Segmenting Filamentous Biological Structures by Automated Tracing

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Abstract

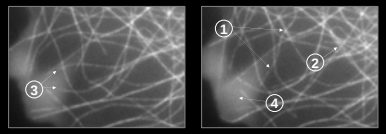
Currently, biological image analysis generally relies on manual segmentation of objects and subsequent quantification of desired features. In the case of filamentous structures, such as cytoskeletal structures, the natural clutter and image variability impose significant challenges to automated segmentation methods. In this work, we describe a method to address these issues. Starting from a point on the target structure, we examine potential directions to extend the trace along the structure. The final trace of the object is selected from the candidates as the best match with the image data.

Our method can be used in collecting statistical features from images of filamentous structures. We demonstrate the method on microtubule image sequences, determining tip positions in image sequences, calculating curvature values along the microtubule body, and estimating microtubule tracks over time.

Given the increased analytical efficiency of our method, we can now acquire sufficient data to employ statistical analysis methods on biological image sets. Additionally, we can now examine quantitative data beyond the traditional manual measurements. For example, bending tendency can be quantified as a function of curviness observed in images, which is impractical to capture manually. Statistics derived from this parameter, such as the spatial distribution of highly curved objects, may raise novel biological questions and hypotheses. In the case of microtubules, curvature may be an invaluable measure in quantifying cell shape changes or changes in the direction of cellular or growth cone migration. Our algorithm can be easily modified to measure various spatial and temporal characteristics to all sorts of filamentous structures.

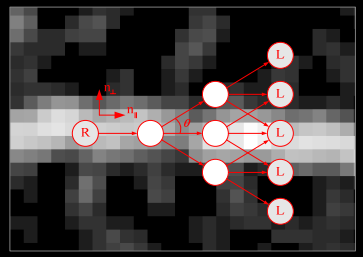
Segmentation challenges

- Quantitative analysis relies on accurate tracing of individual structure body.
- Challenges:
  1. non-uniform intensity along the body,
  2. natural clutter (intersections, bundling),
  3. ends (tips) blending into the background,
  4. excessive and non-uniform noise.
- Microtubules (MT) are considered. Individual tips are tracked over time for the quantitative analysis of MT dynamic instability.

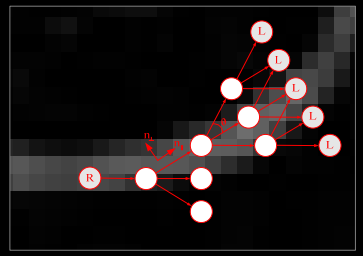


Consecutive (4s apart) MT images. 1-4 show examples of segmentation issues described above.

Tracing a microtubule



- From an initial point on the MT body, construct a graph where paths from the root node (R) to leaf nodes (L) represent potential MT bodies. This graph explores a (θ_r-θ) neighborhood of the parallel direction ($n_\parallel$) to the MT body.



- Select the best orientation on the body to continue to the next point. This is done by matching all possible paths to the observed image, and finding the best path.

Finding the best path

- Initial normal ($n_\perp$) and parallel ($n_\parallel$) directions are calculated by eigenvector analysis along the curve.
- The best path between the nodes R and L_r is calculated by the total edge weights, $E(R,L_r)$:

$$\hat{r} = \arg\max_r \sum_r E(R, L_r)$$

- The next graph is formed by assigning the best child node as the current root node.
- The algorithm is terminated if no edge weights satisfy a predefined threshold.

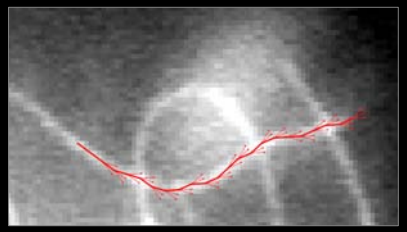
Calculating edge weights

- The weight between the nodes u and v is computed by:

$$E(u,v) = \int_0^1 f\left(uv(1-t), uv(t), \alpha\right) dt$$

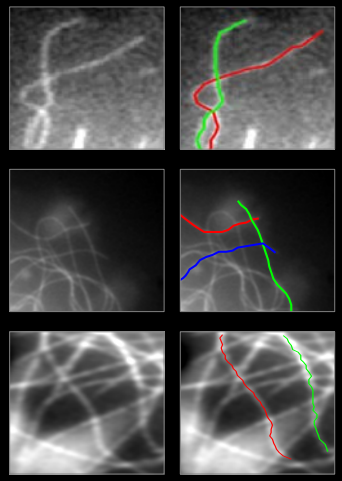
- The $f(x,y,\alpha)$ corresponds to the second derivative of Gaussian filter response along the α direction.

Example tracing run



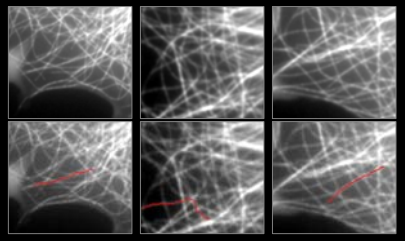
Explored directions and the best path on a MT.

Visual examples on MTs



Original regions (left) and traced MTs (right).

Visual evaluation



Correct (88%) *Wrong (6%)* *Ambiguous (6%)*

Results

- Dataset and ground truth:
  - 1374 microtubule body traces manually approximated by poly-lines
  - available at *www.bioimage.ucsb.edu*
- Error measures are calculated as magnitudes of:
  - error in tip distance (ε_{tip})
  - error in max body distance ($\varepsilon_{body\text{-}max}$)
  - error in mean body distance ($\varepsilon_{body\text{-}mean}$)
  - error in body length ($\varepsilon_{body\text{-}length}$)
- Following conditions are set by biologists for successful tracing:
  - $c_{tip} \equiv \varepsilon_{tip} < 0.792\ \mu m$
  - $c_{body} \equiv \varepsilon_{body\text{-}max} < 0.792\ \mu m$ & $\varepsilon_{body\text{-}mean} < 0.396\ \mu m$
  - $c_{length} \equiv \varepsilon_{body\text{-}length} < 0.792\ \mu m$

Rates of tracing success per condition	
c_{tip} & c_{body} & c_{length}	0.9138
c_{tip}	0.9160
c_{body}	0.9145
c_{length}	0.9220

$E\{\varepsilon_c \mid c\}$ = mean of errors for condition c	
$E\{\varepsilon_{tip} \mid c_{tip}\}$	0.1584 μm
$E\{\varepsilon_{body\text{-}mean} \mid c_{body}\}$	0.0349 μm
$E\{\varepsilon_{body\text{-}length} \mid c_{length}\}$	0.1435 μm

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