Processing Multi-View Selective Plane Illumination Microscopy Data

Stephan Preibisch

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Dresden, Germany

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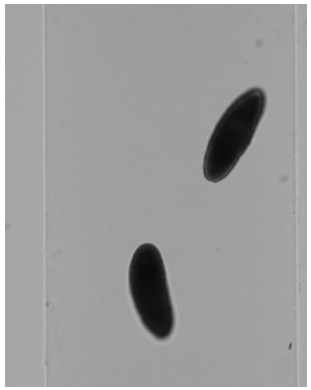
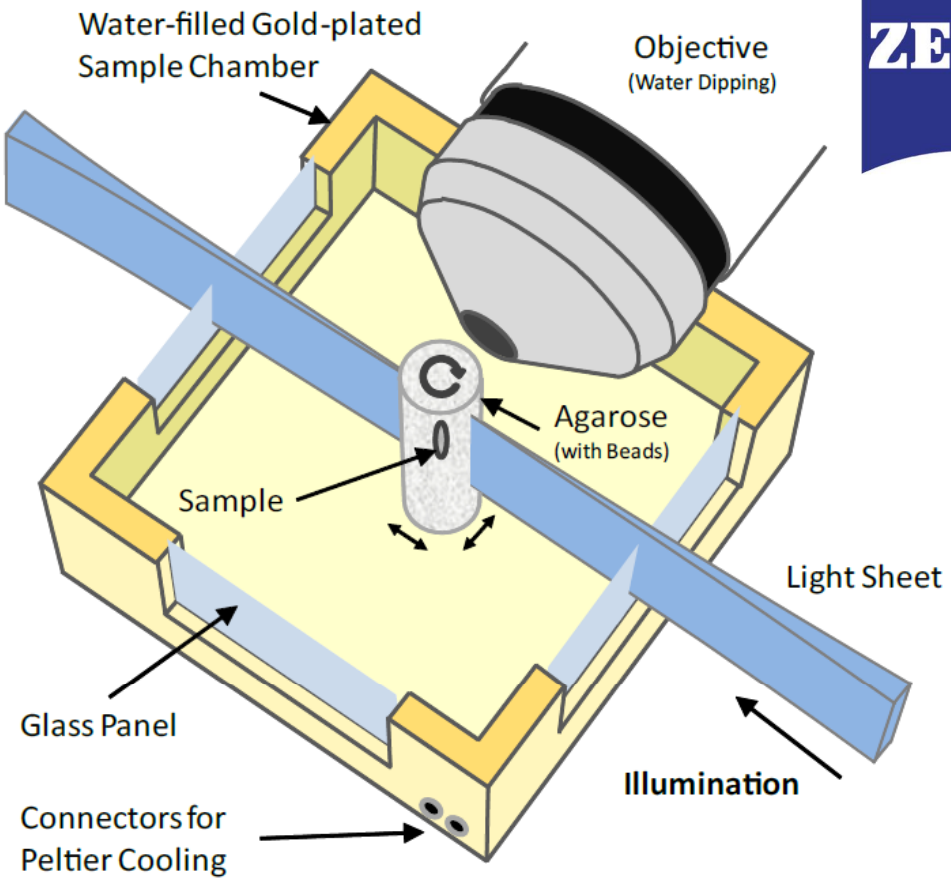
The SPIM

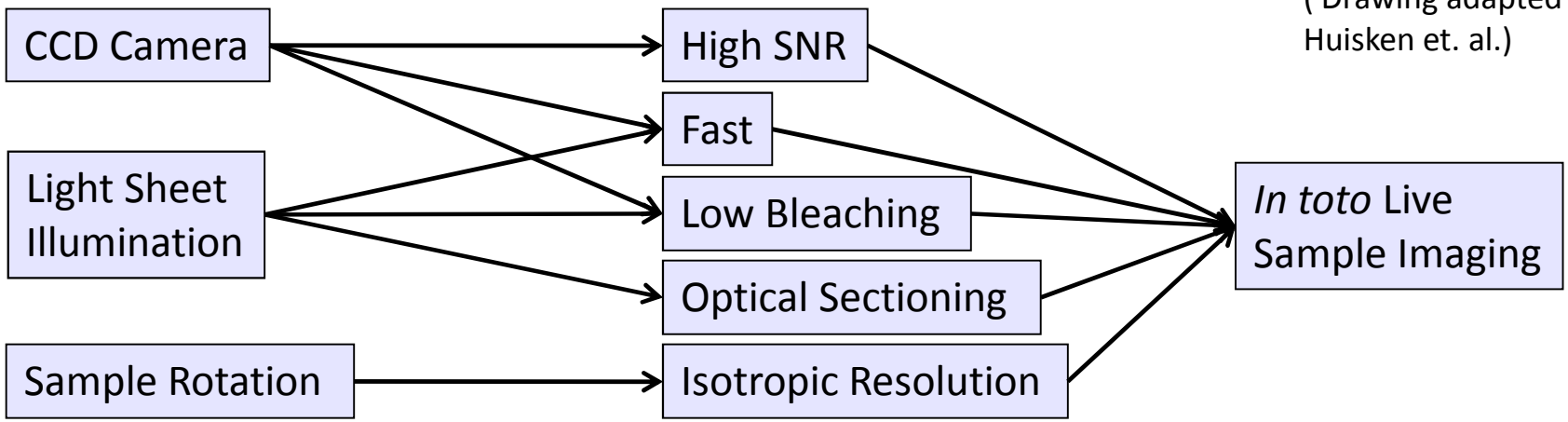
(Selective Plane Illumination Microscope)

J. Huisken, J. Swoger, F. Del Bene, J. Wittbrodt, and E. H. K. Stelzer, *Science* 305, pp. 1007–1009, 2004.

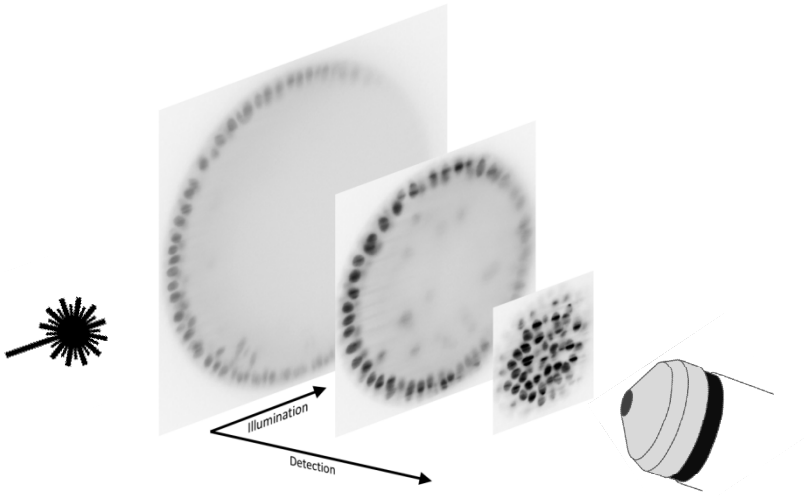




(Drawing adapted from Huisken et. al.)



Registration - A lot of Problems



1. **Signal Degradation**

J. Huisken, "Multiview microscopy and multibeam manipulation for highresolution optical imaging", *Dissertation*, 2004.
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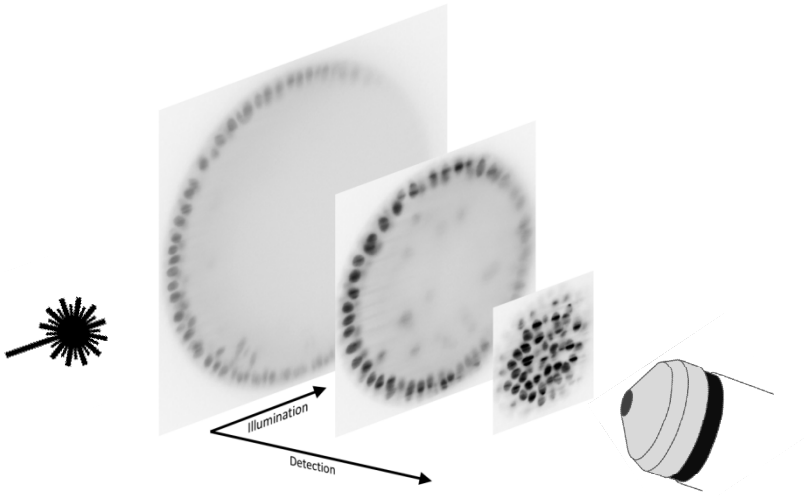

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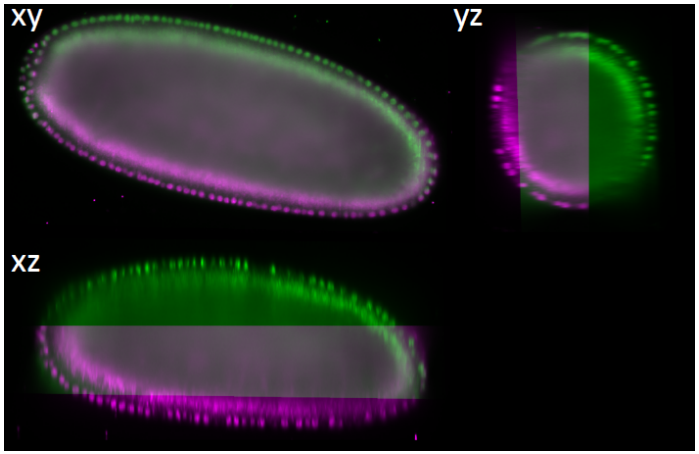

2. **Limited Overlap**

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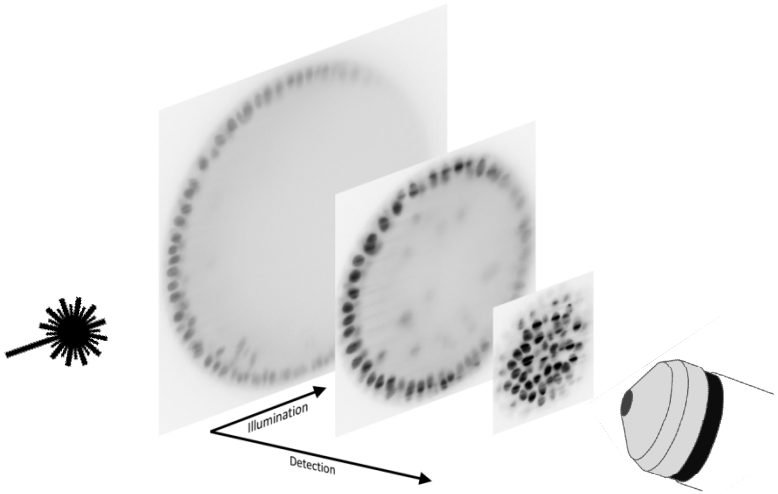

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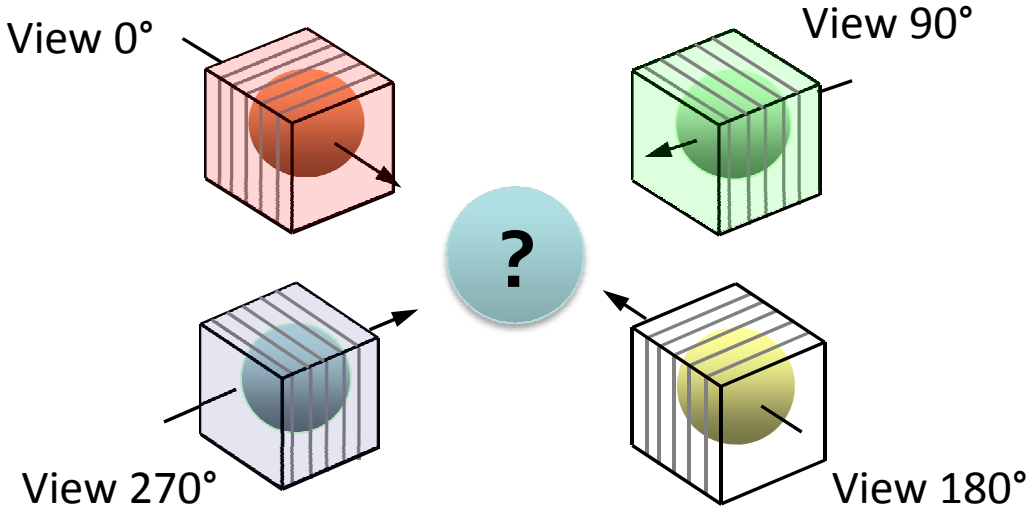
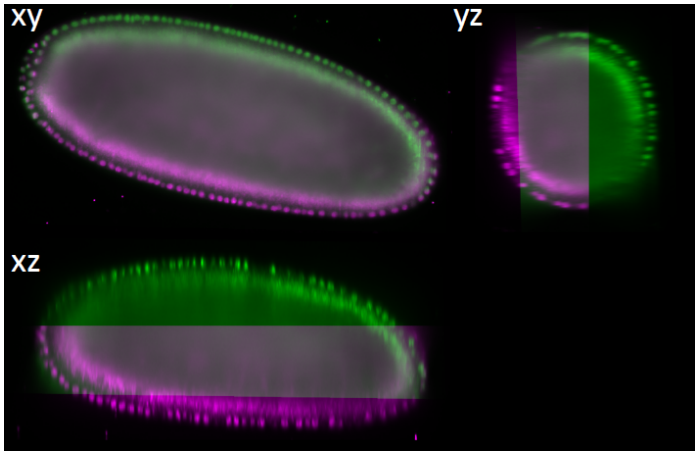


