 + |\nabla u|^2} \nabla . \left(g \frac{\nabla u}{\sqrt{\varepsilon^2 + |\nabla u|^2}} \right), \quad g = g(|\nabla G_\sigma * I^0|) \qquad (2)$$

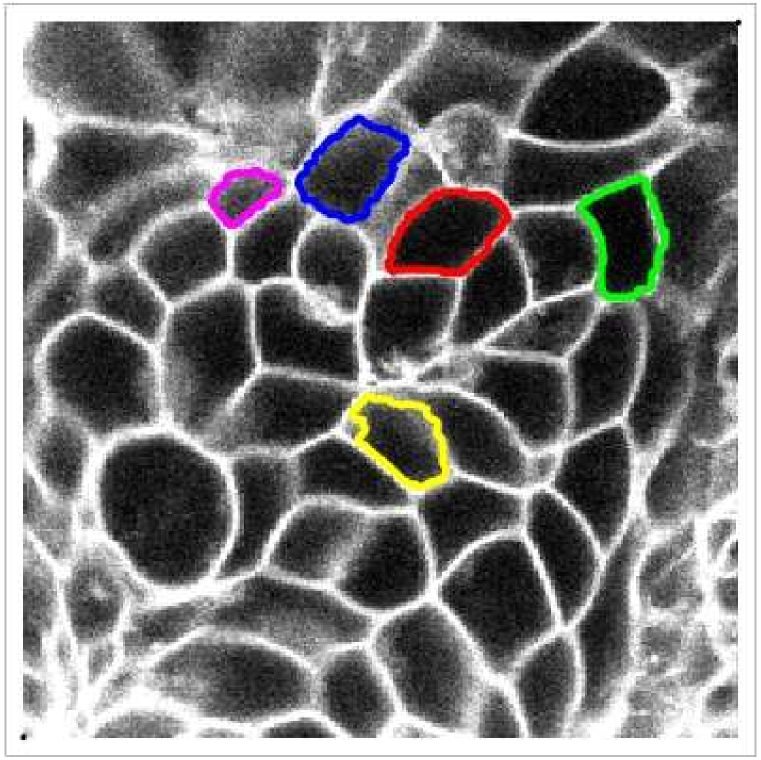
- generalized version with different weigths to advective and diffusive parts - K.M., N.Peyriéras, M.Remešiková, A.Sarti (2008, FVCA5)

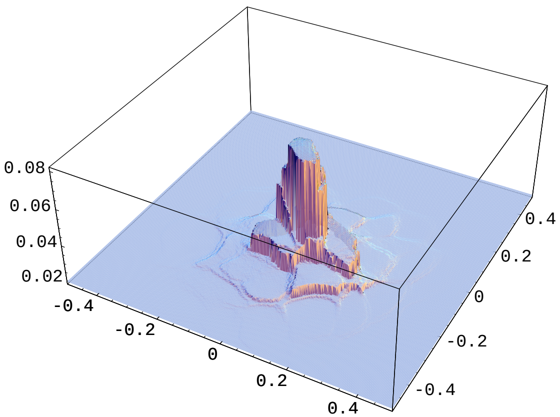
$$u_t = \mu_1 \; g |\nabla u| \nabla . \left(\frac{\nabla u}{|\nabla u|} \right) + \mu_2 \; \nabla g . \nabla u \qquad (3)$$

- efficient 3D implementations using semi-implicit scheme in curvature part and up-wind schemes in advective part - M.Remešiková, R.Čunderlik, K.M.

