BACT-3D: A LEVEL SET SEGMENTATION APPROACH FOR DENSE MULTI-LAYERED 3D BACTERIAL BIOFILMS


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Overview

Introduction

Motivation

BACT 3D

Results & Analysis

Conclusion
Introduction

- Live in dense aggregations: Biofilms
- Cellular contacts;
- Essential ecological processes;
- High antibiotic resistance.

- Shewanella oneidensis MR-1 biofilms, Gahlmann Lab, UVa.
- Limited understanding of individual bacteria in crowded environment.

[2]: https://youtu.be/6Cx62z50Yp0
• Super-resolution Imaging Technique

Traditional optical confocal microscopy  
Super-resolution microscopy

Previous Segmentation Methods

- **Edge Detection** [1]:
  Affected by image noise.

- **Watershed** [2]:
  Sensitive to intensity changes.

- **Vector Field Convolution** [3]:
  Special initialization required.

- **Seeded Watershed** [4]:
  Challenges in dense-community performance.

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• **Chan Vese** [1]:
  Define the image into foreground and background.

• **L2S** [2]:
  Model the inhomogeneity in the images as linear combination of Legendre polynomials.

[3]: Three images demonstrate the failure of Chan Vese in noisy environment are from L2S.
• **Cell splitting methods**

Splitting touching cells based on concave points [1]:


Splitting touching cells based on gradient flow [2]:

• Other integrated methods

\[ \text{weight} = 0.5 \times RAR + 0.5 \times \text{convexity} \]

\[ RAR = \frac{\min(\text{area}_{\text{object}}, \text{ellipseeaarea})}{\max(\text{area}_{\text{object}}, \text{ellipseeaarea})} \]

\[ \text{convexity} = \frac{\text{area}_{\text{object}}}{\text{area}_{\text{convexhull}}(\text{object})} \]


A. Raw data  ➔  B. Deconvolved image  ➔  C. Projection (Watershed)  ➔  D. Reconstruction
Bact-3D

Initial Layer Slice

a. Initialization

Next Slice

b. Seed selection

c. Level set evolution

d. Identification

e. Stopping criterion

f. Biofilm layer change? Yes

No
• **Dataset generation**

A. Multi-layered dense biofilms

B. Construct bacterial structure

C. Simulate fluorescence emission

D. Convolve with Gaussian kernels

Ground Truth

- Three layers
- Densely distributed

↓ z axis
• **Curvature-based seed selection**

  • Evaluating the Hessian of the image:

  \[
  H = \begin{bmatrix}
  I_{xx} & I_{xy} \\
  I_{yx} & I_{yy}
  \end{bmatrix}
  \]

  • Select the most negative eigenvalues with highest curvature
• **Iterative level set evolution**

\[ C = \{(x, y; t) : \phi(x, y; t) = 0\} \]

\[ C_t = VN \quad \phi_t = -V |\nabla \phi| \]

\[ V = \begin{cases} 
0, & \text{if SC} = 1 \\
g \cdot [1 - \varepsilon \kappa] - \beta V g \cdot N, & \text{otherwise}
\end{cases} \]

Local affinity [1]: based on the gray-scale intensity gradient

\[ g(x, y) = e^{-E(x,y)/\gamma}, E(x,y) = \frac{|\nabla I|}{G*|\nabla I| + \gamma} \]

Contrast normalization

High value in areas with low gradients

\( \gamma \): determines the magnitude of \( g \)

\( \gamma \): constant, ensure \( E \) remain limited in some small gradients


• Localization of individual bacteria

Least square fitting by evaluating the conic form of the ellipse:

\[ ax^2 + bxy + cy^2 + dx + ey + f = 0 \]

• **Stopping criterion**

**a:** Original image;

**b:** Stopping criterion is set as the skeleton of *background* that excludes *ellipses*;

**c:** Stopping criterion is efficient for most situations;
• **Layer detection and re-initialization**

  • Stopping criterion is re-initialized, when there is a layer change detected.

  • Layers are automatically detected by identifying *sharp* local minima.
Experimental results
- **Locality**: the contours are always limited to a single-cell region;
- **Trackability**: locations and orientations are available for each individual bacterium.
Comparison of segmentation performance
Dice Coefficient
- Compares similarities

\[ Dice = \frac{2|V_g \cap V_t|}{|V_g| + |V_t|} \]

Mean squared error
- Compares averaged error

\[ MSE = \frac{1}{Z} \cdot \left\| V_g - V_t \right\|_2^2 \]

Cell detection accuracy
- Number of cells detected

\[ CD = \frac{2\min(N_g, N_t)}{N_g + N_t} \]

### Resolution 1

<table>
<thead>
<tr>
<th></th>
<th>Dice</th>
<th>MSE</th>
<th>CD%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bact-3D</td>
<td>0.871</td>
<td>0.084</td>
<td>99.8</td>
</tr>
<tr>
<td>Yan, et al.</td>
<td>0.558</td>
<td>0.240</td>
<td>56.54</td>
</tr>
<tr>
<td>Chan-Vese</td>
<td>0.895</td>
<td>0.073</td>
<td>5.41</td>
</tr>
<tr>
<td>L2S</td>
<td>0.891</td>
<td>0.075</td>
<td>5.27</td>
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</table>

### Resolution 2

<table>
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<tr>
<th></th>
<th>Dice</th>
<th>MSE</th>
<th>CD%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bact-3D</td>
<td>0.861</td>
<td>0.089</td>
<td>99.8</td>
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<tr>
<td>Yan, et al.</td>
<td>0.546</td>
<td>0.245</td>
<td>72.2</td>
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<tr>
<td>Chan-Vese</td>
<td>0.834</td>
<td>0.105</td>
<td>15.7</td>
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<tr>
<td>L2S</td>
<td>0.876</td>
<td>0.087</td>
<td>4.52</td>
</tr>
</tbody>
</table>
Why are Bact-3D’s Dice and MSE not better than the other two?
• New: no layer assumption
• Use Chan-Vese initialization to estimate orientation of cell; take 2D skeletons to make stopping criterion in X, Y, Z (via union of slices)
• Velocity of level set now depends on the distance to nearest stopping criterion (slow down near the stopping criterion)
From active contour to active surface: Bact-3Ds

Old Method:
Choose orange slice to build a "red wall" that separates the touching cells

Improved Method:
Choose orange layers inside to build "red walls" that separate the touching cells
1. Seed Selection: 3D ChanVese

2. Curvature-based active surface

\[ \phi(x, y, z; t) \]

DVF: distance velocity field in geometric active surface
Sliced comparisons

3D viewers (detected No./ total No.)

Bact-3Ds

Bact-3D

Chan-Vese

52/60

17/60

1/60
Conclusion

Super-resolution → Bact-3D

- Separate touching cells
- Reconstruct multilayered bacterial biofilms
- Provide tool for tracking cells and studying group structure
- Modify to be robust for real data

How do cells communicate, share nutrients, discard waste and self-organize?

Andreas Gahlmann
Thank you!
谢谢！