3. **Varying orientations of the optical sections**



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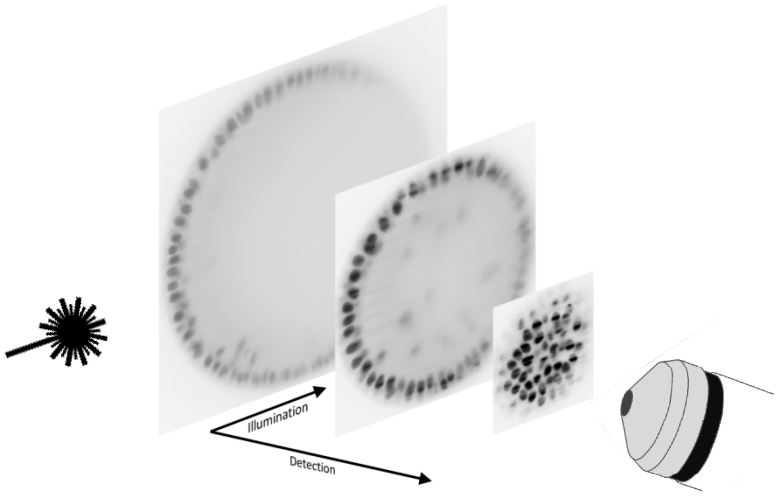

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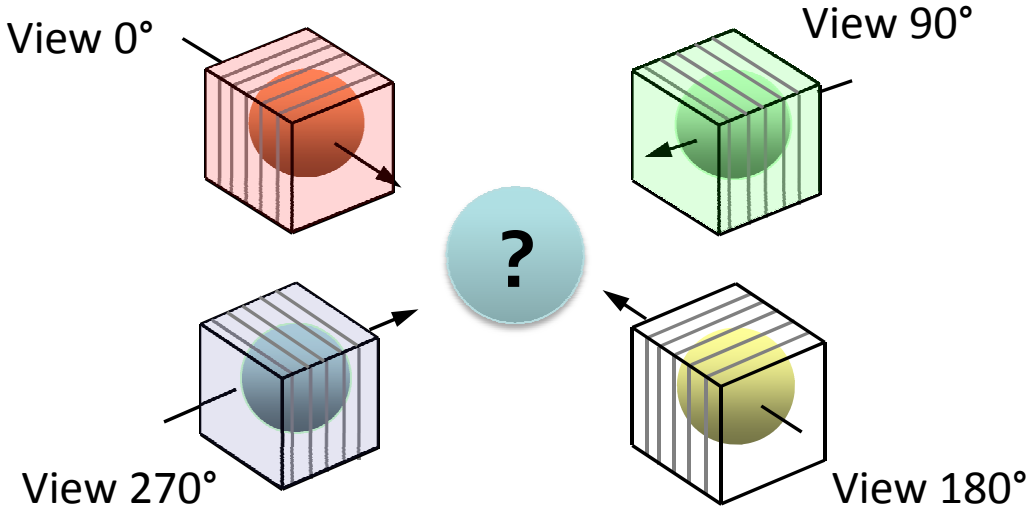
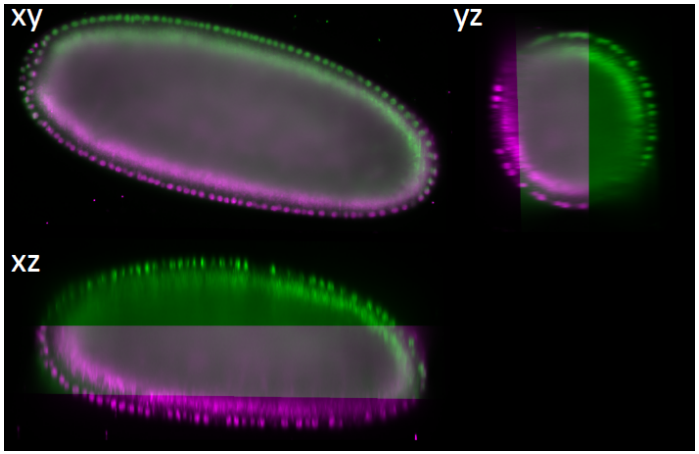


3. **Varying orientations of the optical sections**
4. **Development of the specimen**



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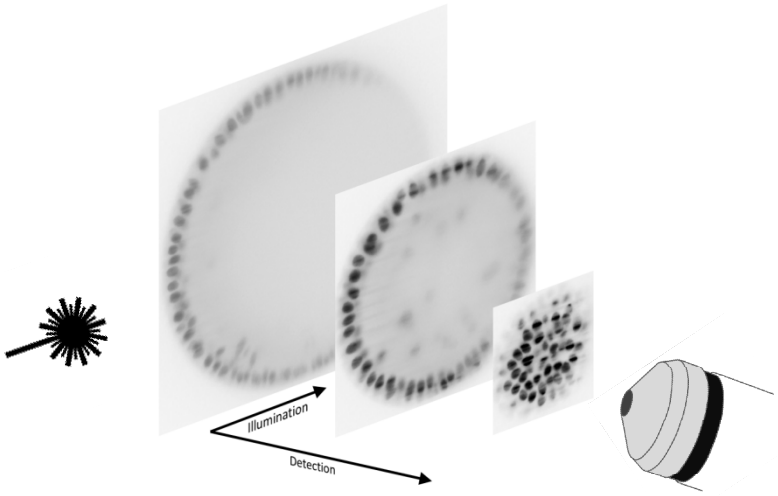

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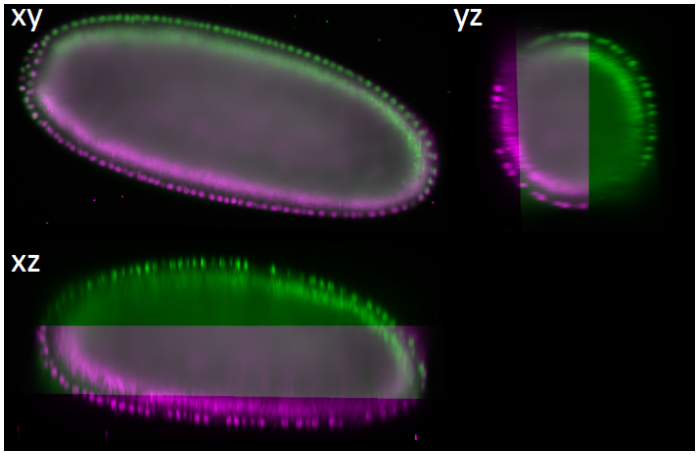

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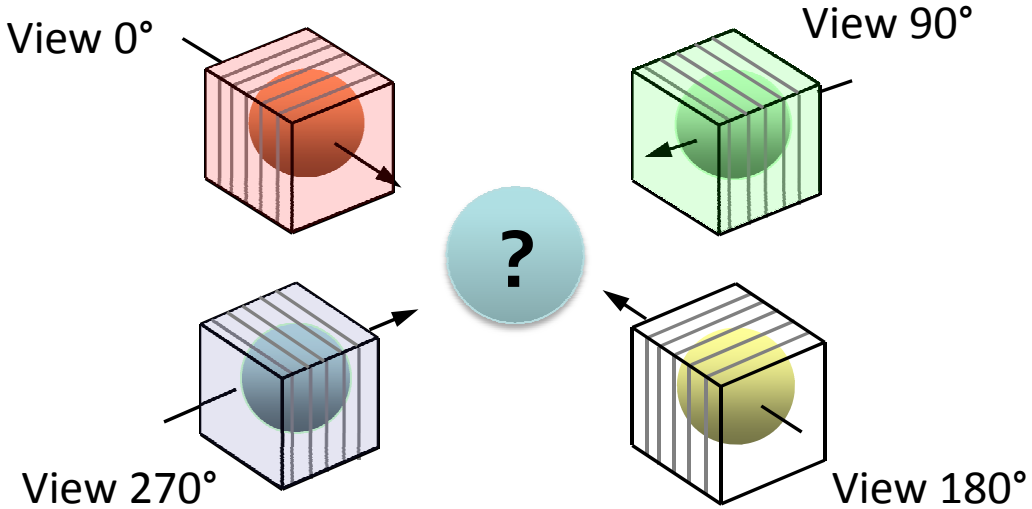

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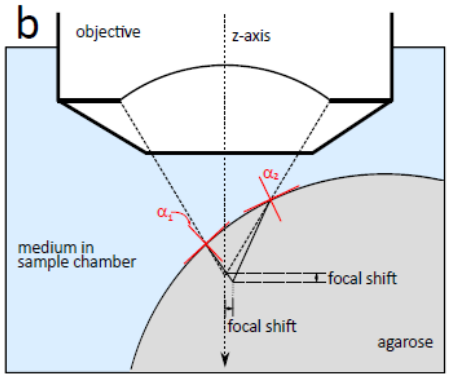

5. **Scaling introduced by refractive index change**

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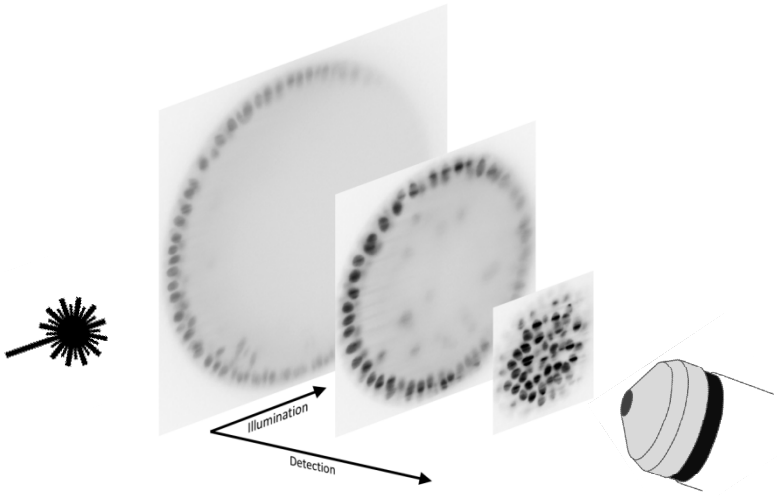

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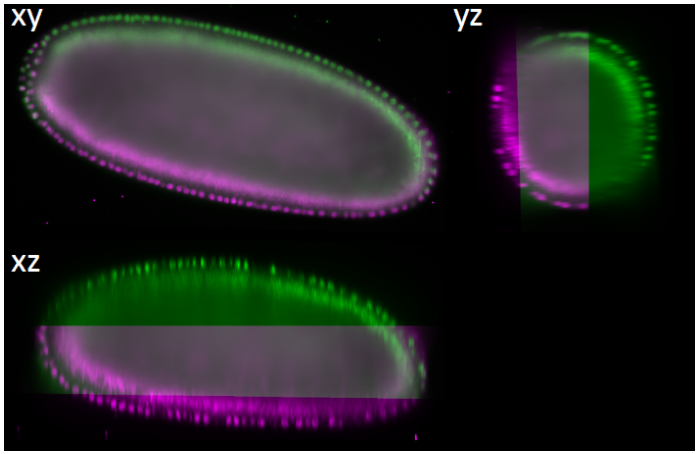

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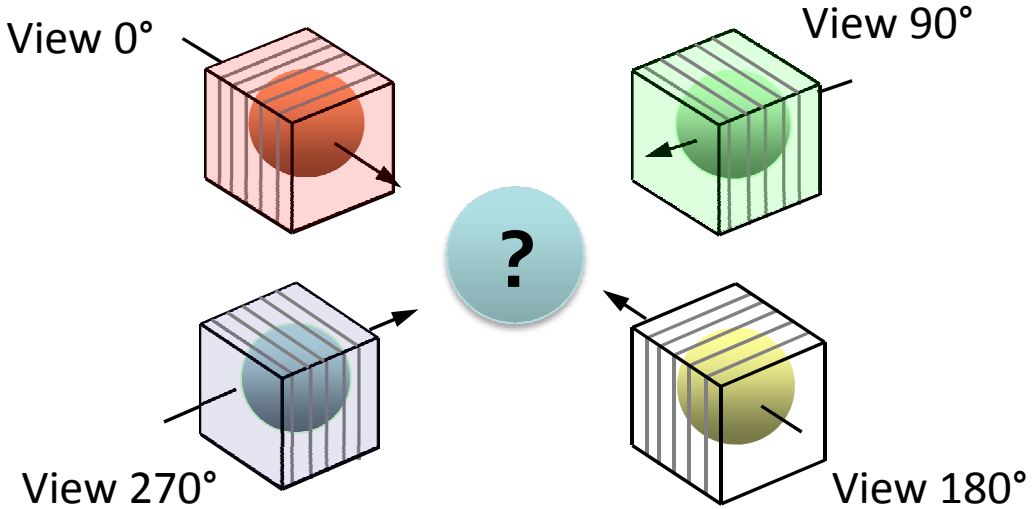

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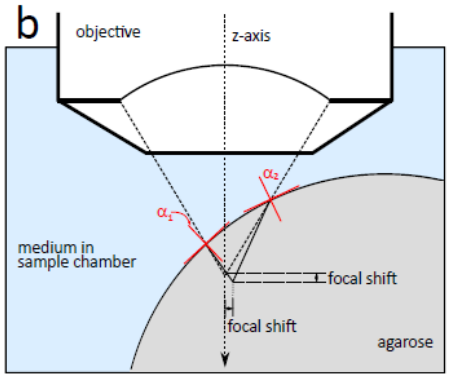

