Particle tracking for cardiovascular function analysis in zebrafish

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In vivo measurements of cardiac function in zebrafish

Why do we care?

- Mechanical properties of cardiac tissue are conserved across species
- Important for establishing of any heart disease model
- Impossible to infer from transmembrane potential or [Ca^{2+}] studies
- Require intact circulation (mechanical properties depend load)

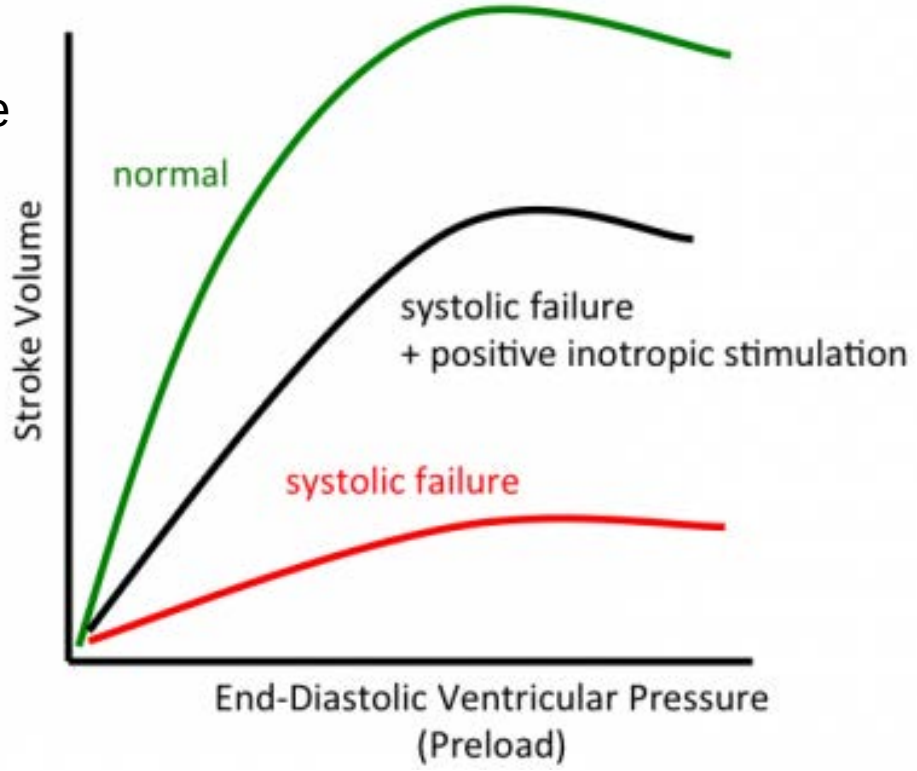
How do we do it?

Observe heart function under microscope

Bright field-based
- Heart rate
- Cardiac output

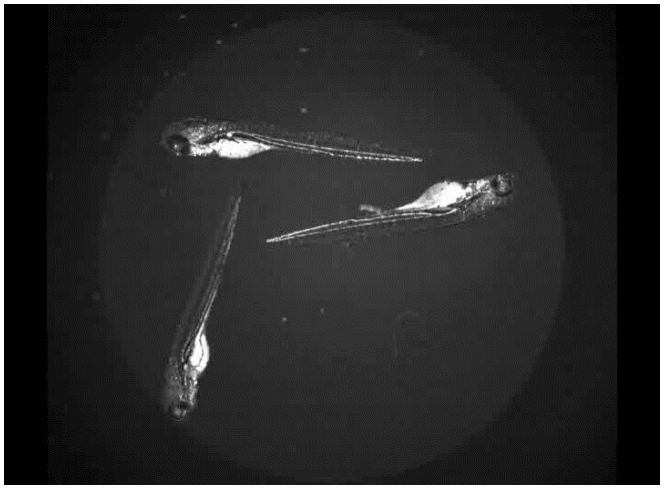
Fluorescence-based
- Edge detection
- Particle tracking

