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

Karol Mikula

Department of Mathematics

Slovak University of Technology, Bratislava, Slovakia

http://www.math.sk/mikula

Joint results with

R.Čunderlik, O.Drbliková, M.Remešiková, M.Smišek, R.Špir (Bratislava)

P.Bourgine (Paris), N.Peyrieras (Gif-sur-Yvette), A.Sarti (Bologna)

Motivation from biology and medicine

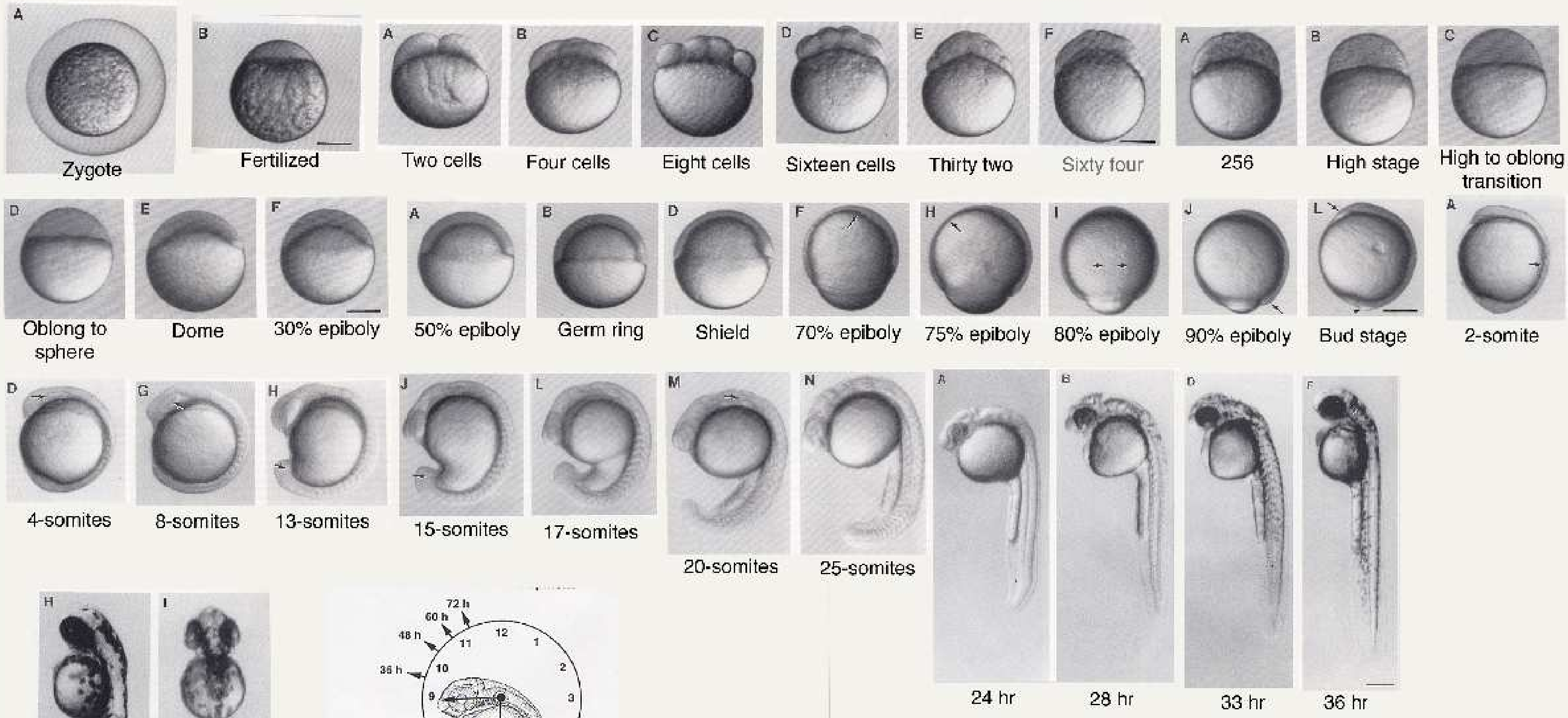
- cooperation with biologists (CNRS - Department of developmental biology, Institute Pasteur and Institute Curie, Paris), bioengineers (University of Bologna), computer scientists (Ecole Polytechnique, Paris) and CNRS supercomputing center (IN2P3 Lyon) - European projects Embryomics and BioEmergences

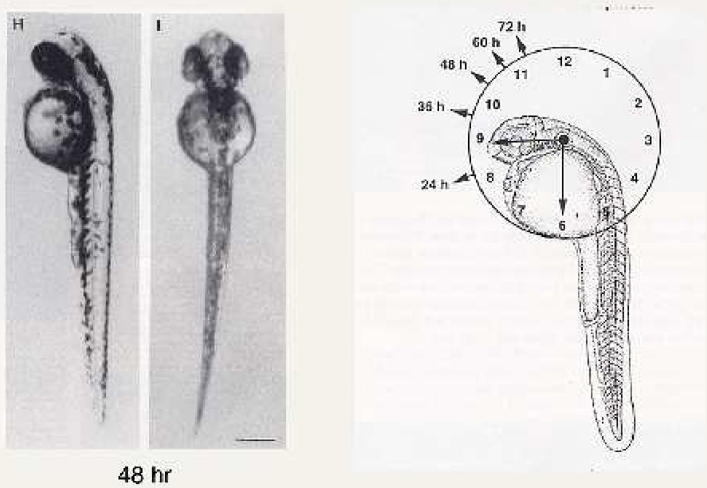
- an automated reconstruction of the vertebrate early embryogenesis in space and time - e.g. zebrafish - transparent for laser microscopes

- extraction of the cell trajectories and the cell lineage tree

- reconstruction of morphogenetic fields

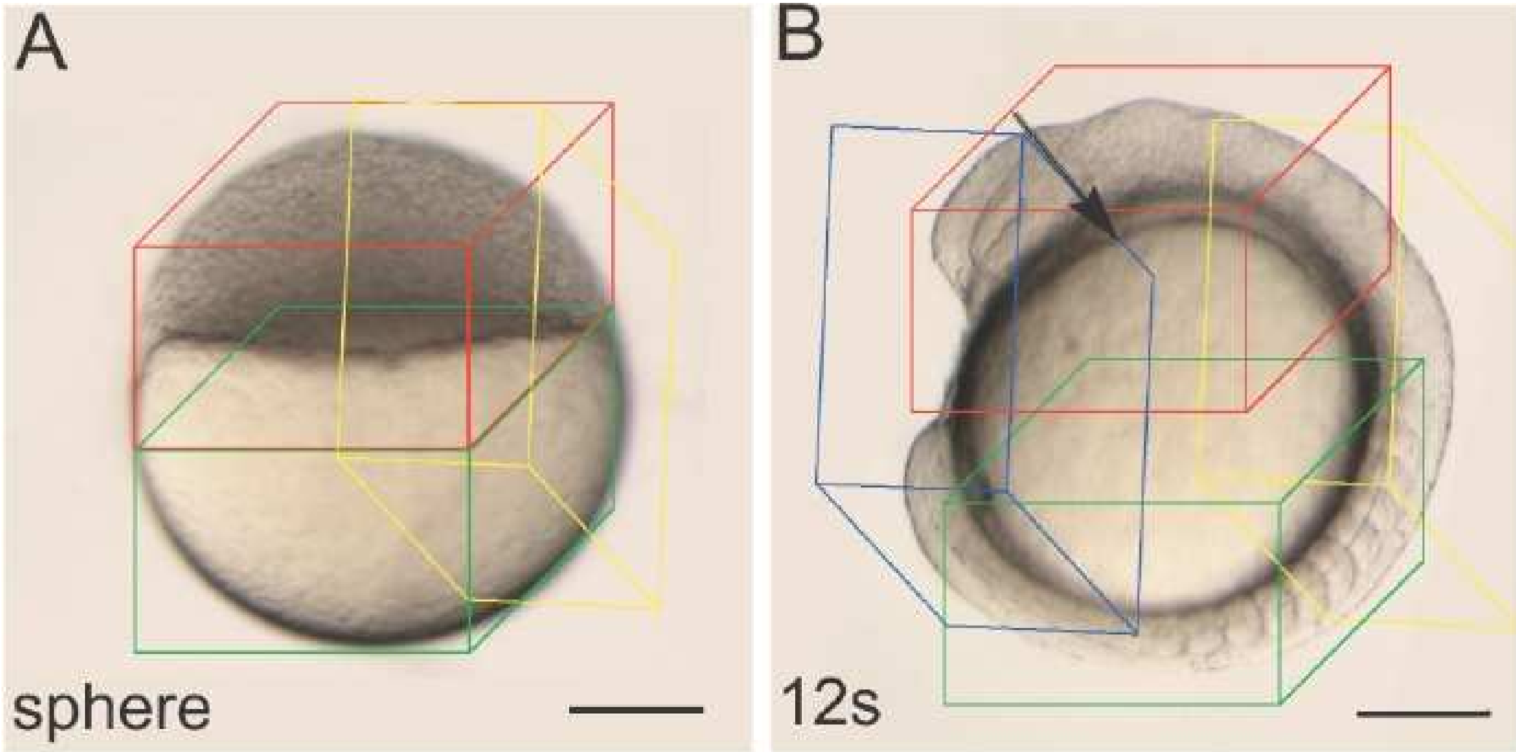
- comparison of untreated and treated cell populations development



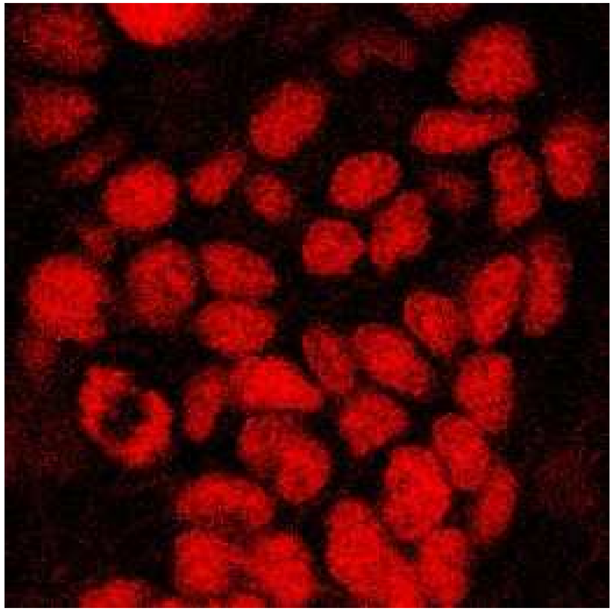
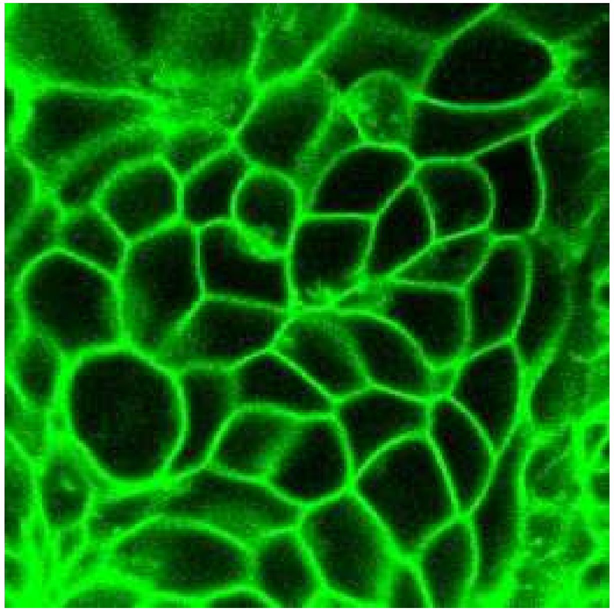
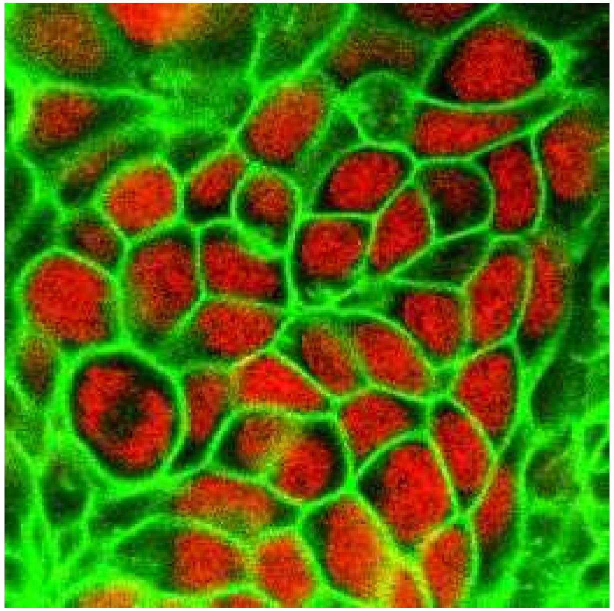


From: Kimmel et al. Stages of embryonic development of the zebrafish Dev. Dyn. 203:253-310, 1995