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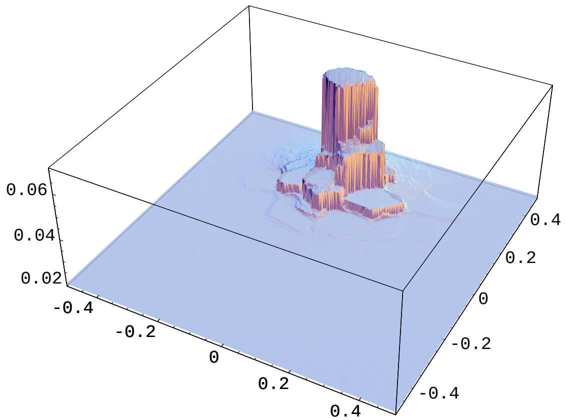
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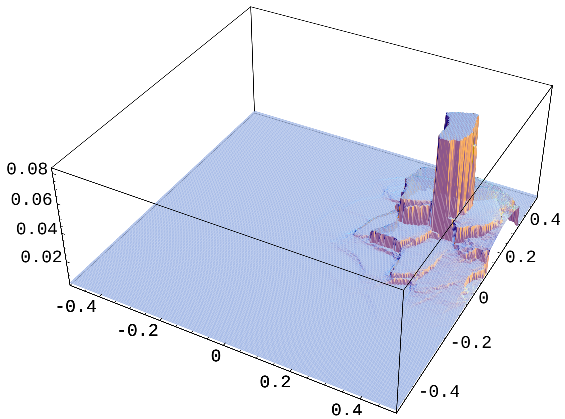
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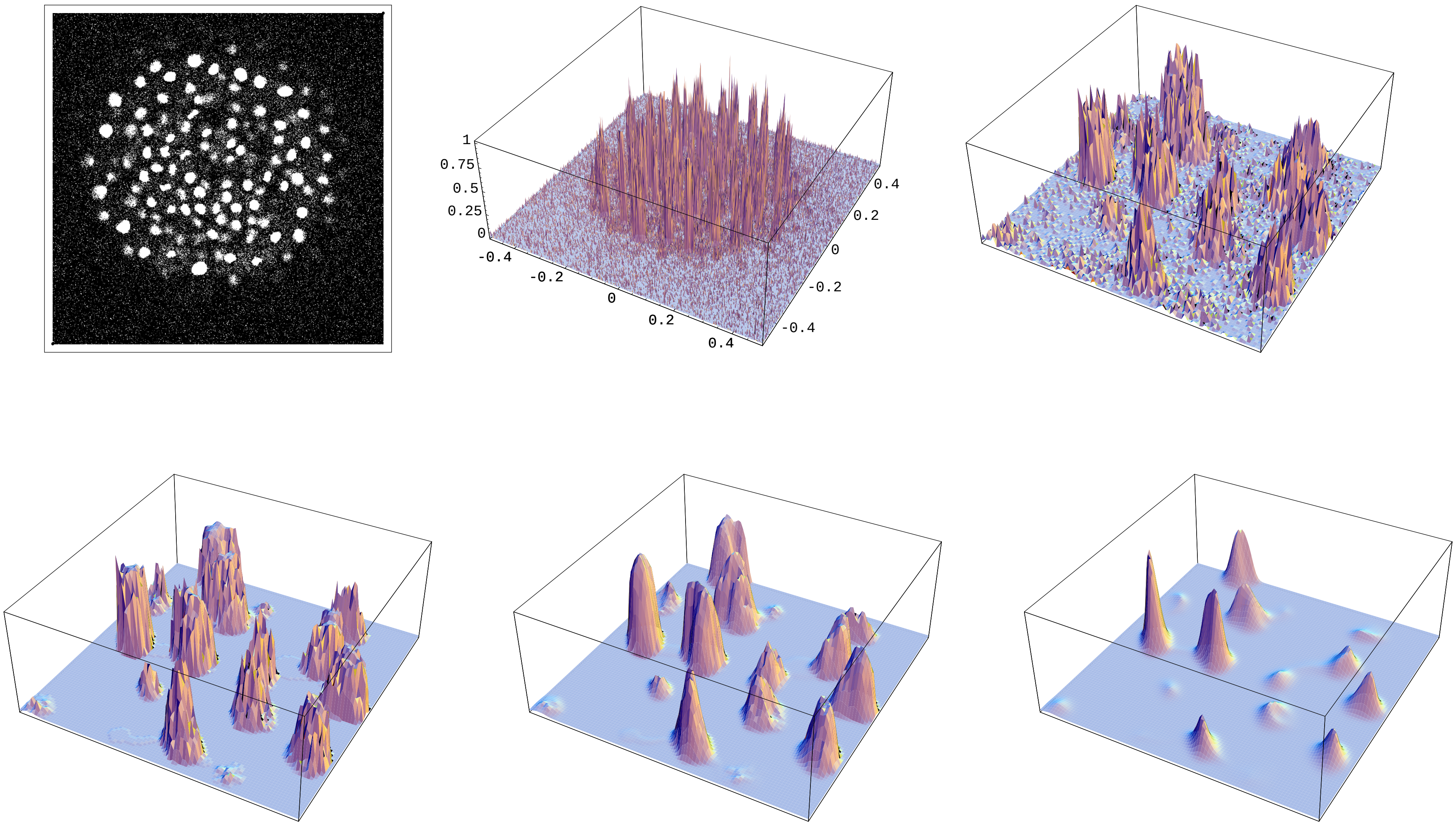
Nuclei center detection

• To get starting points for image segmentation we apply to (filtered) nuclei image intensity geometrical advection-diffusion equation which moves every level set in normal direction by a constant speed δ with a slight regularization by the mean curvature term - P.Frolkovič, K.M., N.Peyriéras, A.Sarti (2007)

$$u_t = \delta \frac{\nabla u}{|\nabla u|} \cdot \nabla u + \mu |\nabla u| \nabla \cdot \left(\frac{\nabla u}{|\nabla u|} \right) \quad \text{h.N.b.c} \tag{4}$$