Frank – Starling law of the heart

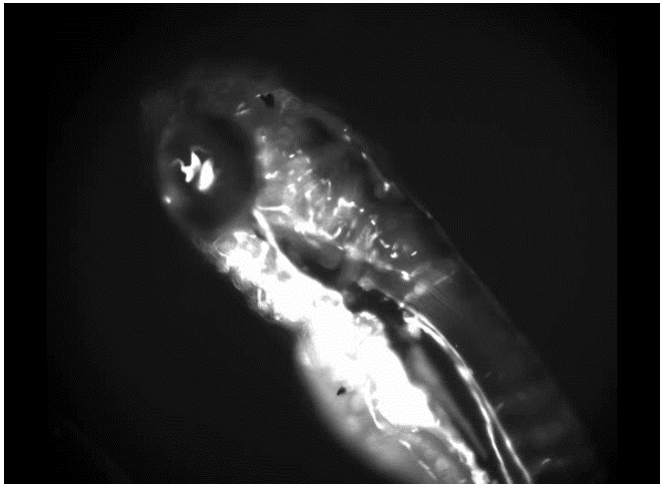


Imaging parameters

- 10x objective
- 0.38x de-magnification at camera port
- Reduce frame (binning does not increase rate)
- 256 x 216 pixels (10 % of available pixels)
- Field 0.44 x 0.37 mm
- Spatial resolution ~ 1.7 µm/pixel (588 pixels/mm)
- Temporal resolution ~ 8 ms per frame (125 fps)



2 X full field

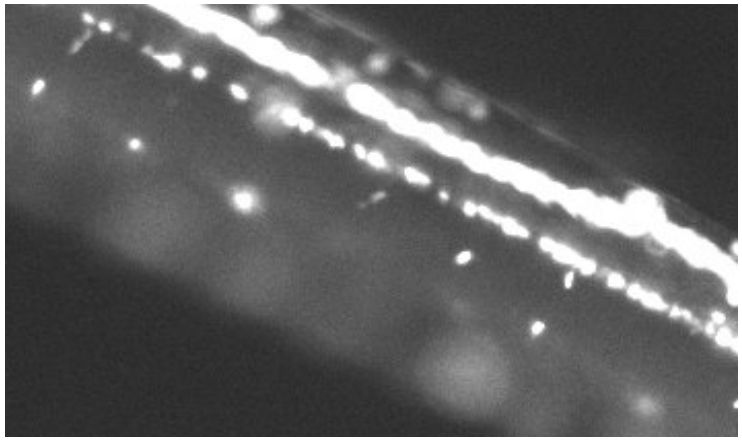


10 X full field

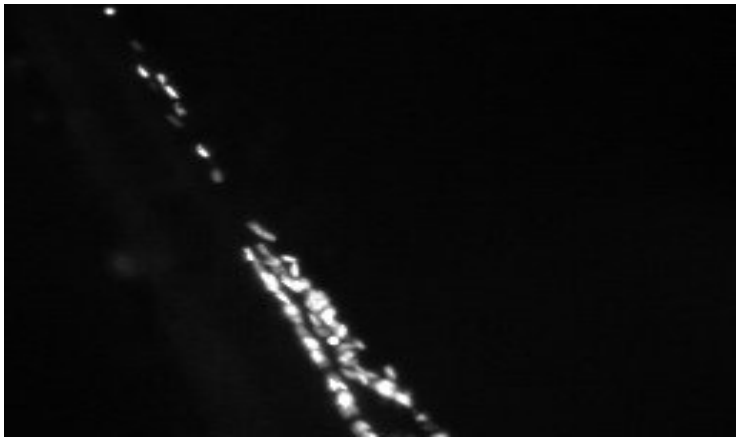
Fast imaging is important

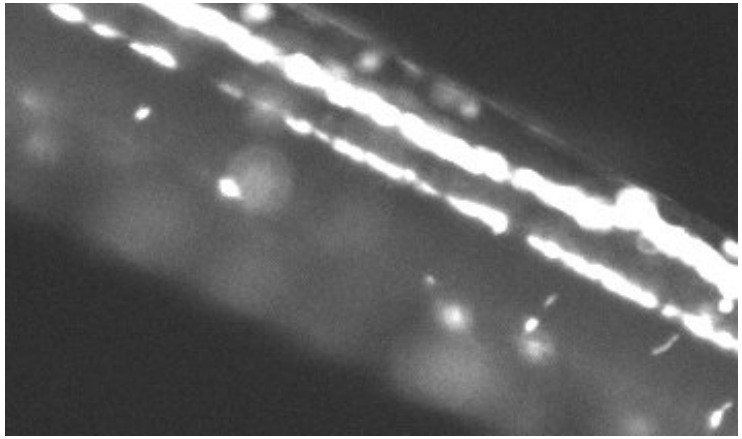
30 ms per frame (896 x 756 pixels)

8 ms per frame (256 x 216 pixels)

