Filament tracking to cell force measurements:

Gaussian polynomial models, gradient descent, random forest classifier

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Outline

1. Motivation
2. Gaussian Polynomial Models
   ▷ Tracking FIlaments at Sub-pixel accuracy
3. Ellipse fitting using Ransac
   ▷ Using Random Forest Classifier for full automation
4. Want Perfect Segmentation?
   ▷ RAF based tool: Ilastik

Motivation

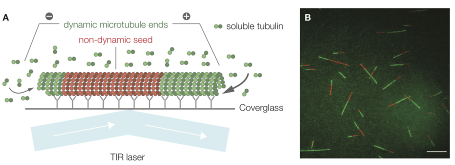
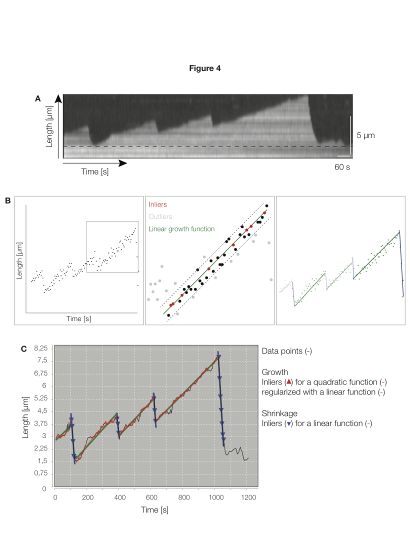


Figure 1 Imaging Microtubule Dynamics by TIRF Microscopy
A Schematic of the experimental design: stabilized microtubule seeds (red) are bound to the coverslip by antibodies and serve as nucleation points for dynamic microtubules (green), imaged using TIRF microscopy. One microtubule end usually shows higher growth rates (+) than the other end (-). **B** Representative TIRF microscopy image of dynamic microtubules (green) grown from GMPCPP-stabilized seeds (red). Scale bar: 10 µm.

- Computer Vision/ Machine Learning algorithms → enables object recognition (MT).
- Cylindrical Protein Polymers → Gaussian Polynomials do the localization.

Trajectory Analysis



Part 1

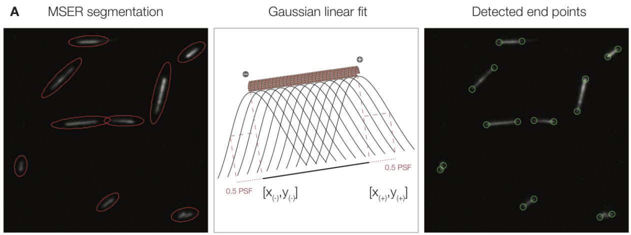
Locating and Localizing MT

$$\downarrow$$

"Computer Vision and Mathematics"

Locate and Localize

Figure 2



Error Stats

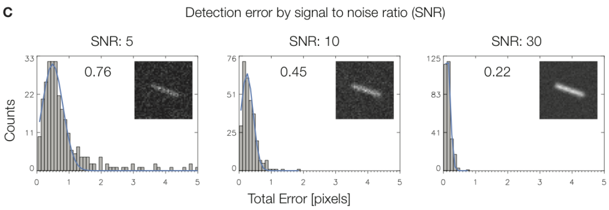


Figure 2 Microtubule Seed Detection

A The default algorithm to identify microtubule seeds as objects is Maximally Stable Extremal Regions (MSER). Microtubule seeds detected by MSER are marked by red ellipses. To determine the exact end points of each microtubule seed, a sum of Gaussians model is fit to the major axis of the ellipsoid. Detected end points are marked by green circles. **B** The detection efficiency of microtubule seeds depends on the distance with a cutoff of 5 pixels. **C** Subpixel accuracy of microtubule detection depends on signal-to-noise ratio (SNR).

Gaussian Line Model

Localizing End points of MT seeds:

We developed Gaussian Line model as sum of Gaussians along a line

$$\downarrow$$

$$G(x,y)\delta(y-mx-c)$$

$$\downarrow$$

$$G(x_i,y_i) = A\sum_i \exp\left[-\left(\frac{x-x_i}{\sigma_x}\right)^2 - \left(\frac{y-y_i}{\sigma_y}\right)^2\right] + B$$

$$\downarrow$$

Parameters of the model $\vec{\theta} = (A, B, (x_s, y_s), (x_e, y_e), \vec{ds} = (dx, dy))$.

Optimization

- Using the model minimize $\chi^2 = \sum_{\text{pixels}}[I_{ij} - F(\vec{x}, \vec{\theta})]^2$.
- I_{ij} is Image intensity at $\vec{x}$.

