3D Deformable Surfaces with Locally Self-Adjusting Parameters – A Robust Method to Determine Cell Nucleus Shapes

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► Motivation

When using deformable models for the segmentation of biological data, the choice of the best weighting parameters for the internal and external forces is crucial. Especially because of blur and signal attenuations, one set of fix weighting parameters is often not sufficient for the segmentation of the whole contour. We are presenting a method for the dynamic adjustment of the weighting parameters, that we evaluate on recordings of two types of cell nuclei.

► Data and Challenges

We are applying our method to the segmentation of two types of cell nuclei, to DAPI stained cultures of *Drosophila* S2 cells from 3D widefield microscopic recordings and to cells in a DAPI stained *Arabidopsis Thaliana* root tip recorded with a confocal laser scanning microscope.
The segmentation of the cell nuclei is challenging. Oftentimes the contour of the object we want to segment is not fully visible because of bad contrast or staining artifacts. E.g. cell nucleoli are often not stained and thus cause holes in the nucleus boundaries, whereas regions of dense chromatin result in very bright image regions and thus hamper a good segmentation.

► Approach

The method we present here is based on the assumption, that the boundaries of the objects we want to segment are in some way similar over the whole object surface. The external, data driven active surface forces are thus strongly weighted if this constraint is fulfilled. If a boundary estimate looks much different from the rest of the boundary, the data is considered deficient and high weights are assigned to the internal active surface forces. Thus we are replacing the classical low level weighting parameters by one high level parameter, which is the ratio of the boundary that is surely not missing in the recording.

► Results

For the *Drosophila* S2 cells, the overall result seems reasonable for most of the 393 nuclei. Due to the strong blurring in z-direction caused by the microscopy technique, it is hard to judge the segmentation in the lower regions. Unfortunately, we don't have ground truth labeled data.

The segmentation of the *Arabidopsis Thaliana* nuclei is more difficult because of the dense tissue. The more central the nuclei lie inside the root, the more difficult is the segmentation with our homogeneity based parameter estimation, because the nucleus boundaries become less and less homogeneous. Also,the nuclei oftentimes touch one another and cell organelles touch the boundaries. Nevertheless, we could achieve a good segmentation for these nuclei as well.

Segmentation with 3D Active Surfaces

3D Active surfaces: $\mathbf{X} : [0,1] \times [0,1] \rightarrow \mathbb{R}^3$

minimize $E(\mathbf{X}) = E_{\text{int}}(\mathbf{X}) + E_{\text{ext}}(\mathbf{X})$

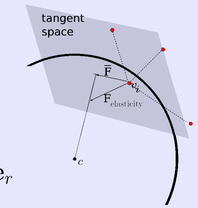
force balance system $\alpha \mathbf{F}_{\text{int}} + \beta \mathbf{F}_{\text{ext}} = 0$

Internal forces: $\mathbf{F}_{\text{int}} = \mathbf{F}_{\text{elasticity}} + \mathbf{F}_{\text{rigidity}}$

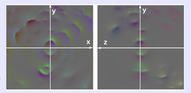
External force field: $\mathbf{F}_{\text{ext}} = \text{GVF}(\mathbf{A})$

$\mathbf{A}(\mathbf{x}) = \langle \nabla s(\langle \nabla I, \mathbf{e}_r \rangle)(\mathbf{x}), \mathbf{e}_r \rangle \cdot \mathbf{e}_r$

set to 0 if ≤0 — radial unit vector

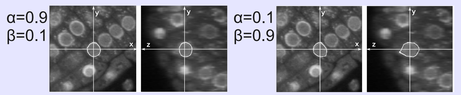


$\mathbf{F}_{\text{ext}}$



Adaption of the Weighting Parameters

Impact of the weighting parameters:



Dynamic parameter adjustment:

Use radial grayvalue profile $\mathbf{r}_{\mathbf{x}_i}$ of each vertex v_i at position $\mathbf{x}_i$.

profile PDF $f(\mathbf{r}_{\mathbf{x}_i}) = \frac{1}{(2\pi)^{l/2}|\Sigma|^{1/2}} \cdot \mathrm{e}^{-\frac{1}{2}(\mathbf{r}_{\mathbf{x}_i}-\mu_{\mathbf{r}})^T \Sigma^{-1} (\mathbf{r}_{\mathbf{x}_i}-\mu_{\mathbf{r}})}$

PDF of $P(\mathrm{B}) \cdot |\mathrm{V}|$ most probable profiles $g_f(\mathbf{r}_{\mathbf{x}_i})$ used to compute $p(\mathbf{r}_{\mathbf{x}_i}|\mathrm{B})$