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6. **Size of the datasets (up to terrabyte range)**

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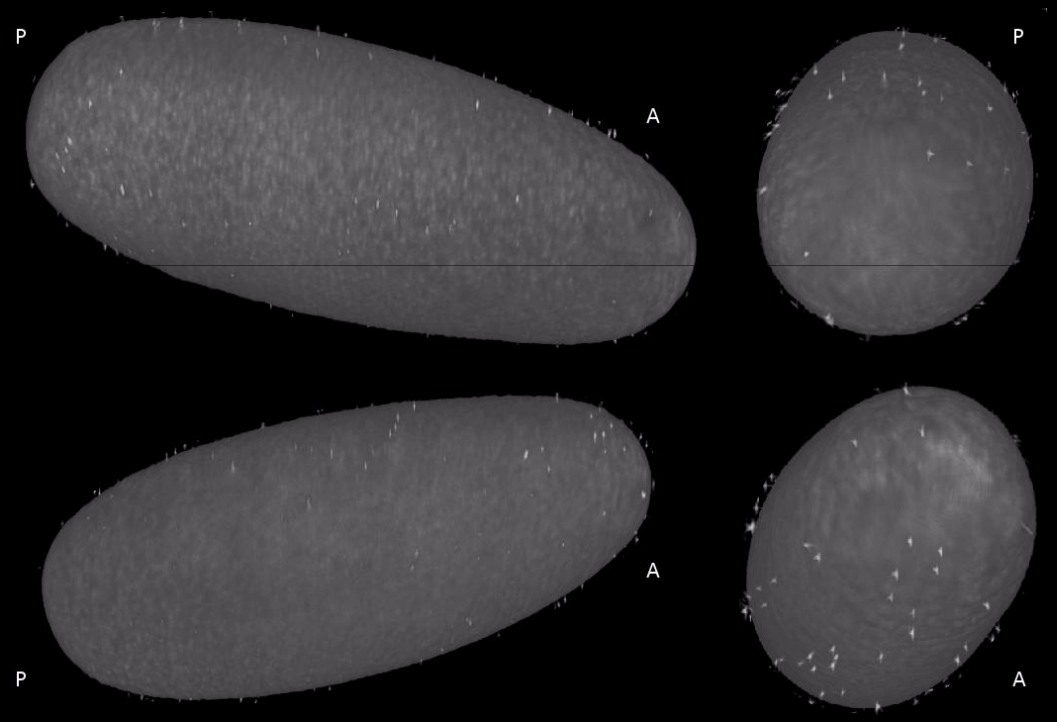
Compensate for incomplete sample coverage

Achieve in toto imaging



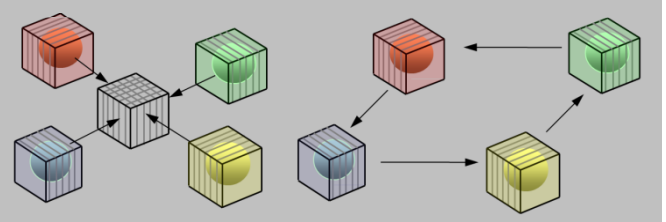
Preibisch S., Saalfeld S., Schindelin J., Tomancak P., "Software for bead-based registration of selective plane illumination microscopy data", *Nature Methods* **6(**7), 2010.

Potential Registration Approaches

Intensity based

- No embedding necessary
- Sample independent

- Typically slow
- Hard to cope with developing samples
- Result hard to verify automatically



P. Shaw et al., in *Biophysical Journal* ***55***, 1989.

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P. Verveer et al., in *Nature Methods* ***4***, 2007.

W. Wein et al., in P*roceedings of MICCAI*, 2007.

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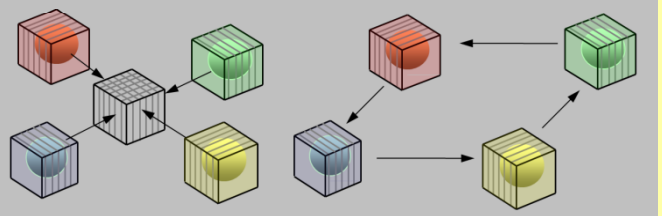

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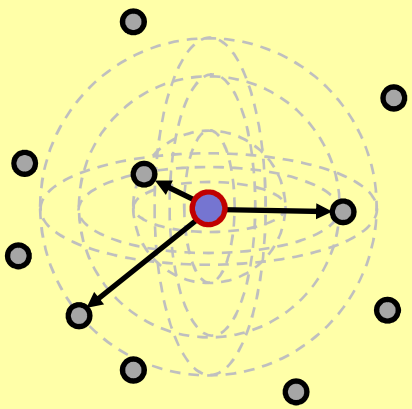
Bead based

- Very fast
- Sample independent
- Easy use with developing samples
- Automatic verification

- Embedding in rigid medium



S. Preibisch, S. Saalfeld T. Rohlfing, P. Tomancak, in *SPIE Medical Imaging 2009.*

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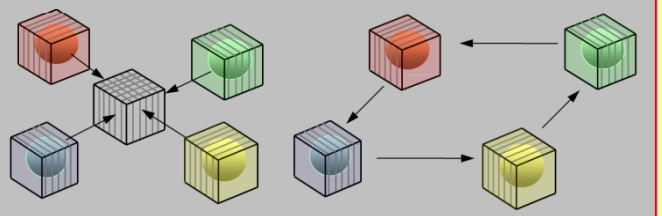

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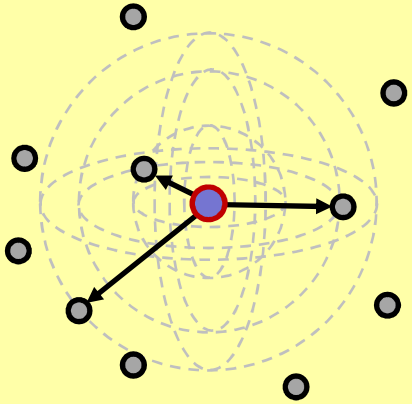

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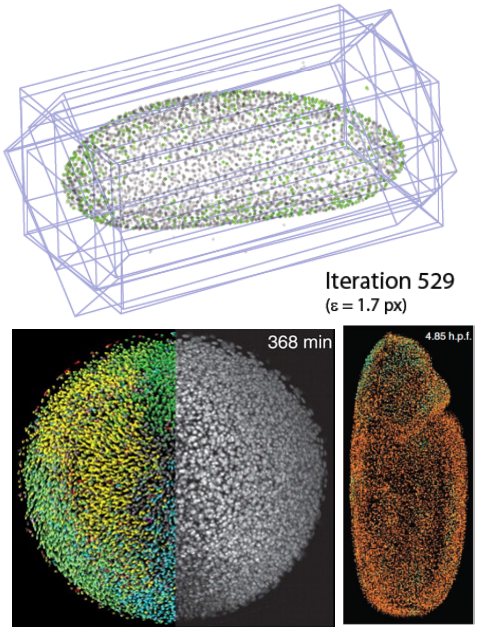
Segmentation based

- Potentially fast
- Automatic verification possible
- No embedding necessary

- Staining dependent
- Hard to cope with developing samples



P. Keller et al., in *Science*, 2008.

S. Preibisch, in *PhD Thesis*, 2010.

P. Keller et al., in *Nature Methods* ***8***, 2010.

Bead based Registration Framework

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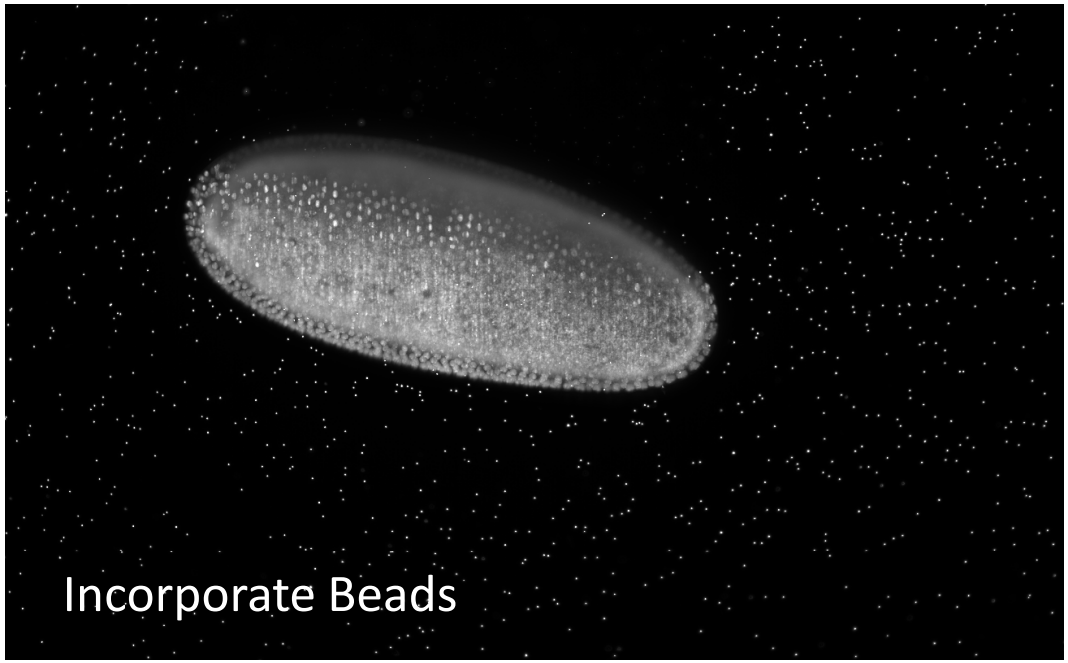

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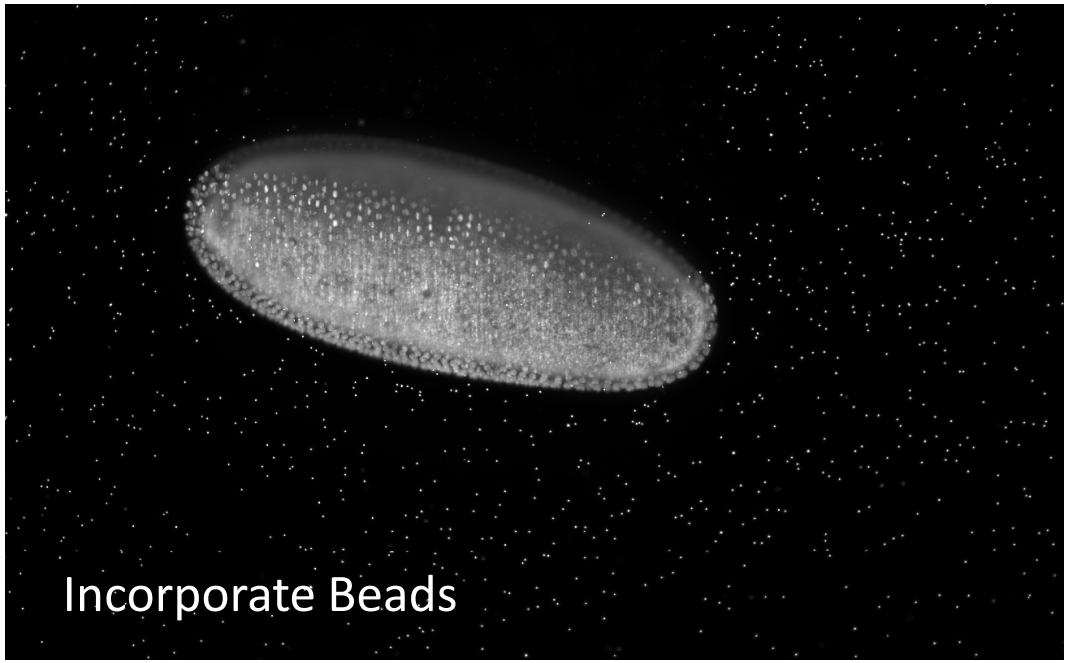