• 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

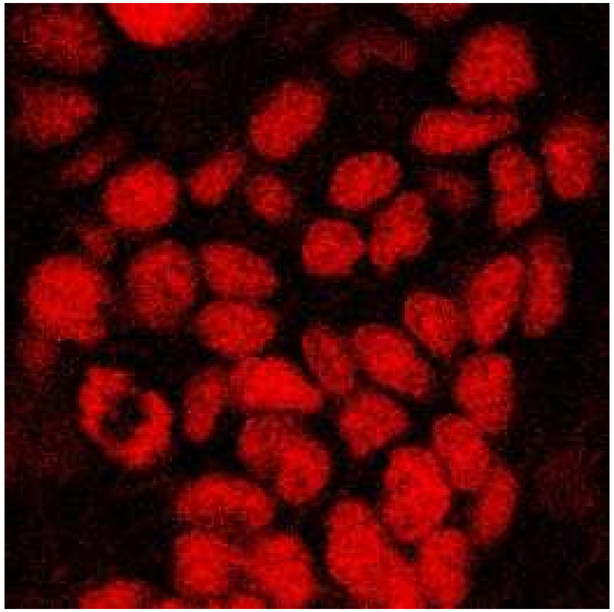
- **cell nuclei center detection** - by convection-diffusion level set equation $\longrightarrow$ approximate number of cells (proliferation rate), detected nuclei centers are starting points for the image segmentation

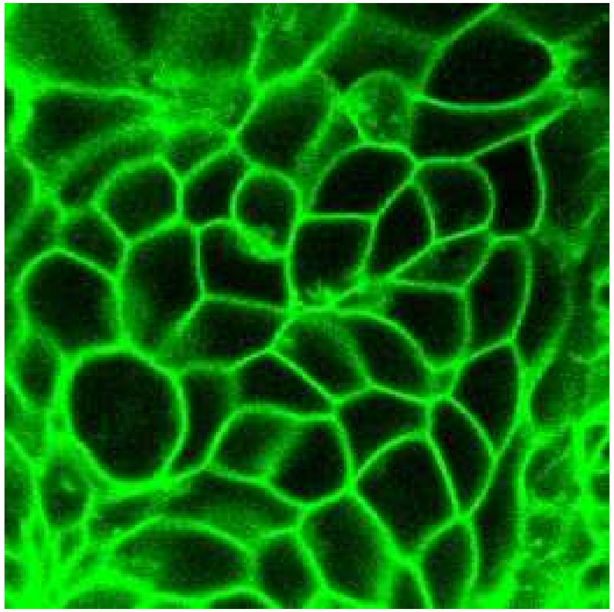
- **cell nuclei segmentation** - by the generalized subjective surface method (geometrical PDE) $\longrightarrow$ 3D nuclei shapes during development, correction of number of cells and positions of the nuclei centers - basis for cell tracking and cell trajectories extraction

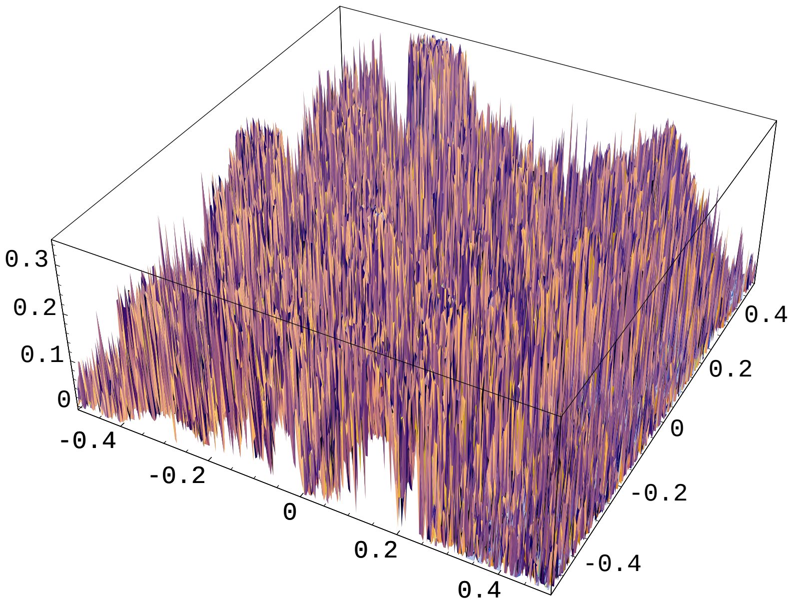
- **whole embryo segmentation** $\rightarrow$ cell density evolving in time

- **cell membranes segmentation** $\rightarrow$ 3D cell shapes during development

- **cell tracking and cell trajectories extraction** - by finding centered paths in 4D spatio-temporal segmented tree structure by steepest descent of potential built by a proper combination of constrained distance functions computed inside the 4D segmentation






0.3
0.2
0.1
0
-0.4
-0.2
0
0.2
0.4
0.4
0.2
0
-0.2
-0.4

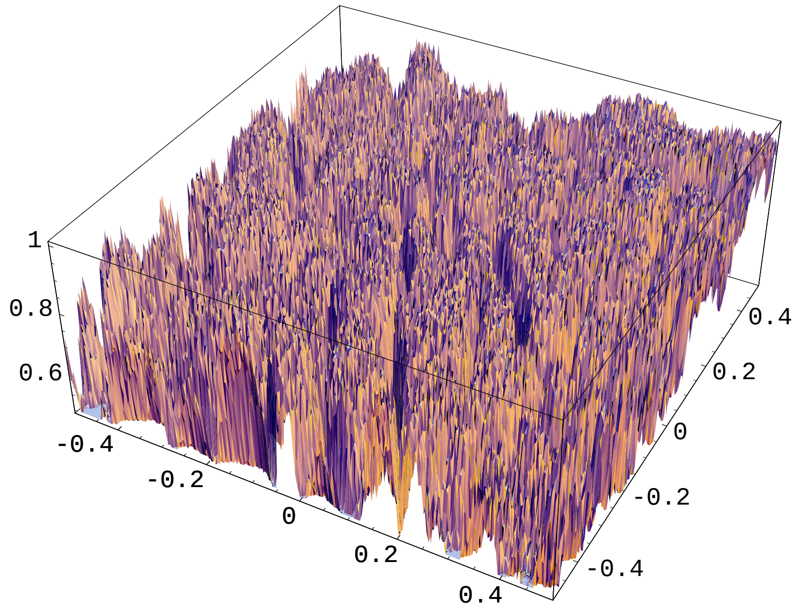
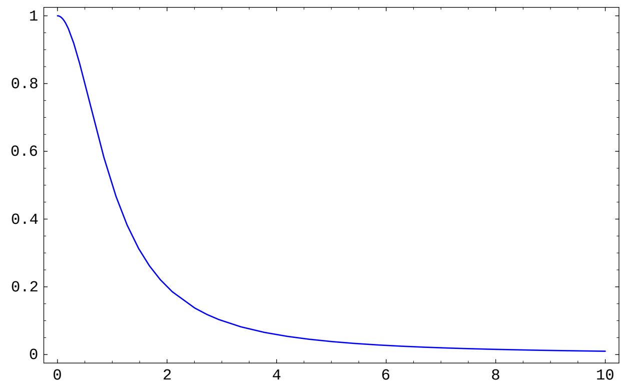

1
0.8
0.6
-0.4
-0.2
0
0.2
0.4
0.4
0.2
0
-0.2
-0.4

Image filtering

- **Geodesic mean curvature flow equation**

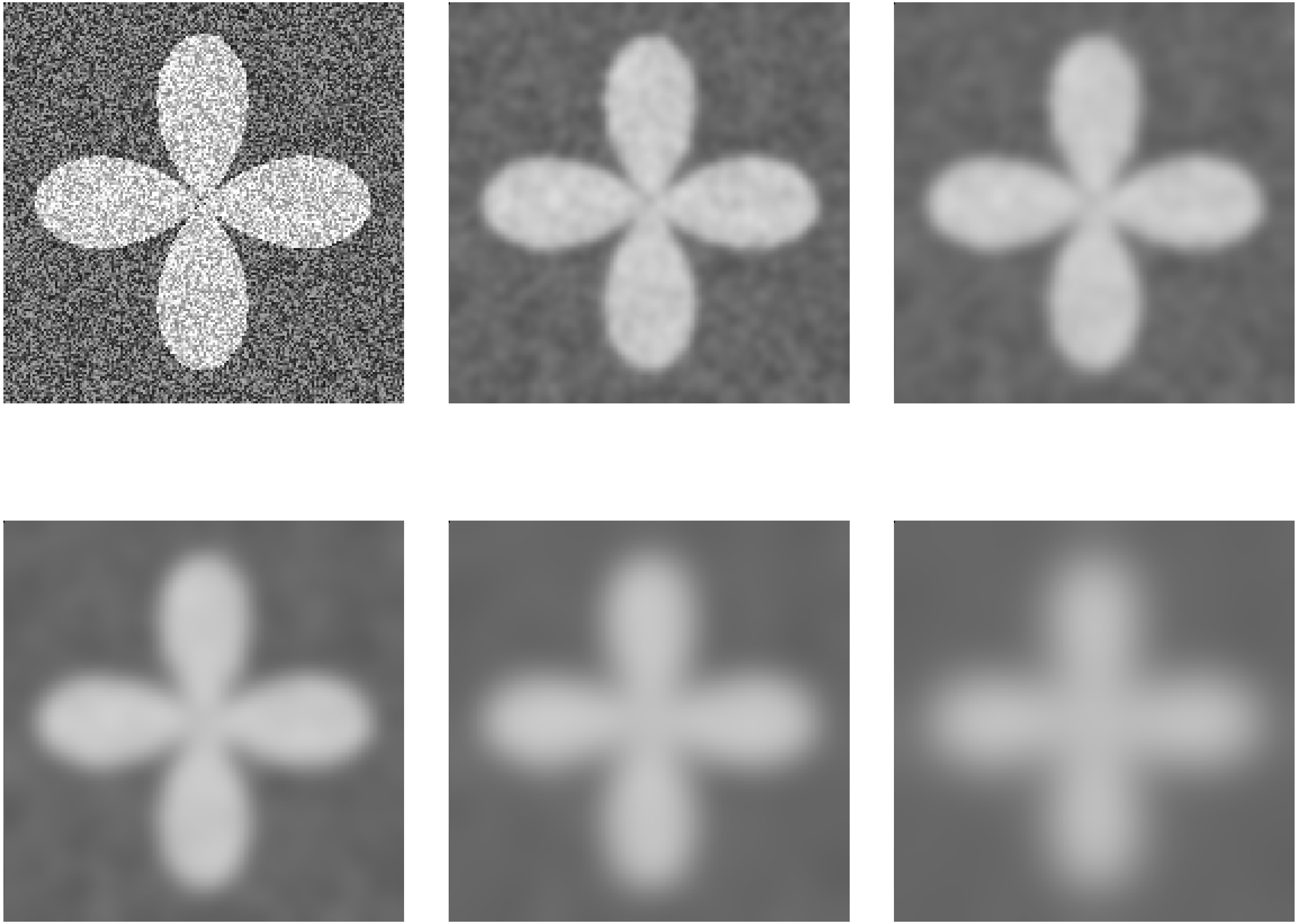
(Caselles, Kimmel, Sapiro and Chen, Vemuri, Wang)

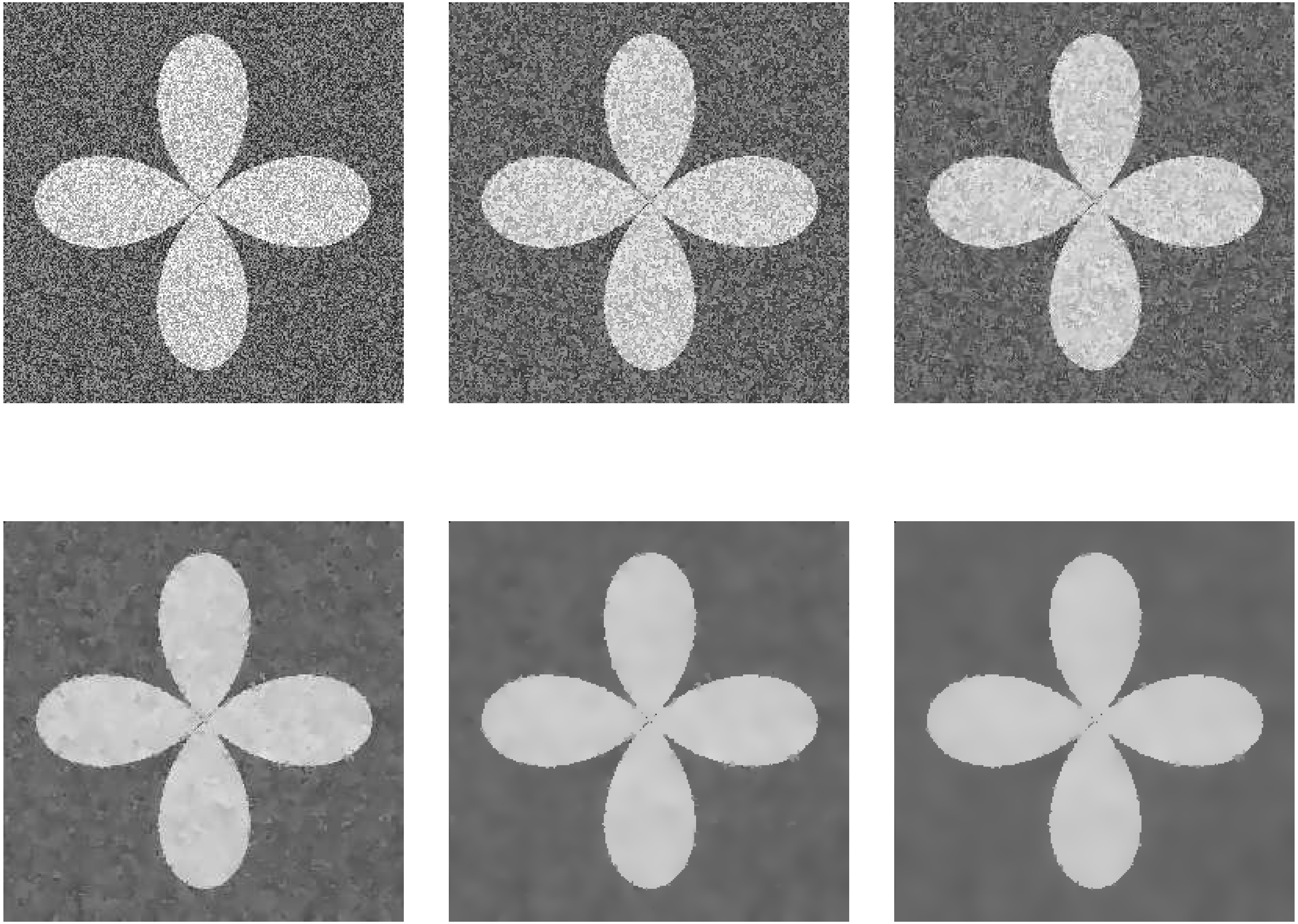
$$u_t = |\nabla u| \nabla . \left(g(|\nabla G_\sigma * u|) \frac{\nabla u}{|\nabla u|} \right) \quad u(0,x) = I^0(x), \quad \text{h.N.b.c} \qquad (1)$$