Optimization

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- I_{ij} is Image intensity at $\vec{x}$.

Minimization is done using Levenberg-Marquardt solver

$\downarrow$

Improve answer by doing weighted centre of mass fit using N Gaussians

$\downarrow$

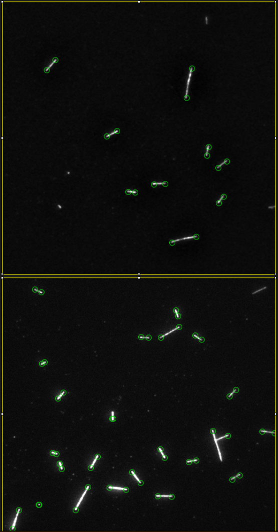
$$(x_c, y_c) = \frac{\sum_{ij} X_{ij} I_{ij} W_{ij}}{\sum_{ij} I_{ij}}$$

$$W_{end}(x, y) = \sum_i \exp\left[-\left(\frac{\vec{x}+\vec{ds}_i-\vec{x}_c}{\vec{\sigma}}\right)^2\right]$$

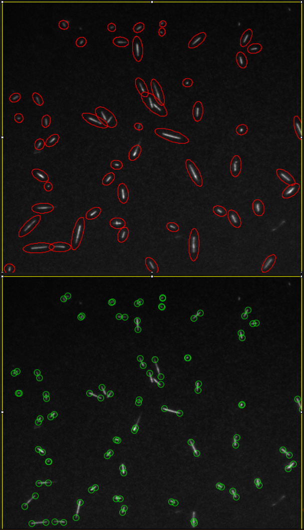
$$W_{start}(x, y) = \sum_i \exp\left[-\left(\frac{\vec{x}-\vec{ds}_i-\vec{x}_c}{\vec{\sigma}}\right)^2\right]$$

Real Examples

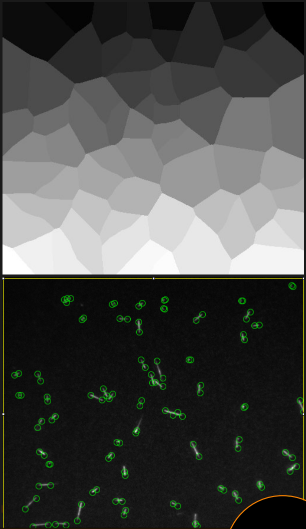
MSER



MSER



Watershed + Hough



Location and Localization (t)

From biology to mathematics

- MTs are cylindrical polymers, as an Image appear as Gaussian Polynomials.

- Now we need to upgrade the Line model to a Polynomial model
- $G(x, y)\delta(y - dx^3 - ex^2 - mx - c)$

The polynomial Models

Localization in time

$$\downarrow$$

$$G(x,y)\delta(y - dx^3 - ex^2 - mx - c)$$

$$\downarrow$$

$$G(x_i,y_i) = A\sum_i \exp\left[-\left(\frac{x-x_i}{\sigma_x}\right)^2 - \left(\frac{y-y_i}{\sigma_y}\right)^2\right] + B$$

$$\downarrow$$

Parameters of the model $\vec{\theta} = (A, B, (x_s, y_s), (x_e, y_e), \vec{ds} = (dx, dy), d, e)$.

Real-Time tracking

Click

Ransac Fits

- RANSAC is a technique to find outliers in data: Iterative regression using a function.

RANSAC on a Random walker

↓

Random Walk trajectories appear like polynomials (zig-zag curves).

↓

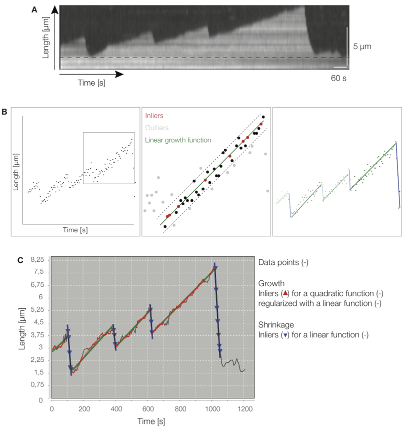
We use second/ third order regularized polynomial to find inliers.

↓

On those inliers we fit a line to determine rates (min 3 time-points).