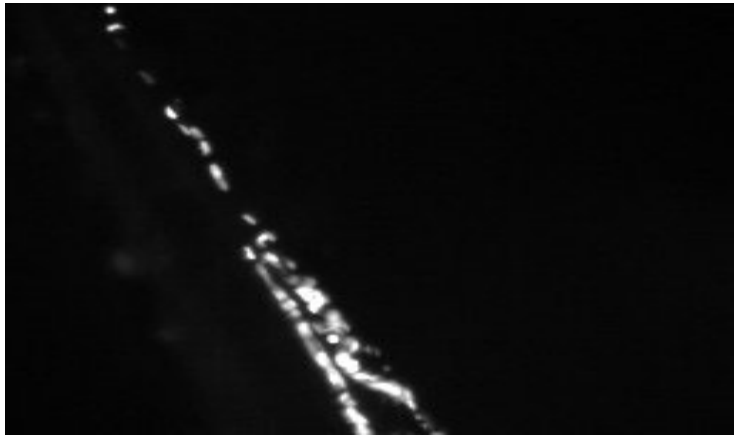


Diastole



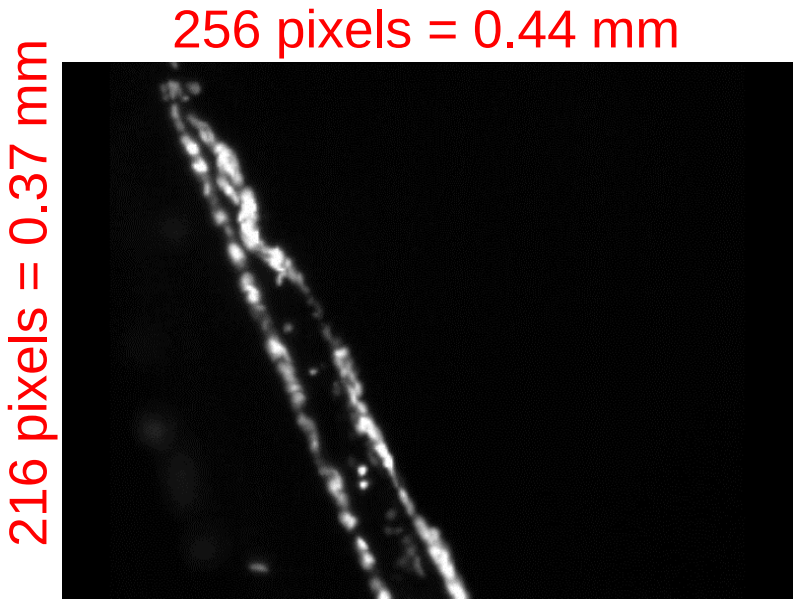


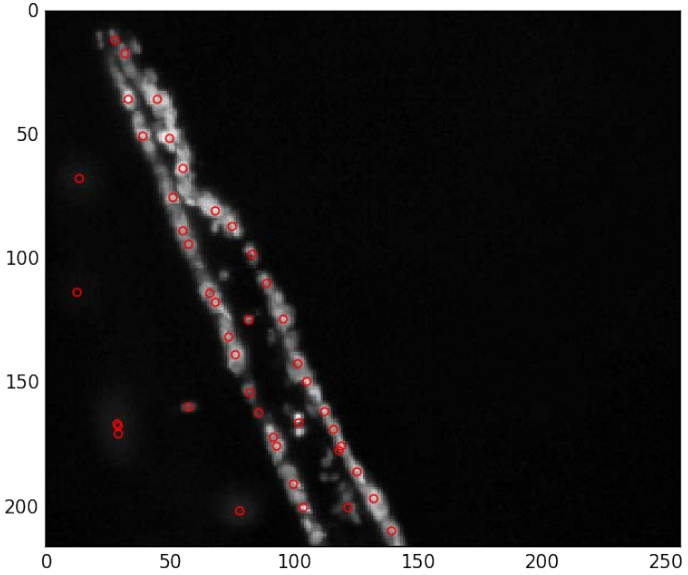
Systole

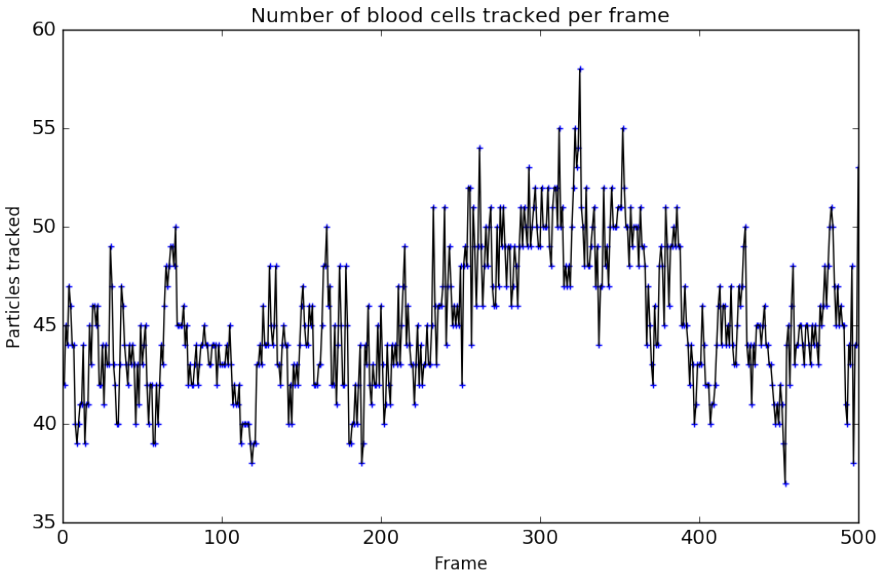


Blood cells can get lost if exposure times are too large.