• in advective part - motion in normal direction - flux-based finite volume level set method - in curvature part - semi-implicit scheme - P.Frolkovič, K.M., APNUM 2007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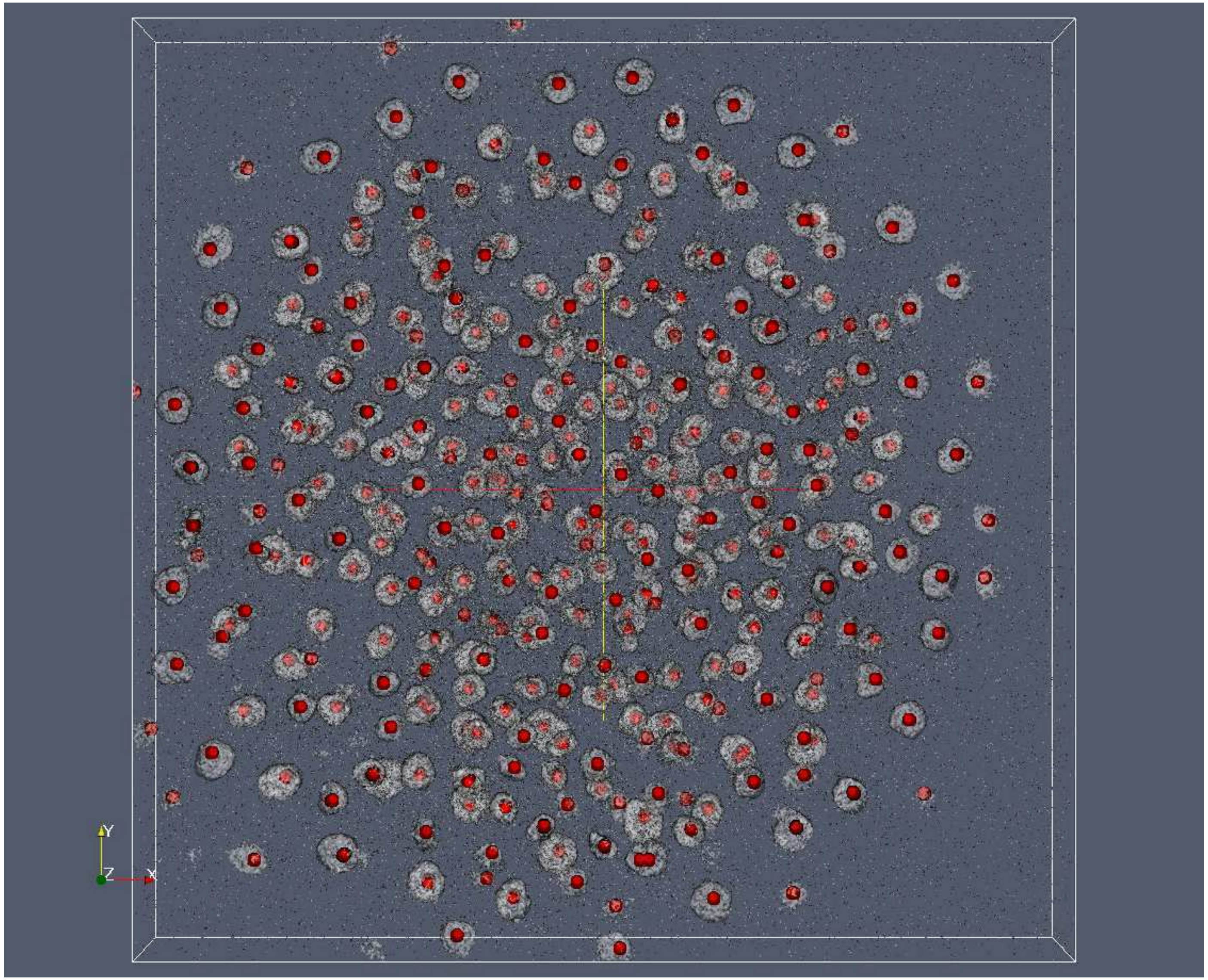
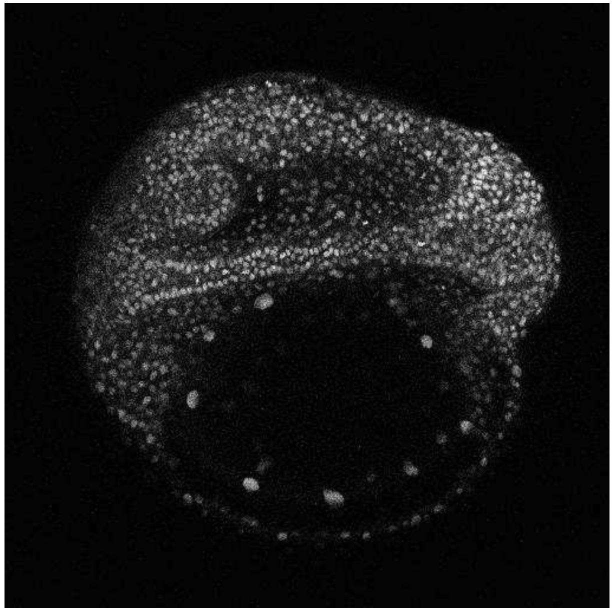
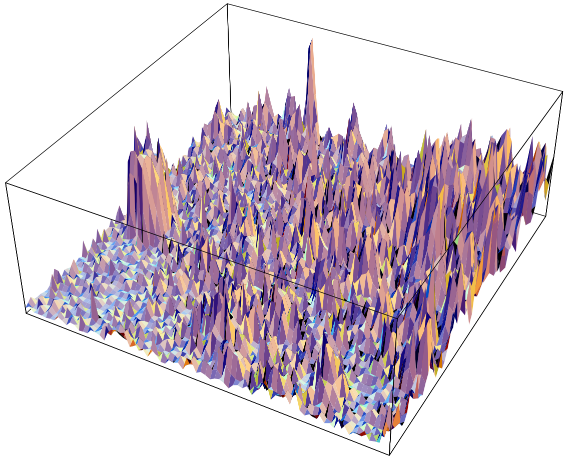
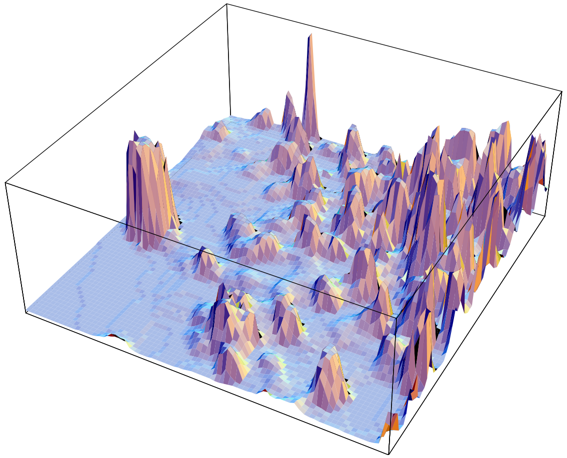
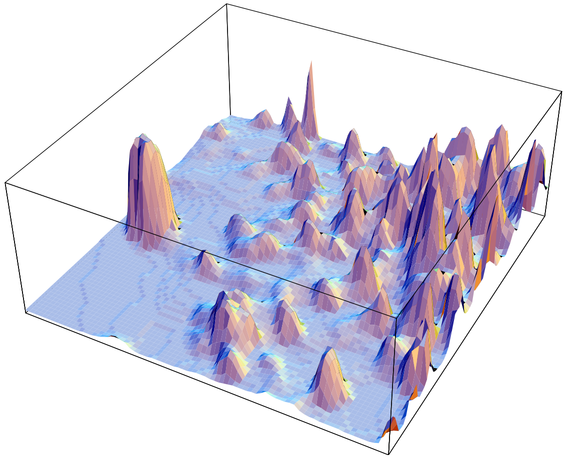