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

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





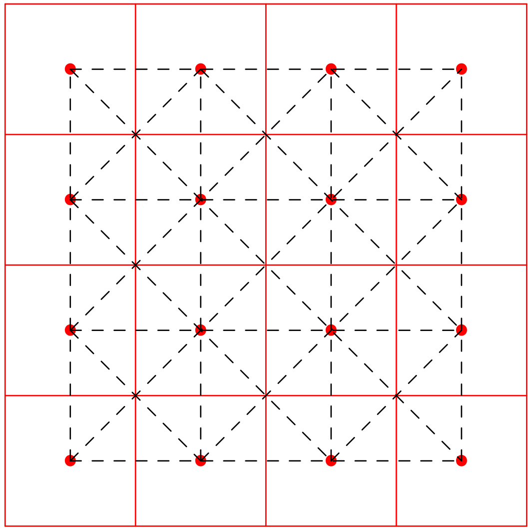
Numerical solution of nonlinear PDEs

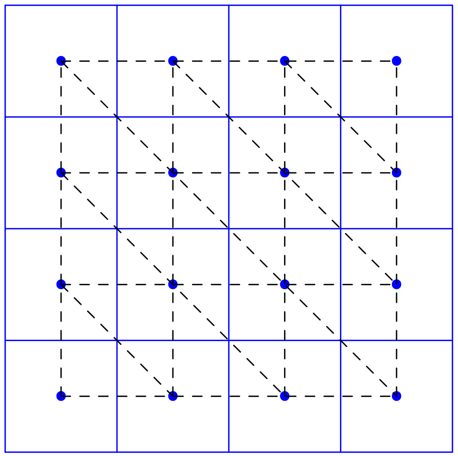
- **Semi-implicit schemes - J.Kačur, K.M. (1995), J.Weickert (1995), A.Handlovičová, K.M., F.Sgallari (2003, 2006)**

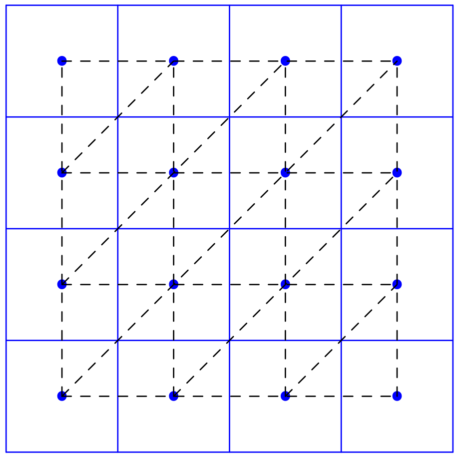
Let k and σ be fixed numbers and $u^0 = I^0$. For every $n = 1, \ldots N$, we look for a function u^n, a solution of the equation

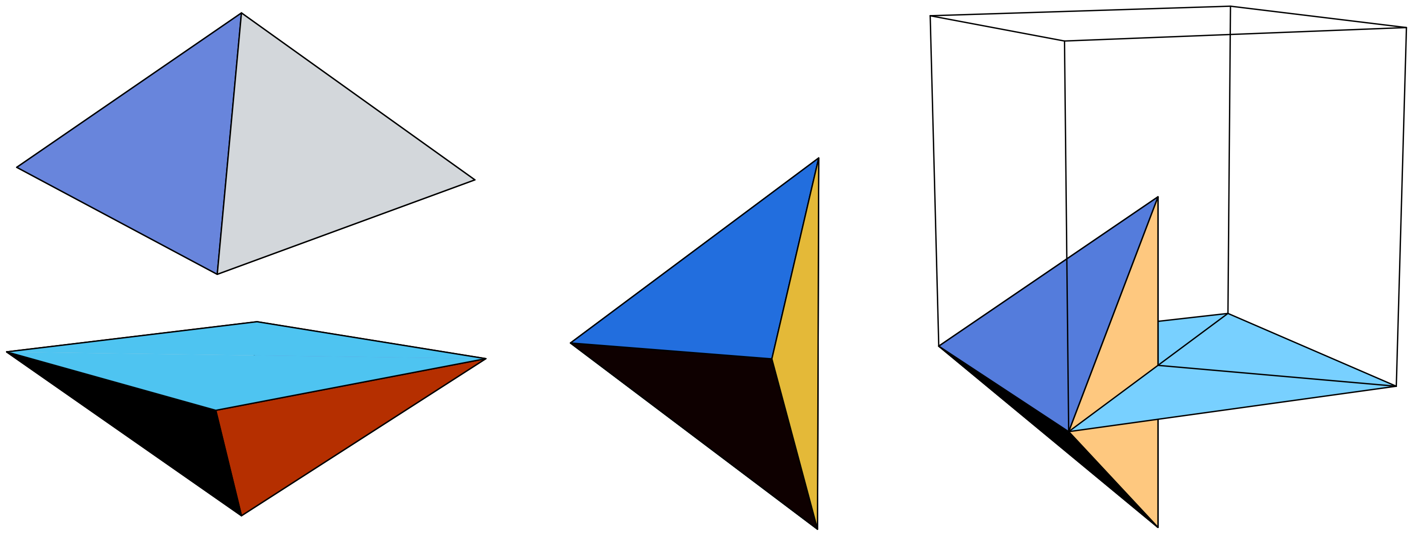
$$\frac{1}{|\nabla u^{n-1}|}\frac{u^n - u^{n-1}}{k} - \nabla . \left(g(|\nabla G_\sigma * u^{n-1}|) \frac{\nabla u^n}{|\nabla u^{n-1}|} \right) = 0. \qquad (2)$$

- space discretization - **finite volume** or **co-volume methods** on uniform (by image given) grids $\longrightarrow$ solving linear systems in every filtration or segmentation step - SOR method - naturally parallelizable in 3D (**MPI implementation**)









- 3D implementation - every cubic voxel is splitted into 6 pyramids. The neighbouring pyramids of neighbouring voxels are joined together to form octahedron (**diamond cell** for the face) which can be itself used to evaluate gradients of solution on the face or it can be further split into 4 tetrahedras, elements of 3D triangulation on which we can evaluate nonlinearities depending on gradients.

- S.Corsaro, K.M., A.Sarti, F.Sgallari, SIAM J. Sci. Comp., 2006

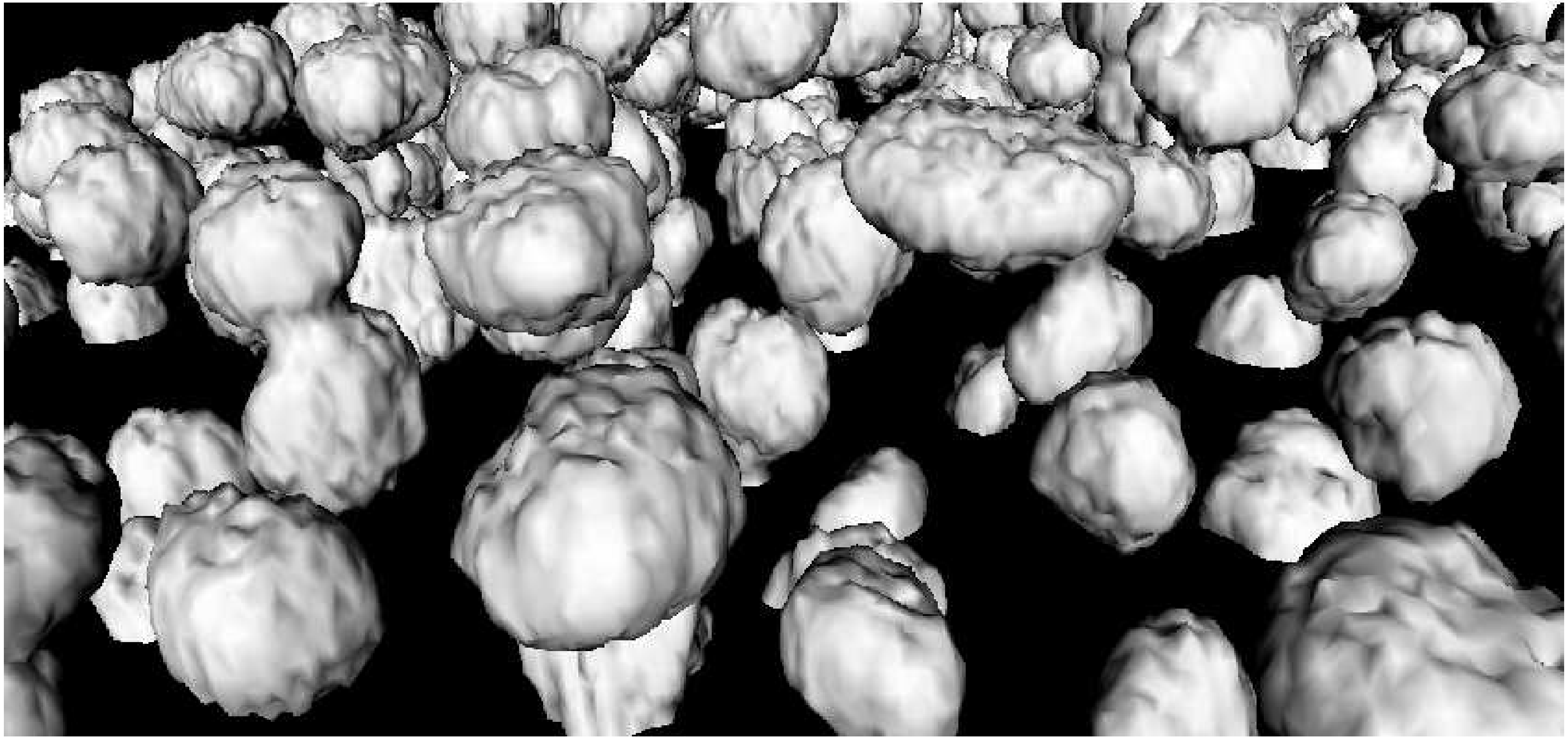
- **unconditional** L_∞ - **stability**, no spurious oscillations, no restriction on time step

- **convergence** of the finite volume schemes and error estimates for the Perona-Malik-type equations - K.M., N.Ramarosy, Num. Math., 2001, A.Hadlovičová, Z.Krivá, AMUC, 2005

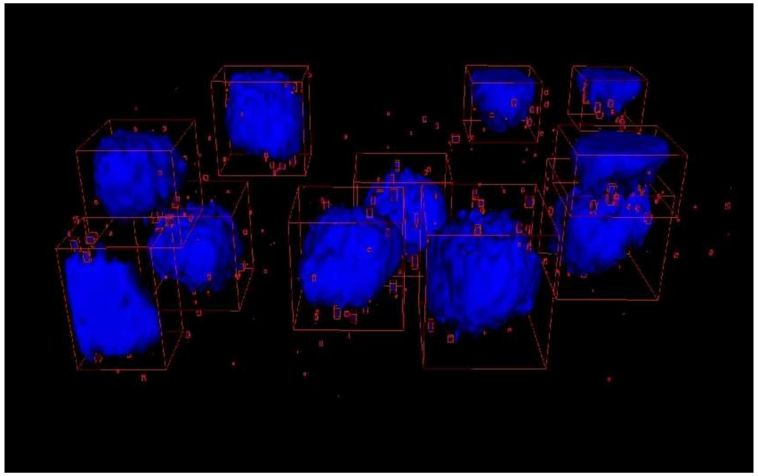
- **convergence** of the finite volume schemes and error estimates for Weickert's model of nonlinear tensor-driven anisotropic diffusion - O.Drbliková, K.M., SIAM J. Numer. Anal., 2007, O.Drbliková, A.Hadlovičová, K.M., APNUM, 2009

