Microtubule Filament Tracing and Estimation

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- Microtubules are filamentous cytoskeletal structures composed of tubulin protein subunits.
- These subunits can add on, or dissociate from, the tubulin polymer rapidly, making them highly dynamic.
- Microtubules are critically involved in many essential cellular functions, such as chromosome segregation at mitosis and intracellular cargo transport.

Introduction
Motivation

- Microtubules are generally studied using three dimensional fluorescence microscopy.
- The output is a 3D image of the microtubules, blurred due to
  - the lenses
  - the imaging device,
  - sampling and digitization
  - finite size of microtubules

Introduction
Aim

- To automatically trace each microtubule filament in the 3D microscope image
- The traced image will be used to estimate statistics, like
  - Number of filaments
  - Average length
  - Distribution of length

Background

- The microtubules originate from a common center and grow outwards — density of filaments decreases from center to periphery
- Filaments grow in a straight line unless an obstacle exists — minimum curvature constraint can be imposed to prevent wrong tracing of the tubules.
- Points of intersection of microtubules glow twice as bright as any other point on a single microtubule.

Theory
Deblurring

- The input images in the tests are noise free.
- Actual images will have Poisson noise.
- Richardson-Lucy deconvolution algorithm can be used.

Examples
Single Filament

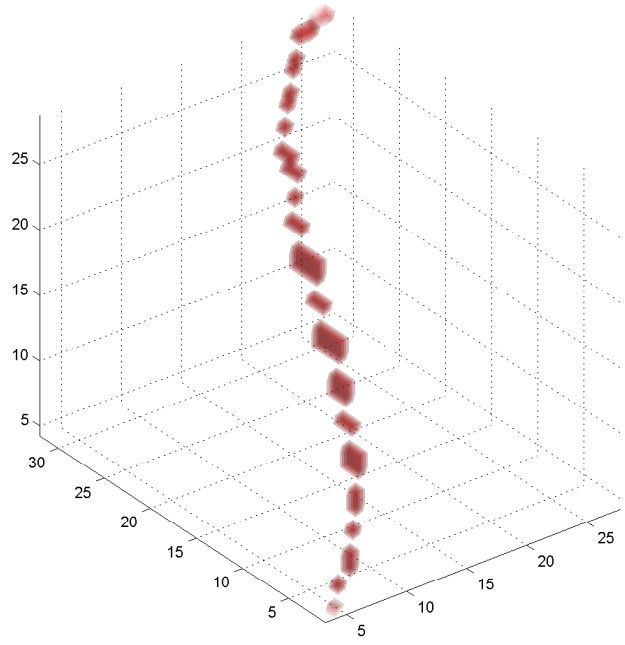

Figure: Single Filament — Original Image

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Examples
Single Filament

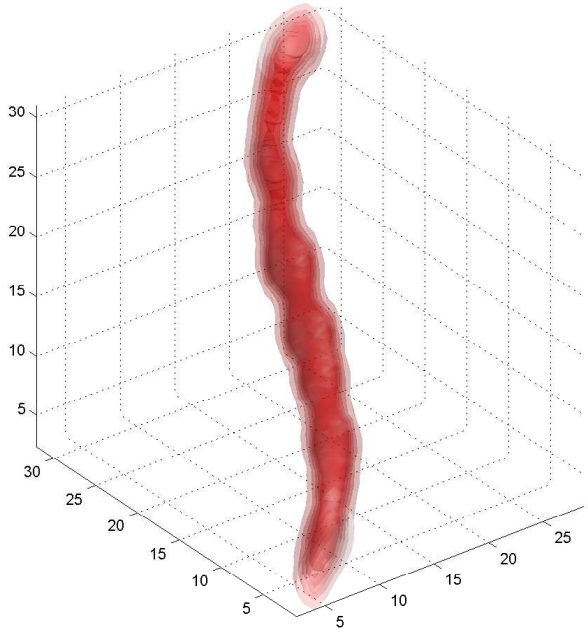

Figure: Single Filament — Input Image

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Deblurring Example

Single Filament

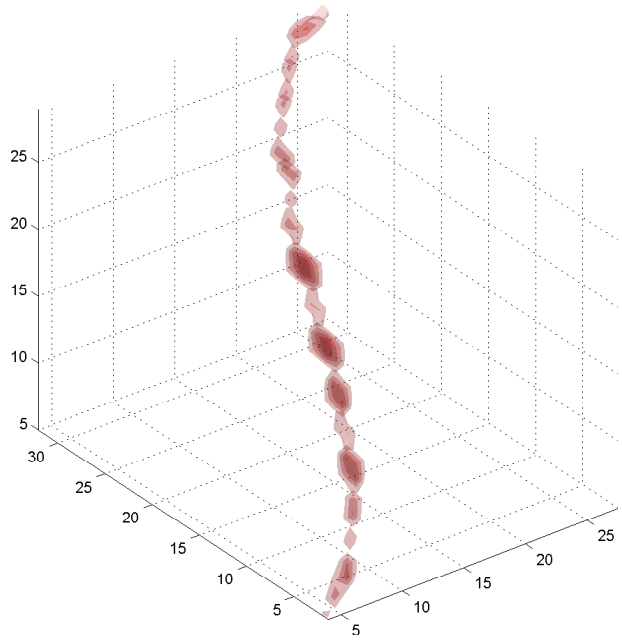

Figure: Single Filament — Deconvolved Image

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Theory
Direction of Extension

- After deconvolution, we are not guaranteed a thin image.