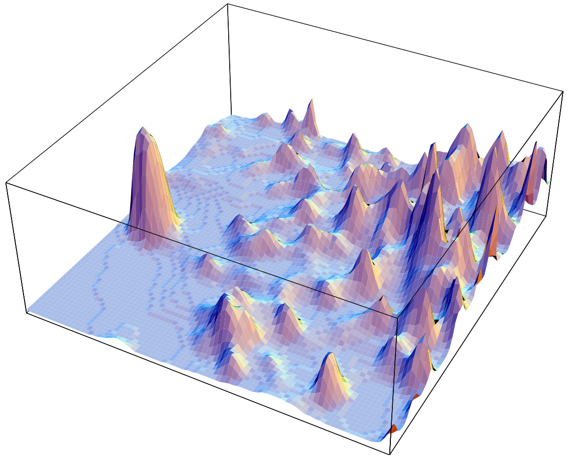
- error manually checked by biologists - less than 0.5%

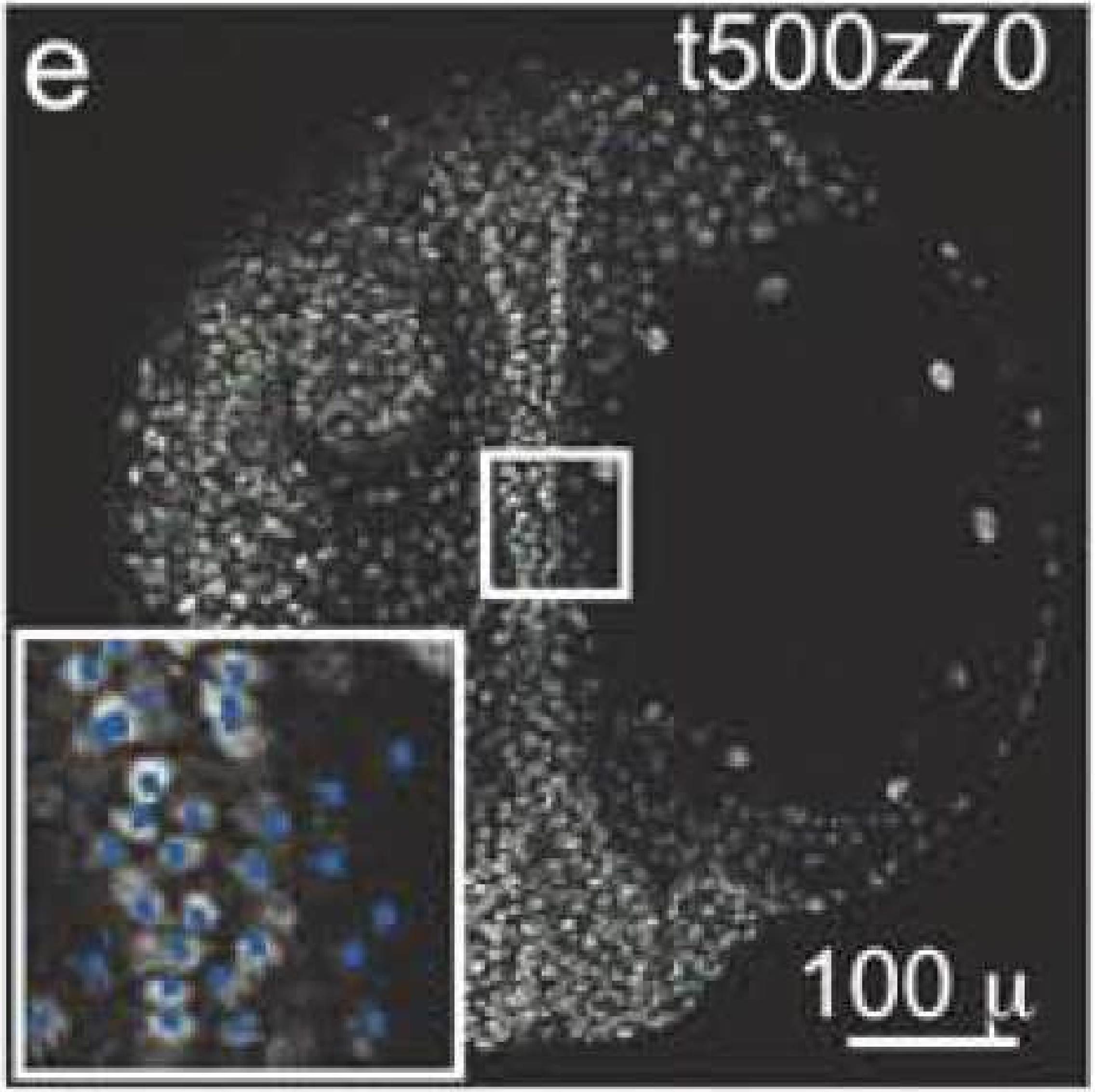








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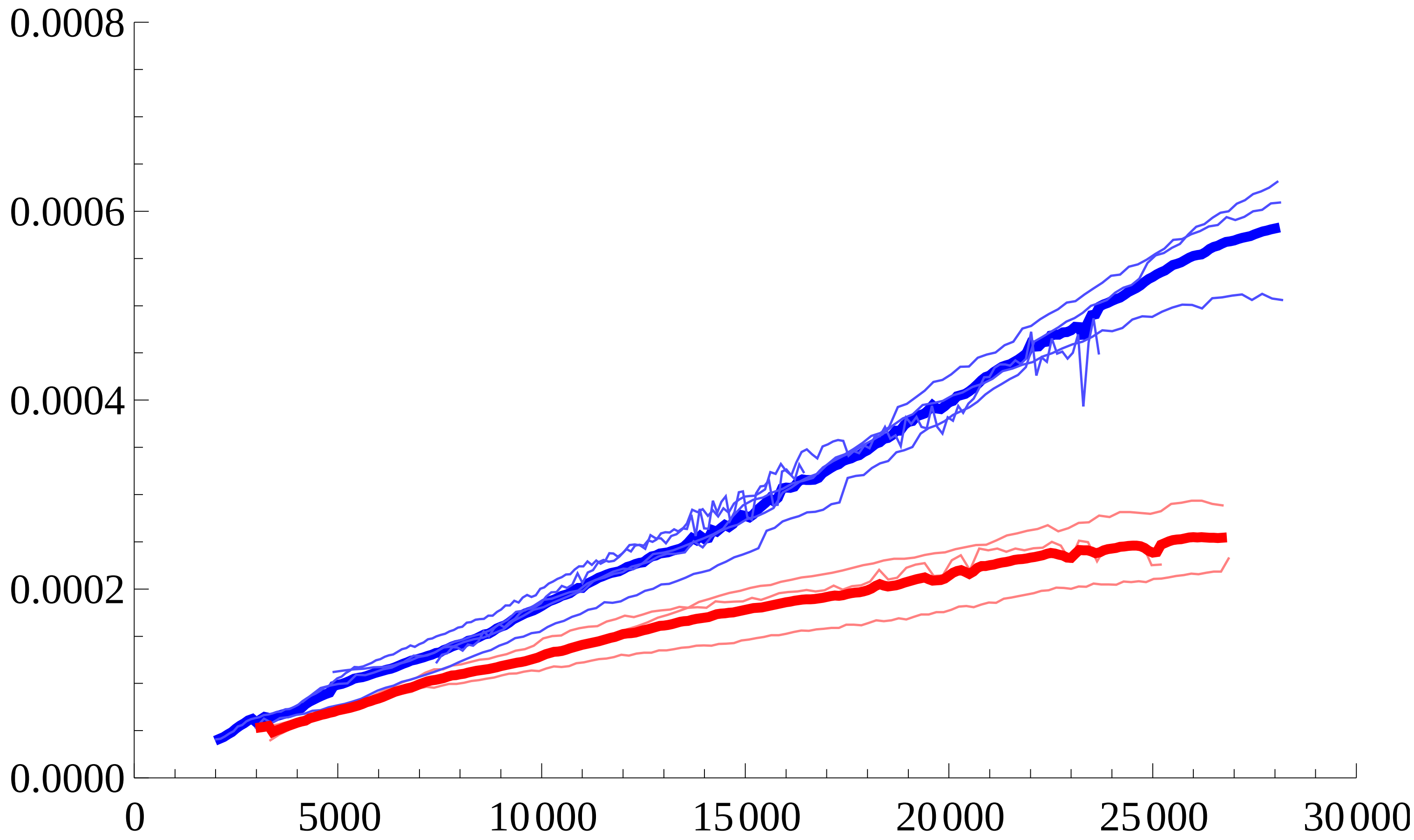