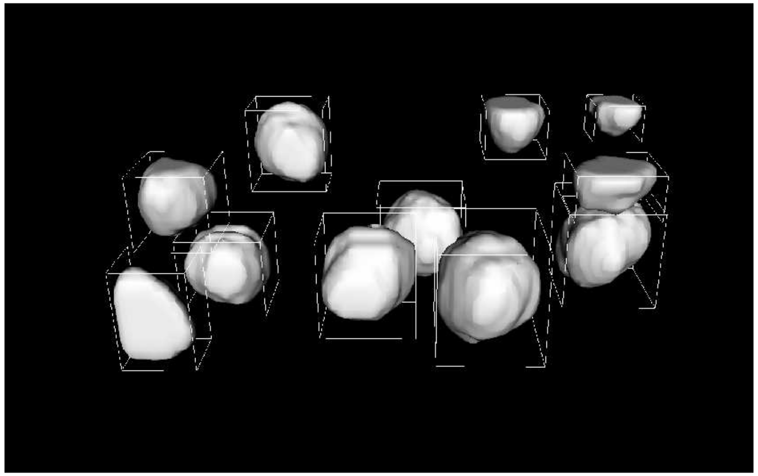
- **consistency** of co-volume schemes for regularized curvature level set equation - K.M., A.Handlovičová, Appl. Math., 2008

- **convergence** of finite volume schemes for regularized curvature level set equation - R.Eymard, K.M., A.Hadlovičová, IMAJNA 2011



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





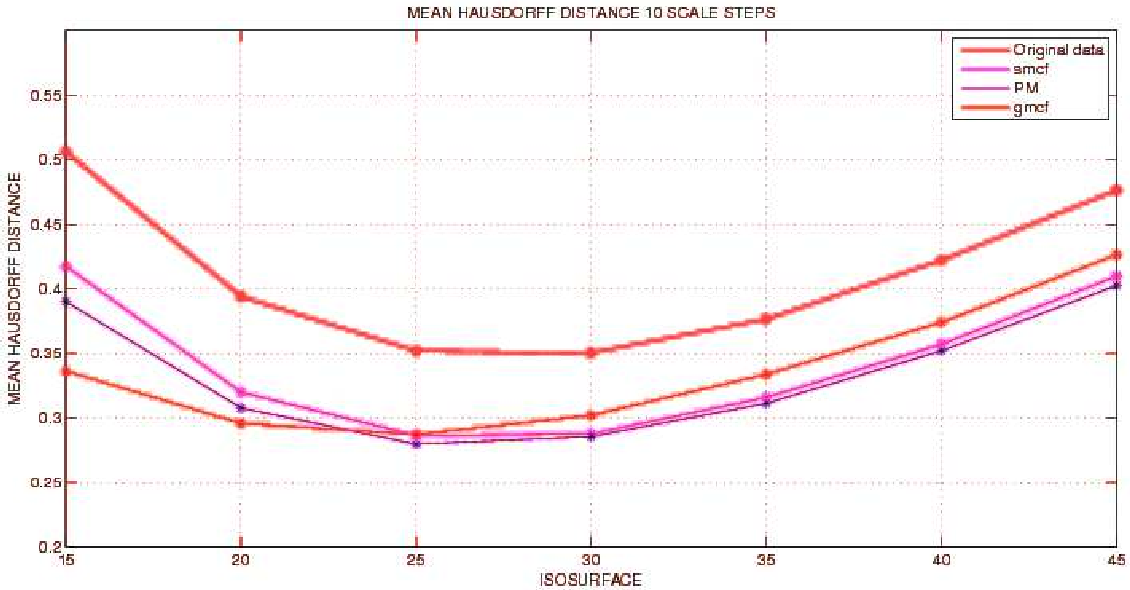


Image segmentation

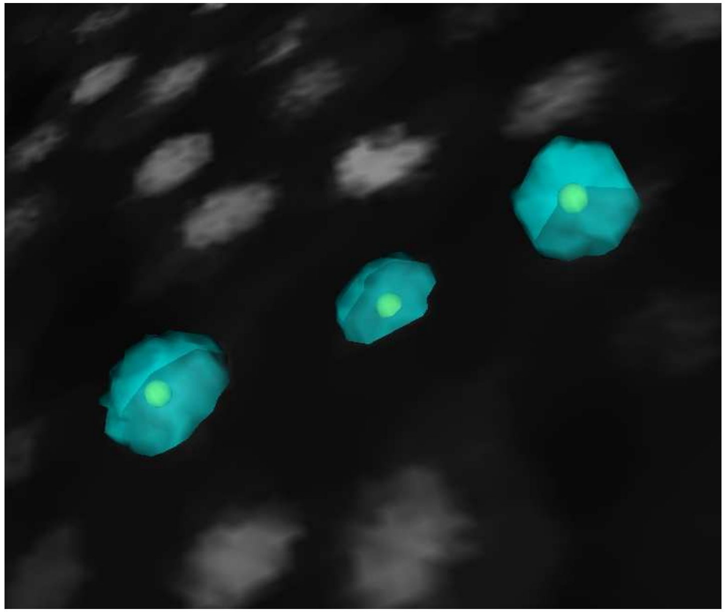
• subjective surface method due to Sarti, Malladi, Sethian (2000) - ε-regularization of the geodesic mean curvature flow equation

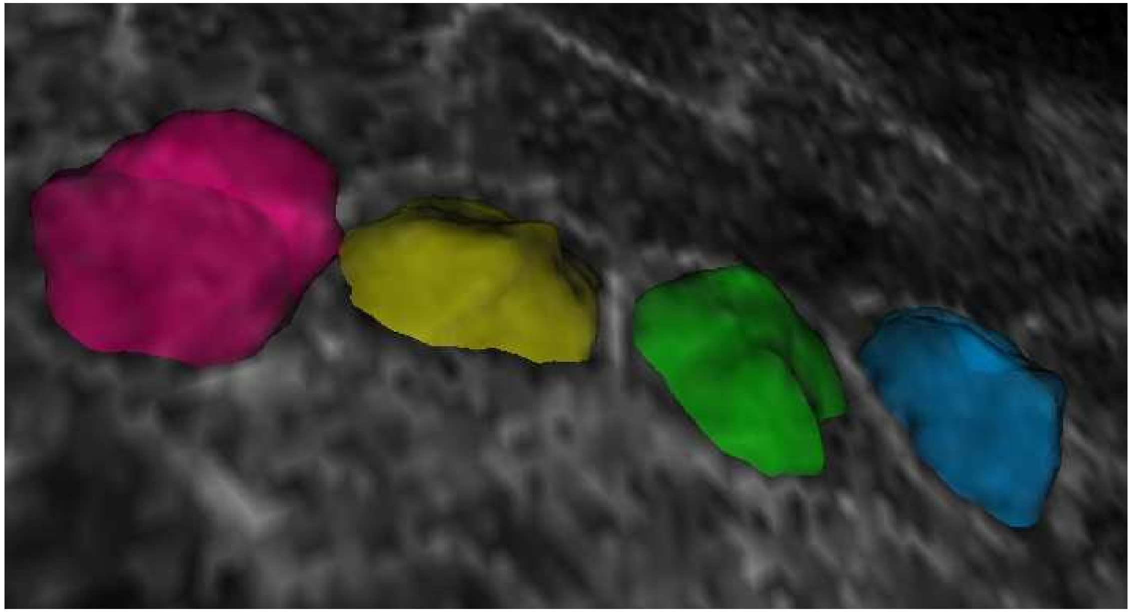
$$u_t = \sqrt{\varepsilon^2 + |\nabla u|^2} \nabla . \left(g \frac{\nabla u}{\sqrt{\varepsilon^2 + |\nabla u|^2}} \right), \quad g = g(|\nabla G_\sigma * I^0|) \qquad (3)$$

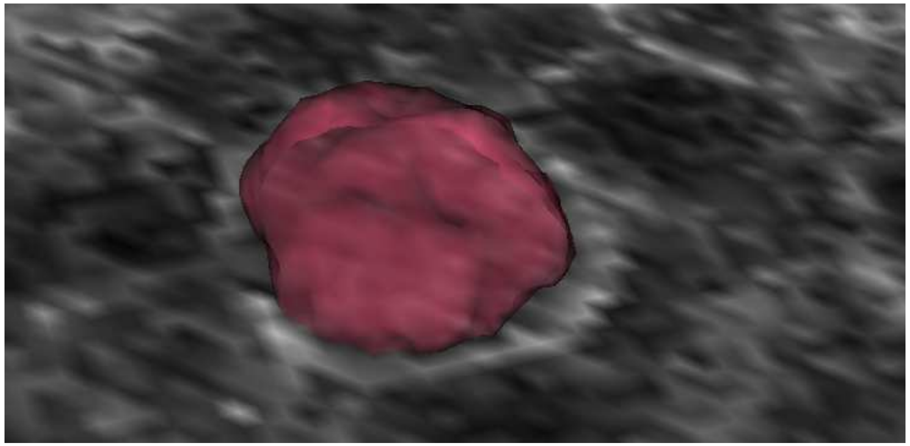
• generalized version with different weigths to advective and diffusive parts - K.M., N.Peyriéras, M.Remešiková, A.Sarti (2008, FVCA5) and C.Zanella et al.(2010, IEEE TIP)

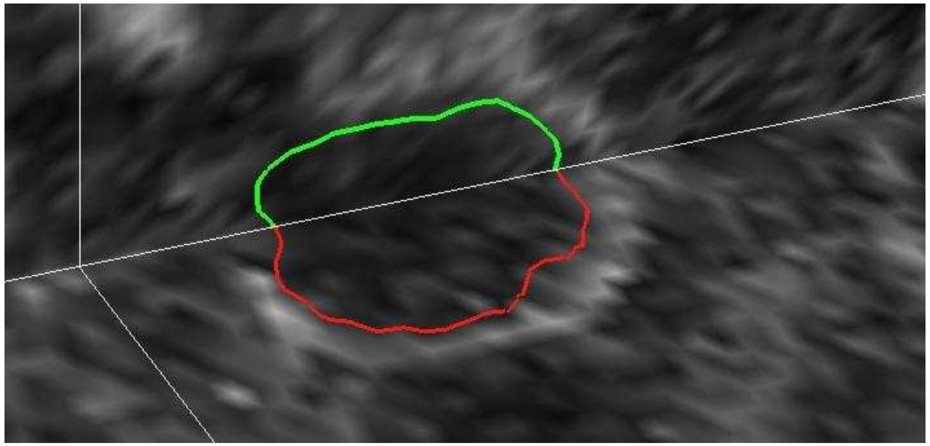
$$u_t = \mu_1 \, g|\nabla u| \nabla . \left(\frac{\nabla u}{|\nabla u|} \right) + \mu_2 \, \nabla g . \nabla u \qquad (4)$$

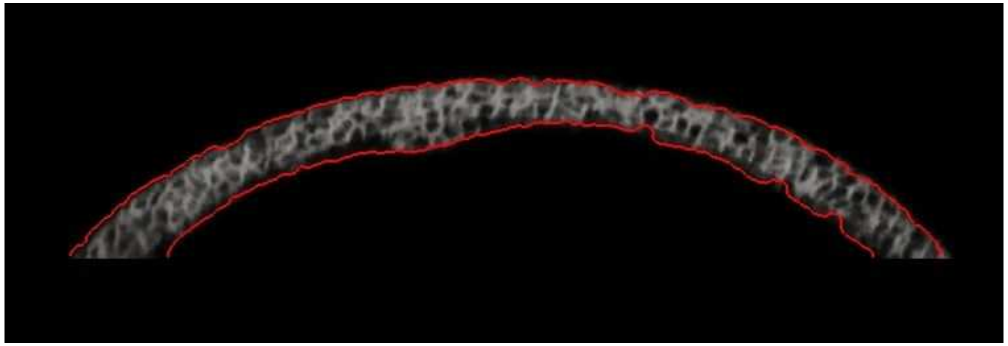
• efficient 3D implementations using semi-implicit scheme in curvature part and up-wind schemes in advective part - M.Remešiková, R.Čunderlik, K.M.