- While thinning as well as tracing filaments, it is essential to know the direction that the filament at that point has grown from, and the direction it is growing in.
- The Hessian is often used to determine this.

Theory
The Hessian

- For an n-dimensional image $I(x_1, x_2, \ldots, x_n)$, the Hessian is

$$H = \begin{pmatrix} \frac{\partial^2 I}{\partial x_1^2} & \frac{\partial^2 I}{\partial x_1 \partial x_2} & \cdots & \frac{\partial^2 I}{\partial x_1 \partial x_n} \\ \frac{\partial^2 I}{\partial x_2 \partial x_1} & \frac{\partial^2 I}{\partial x_2^2} & \cdots & \frac{\partial^2 I}{\partial x_2 \partial x_n} \\ \vdots & \vdots & \ddots & \vdots \\ \frac{\partial^2 I}{\partial x_n \partial x_1} & \frac{\partial^2 I}{\partial x_n \partial x_2} & \cdots & \frac{\partial^2 I}{\partial x_n^2} \end{pmatrix}$$

- Symmetric, real eigenvalues.
- Direction of extension of filament is given by eigenvector corresponding to minimum magnitude eigenvalue.
- For the discrete case, finite-difference version has to be implemented.

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Theory
Thinning

- Thinning of image achieved by non-maximal supression.
- Checks if a point is a local maximum along directions perpendicular to the direction of extension, and puts it to zero if it isn't.
- Quantize the angle, find perpendicular directions, check 4 values.
- Thinning can be used before deconvolution, but his will not work in presence of noise.

Thinning Example

Single Filament

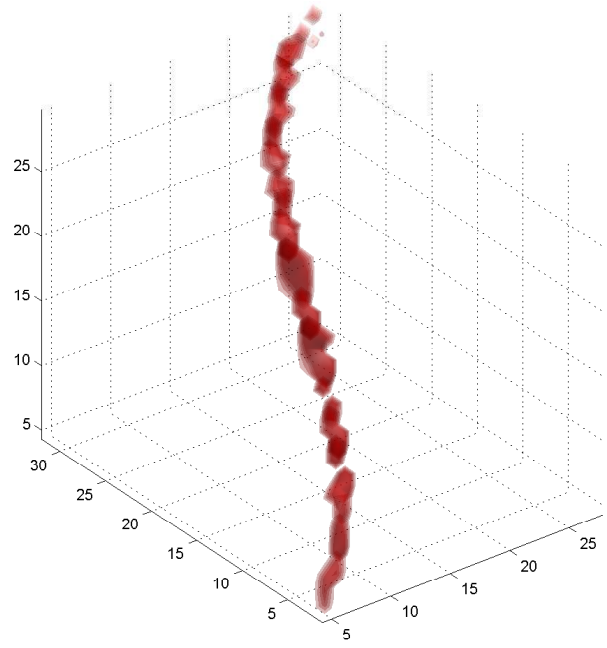

Figure: Single Filament — Thinned Image

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Theory
Thresholding

- Intensity data along the length of the filament can be used in future to detect intersections.
- Hysteresis thresholding is used as the basic idea. It completes broken filaments.
- 3 different intensity levels, and correspondingly 4 different threshold levels
- Anything below level 1 is 0, anything above level 4 is 2, anything between levels 2 and 3 is 1.
- Anything between levels 1 and 2 is 1 if one of its *26*-neighbors is above level 2, 0 otherwise.
- Anything between levels 3 and 4 is 2 if atleast 2 of its 26-neighbors are 1.

