Markerless 3D spatio-temporal reconstruction of microscopic swimmers from video

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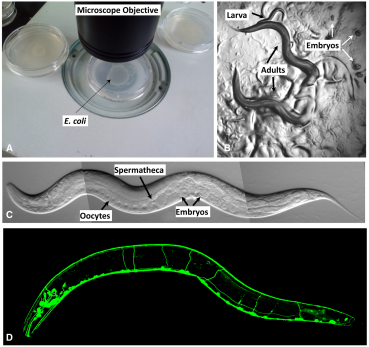
Engineering and Physical Sciences Research Council

A very special microswimmer: *C. elegans*



Corsi *et al.*, 2005. doi:10.1895/wormbook.1.177.1

Why?

- *C. elegans*, a 1mm roundworm
- Model organism
- Study of animal behavior
- Postures, kinematics, biomechanics

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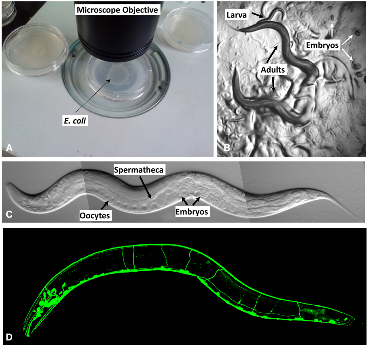

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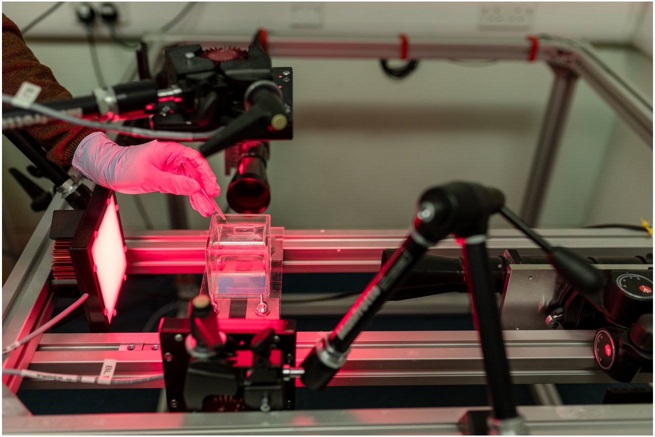
Our objectives

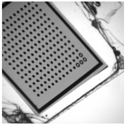
- 3D microscopy
- Resolve postures
- Large field of view
- Capture long trajectories

Camera set-up

Challenges: Depth of fields vs resolution, aspect ratio, transparent living object



Pipeline overview



Input

- Calibration images (about 100 triplets)

Pipeline overview

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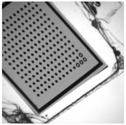
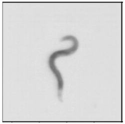
- Calibration images (about 100 triplets)
- Grayscale videos ($3 \times 2048^2 \times 8$ bit, 25fps)
- Single worm ($\sim$1 mm or ≤ 200 px) $+$ blur





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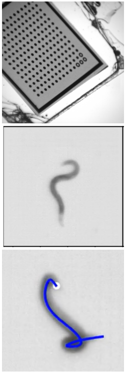

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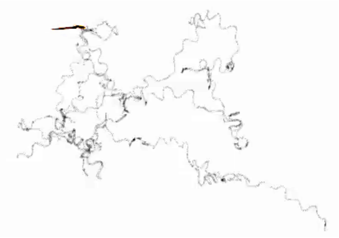


Output

- Worm midline over time
- Real world coordinates

Video example

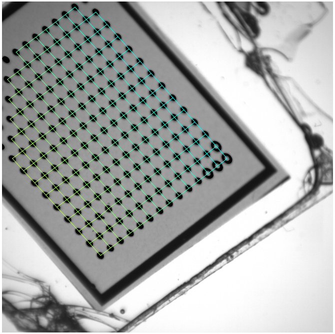
Video example

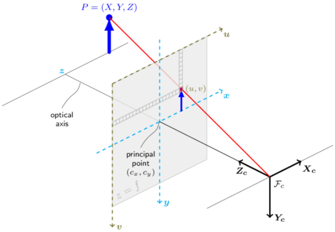
Key steps

a) Calibration of the camera setup using calibration images taken before the experiment.
b) Image normalization, object tracking and triangulation.
c) 2D image segmentation to find midlines using a trained equivariant convolutional neural network.
d) Correlation-based fine tuning of the camera calibration along moving object.
e) Space carving with three-way majority voting to obtain a discrete skeleton.
f) Curve fit optimization using a finite element formulation and weighted candidate points.