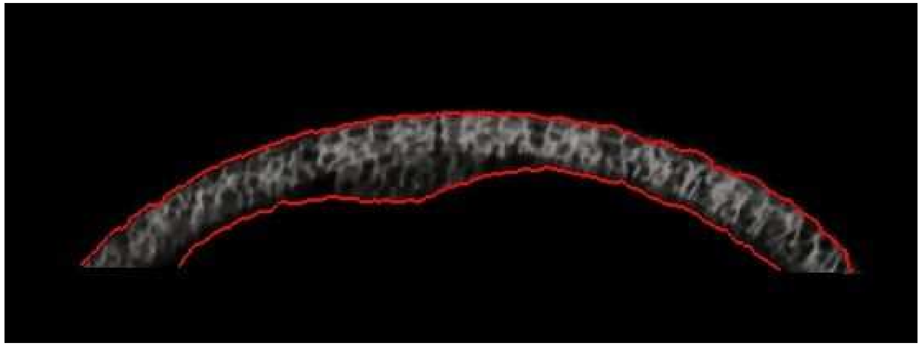


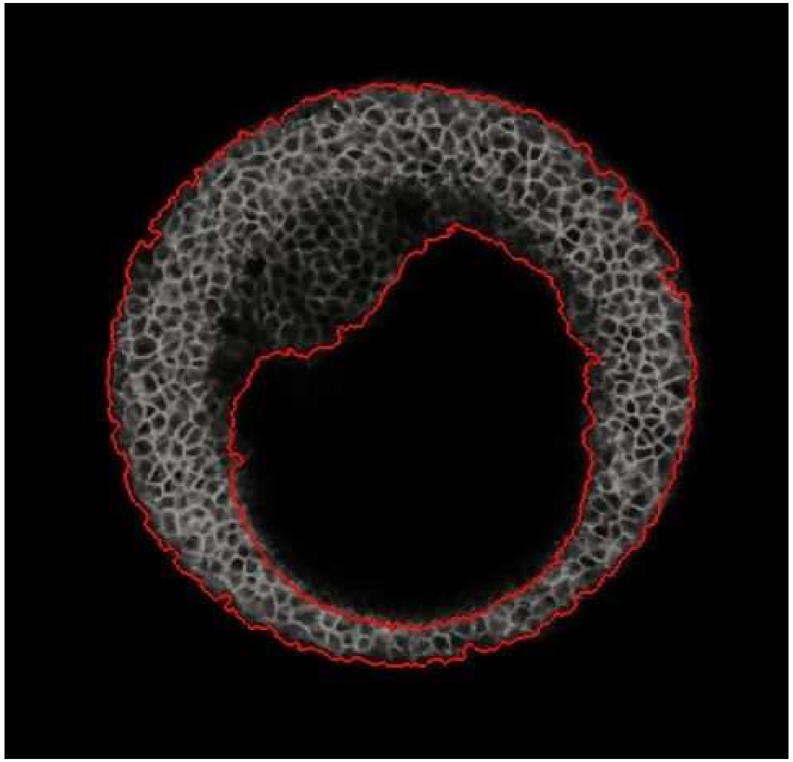


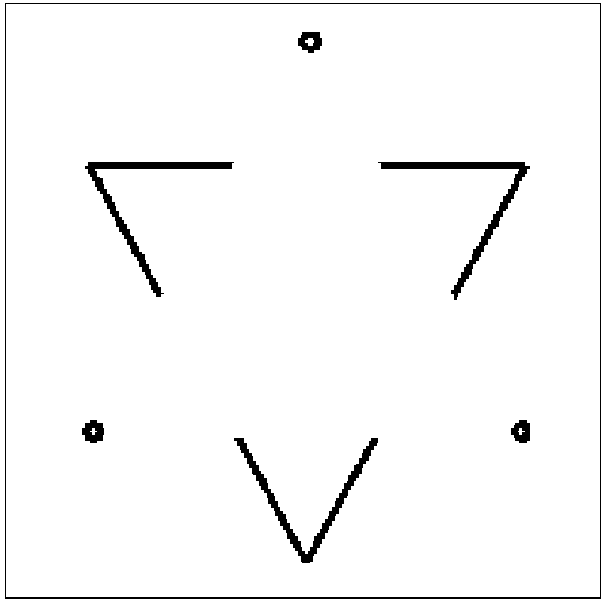




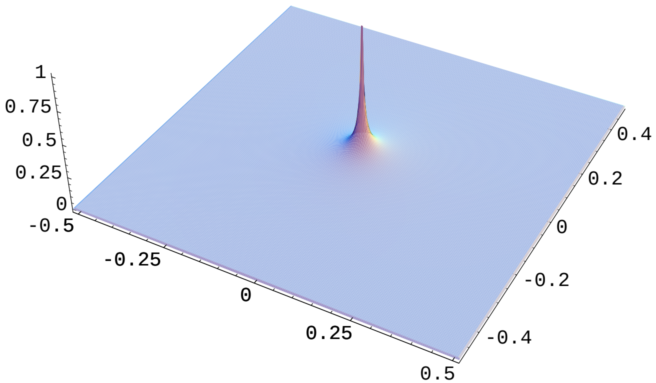


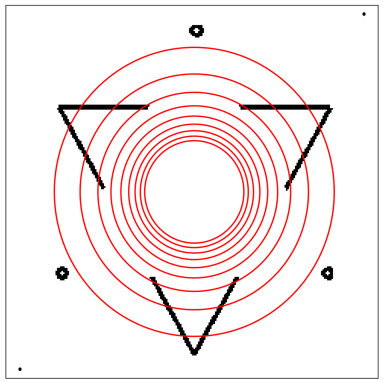


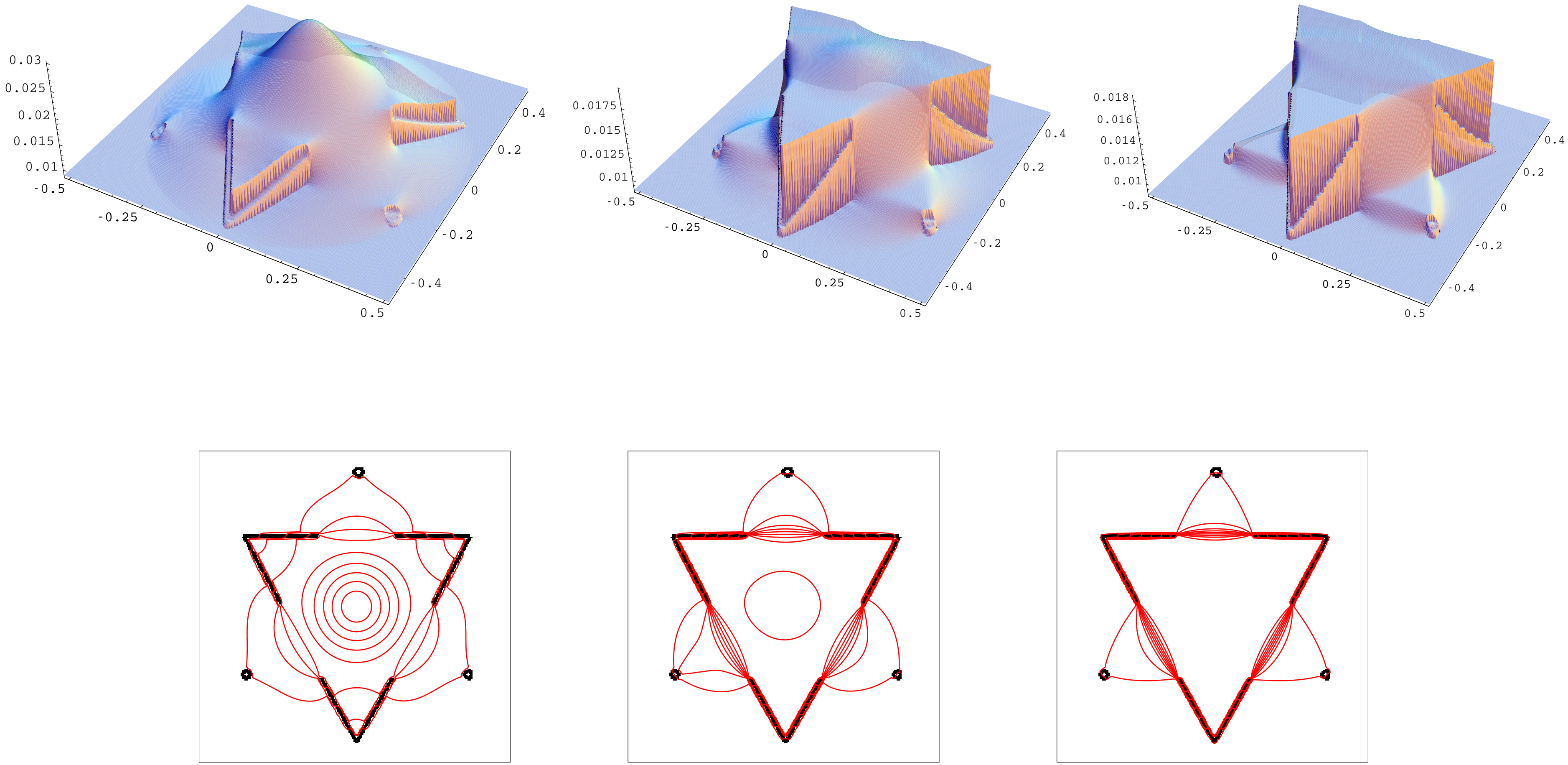




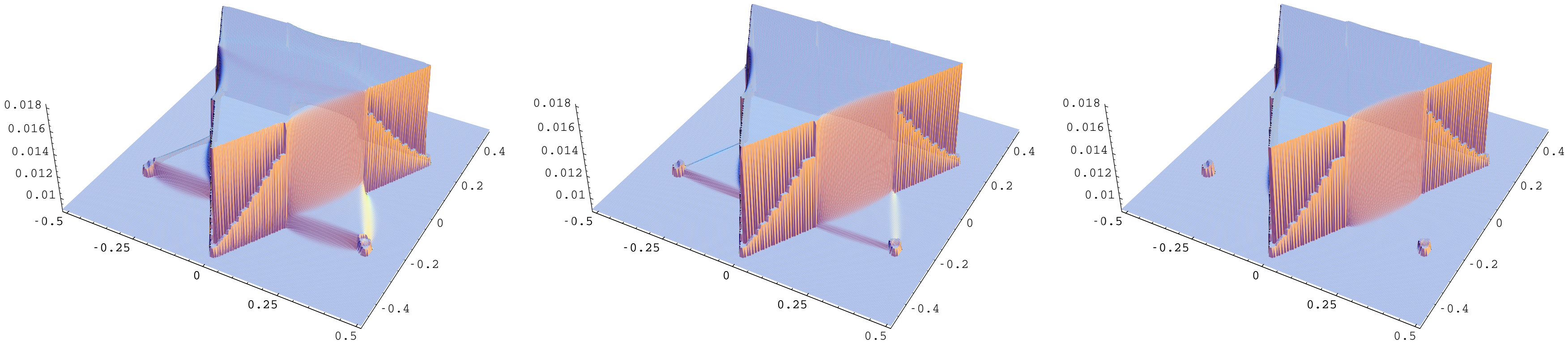
Finding the subjective contours in double-Kanizsa-triangle image

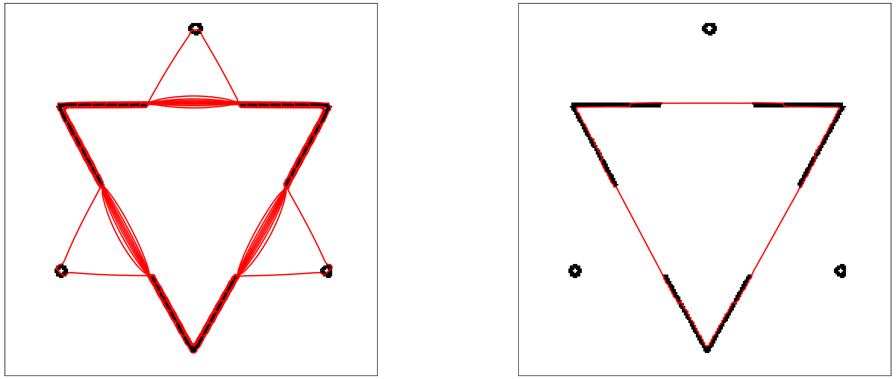




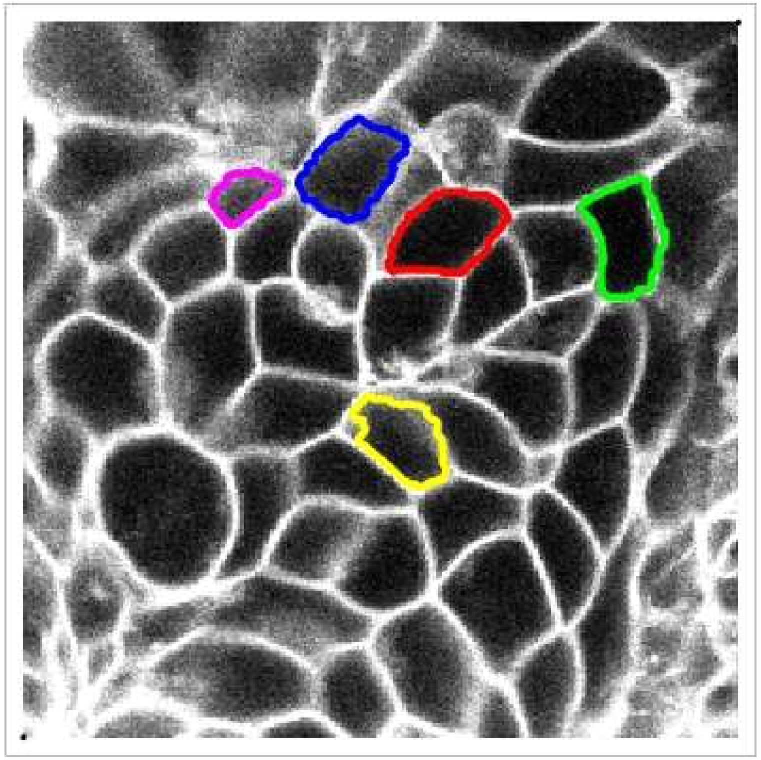


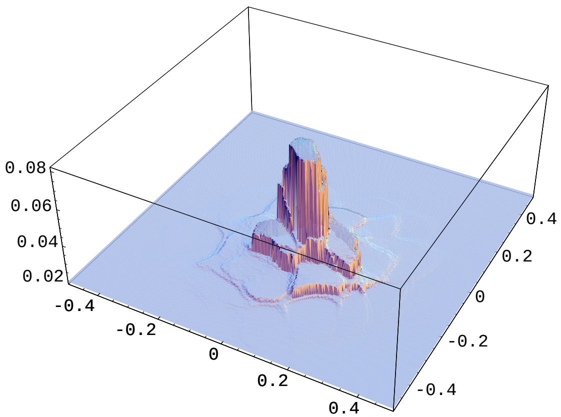
Level lines and 3D graphs of segmentation function after 10, 30, 60 time steps, $\varepsilon = 10^{-5}$.