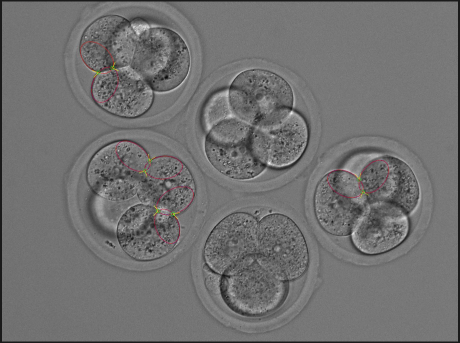
Ransac Fits

Figure 4

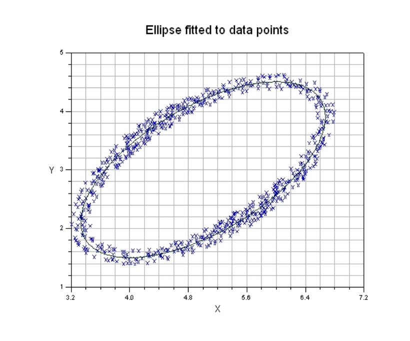


Part 2

Ellipse Fitting to determine embryonic forces



Ransac for 3D ellipsoid fitting



- Require closest distance of a point from the ellipsoid. Code here
- Regression based ellipse fit on the inliers. Code here

Finding points of intersection

$A(x, y) = a_0 + a_1 x + a_2 y + a_3 x^2 + a_4 xy + y^2 = 0$
$B(x, y) = b_0 + b_1 x + b_2 y + b_3 x^2 + b_4 xy + y^2 = 0$
Requires solving quartic equation

$$w^2 + (a_0 + a_1 x + a_3 x^2) - (a_2 + a_4 x)^2 = 0,$$

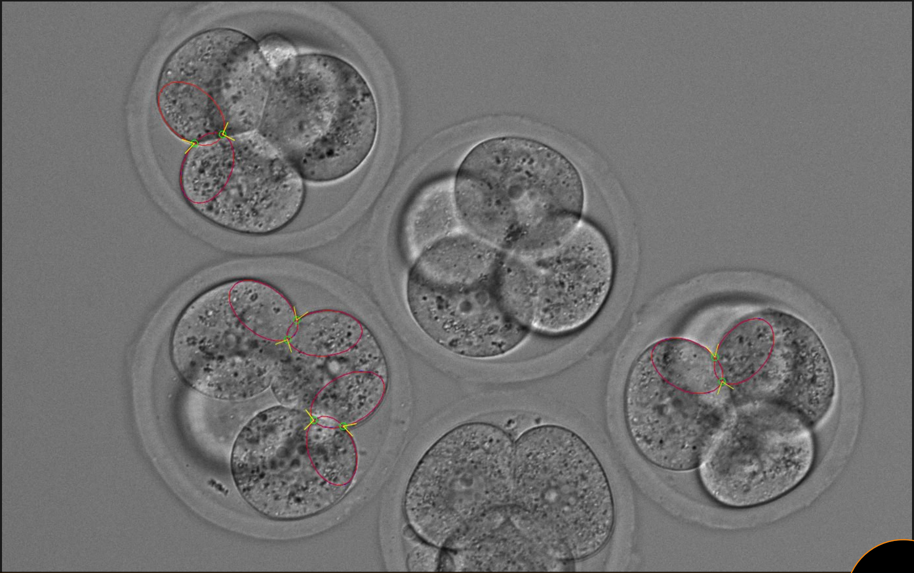
$$(d_2 + d_4 x)w + e_0 + e_1 x + e_2 x^2 = 0,$$

$$d_i = a_i - b_i,$$

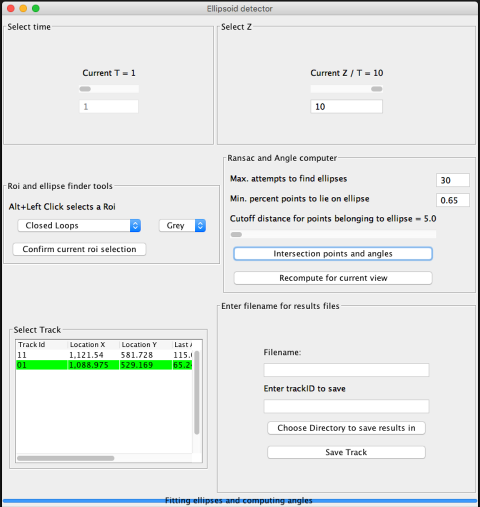
$$e_0 = d_0 - a_2 d_2/2, e_1 = d_1 - (a_2 d_4 + a_4 d_2)/2, e_2 = d_3 - a_4 d_4/2$$