a) Calibration of the camera set-up

Task: recover camera positions and parameters from images of fixed grid





OpenCV 3D pinhole camera model

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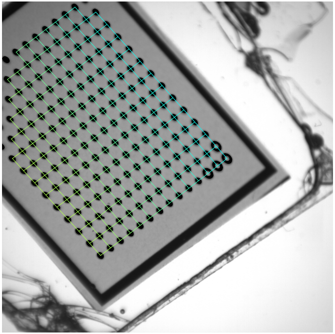
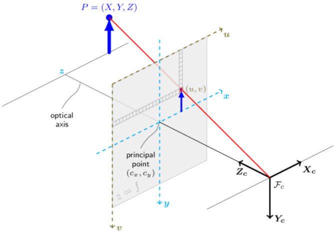




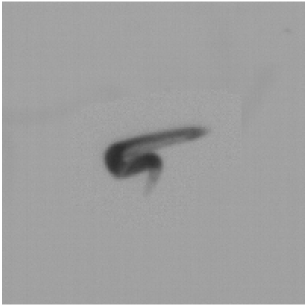
OpenCV 3D pinhole camera model

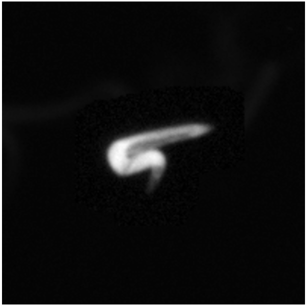
Problems:

- Accuracy issues: focus, distortion, gel properties but good enough for tracking

b) Image normalization, object tracking and triangulation.

- Static background subtraction
  - background: maximum brightness over time.
  - caveat: moving bubbles
- Invert brightness, range normalisation





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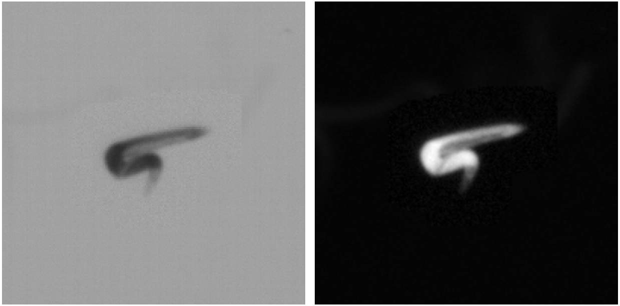
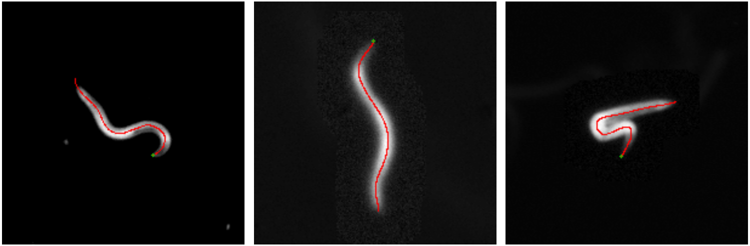


- Identify candidate silhouettes in 2D
- Triangulate all, pick lowest reprojection error
- In subsequent frames: pick nearest silhouettes

c) Image segmentation to find 2D midlines using a CNN

- Equivariant CNN, autoencoder/decoder architecture
- Manual annotations



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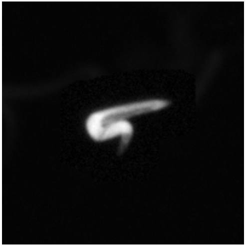

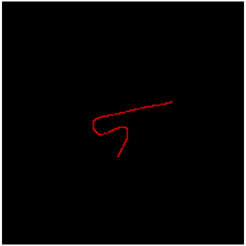

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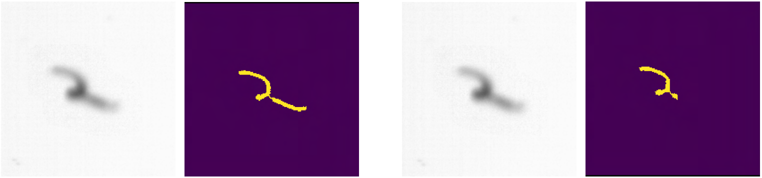