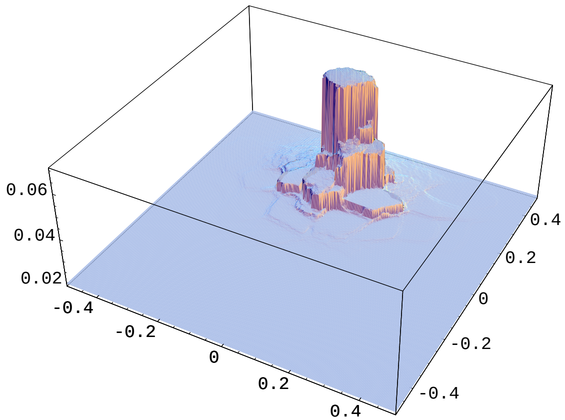


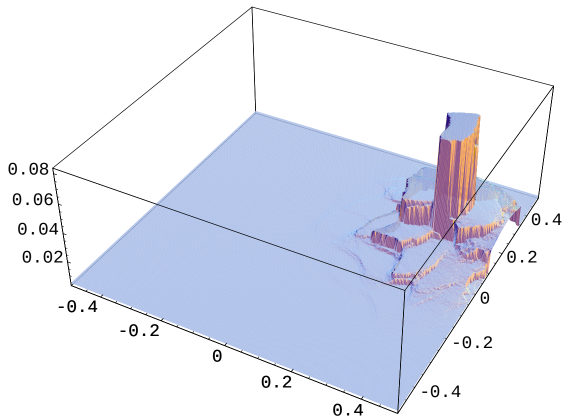


Level lines and 3D graphs of segmentation function after 100, 300, 800 time steps, $\varepsilon = 10^{-5}$.




0.08
0.06
0.04
0.02
-0.4
-0.2
0
0.2
0.4
0.4
0.2
0
-0.2
-0.4


0.06
0.04
0.02
-0.4
-0.2
0
0.2
0.4
0.4
0.2
0
-0.2
-0.4

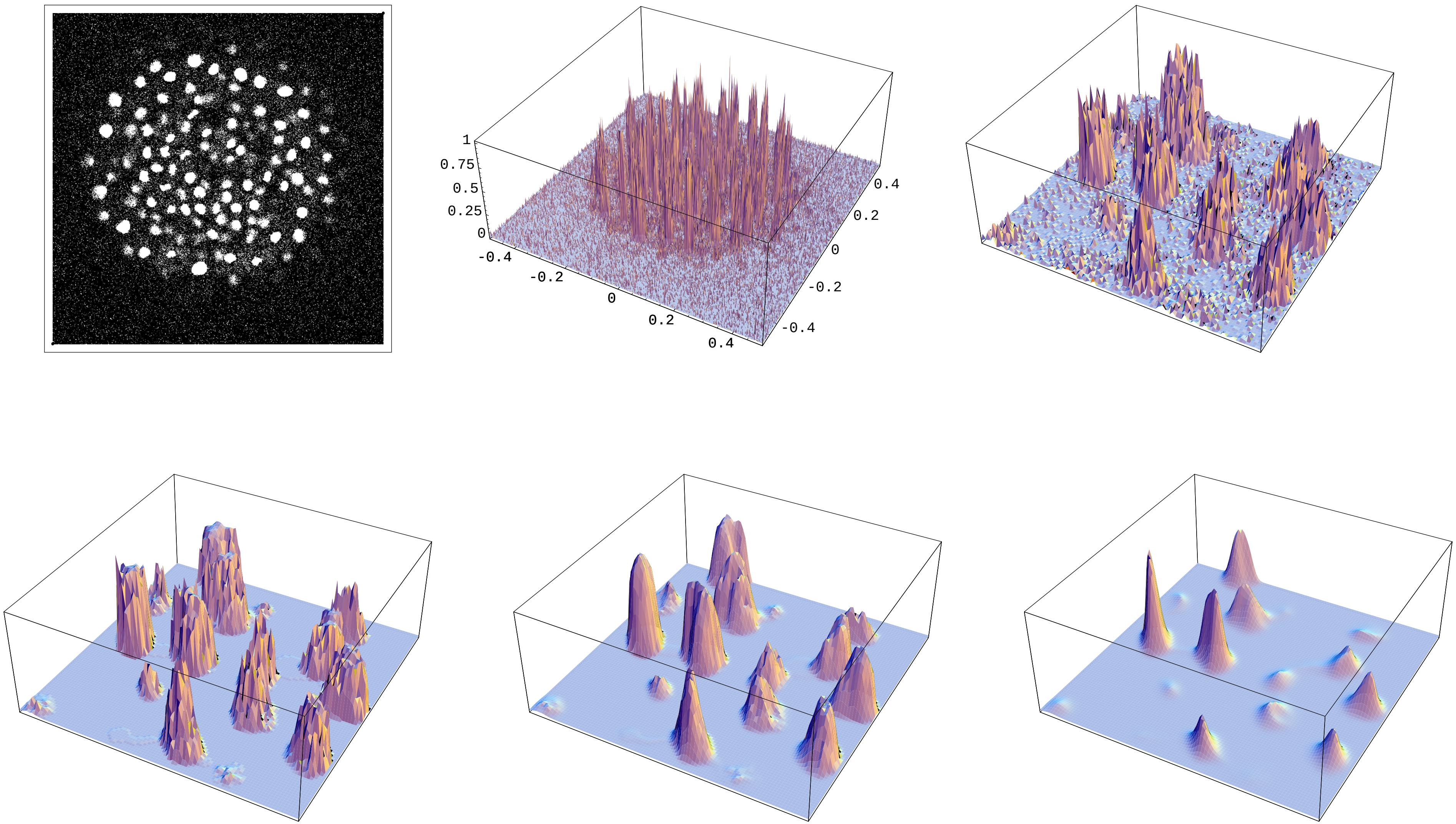

0.08
0.06
0.04
0.02
-0.4
-0.2
0
0.2
0.4
0.4
0.2
0
-0.2
-0.4

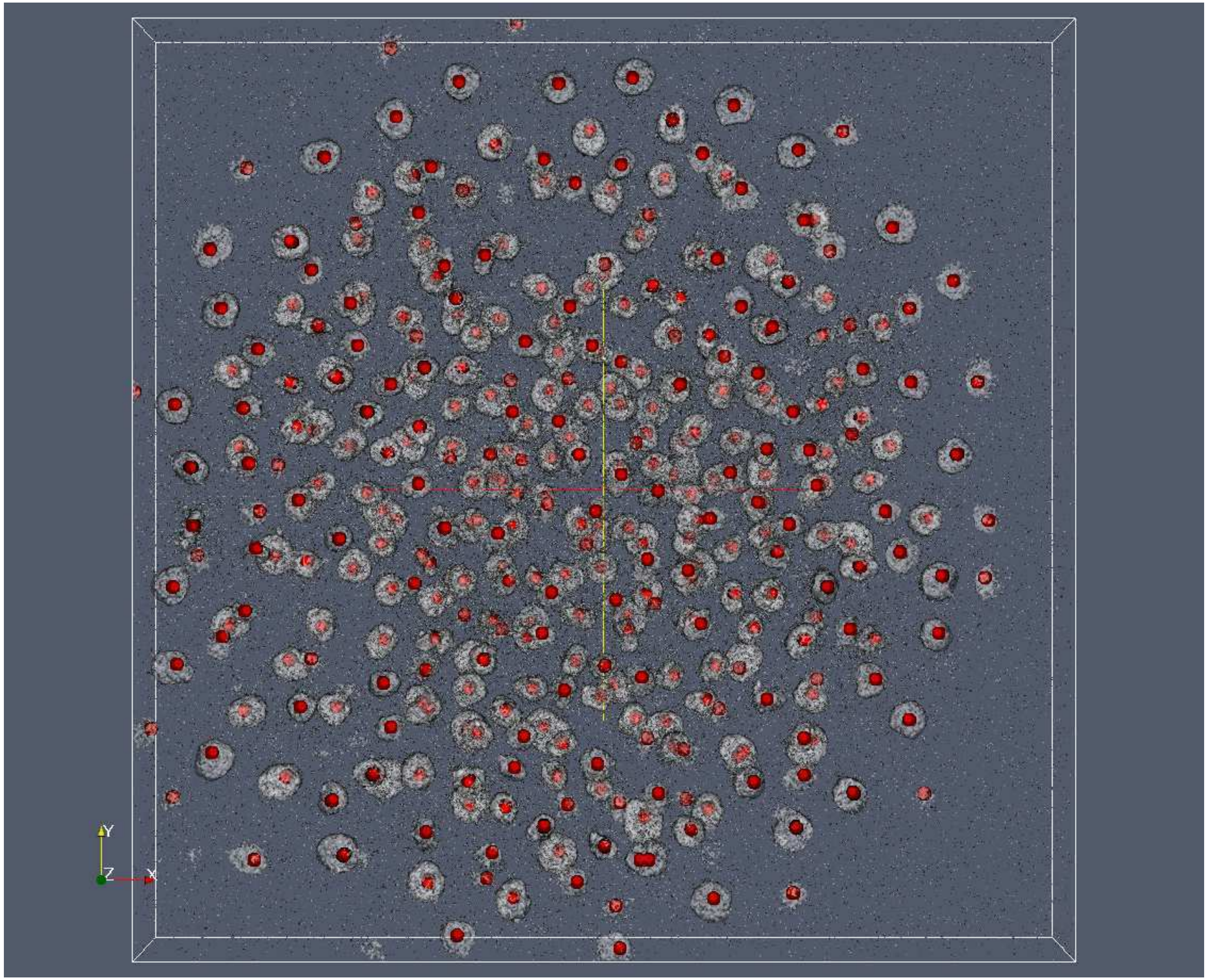
Nuclei center detection

• 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)

$$u_t = \delta \frac{\nabla u}{|\nabla u|} \cdot \nabla u + \mu |\nabla u| \nabla \cdot \left(\frac{\nabla u}{|\nabla u|} \right) \quad \text{h.N.b.c} \qquad (5)$$