Cell tracking: feature extraction



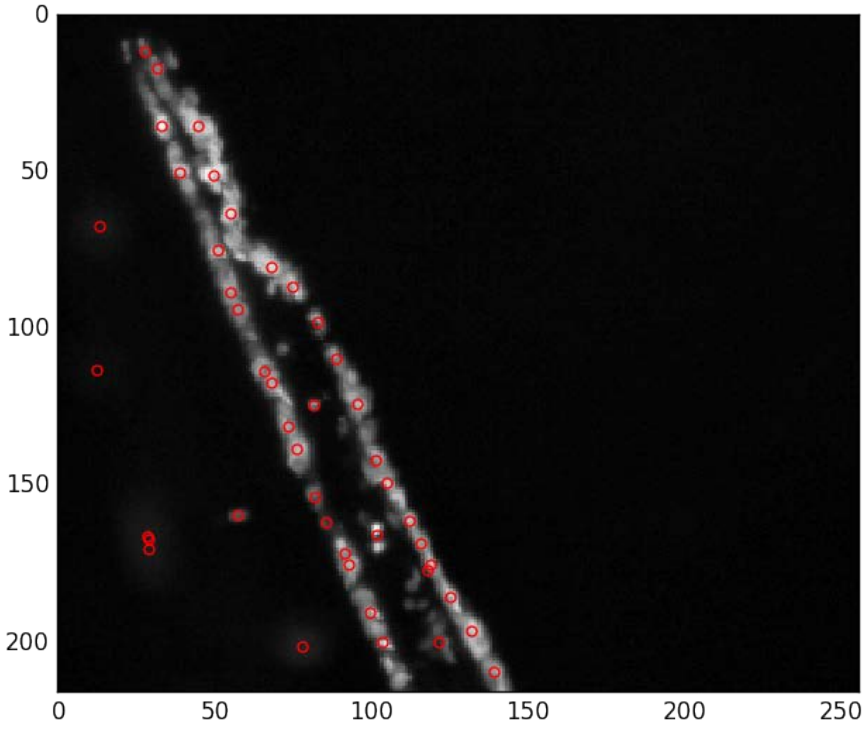




Cell identification

- 40 ROIs per frame
- 2000 ROIs for 500 frames (4 s)
- Next step: linking

Cell tracking: feature linking

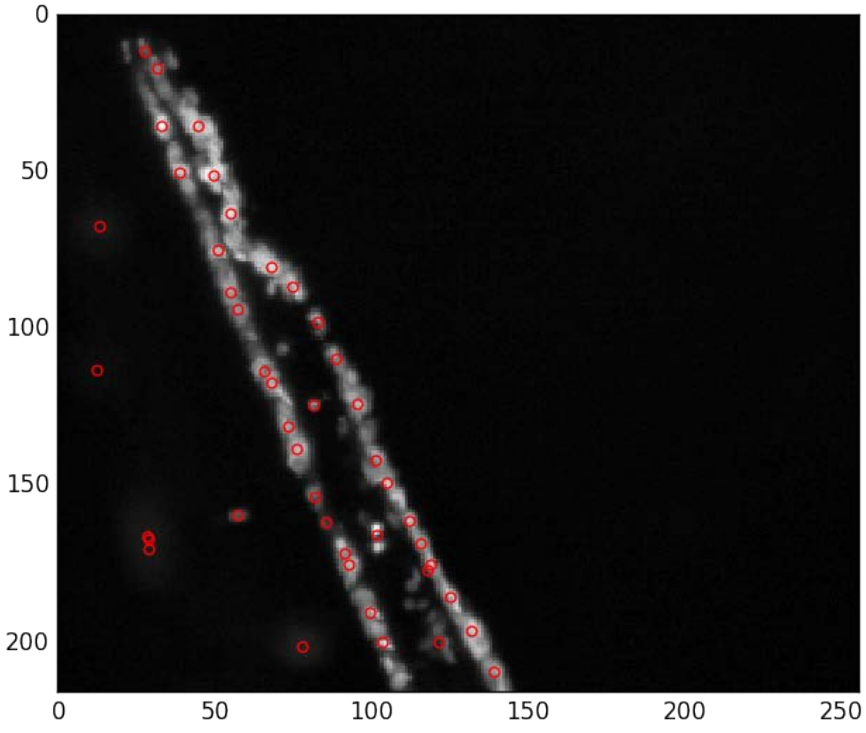


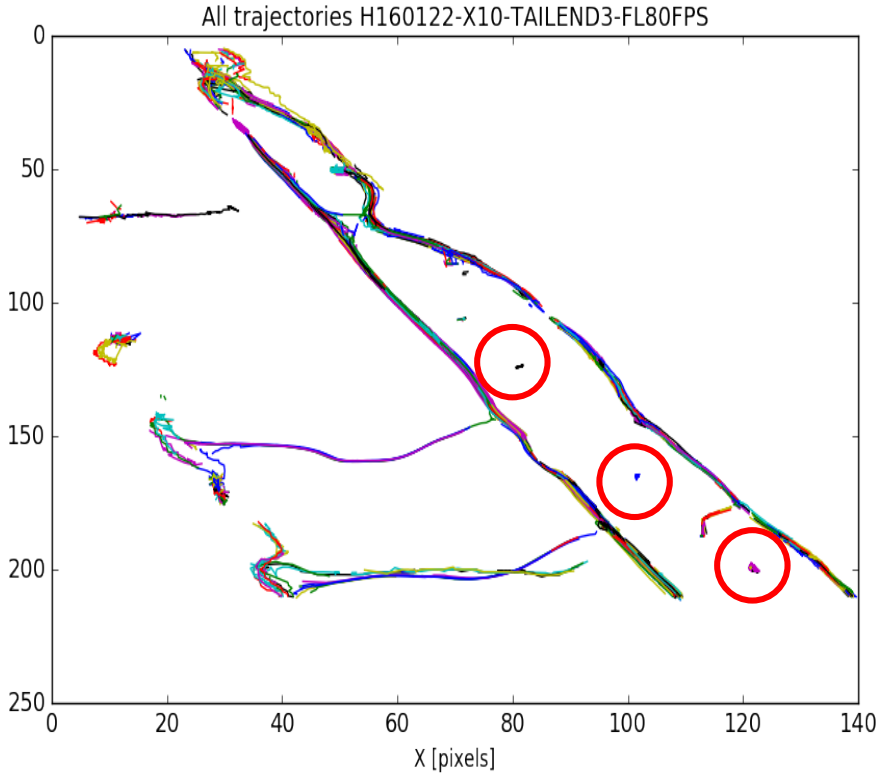
20,000 features (40 x 500)

- > 1000 trajectories Tr (x,y,t)
- ~ 300 persist for < 5 frames
- ~ 25% are shorter than 20 µm

Some of these circles are wrong; bright spots that are not blood cells.
But they do not move (or not very far) during the measurement.