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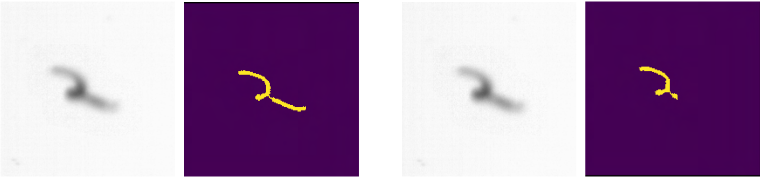
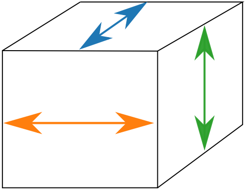


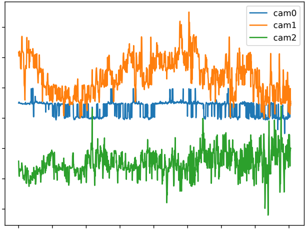
- Reiterate?

d) Fine tuning of the camera calibration along the moving object.

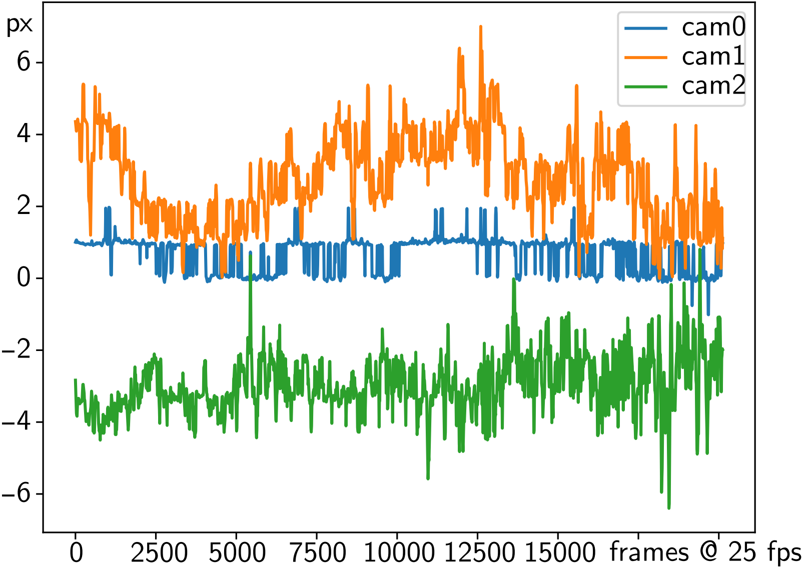
Problem: Calibration not perfect and changes in time – need to align images locally
Idea: Update local in time shift maximizing correlation using stochastic gradient descent on 20 frame batches.

$$\text{Correlation} = \max_{p \in \mathbb{R}^3} \int_{\text{cube}} \prod_i v(\text{shift}_p^i(\text{cam}_i(x)))\mathrm{d}x$$



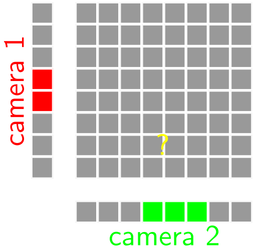


Example camera correction for 13 minute clip



e) Space carving with three-way majority voting to obtain a discrete skeleton.

Challenge:
2d midline pixels →
find 3d midline voxels



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Steps:

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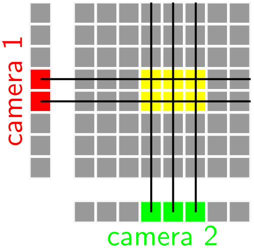

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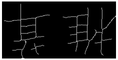
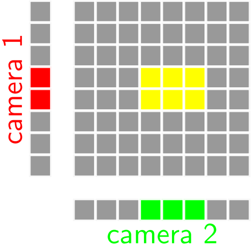
- Skeletonise in 2D (Guo-Hall) and 3D (Lee)





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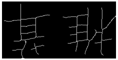
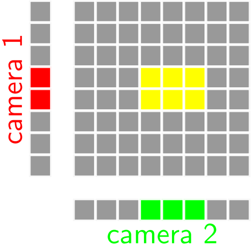

- Some majority voting to counter drop-out



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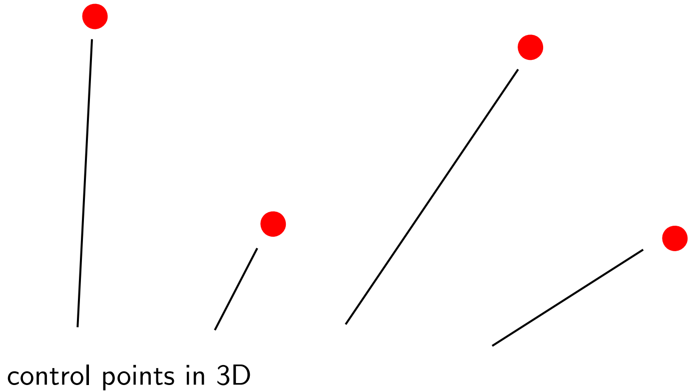