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Thresholding Example

Single Filament

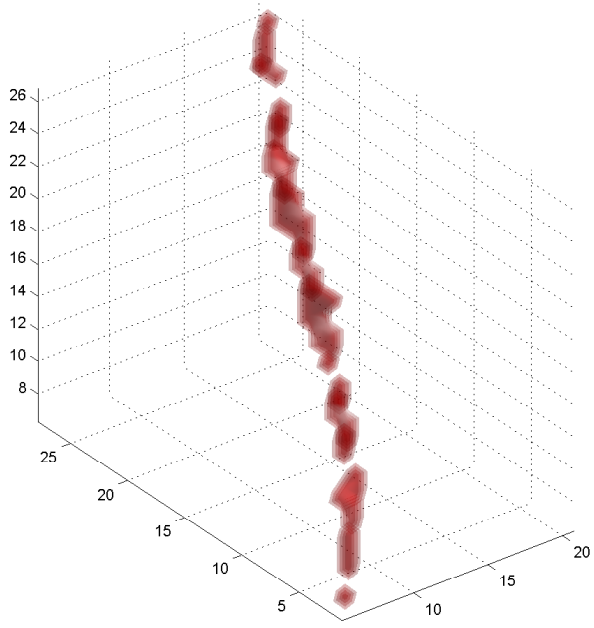

Figure: Single Filament — Thresholded Image

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Results
Intersecting Filaments

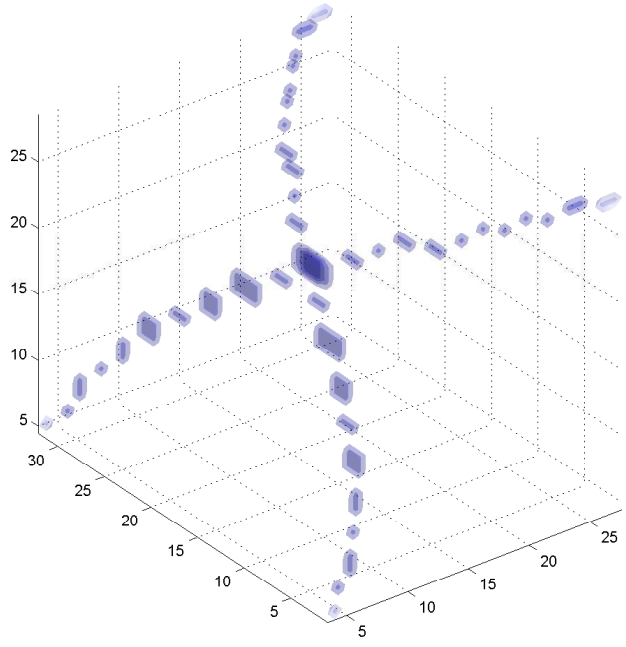

Figure: Intersecting Filaments — Original Image

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Results
Intersecting Filaments

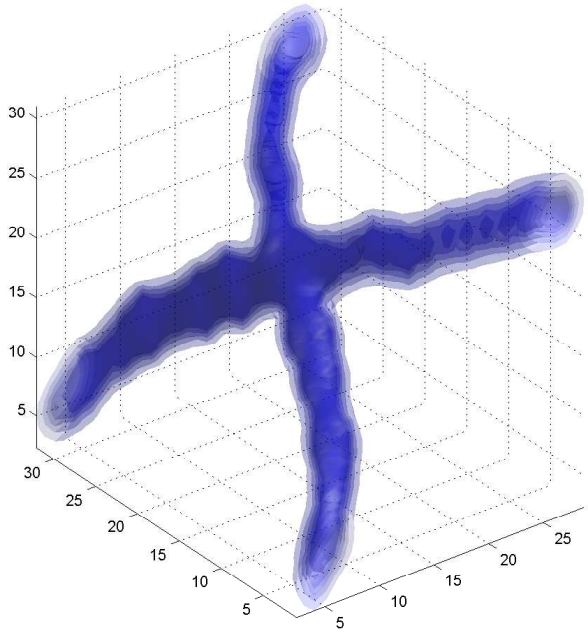

Figure: Intersecting Filaments — Input Image

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Results
Intersecting Filaments