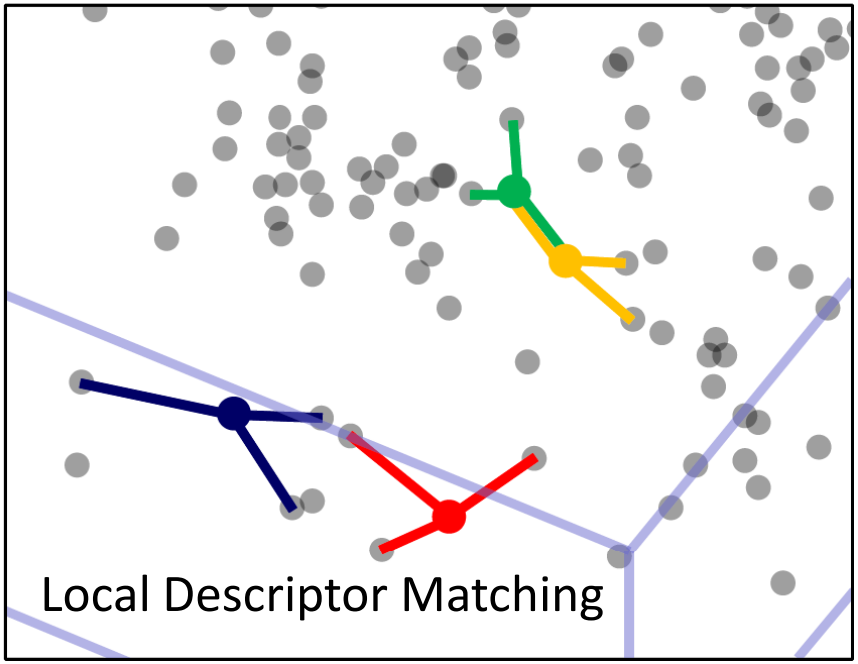

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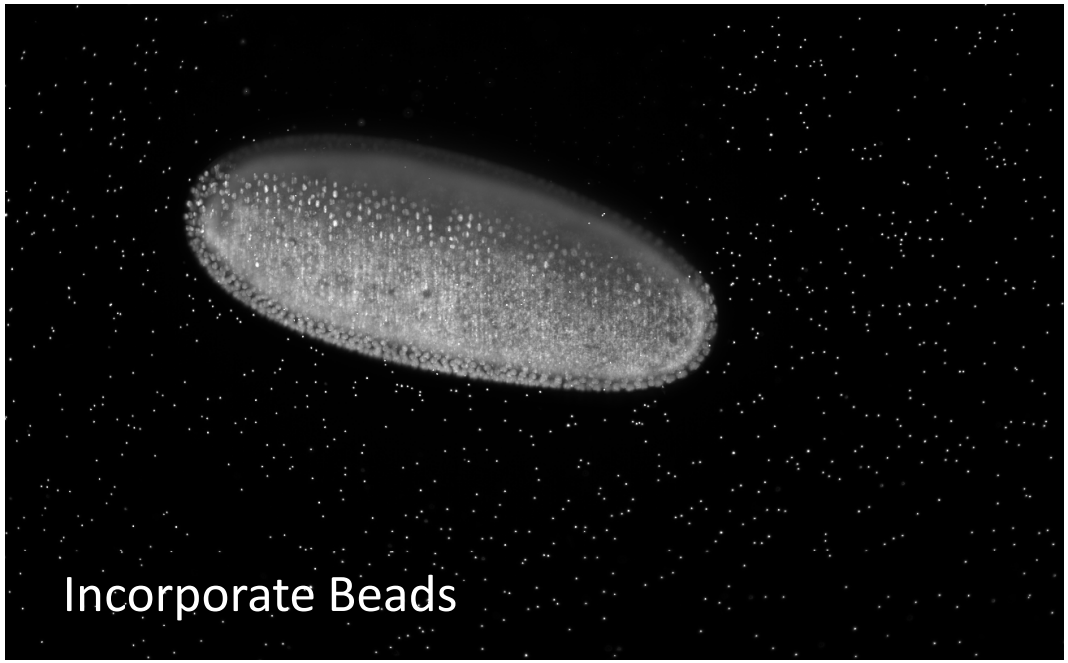

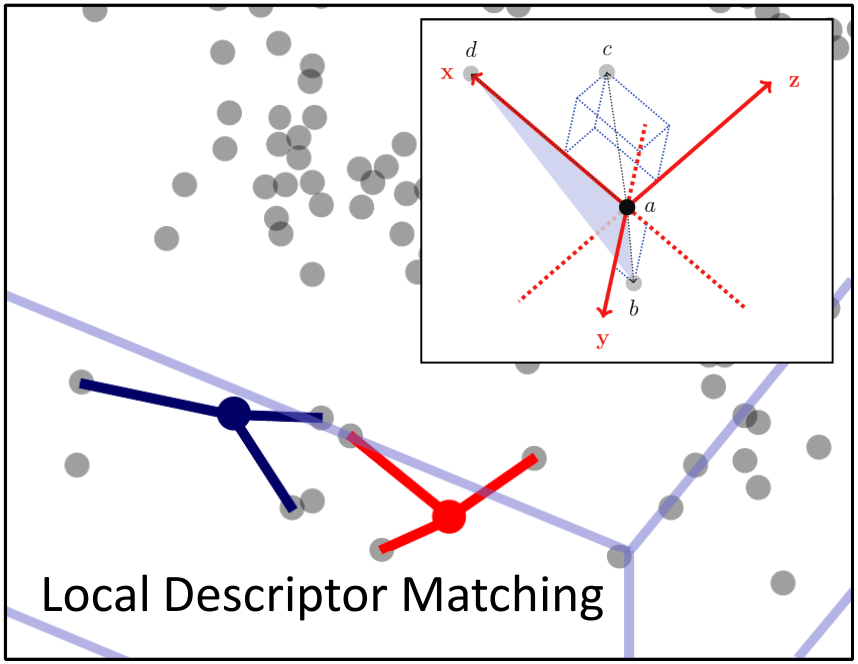

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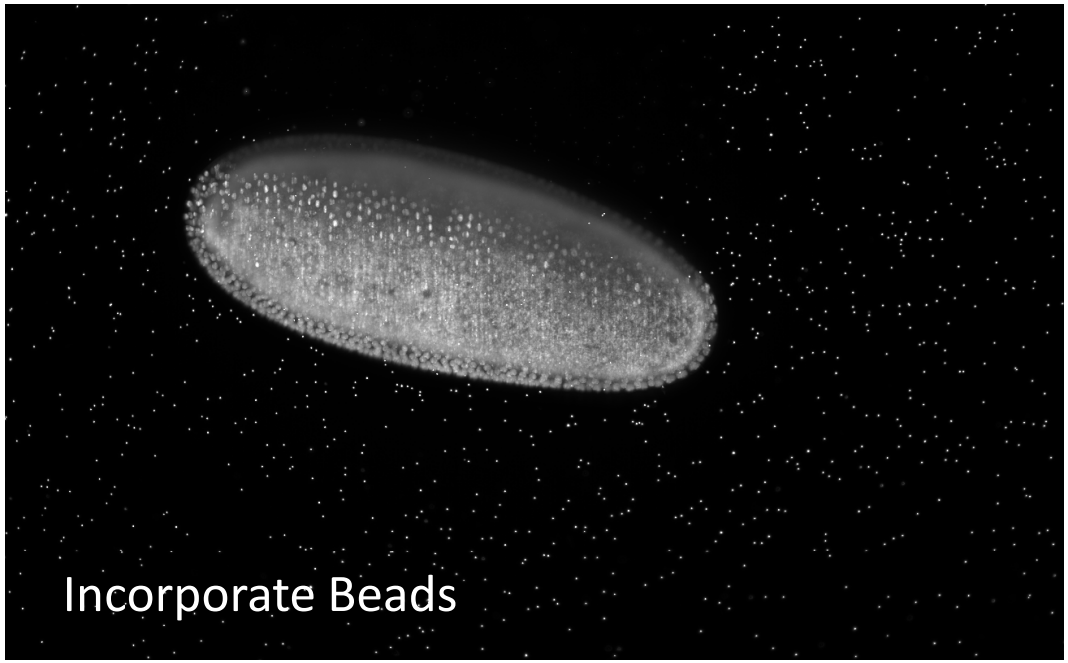

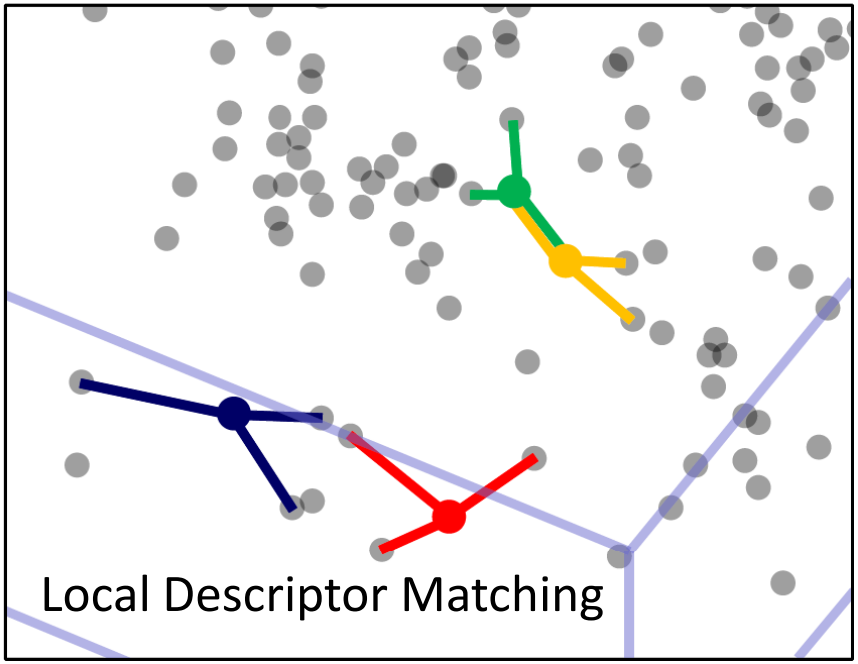

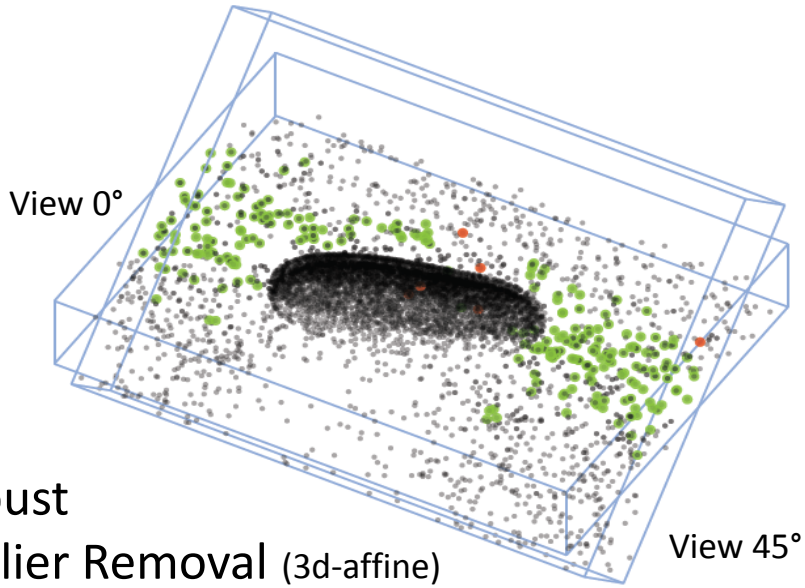

Robust
Outlier Removal (3d-affine)

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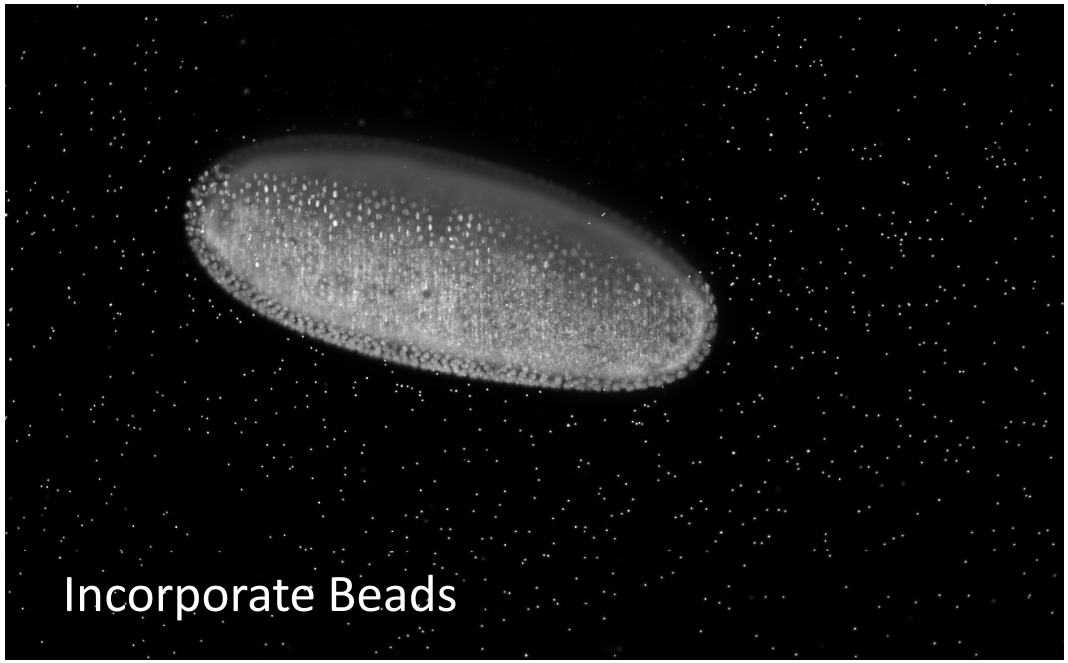