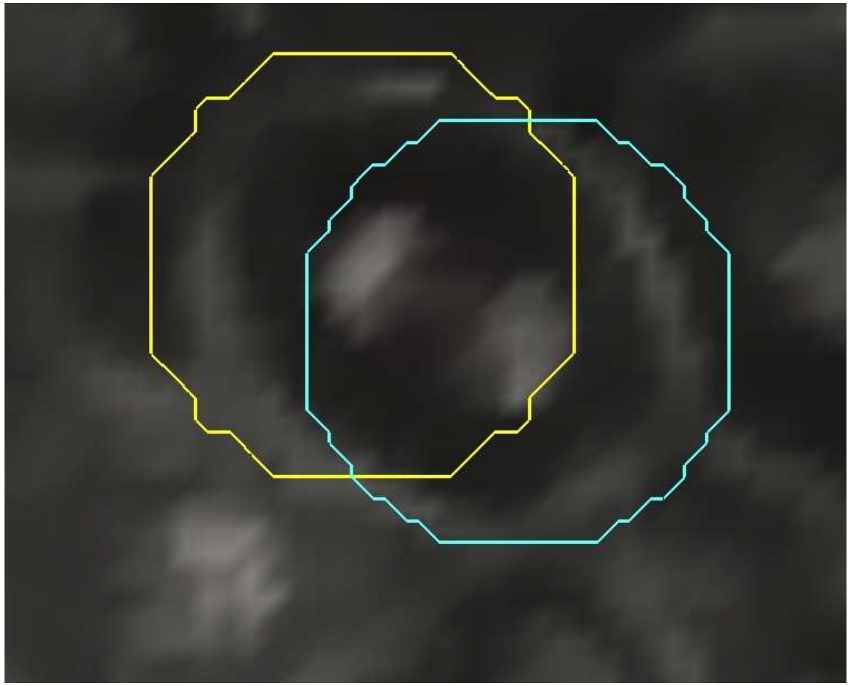
- anticancer drug testing using cell density curves = (number of cells) / (segmented volume of the imaged part of embryo) evolved in time