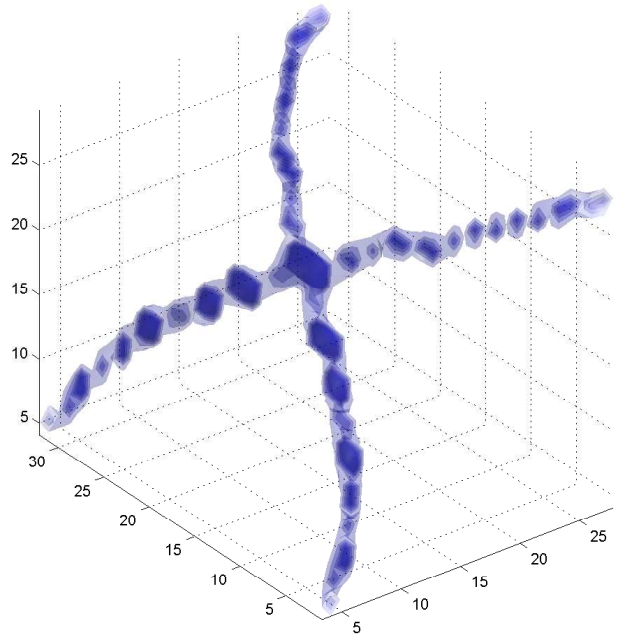

Figure: Intersecting Filaments — Deconvolved Image

Results
Intersecting Filaments

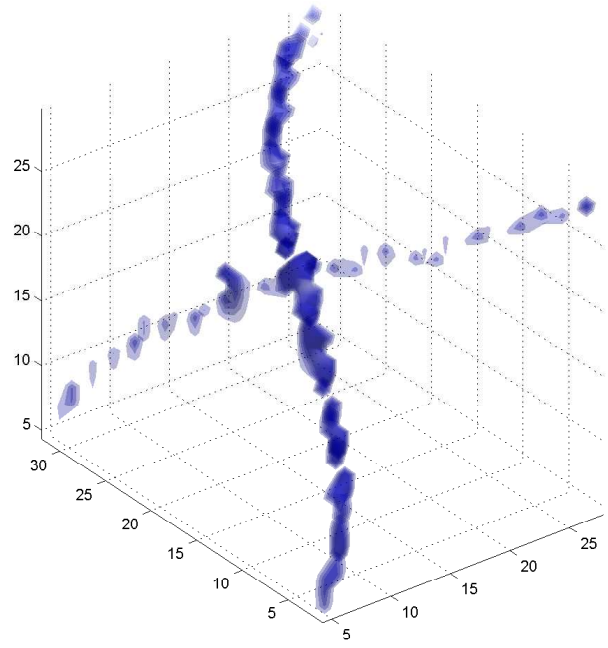

Figure: Intersecting Filaments — Thinned Image

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Results
Intersecting Filaments