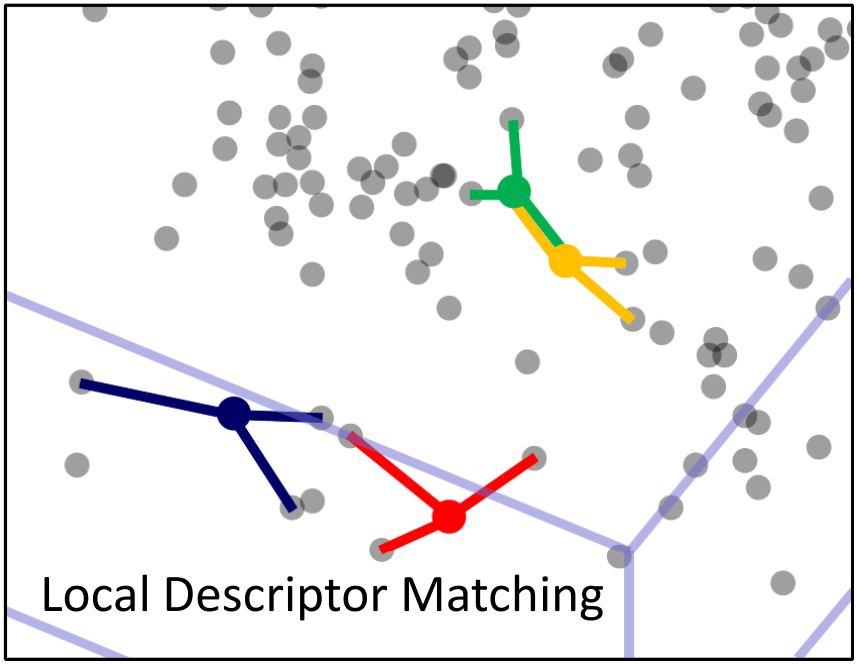

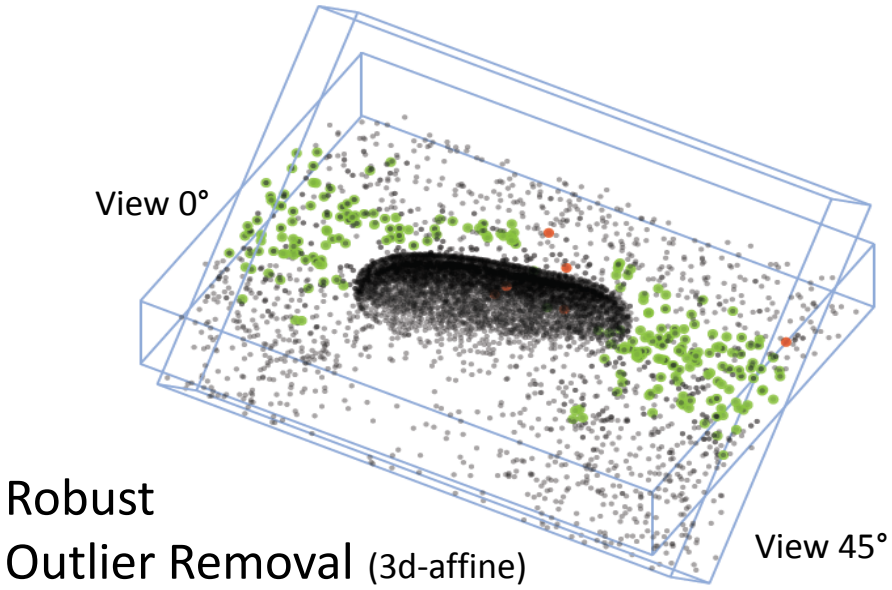

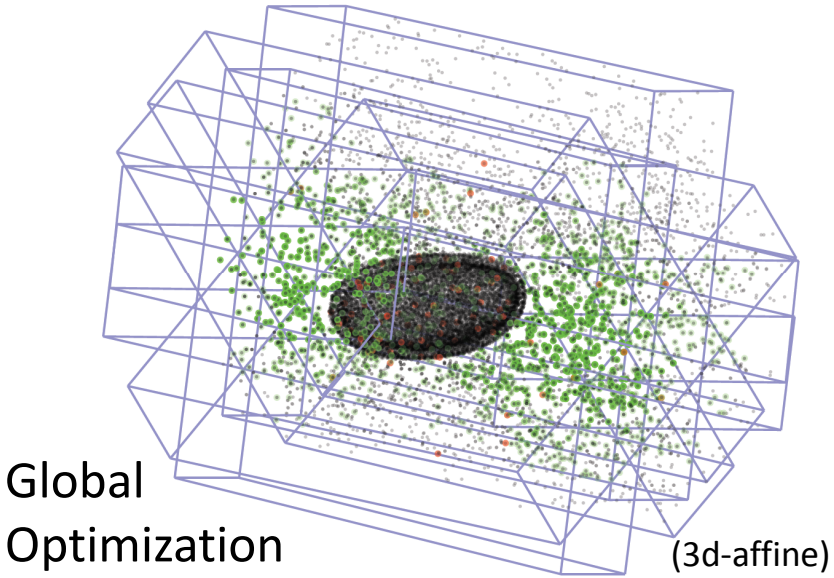

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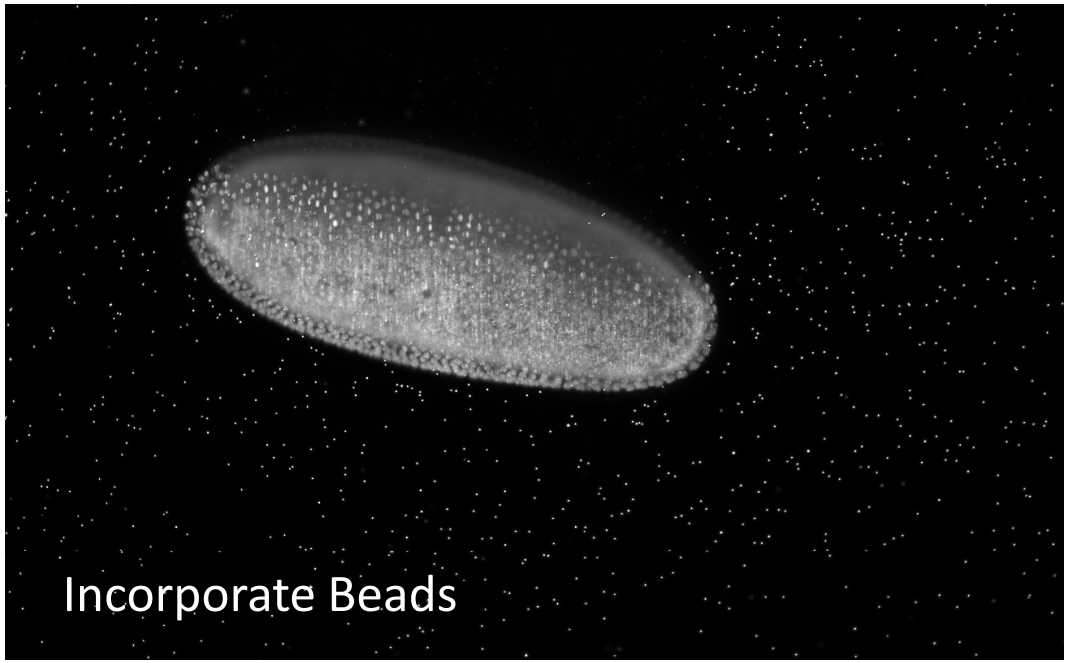
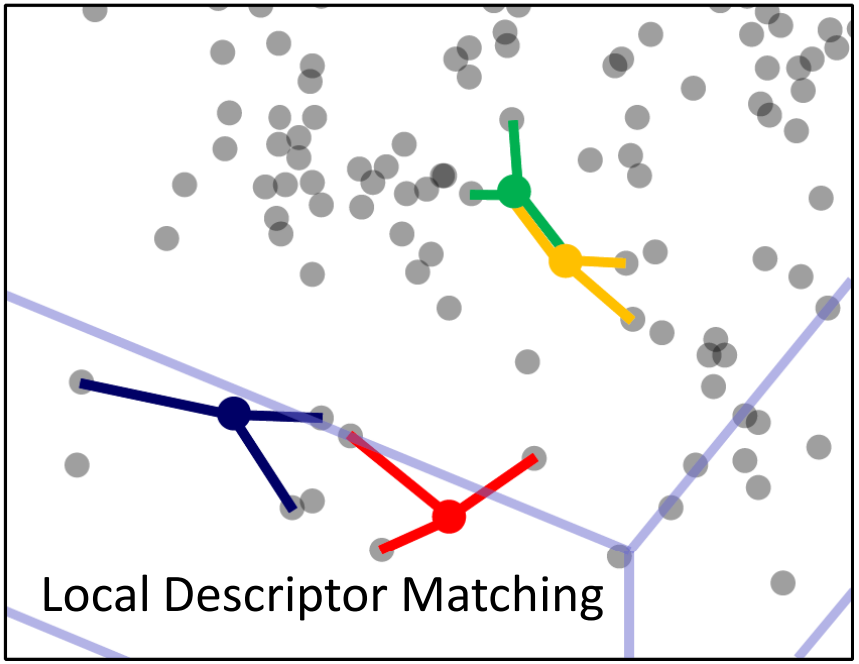
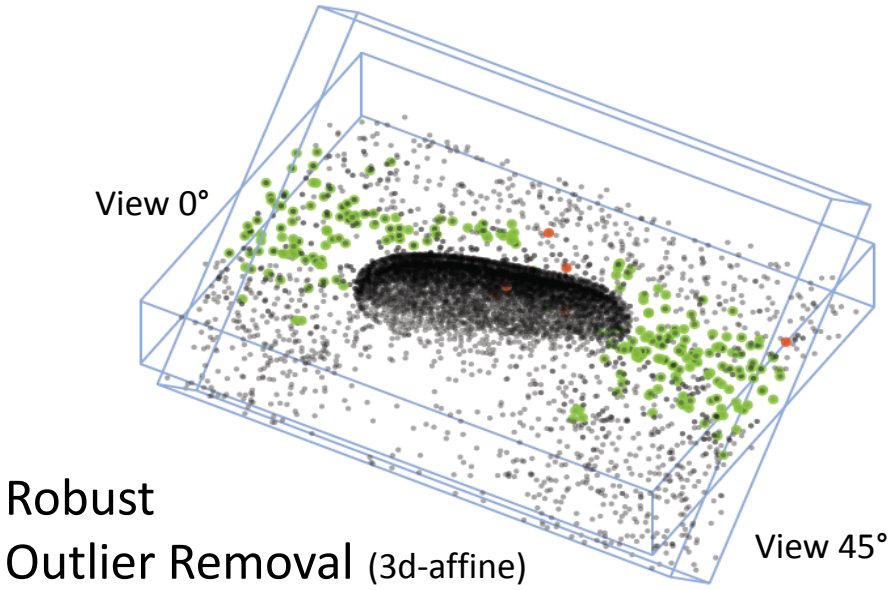
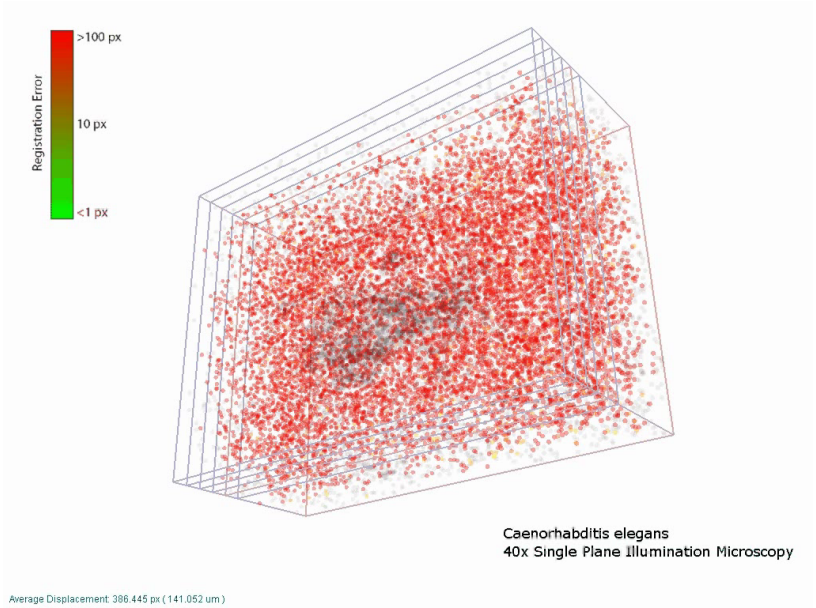






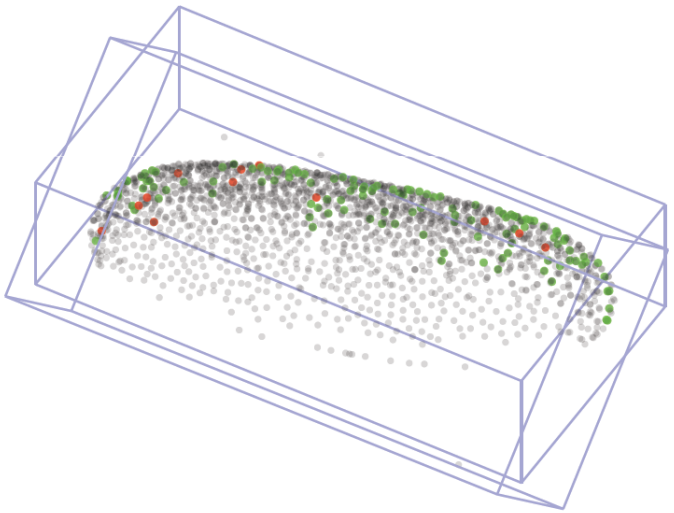


Nuclei based Registration

RANSAC

Drosophila

20x/0.5NA



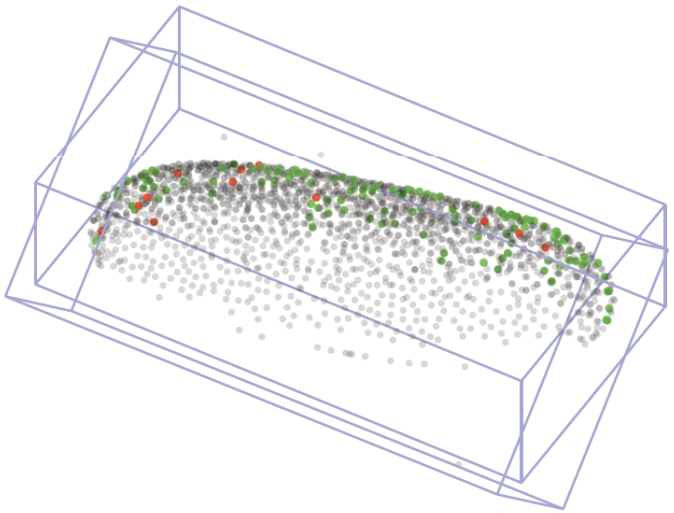
Correspondences	122
Average Error	**1,37 px**
Computation Time	112 seconds