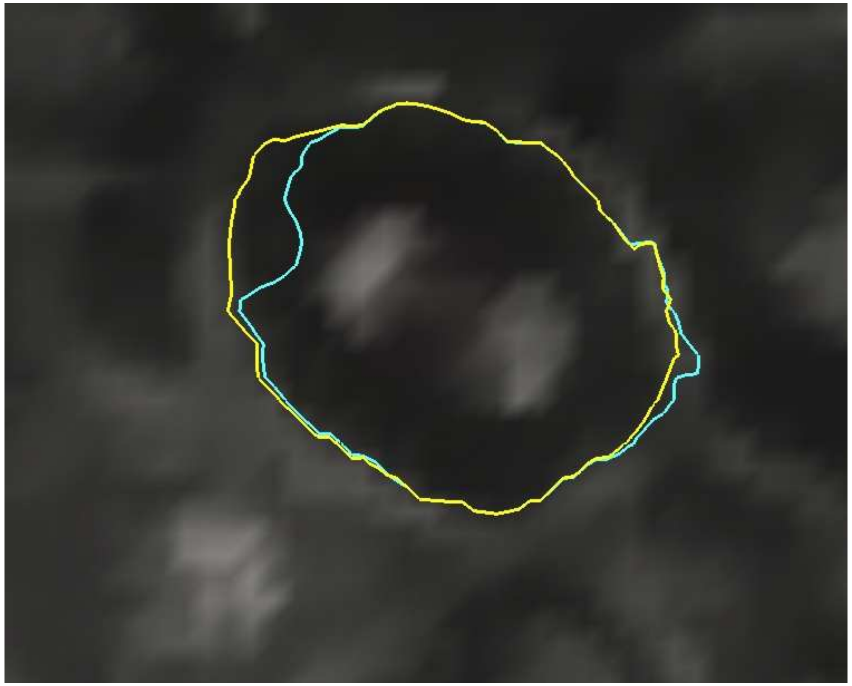


- blue - untreated embryos, red - after drug application

Mitosis detection

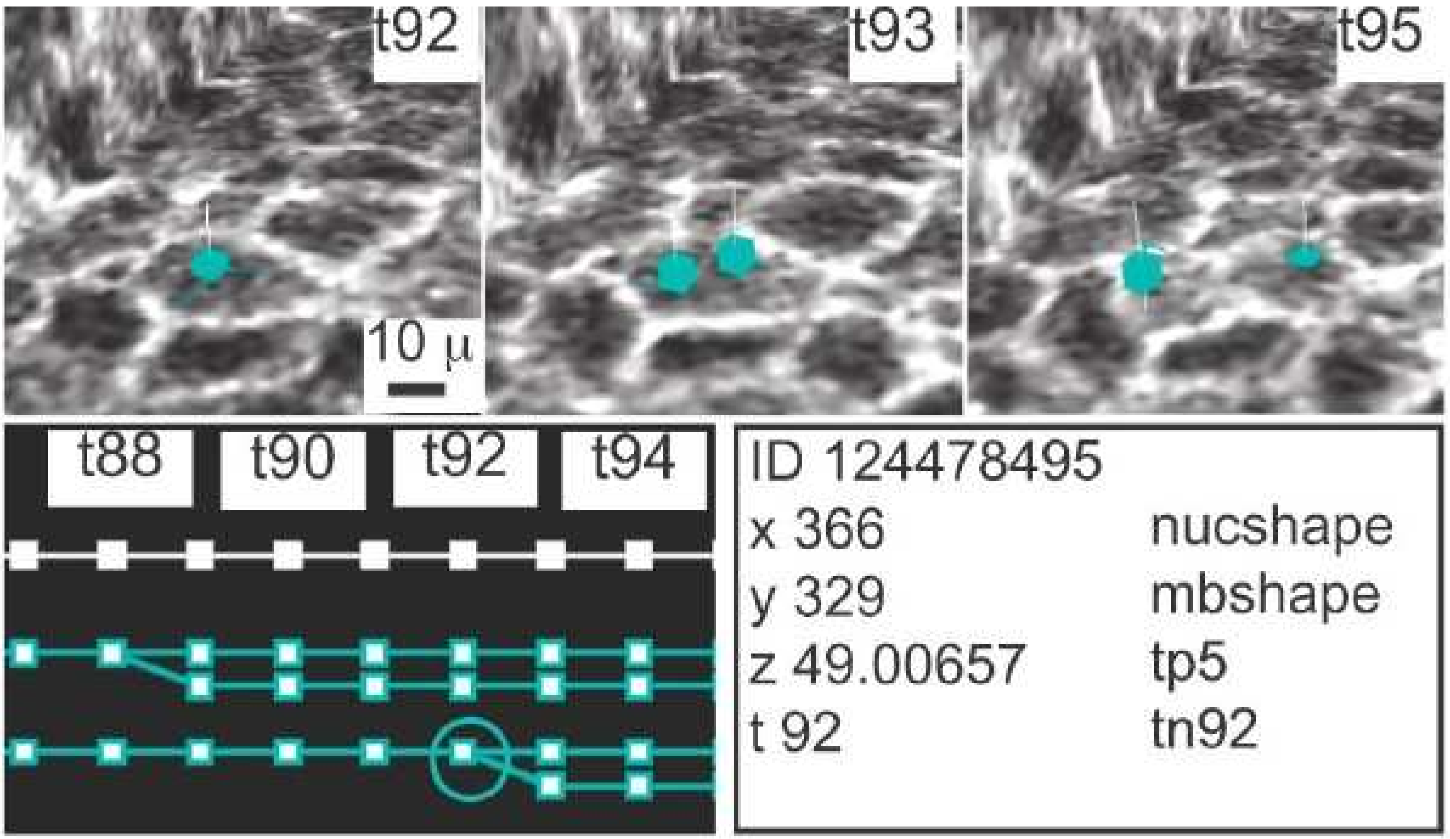
- if we start segmentation from two close nuclei centers and we get the similar result of cell membranes segmentation we detect candidates for mitosis