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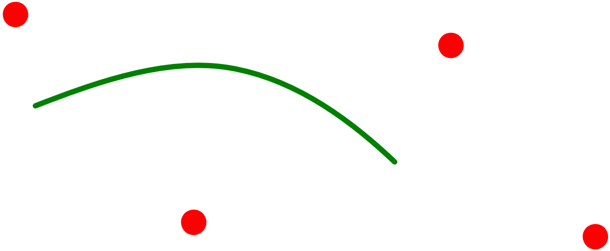

short initial guess for midline curve

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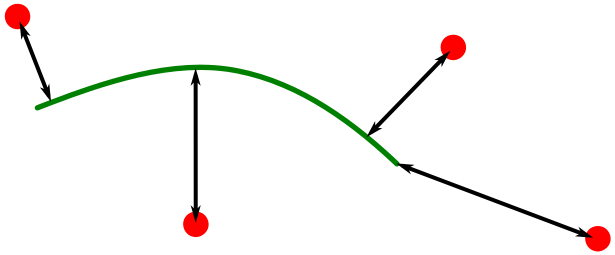

physics based model with elastic curve
and forces from control points

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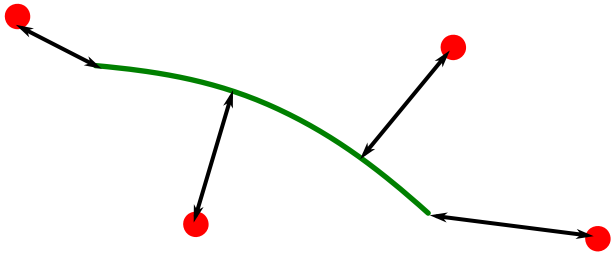

use gradient flow to balance forces (length fixed)

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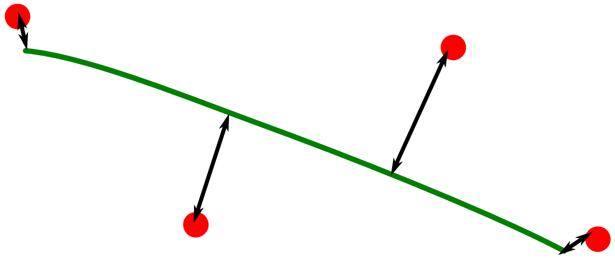

increase length and reduce stiffness for best fit

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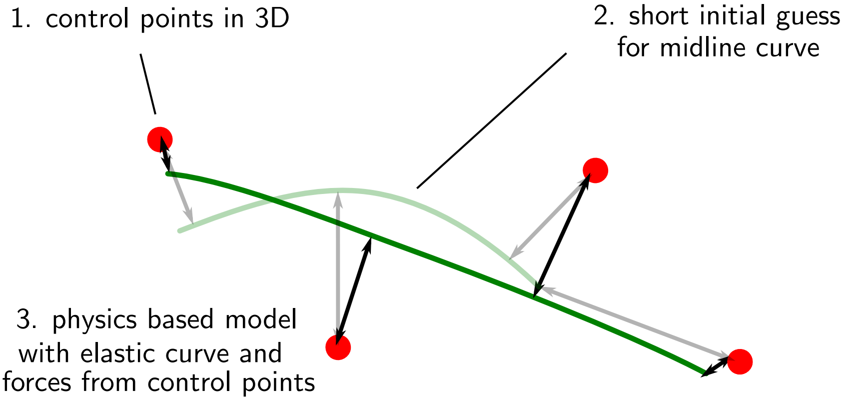

4. use gradient flow to balance forces (length fixed)

5. increase length and reduce stiffness for best fit

Ranner, 2020. doi:10.1016/j.apnum.2020.05.009

Summary

- Studying *C. elegans* locomotion in 3D presents many technical and experimental challenges
- Data capture requires careful experimental set-up
- Camera (re)calibration is essential
- CNNs are useful for segmenting image data
- Finite-element methods enable physics inspired shape fitting
- Our set-up recovers:
  - Trajectory of the swimmer's center-of-mass
  - Parametrized postures (3D midline curves) for each frame
- Analysis of the data is underway

Thank you for your attention!