Nuclei based Registration

RANSAC

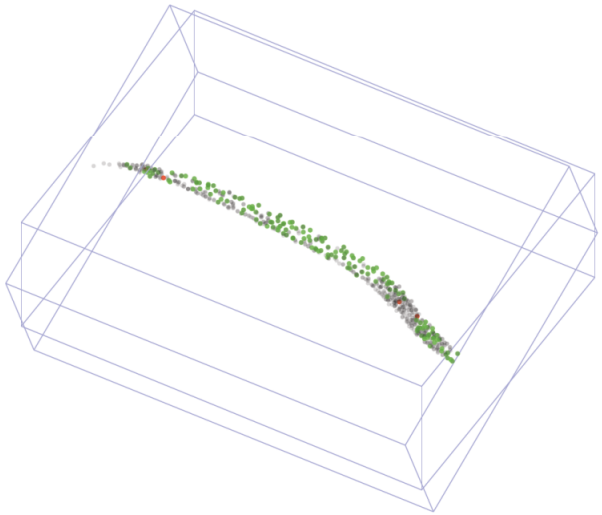
Drosophila

20x/0.5NA



C. elegans

100x/1.0NA

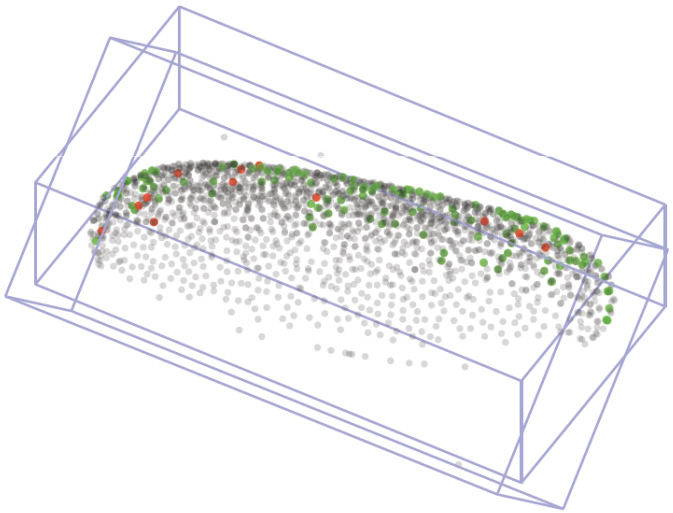


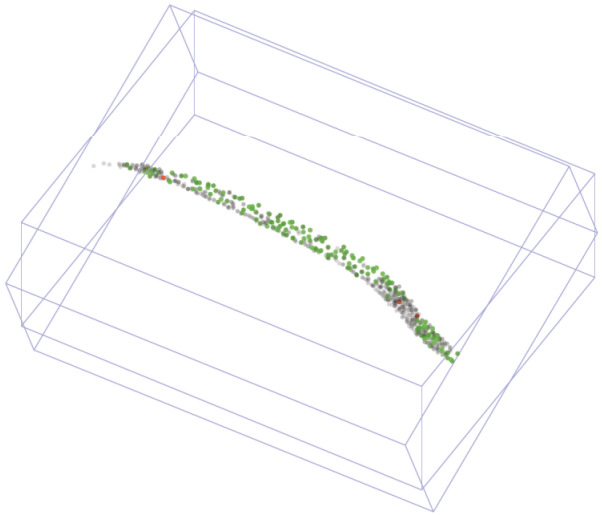
	Drosophila	C. elegans
Correspondences	122	156
Average Error	**1,37 px**	**1,80 px**
Computation Time	112 seconds	97 seconds

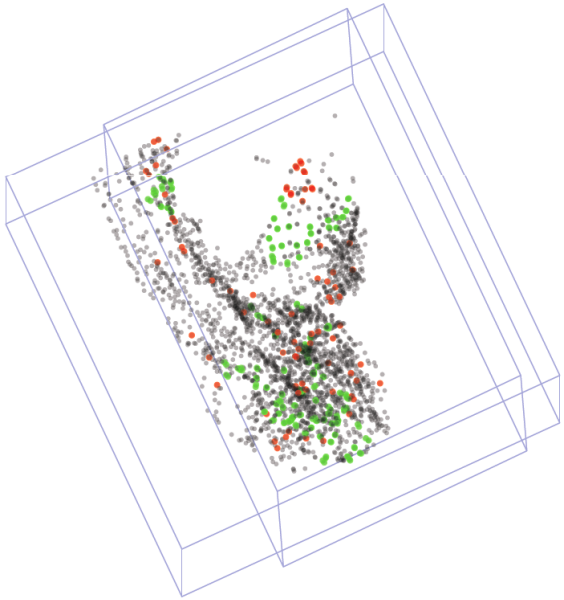
Nuclei based Registration

RANSAC

	Drosophila	C. elegans	Zebrafish 6dpf
	20x/0.5NA	100x/1.0NA	20x/0.5NA



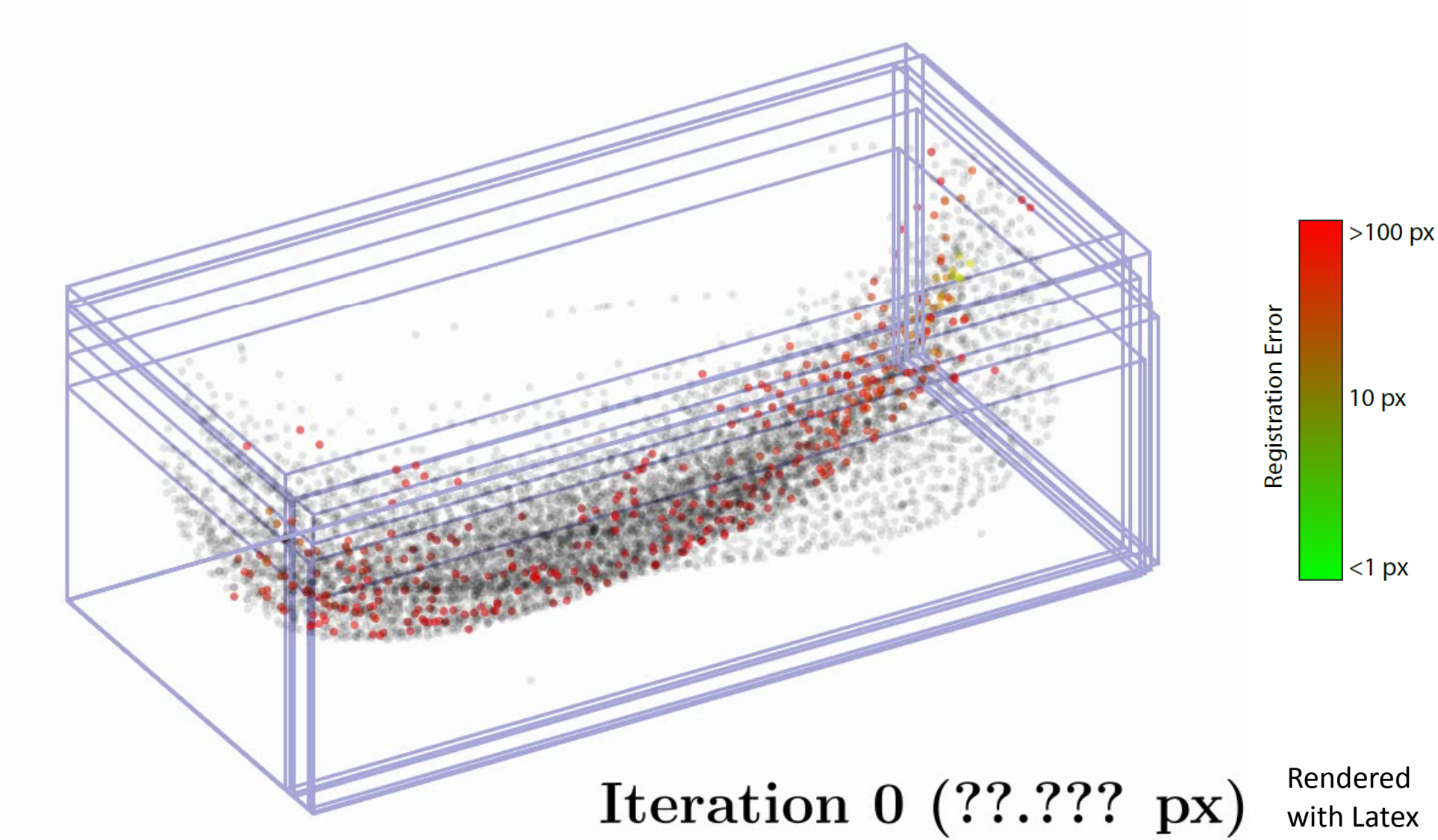




	Drosophila	C. elegans	Zebrafish 6dpf
Correspondences	122	156	92
Average Error	**1,37 px**	**1,80 px**	**2,97 px**
Computation Time	112 seconds	97 seconds	110 seconds