Cell tracking: feature linking





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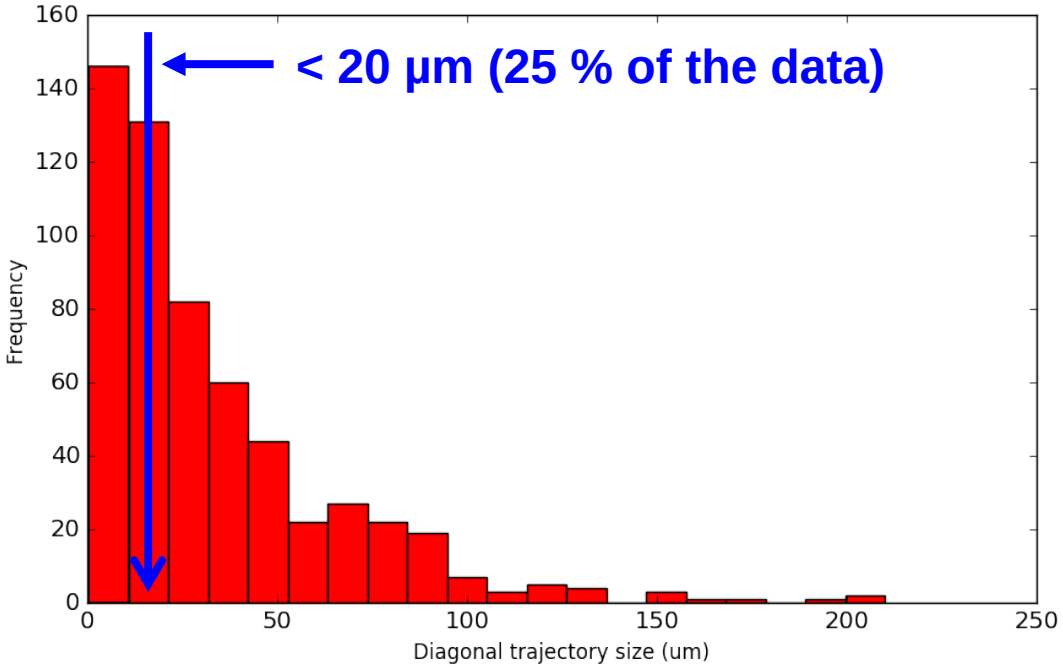
Filter trajectories by size to remove artifacts

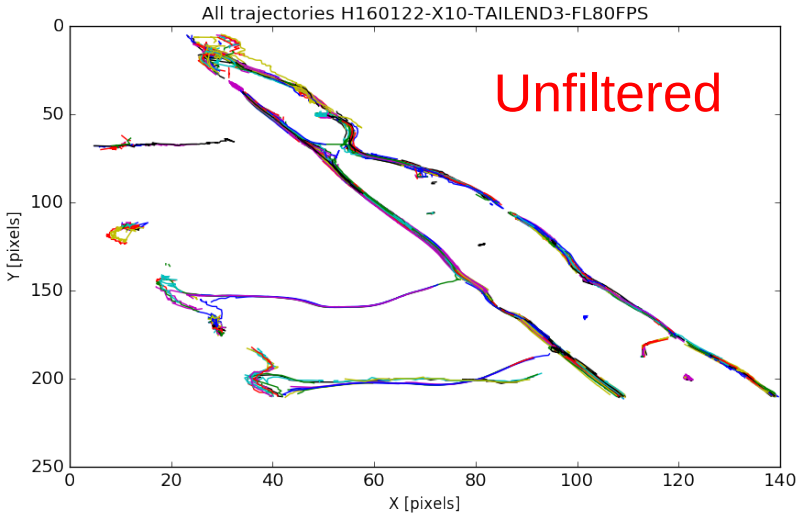


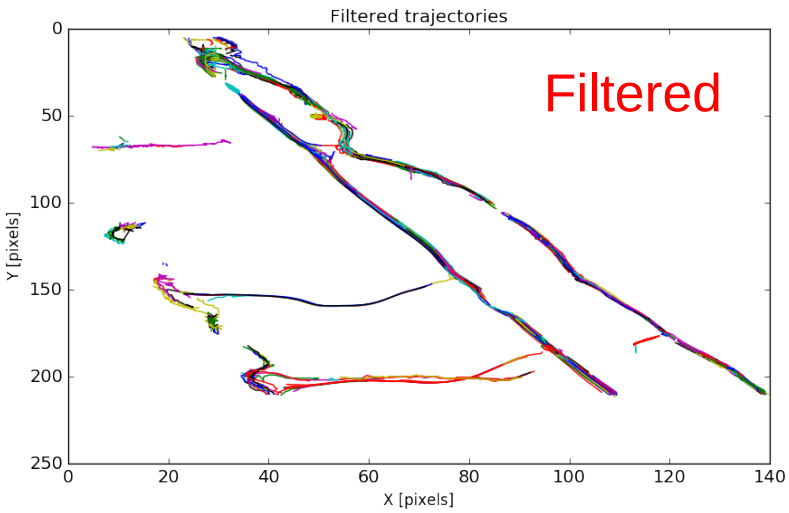
Linking of 23,281 features

- > 1000 trajectories Traj (x,y,t)
- ~ 300 persist for < 5 frames
- ~ 25% are shorter than 20 µm