Code here: Java implementation

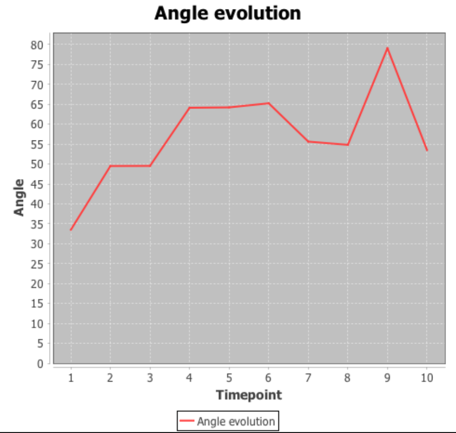
Draw Tangents

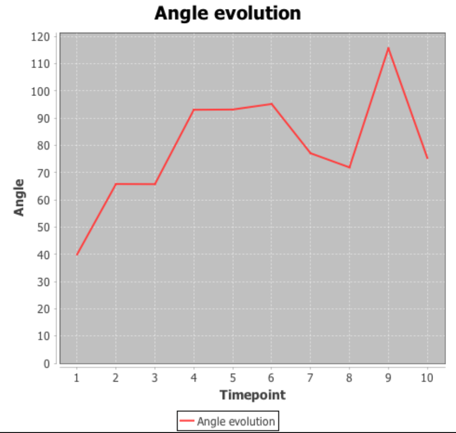


ETrack


Ellipsoid detector
Select time
Current T = 1
1
Select Z
Current Z / T = 10
10
Ransac and Angle computer
Max. attempts to find ellipses
30
Min. percent points to lie on ellipse
0.65
Cutoff distance for points belonging to ellipse = 5.0
Intersection points and angles
Recompute for current view
Roi and ellipse finder tools
Alt+Left Click selects a Roi
Closed Loops
Grey
Confirm current roi selection
Select Track
Track Id
Location X
Location Y
11
1,121.54
581.728
01
1,088.975
529.169
Enter filename for results files
Filename:
Enter trackID to save
Choose Directory to save results in
Save Track
Fitting ellipses and computing angles

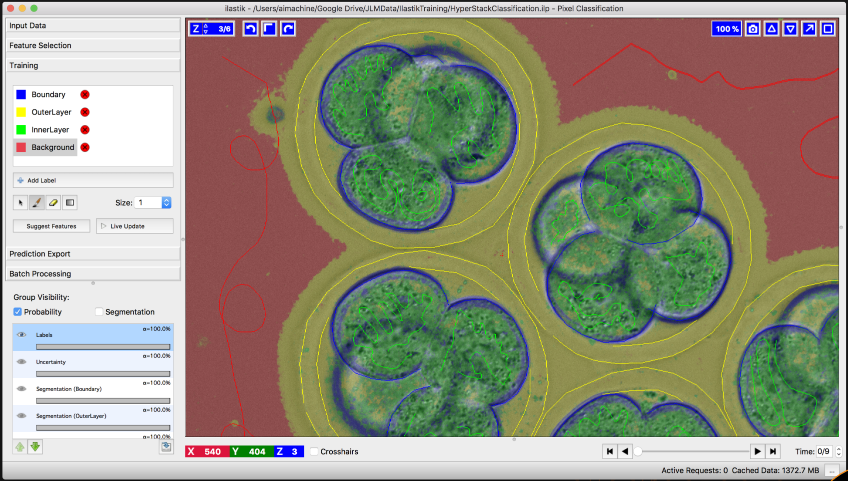
Angles


Angle evolution
Angle
Timepoint
0
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75
80
1
2
3
4
5
6
7
8
9
10
Angle evolution


Angle evolution
Angle
Timepoint
0
10
20
30
40
50
60
70
80
90
100
110
120
1
2
3
4
5
6
7
8
9
10
Angle evolution

Random Forest Classifier

- How to get Candidate points for ellipse fitting automatically?