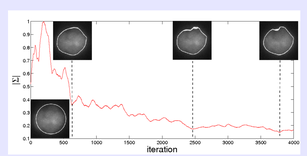
probability that the vertex lies on the boundary given its profile $p(\mathrm{B}|\mathbf{r}_{\mathbf{x}_i}) = \frac{p(\mathbf{r}_{\mathbf{x}_i}|\mathrm{B}) \cdot P(\mathrm{B})}{P(\mathbf{r}_{\mathbf{x}_i})}$

Estimated parameters for vertex v_i

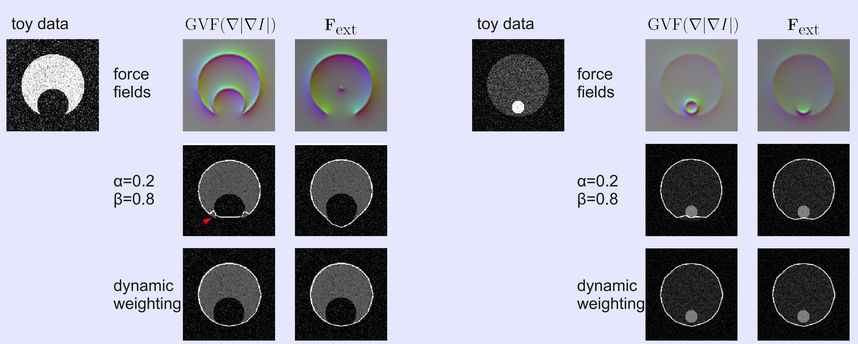
$\alpha_i = 1 - p(\mathrm{B}|\mathbf{r}_{\mathbf{x}_i^{\text{ext}}})$
$\beta_i = p(\mathrm{B}|\mathbf{r}_{\mathbf{x}_i^{\text{ext}}})$

Additional convergence criterion:

stop at step *t* if $\frac{|\Sigma|_t}{\max(|\Sigma|_{1,\ldots,t})} \leq 0.3$



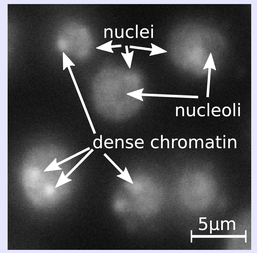
Results on Toy Data



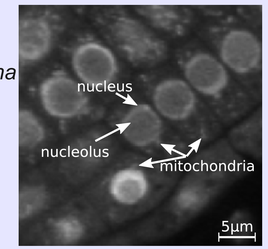
Cell Nucleus Segmentation

Data

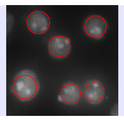
Drosophila S2 Cells (DAPI staining, Widefield Fluorescence Microscopy)



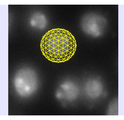
Arabidopsis Thaliana Root Tip (DAPI staining, Confocal Laser Scanning Microscopy)



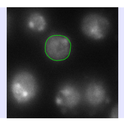
Detection with voting based Hough Transform



Initialize Active Surface



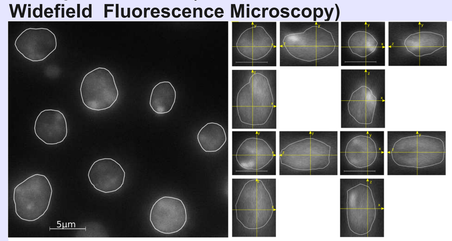
Segmentation



Results

Single cell nuclei:

Drosophila S2 Cells (DAPI, Widefield Fluorescence Microscopy)

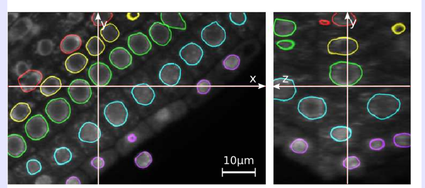


Overview of the segmentation in one recording

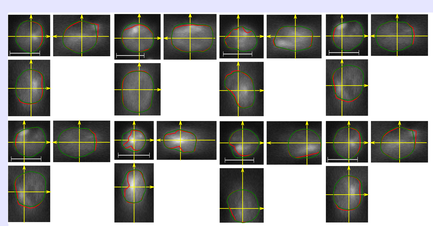
Orthogonal views of the 3D segmentation results. Scale bars indicate the length of 5µm.

Cell nuclei in dense tissue:

Arabidopsis Thaliana Root Tip, (DAPI, Confocal Laser Scanning Microscopy)



Orthogonal views of the 3D segmentation results. The colors indicate the cell layer in the root.



Orthogonal views of 3D segmentation results. The contours found with fixed parameters (α = 0.3, β = 0.8) are drawn in red, the contours found with the new dynamic parameter estimation in green. The results with our new method are less susceptible to data deficiencies.

- Precise Segmentation in x-y-direction after visual inspection
- Good segmentation in z-direction but there are artifacts caused by the PSF
- Regions with weak staining do not cause artifacts any more
- Bright spots do not attract the surface inside the nucleus
- Good segmentation even in dense tissue