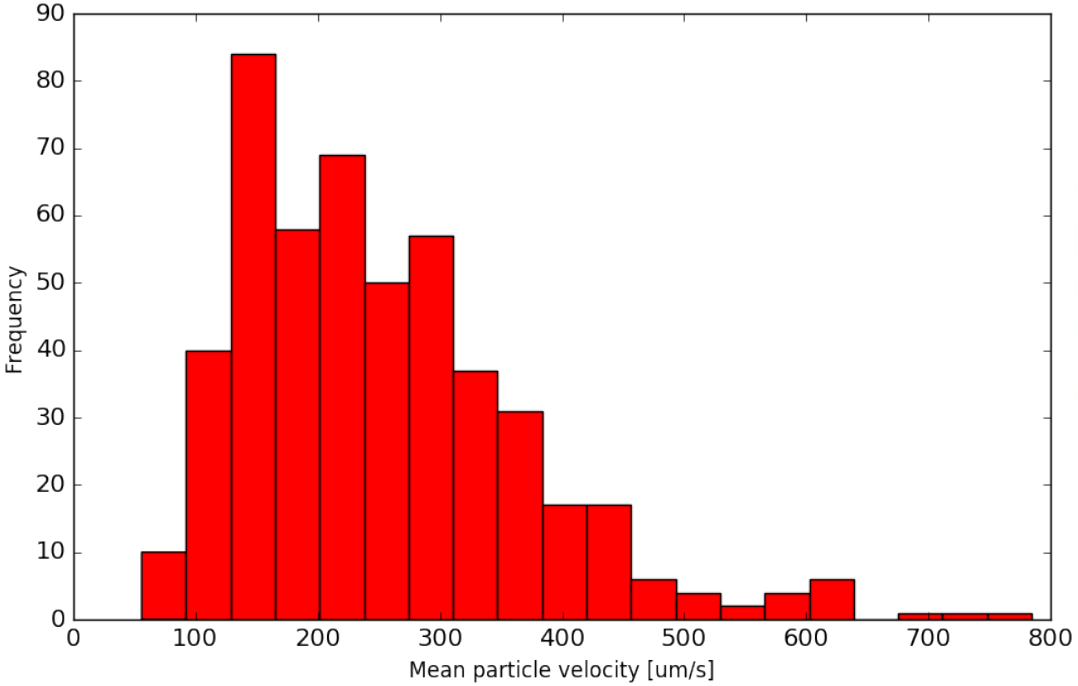
Filtering by q1 of diagonal length

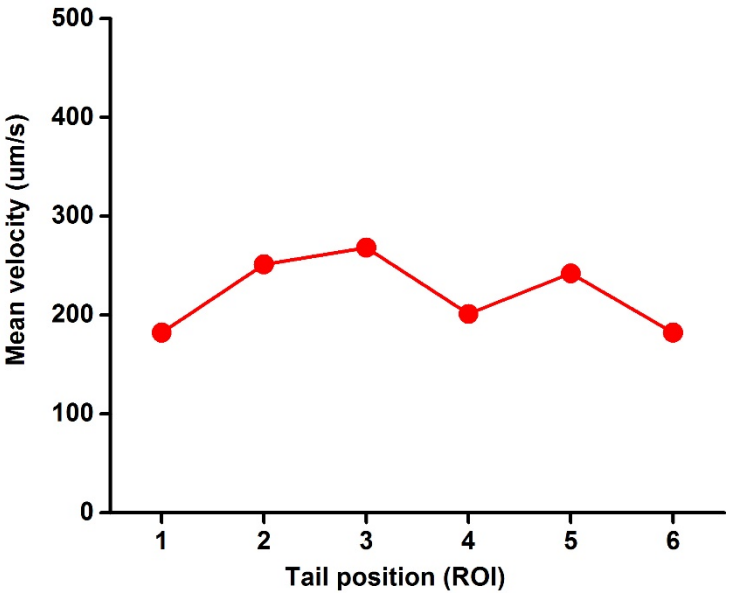
- ~ 500 trajectories
- Cells that move 20 µm or more