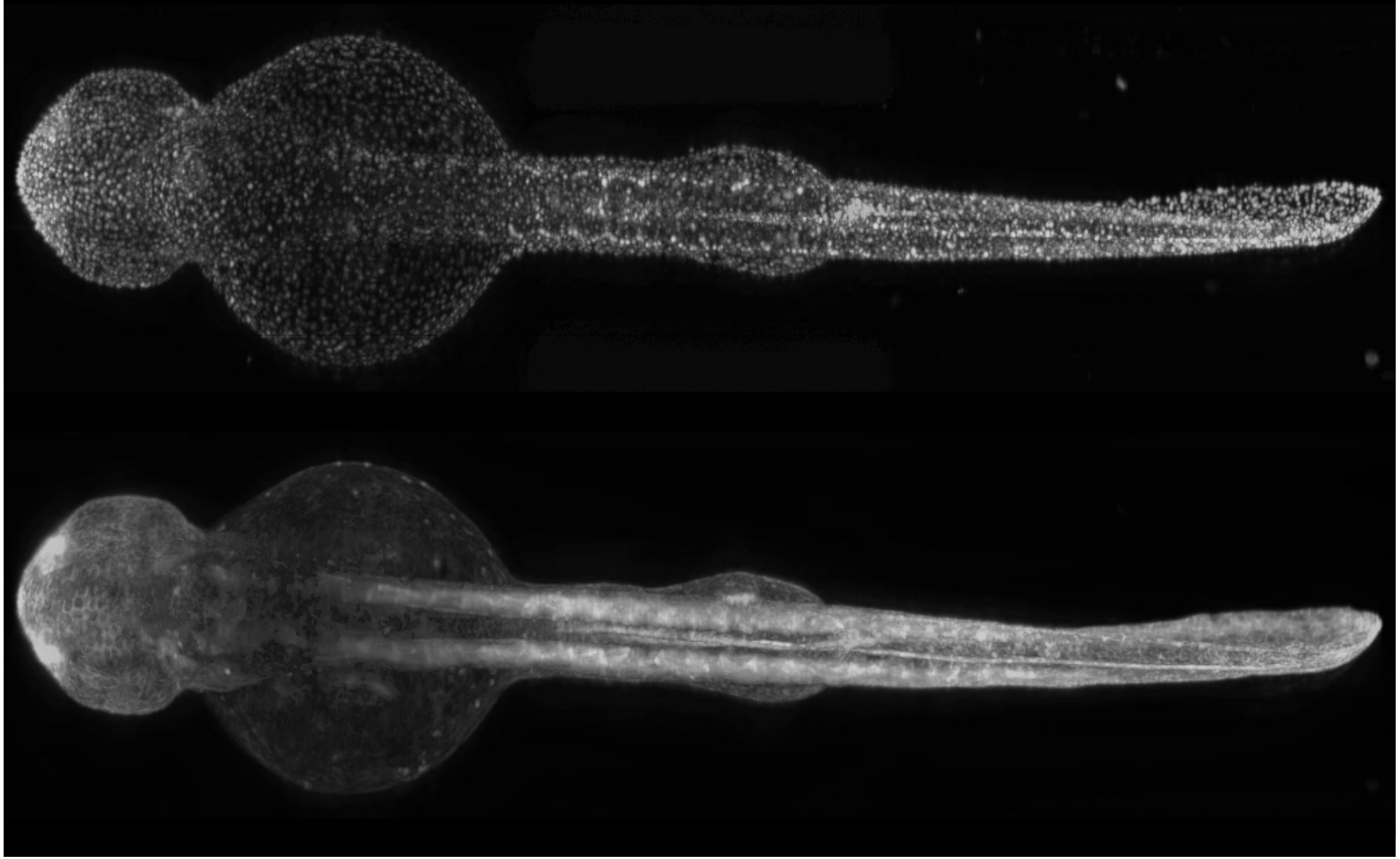
Nuclei based Registration

Global Optimization

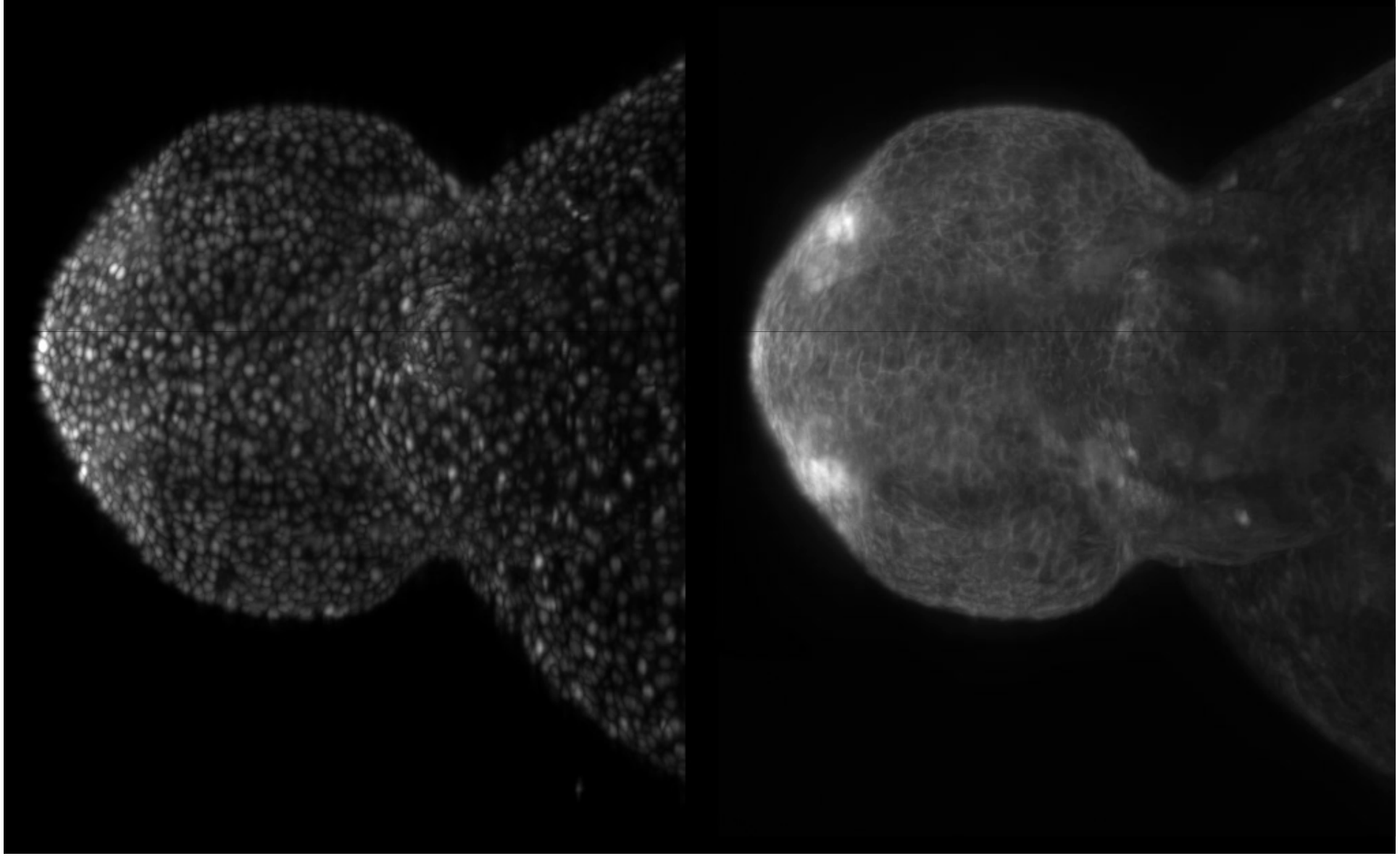


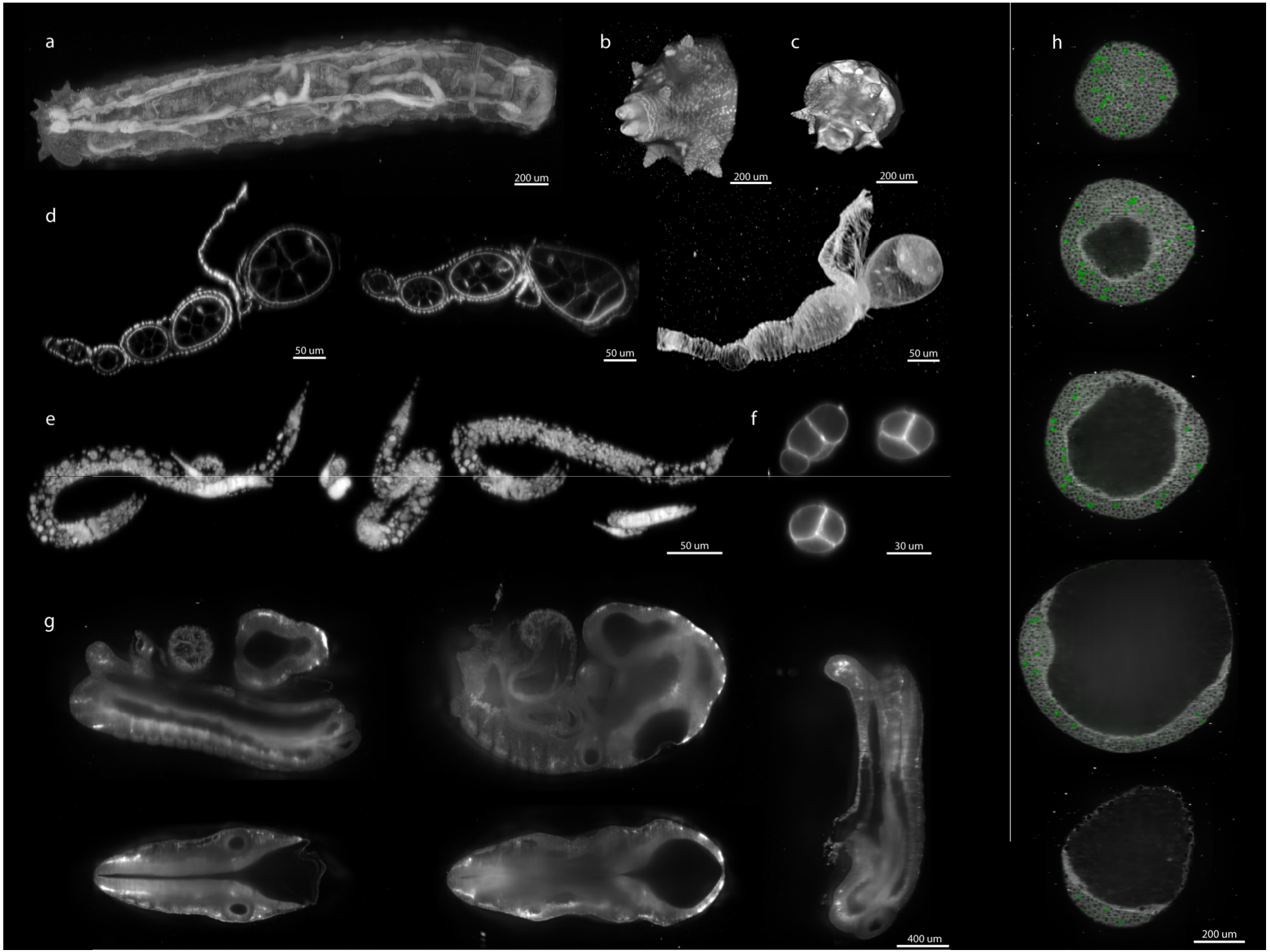
Rendered with Latex

Zebrafish 48 hours post fertilization



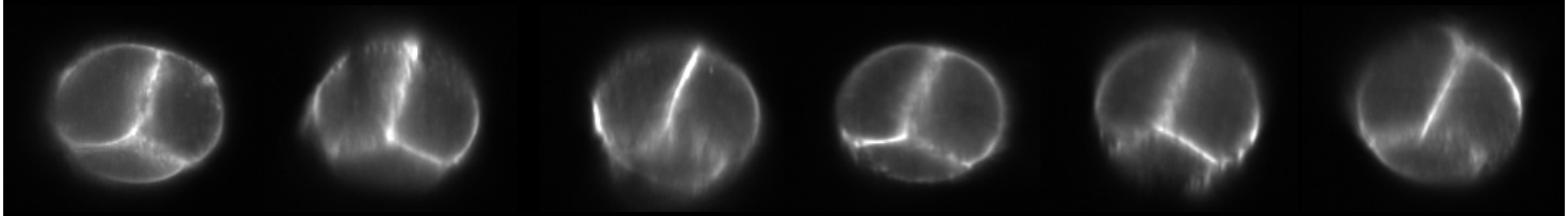
Zebrafish 48 hours post fertilization




a
200 um
b
200 um
c
200 um
d
50 um
50 um
50 um
e
50 um
f
30 um
g
400 um
h
200 um

Multi-View Deconvolution

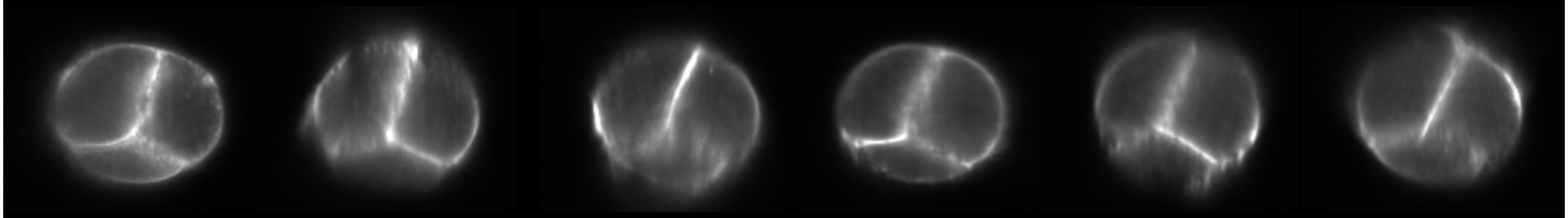
C. Elegans embryo
4-cell stage
Ph-GFP Lipid binding domain



Angle 0 | Angle 60 | Angle 120 | Angle 180 | Angle 240 | Angle 300

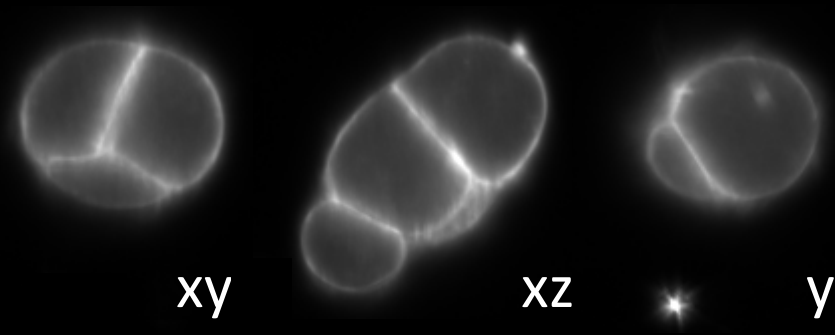
Multi-View Deconvolution

C. Elegans embryo
4-cell stage
Ph-GFP Lipid binding domain



Angle 0 Angle 60 Angle 120 Angle 180 Angle 240 Angle 300

Content based Fusion

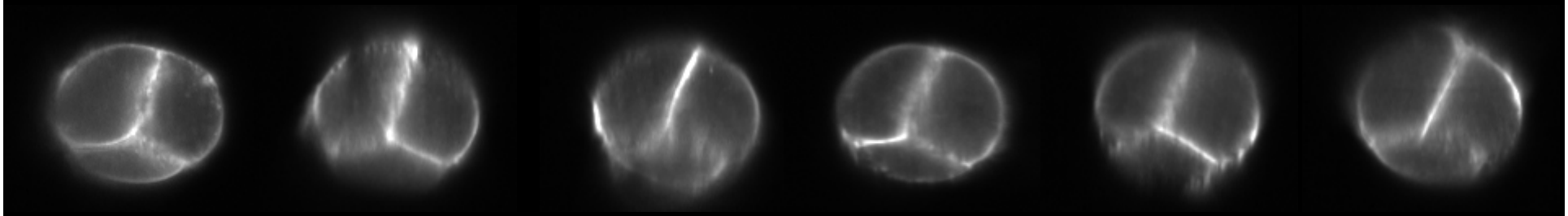


xy xz yz

S. Preibisch et.al. *Nature Methods* 2010.

Multi-View Deconvolution

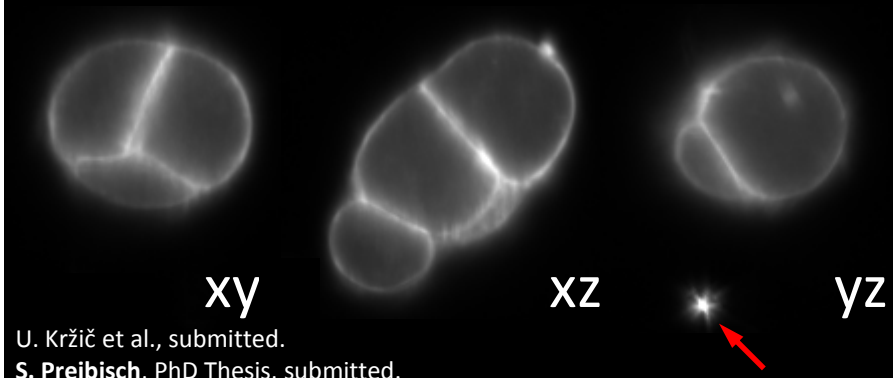
C. Elegans embryo
4-cell stage
Ph-GFP Lipid binding domain