• 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

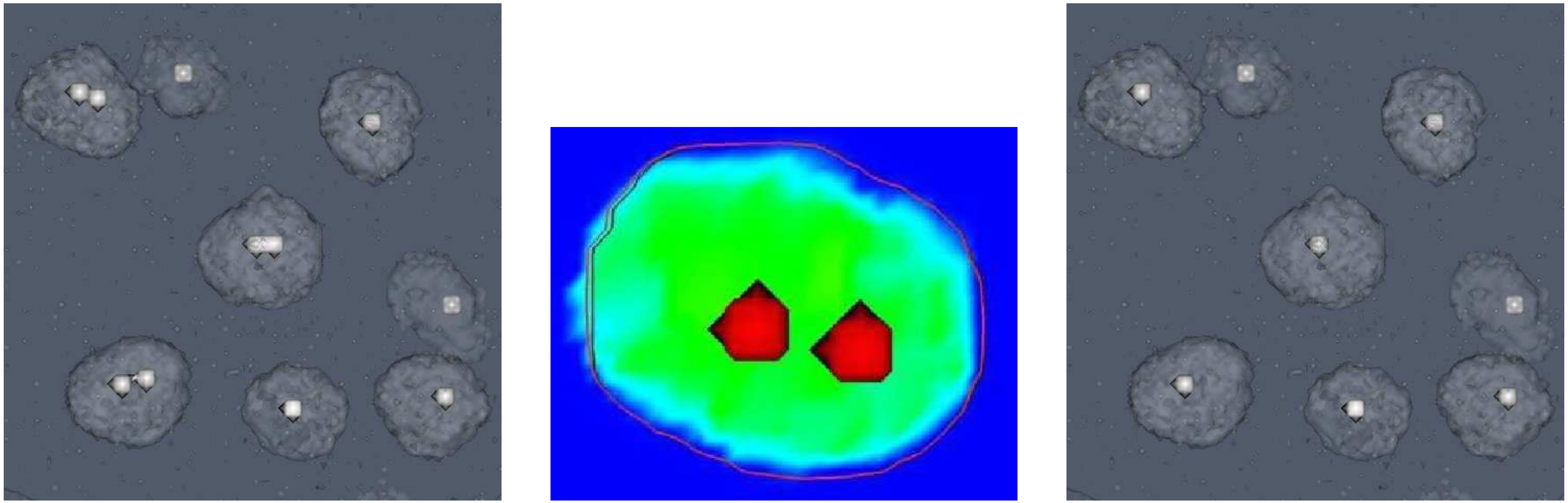

1
0.75
0.5
0.25
0
-0.4
-0.2
0
0.2
0.4
0.4
0.2
0
-0.2
-0.4



- error manually checked by biologists - less than 0.5%

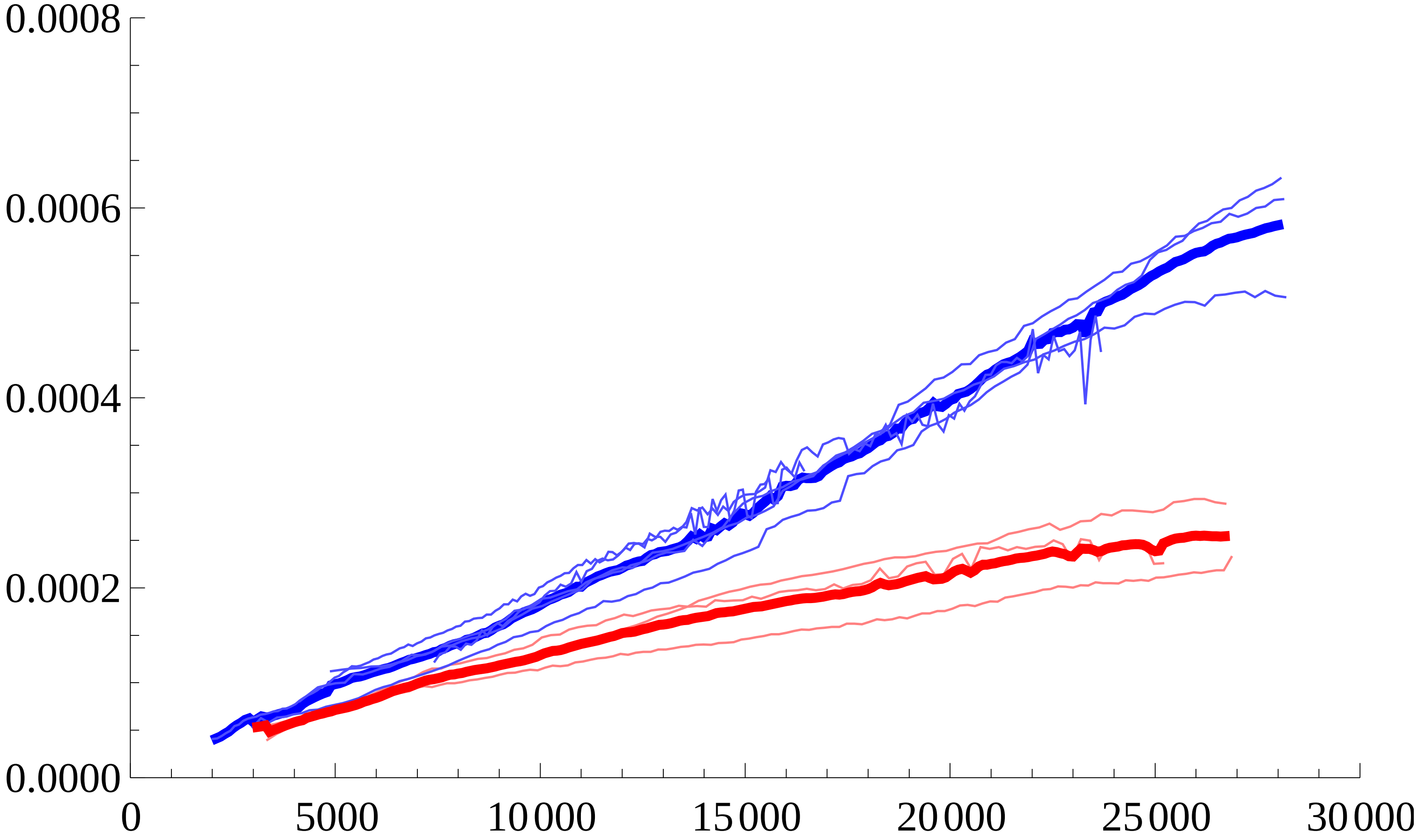
Nuclei center correction

• in large and very noisy data sets we stop the center detection process a bit earlier - better is to detect more centers than lose some of the nuclei - then, if the nuclei segmentation process starting from two centers finishes by the same shape we can relialably remove the superfluous center



• error manually checked by biologists - less than 0.5%

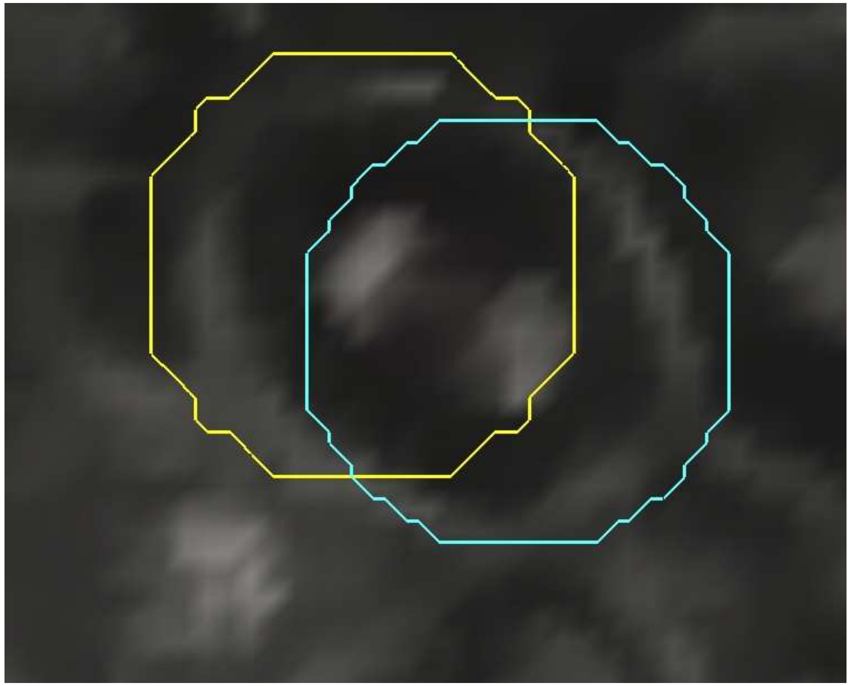
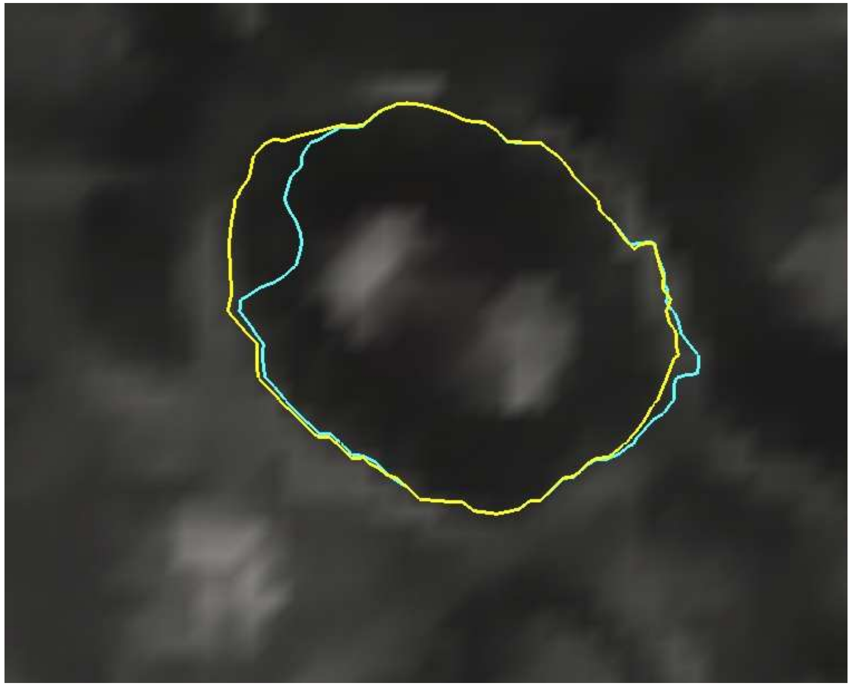
- anticancer drug testing using cell density curves = (number of cells) / (segmented volume of the imaged part of embryo) evolved in time



- 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

- the basis is a 4D space-time segmentation in the form of 4D tree like structures inside 4D image

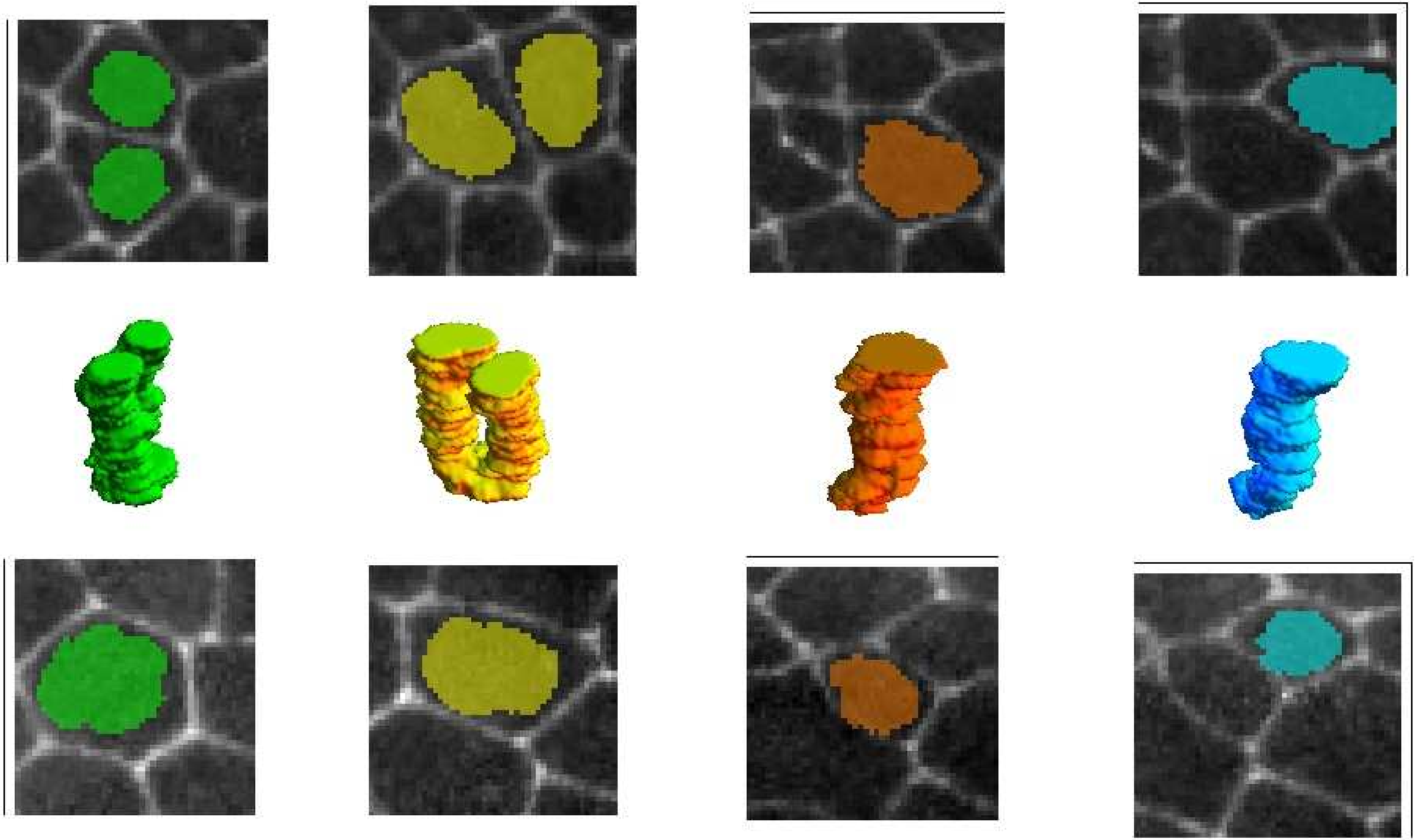
- 4D distance function from the "root" cells is computed ($|\nabla u| = 1$) inside 4D segmentation $\rightarrow$ first estimate of cell trajectories

- 4D distance function from the boundaries of 4D segmentation $\rightarrow$ centering of cell trajectories

- building a potential - difference of distance functions

- steepest descent travers of potential $\rightarrow$ extraction of cell trajectories going backward in time - merging trajectories indicate mitosis

- general approach - we compute the distance functions from cell identifiers at every time step looking forward and perform steepest descent from every time step going backward $\rightarrow$ extraction all possible, also partial trajectories



Space-time segmentations (one branch, "trousers", ..., tree, "forest")

Cell tracking

- the basis is a 4D space-time segmentation in the form of 4D tree like structures inside 4D image

- 4D distance function from the "root" cells is computed inside 4D segmentation $\longrightarrow$ first estimate of cell trajectories

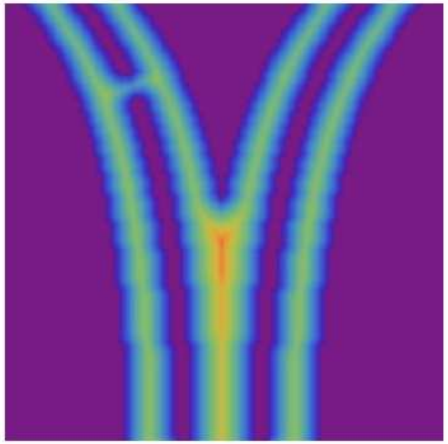
- 4D distance function from the boundaries of 4D segmentation $\longrightarrow$ centering of cell trajectories