Tracking statistics: velocities





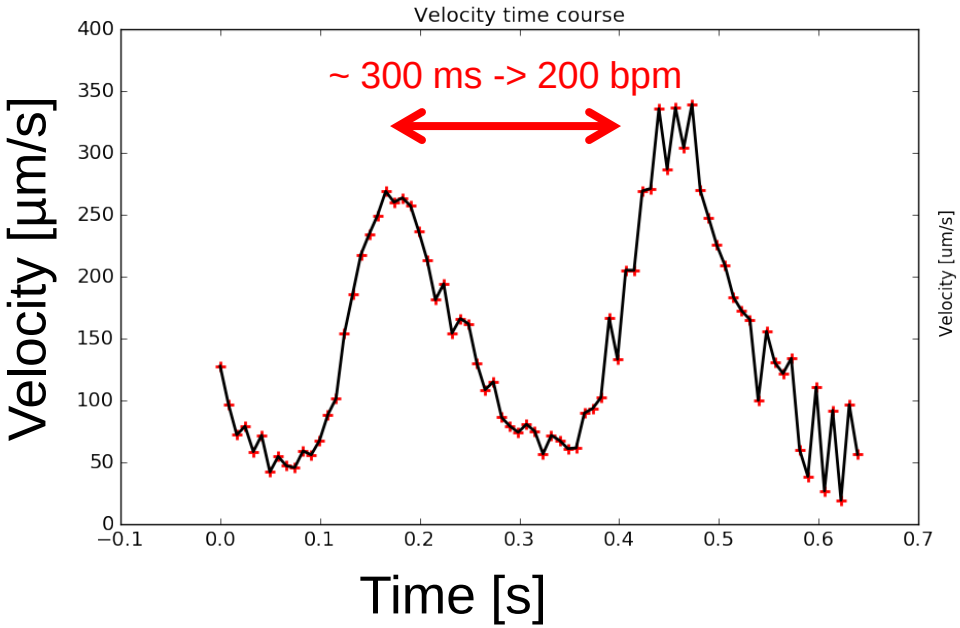
Mean velocity: 251 µm/s

Maximum velocity : 784 um/s

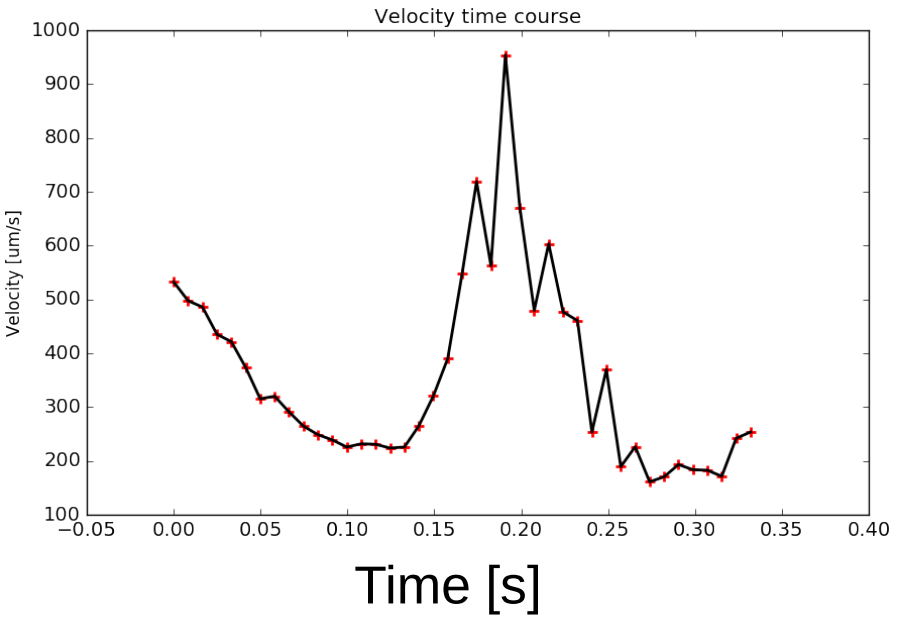
It does not matter where the flow is measured (tail).

Tracking statistics: heart rates

Long trajectory (very few)



Short trajectory



Statistics reveals important limitation

- Most particle trajectories are short
- 75% shorter than 100 µm
- Velocities smaller than 300 µm/s

Possible explanation

- Particles get lost at high velocities
- Insufficient frame rate

Solution:

Increase frame rate!

Advantages of the custom particle tracking algorithm

Microscopy software

- MetaMorph, ImageJ
- Expensive
- ‘Black box’
- Very limited filtering capabilities
- No visualizations
- No trajectories Tr (x,y,t)
- No heart rates

Custom algorithm

- External
- Established python algorithm
- Transparent

(we know what it is doing)

- Powerful statistics
- Complete visualization
- Heart rates from Tr (x,y,t)
- Customizable
- Fast

(experimental conditions)

Summary and outlook

Blood flow analysis

- Objective measurement
- No manual analysis required
- Fully automated
  - Complete flow statistics
  - Blood cell densities
  - Heart rates
- Suitable for high content screening
- Better alternative to CO measurements

Requirements

- Fluorescence microscope
- Fast camera (> 100 fps)
- Labelled blood cells