Angle 0 | Angle 60 | Angle 120 | Angle 180 | Angle 240 | Angle 300

Deconvolution makes use of
Multiple Observations of the same Scene

Content based Fusion

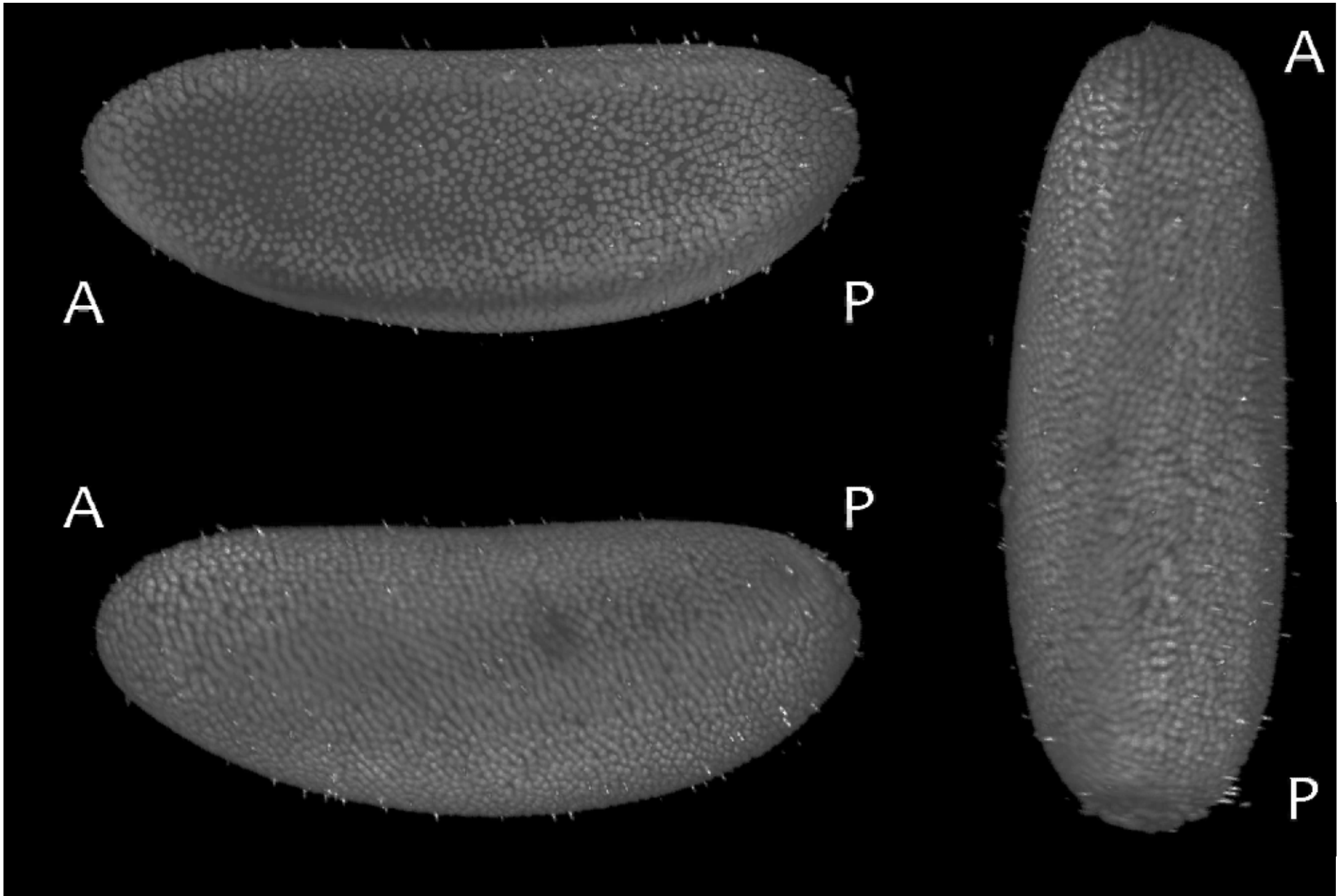


xy xz yz

Multi-View Deconvolution

xy xz yz

U. Kržič et al., submitted.
S. Preibisch, PhD Thesis, submitted.



Bead-based time-lapse registration

One machine with 8 cores, 64 GB RAM

12 hours for 249 time points

Stephan Saalfeld

Implementation in ImgLib

Tobias Pietzsch

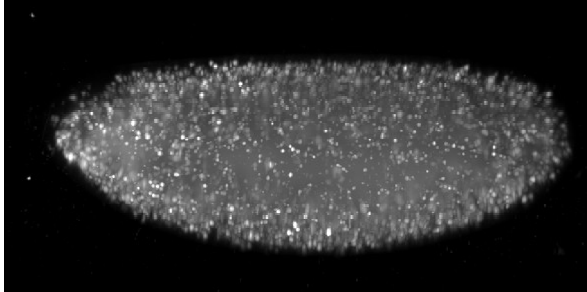
- Open-Source + Plugins available through Fiji
- **ImgLib** – Generic Image Processing in Java
- Dimension-, storage- and datatype independent
- Generic, more algorithm-like programming
- Framework underlying the new NIH-funded **ImageJ2**

Preibisch, Tomancak, Saalfeld , "Into ImgLib – Generic Programming in Java", *Proceedings of the ImageJ User and Developer Conference, 2010.*

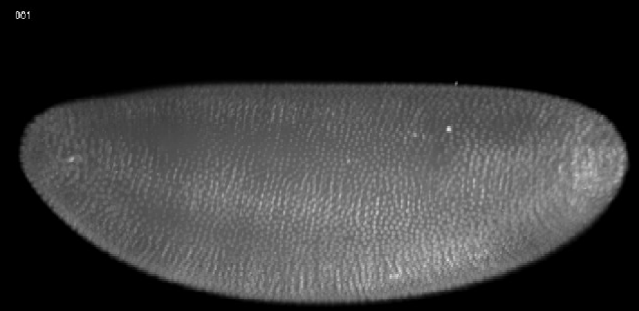
What are we doing now with SPIM?

Fluorescent reporters

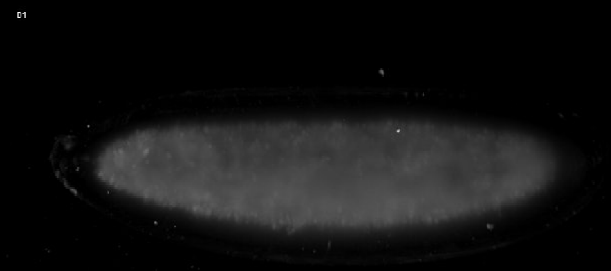
Bicoid
(patterning)



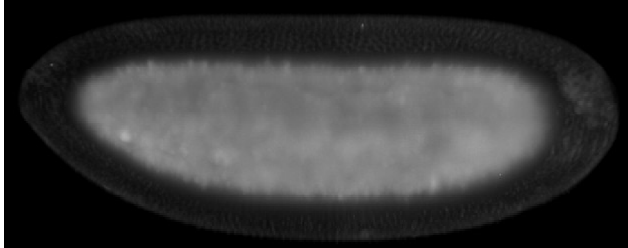
Polo Kinase
(mitotic activity)



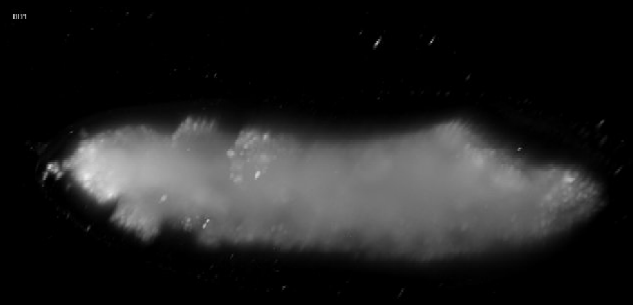
Senseless
(peripheral nervous system)



Cysteine String Proteine
(membrane protein)



Neuronal Synaptobrevin
(neurotransmitter release)



Ejsmont, R. K., Sarov, M., Winkler, S., Lipinski, K. A., Tomancak, P., *Nature Methods* **6**, 435–437 (2009).
Preibisch S., Saalfeld S., Schindelin J., Tomancak P., *Nature Methods* **7**(6), 418-419 (2010).

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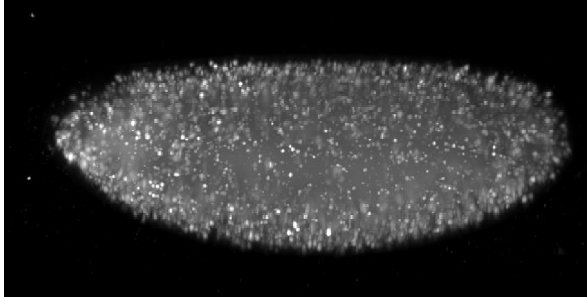

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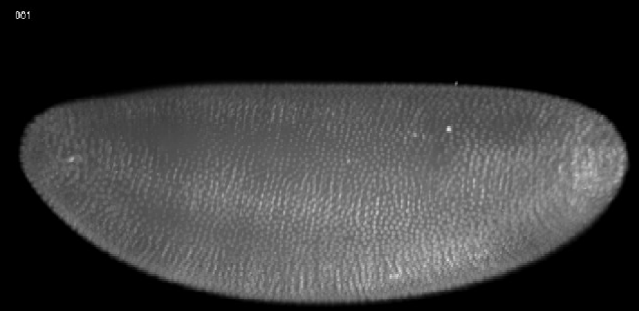
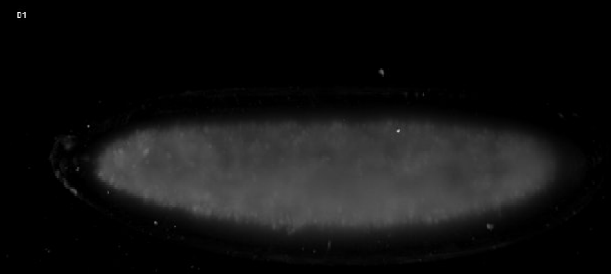


Senseless
(peripheral nervous system)



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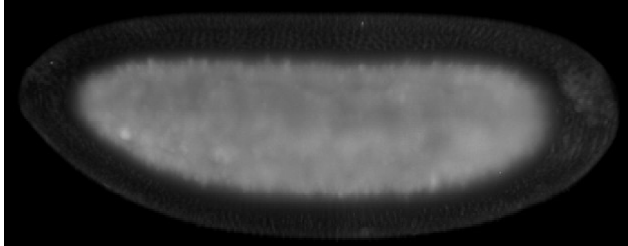

Neuronal Synaptobrevin
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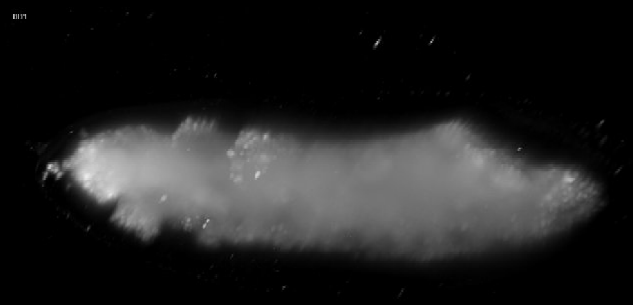

Omero!

Ejsmont, R. K., Sarov, M., Winkler, S., Lipinski, K. A., Tomancak, P., *Nature Methods* **6**, 435–437 (2009).
Preibisch S., Saalfeld S., Schindelin J., Tomancak P., *Nature Methods* **7**(6), 418-419 (2010).

What is new, what should be stored?

- Multi-View acquisitions
- Multi-Channel acquisitions
- Time-lapse acquisitions
  - Preview
  - Compression?
- Acquisition speed is very high over 24 hours
  - 5-50 fps @ 1 Megapixel (soon 4-8 Megapixel)
    - 0,5-4 TB raw data per experiment
  - 100s of terabytes of data
- Good news: Data is usually accessed sequentially

What is new, what should be stored?

- Registration
  - Rotation axis
  - Rotation angle (if known)
  - At least affine transformation models for each view
  - Higher-order transformation models?
  - Point detections for each view?
  - Exemplary overlays to visualize registration quality
  - Keep the original stacks!

What is new, what should be stored?

- Fusion
  - Illumination direction (modeling of light)
  - Light sheet type (gauss, bessel, single/multi-photon)
  - Light sheet thickness
  - Embedding medium
  - Point spread functions of each view
  - Point spread function is variant
  - Fused output image + preview (3d renderings?)

What is the future of SPIM?

- Many research groups build their own SPIM microscopes or derivatives
- Own conference series
- Focus starts to turn from microscope development into normal application
- There is going to be a commercial microscope by Zeiss at some point
- Great chance if OMERO supports such image data – currently it is more or less a mess to deal with those amounts of data

Acknowledgements

Pavel Tomancak's group
- **Stephan Saalfeld**
- **Tobias Pietzsch**
- **Michael Weber**

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- **Johannes Schindelin**
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Jan Huisken

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- Chris Power
- Olaf Selchow

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- Jan Peychl

LOCI Team
- Curtis Rueden
- Kevin Eliceiri

Uros Krzic (EMBL)

Janelia Farm, EMBL, ETH Zürich, MPI-CBG, LOCI Madison for facilitating Hackathons

http://pacific.mpi-cbg.de/