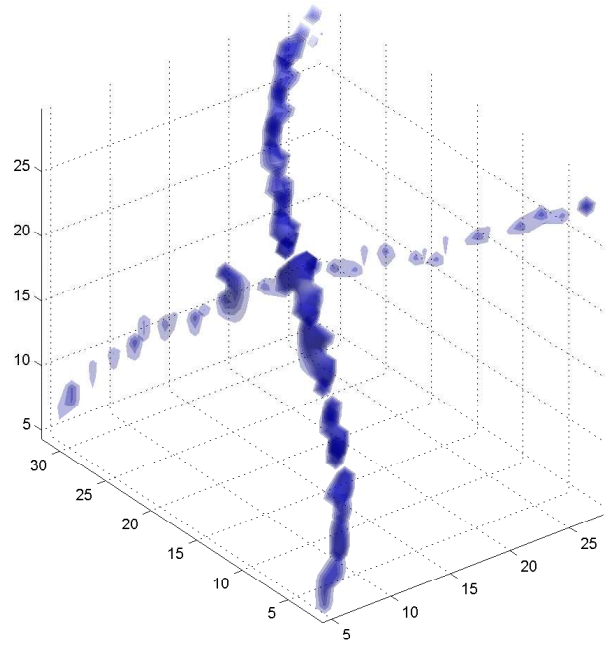

Figure: Intersecting Filaments — Thresholded Image

Results
Simulation

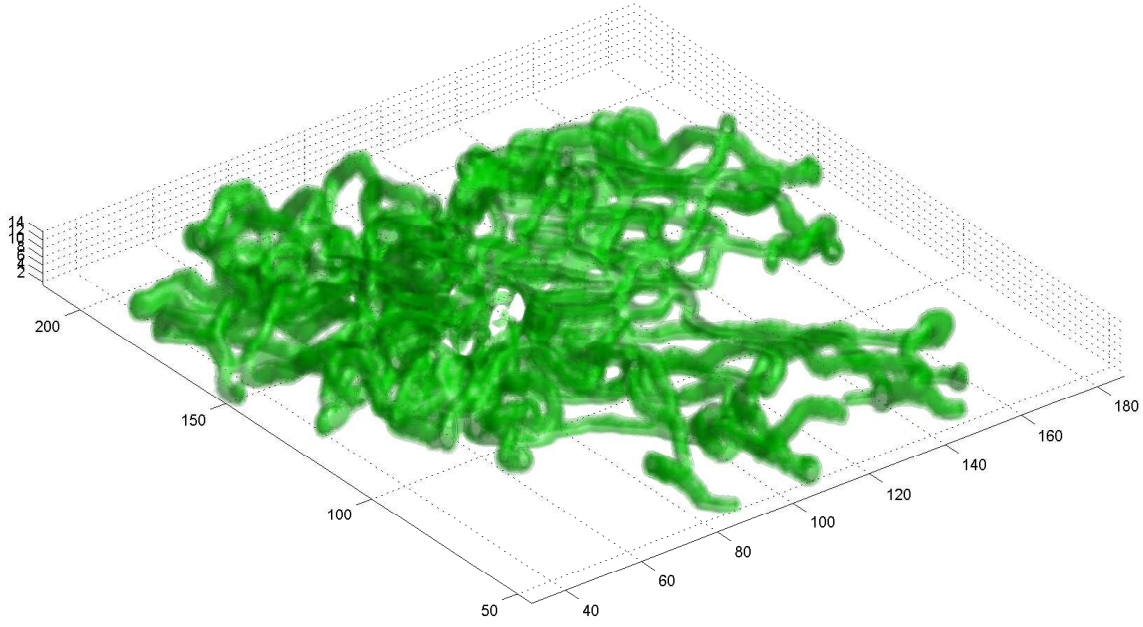

Figure: Simulation — Input Image

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Results
Simulation

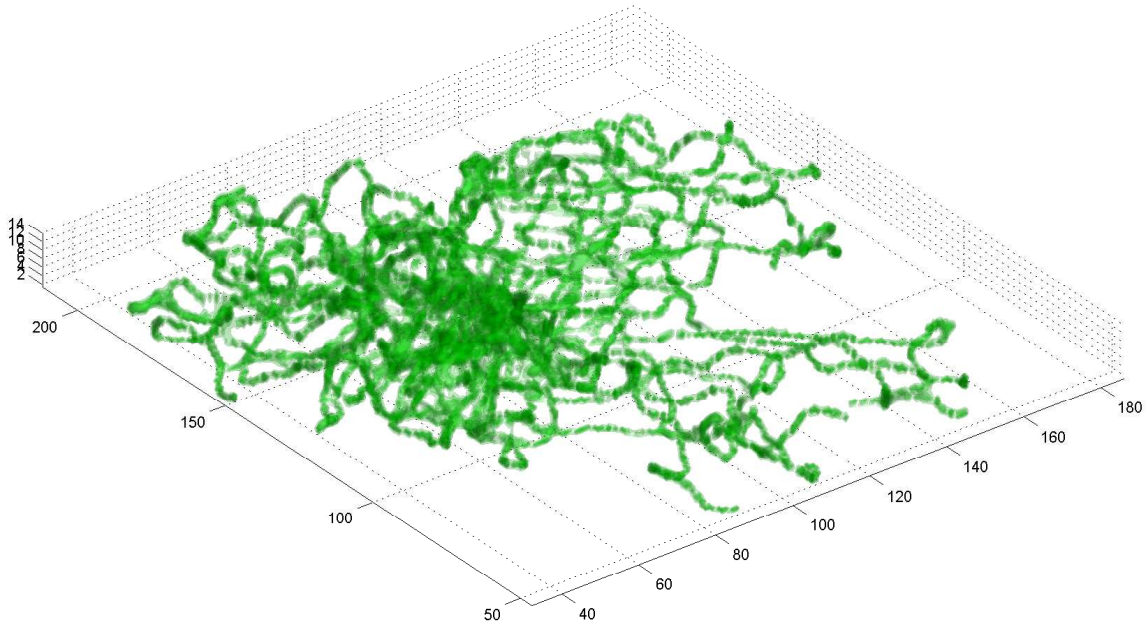

Figure: Simulation — Deconvolved Image

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Results
Simulation

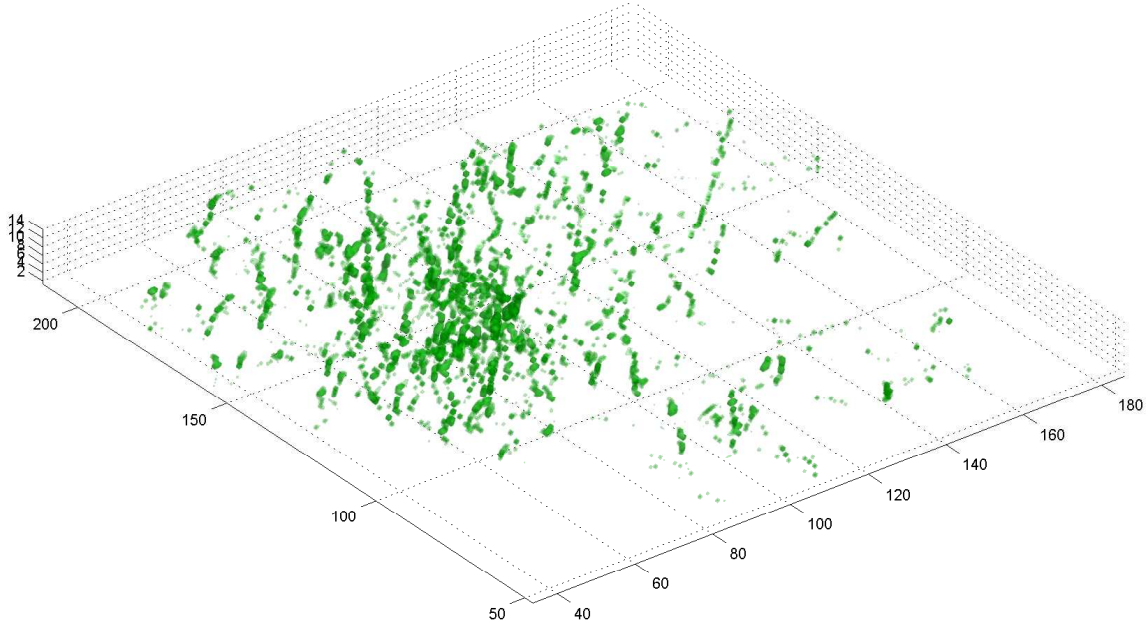

Figure: Simulation — Thresholded Image

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Discussion

- The non-maximal suppression works well on input images without noise, but the results are not so good with deconvolved images.
- Deconvolution step is essential to remove noise.
- Additional step of convolving the deconvolved image with the same or different PSF should give an noise-free blurred image
- Given PSF is elongated in z-direction, which may cause problems in finding direction of extension.

Future Work
Tracing the Filaments

- The key idea used is the same as that of connected components labeling.
- Connected component labeling uses $X_{k+1} = (X_k \oplus B) \cap A$ iteratively.
- In this case, image should be dilated only in the direction of extension of the filament, not isotropically.
- Only needed near intersection of two filaments, already pinpointed in the preprocessed image.
- The initial pixels can be found out by searching inwards from the periphery.

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Conclusion

- Preprocessing of the image is one of the most challenging aspects of automatization of this task.
- Future work will involve tracing the filaments.
- After implementing on simulated images, the algorithm can be tested on actual images obtained from fluorescence microscopy.