- building a potential - difference of distance functions

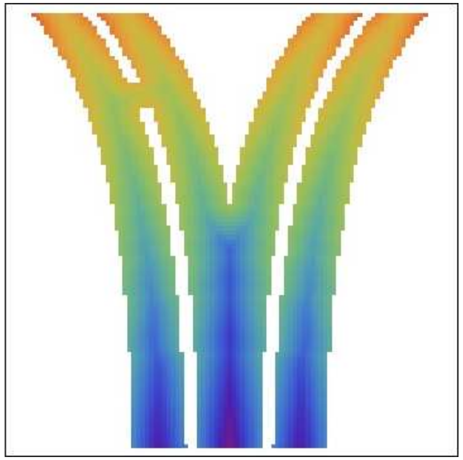
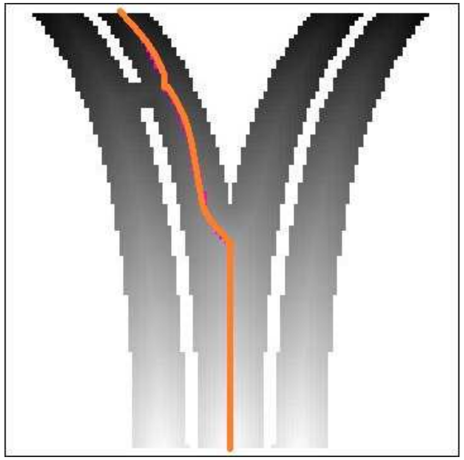
- steepest descent travers of potential $\longrightarrow$ extraction of cell trajectories going backward in time - merging trajectories indicate mitosis

- general approach - we compute the distance functions from cell identifiers at every time step looking forward and perform steepest descent from every time step going backward $\longrightarrow$ extraction all possible, also partial trajectories

Cell tracking

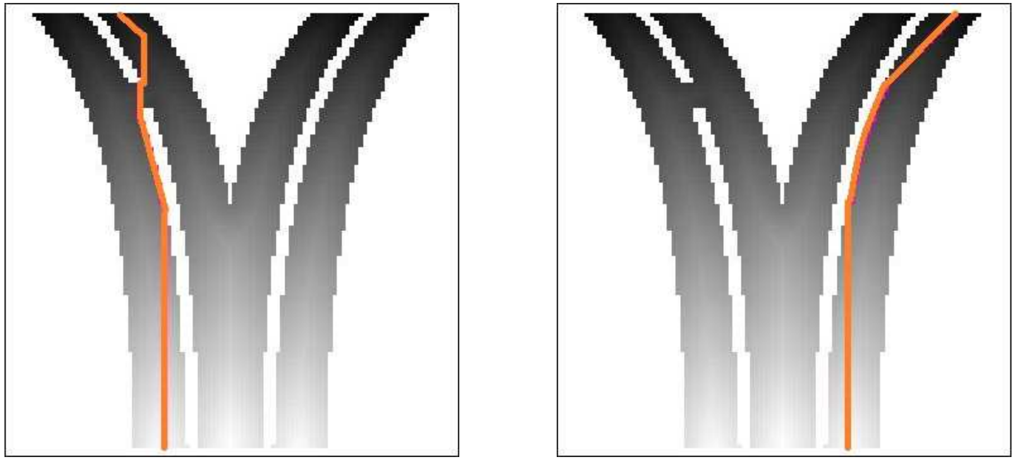
 

left - first distance function, right - second distance function

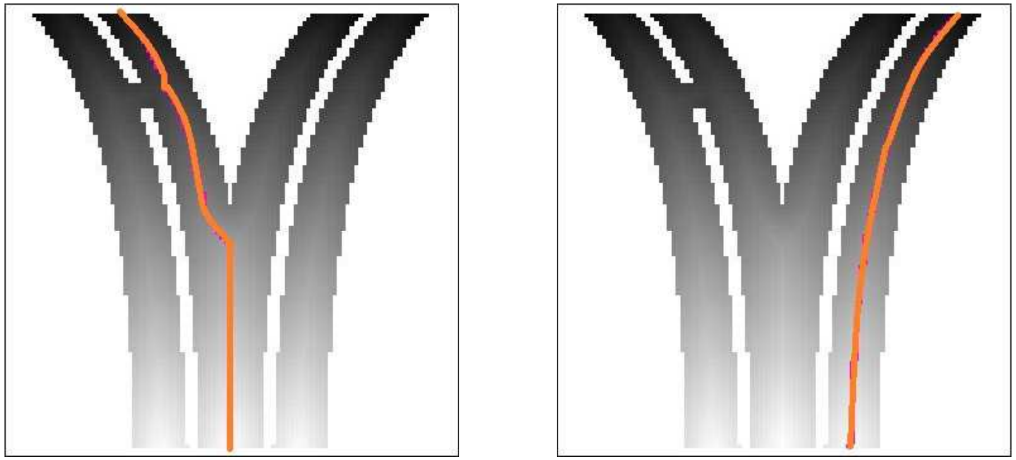
 

first distance **minus** second distance → **centering the trajectory**

Cell tracking



only distance from initial cell identifiers and steepest descent



using both distances from initial cells and from boundaries

Cell tracking

- the basis is a 4D space-time segmentation in the form of 4D tree like structures inside 4D image

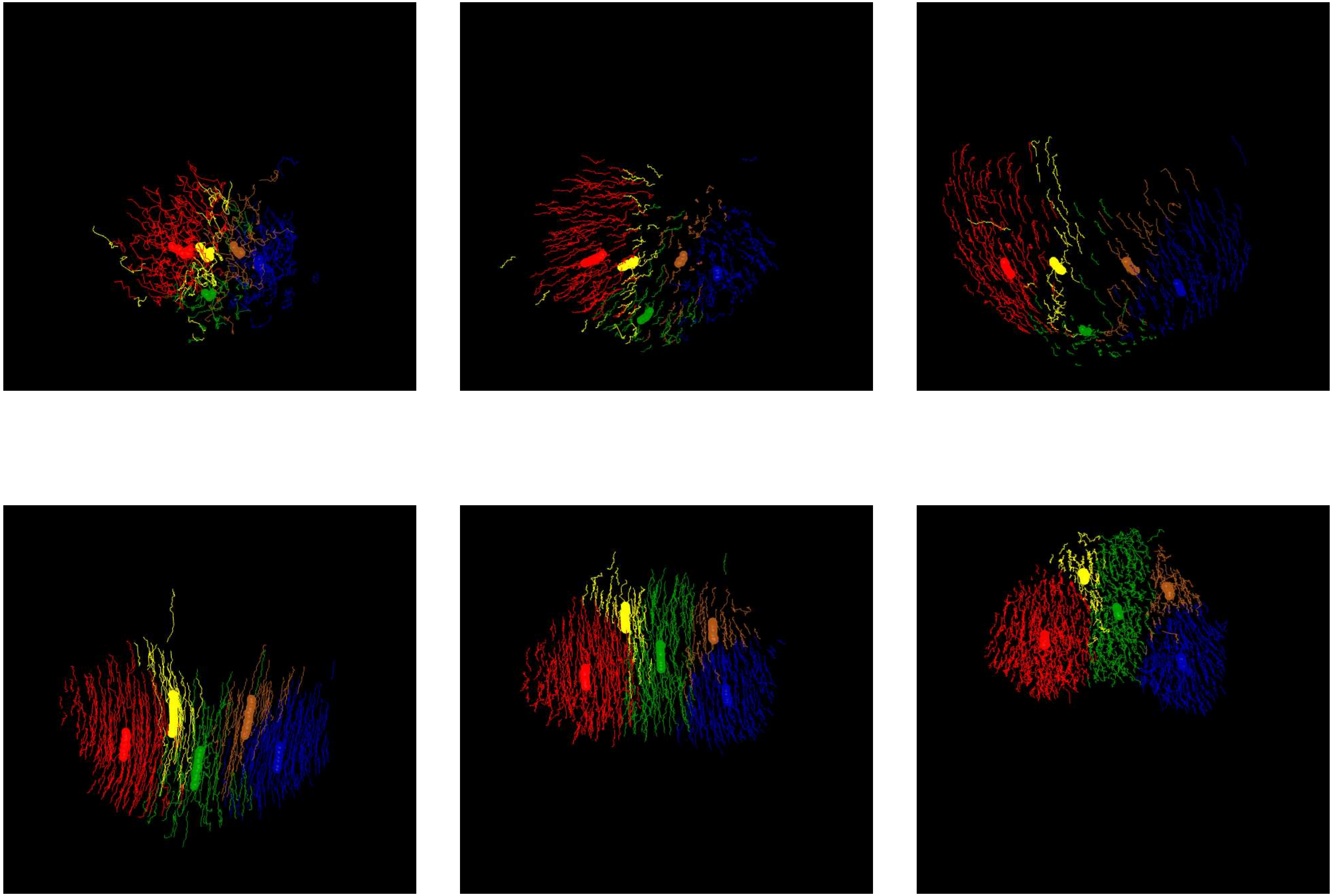
- 4D distance function from the "root" cells is computed inside 4D segmentation $\longrightarrow$ first estimate of cell trajectories

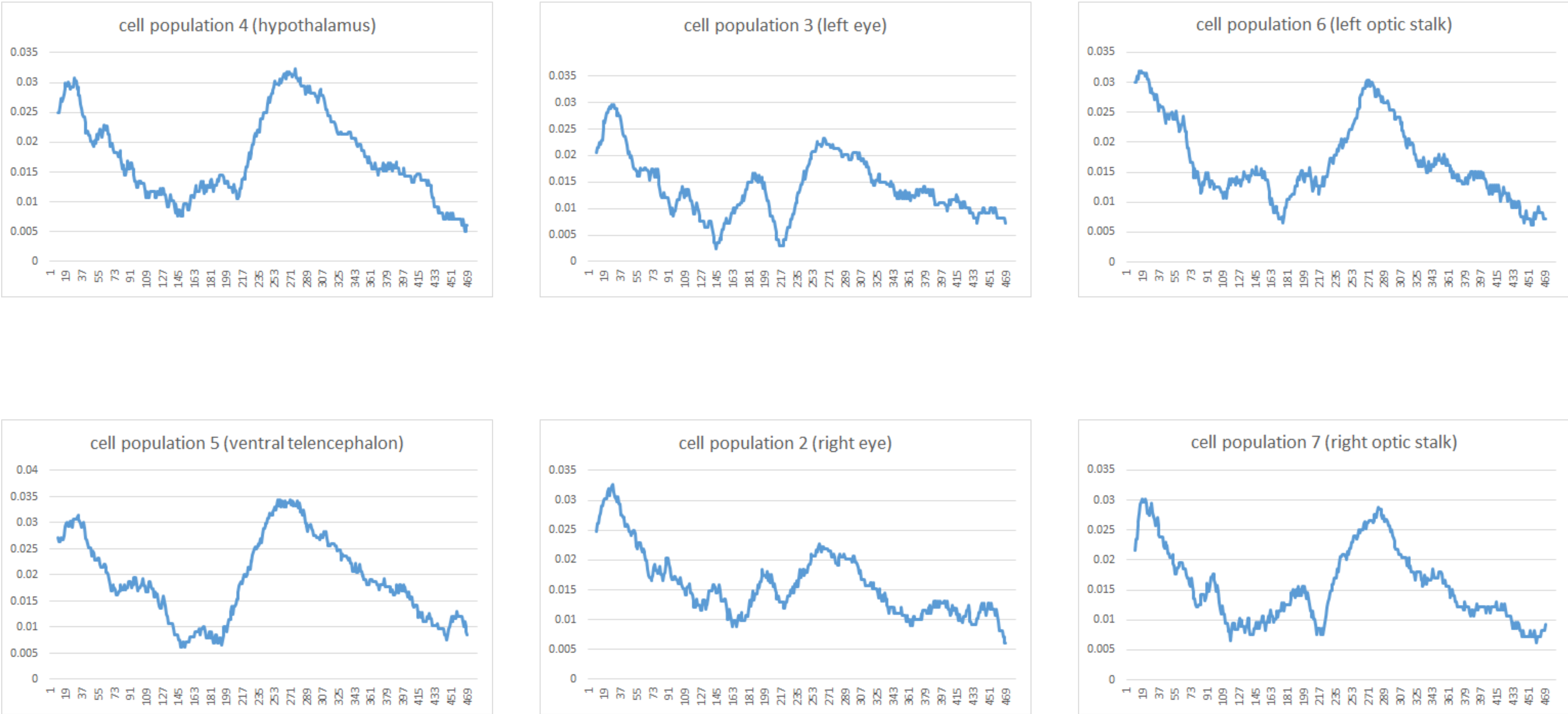
- 4D distance function from the boundaries of 4D segmentation $\longrightarrow$ centering of cell trajectories

- building a potential - difference of distance functions

- steepest descent travers of potential $\longrightarrow$ extraction of cell trajectories going backward in time - merging trajectories indicate mitosis

- general approach - we compute the distance functions from cell identifiers at every time step looking forward and perform steepest descent from every time step going backward $\longrightarrow$ extraction all possible, also partial trajectories





mean velocity of cell populations in $\mu m/sec$

Videos of embryogenesis reconstruction

Thanks for your attention