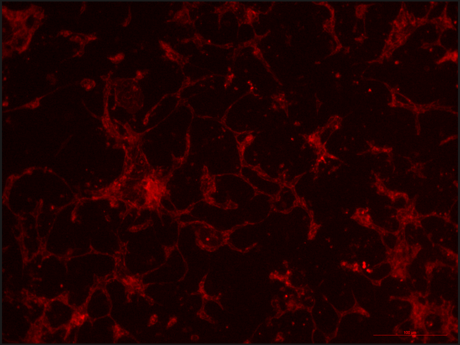


Take 1

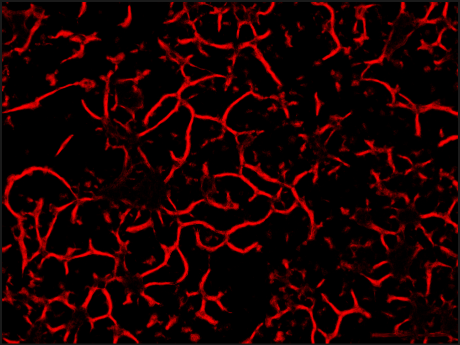
Click

Probability Maps

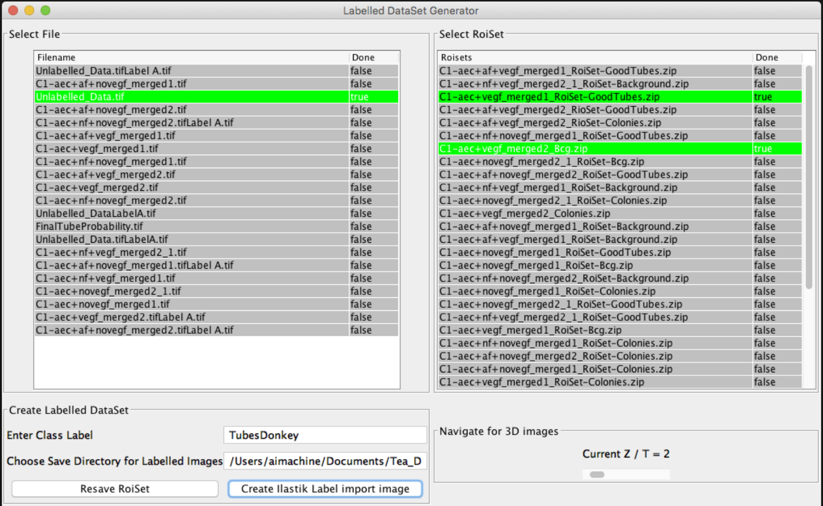
Raw Image



Tube Probablity Pixels

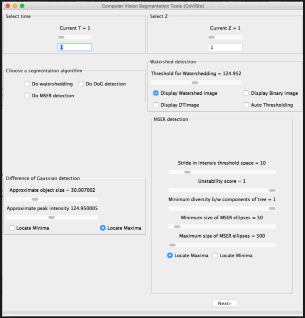


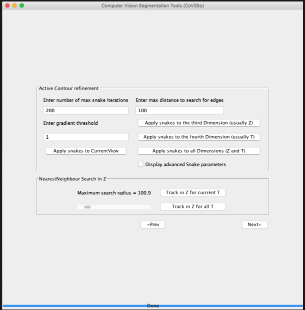
Labelled DataSet Generator

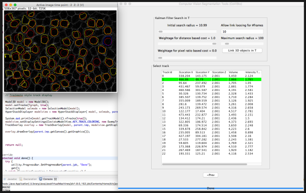


CoViSTo

Computer Vision Segmentation tools (CoViSTo).







Job

UNIVERSITÄT BERN

Together with the Scientific IT Support (scits.unibe.ch) of the Faculty of Science, the Microscopy Imaging Center (mic.unibe.ch) of the University of Bern opens a position for an

Image Analysis Expert for Microscopy (80-100%)

Tasks

- Optimise and deploy image and data analysis pipelines for current and new instruments at the Microscopy Imaging Center (MIC)
- Support MIC users with image processing and analysis challenges
- Support design of IT infrastructure (hardware and software) for new MIC instruments
- Organise and implement MIC trainings on image processing, IT infrastructure usage and application software
- Participate in MIC related research and development projects

Conditions

- Salary according to qualifications and cantonal regulations
- Begin 2018-05-01 or by agreement
- Two years with possible extension

The Microscopy Imaging Center at the University of Bern (MIC) is the interdepartmental platform for high-end microscopy. MIC provides access to state-ofthe-art imaging techniques for researchers from 12 institutes of 3 departments of the University of Bern as well as for external visitors. It collaborates closely with the Science IT Support (ScITS) unit from the Faculty of Science. The successful candidate will be embedded in the ScITS team which maintains university-wide, national and international knowledge networks and is associated with the Mathematical Institute. The ScITS working atmosphere is academic, responsive and cooperative. The office location is next to the Bern central railway station.
The successful candidate will be member of the MIC commission. Questions regarding the position can be directed to the ScITS coordinator Dr. Sigve Haug or the MIC coordinator Dr. Ruth Lyck (sigve.haug@math.unibe.ch, ruth.lyck@tki.unibe.ch). Women are encouraged to apply.

Please send your full application with transcripts of diplomas, CV, two references, relevant publications and motivation letter as a single PDF to sigve.haug@math.unibe.ch until March 26, 2018.

Universität Bern, Mathematisches Institut, Sidlerstrasse 5, 3012 Bern
sigve.haug@math.unibe.ch, www.unibe.ch

Conclusions

```
Boolean followme = true;
if(followme){
https://github.com/kapoorlab
https://twitter.com/EntraCod
}
```