Cell tracking

- correct nuclei centers detected in time in every 3D nuclei image (+ mitosis detection) is the basis for tracking of cell trajectories and extraction of the binary **cell lineage tree** - minimization of a global functional, which contains nuclei centers distances in subsequent time steps, by a simulated annealing algorithm



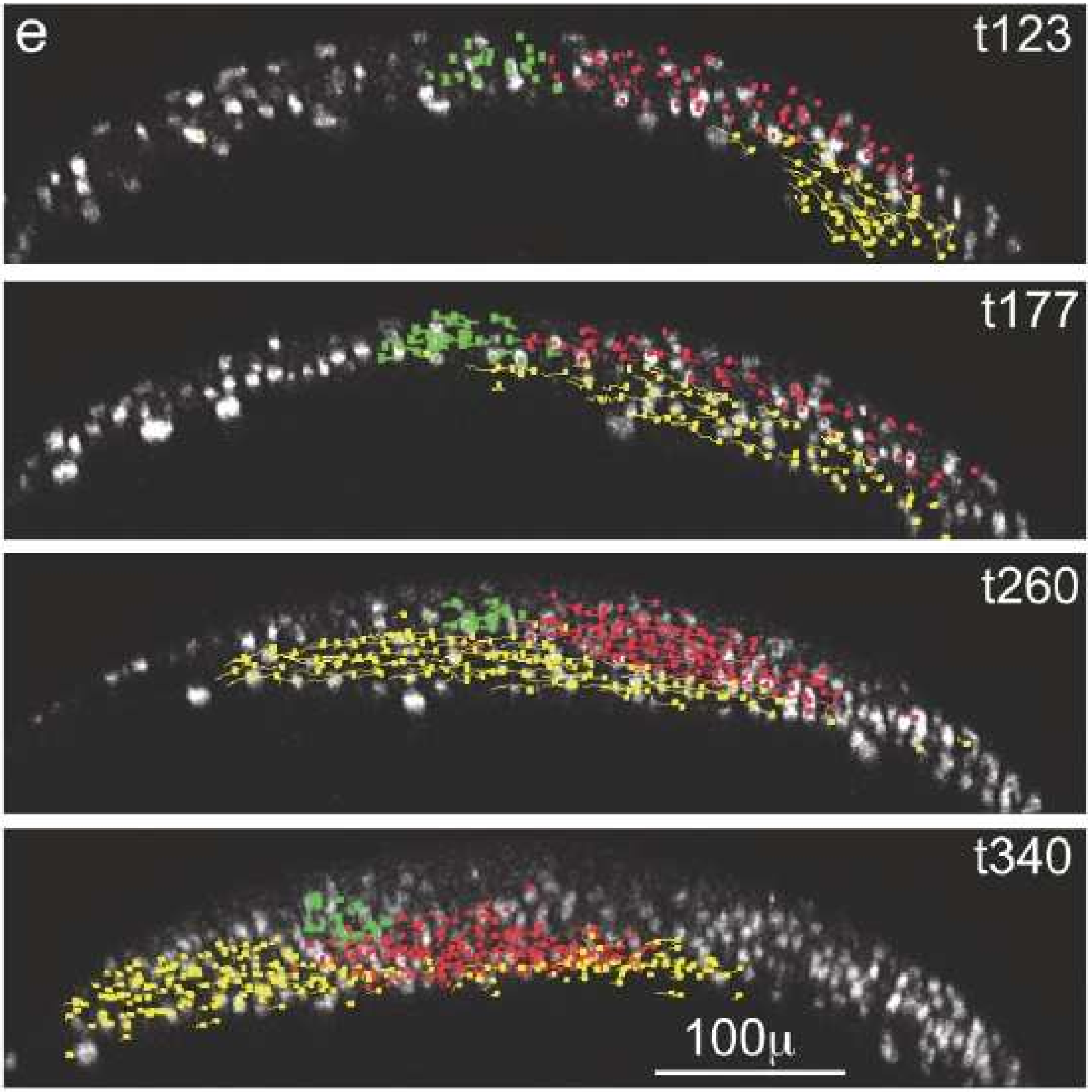
- I - set of all detected centers in all time steps, X_i, $i \in I$ their positions (or further cell features), $f : I \to I$ - unknown binary tree

- a heuristic energy functional which is minimized by the simulated annealing algorithm

$$E(f) = \sum_{i \in I} \|X_{f(i)} - X_i\|_1 + \sum_{i \in I} \sum_{j \in \mathsf{Neigh}(i)} \|(X_{f(i)} - X_i) - (X_{f(j)} - X_j)\|_2$$

- biological coherence of the tree is checked - discontinuities in trajectories $\to$ false negative centers , cells living only a few time steps $\to$ false positive centers

- manual checking of 15000 links in the tree by biologists - until time 300 - less than 2% error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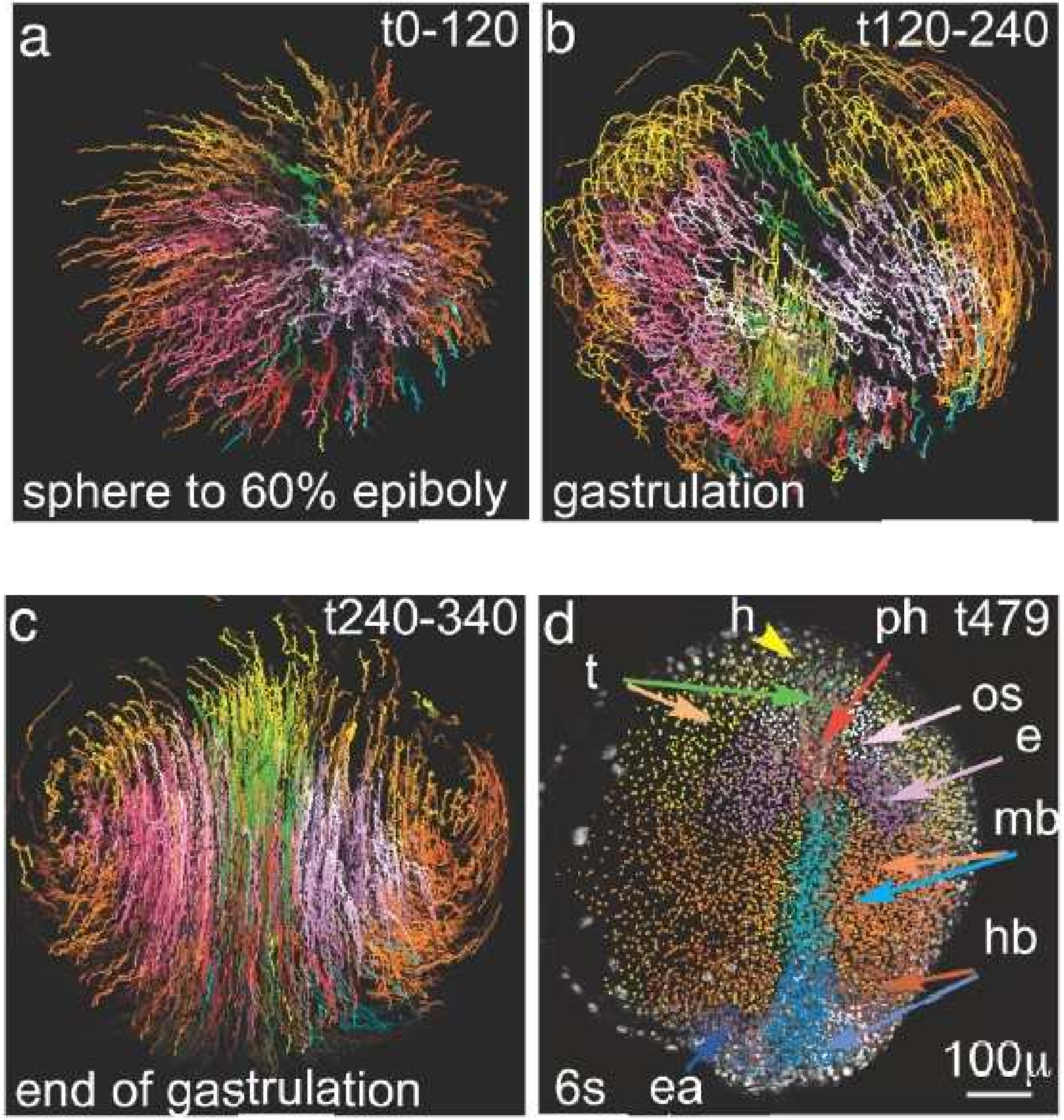
a
t0-120
sphere to 60% epiboly
b
t120-240
gastrulation
c
t240-340
end of gastrulation
d
h
ph
t479
t
os
e
mb
hb
6s
ea
100μ

Videos of embryogenesis reconstruction

Thanks for your attention

- optimal choice of parameters - gold standard + Hausdorff distance - B.Rizzi, Z.Krivá, K.M., N.Peyriéras, A.Sarti