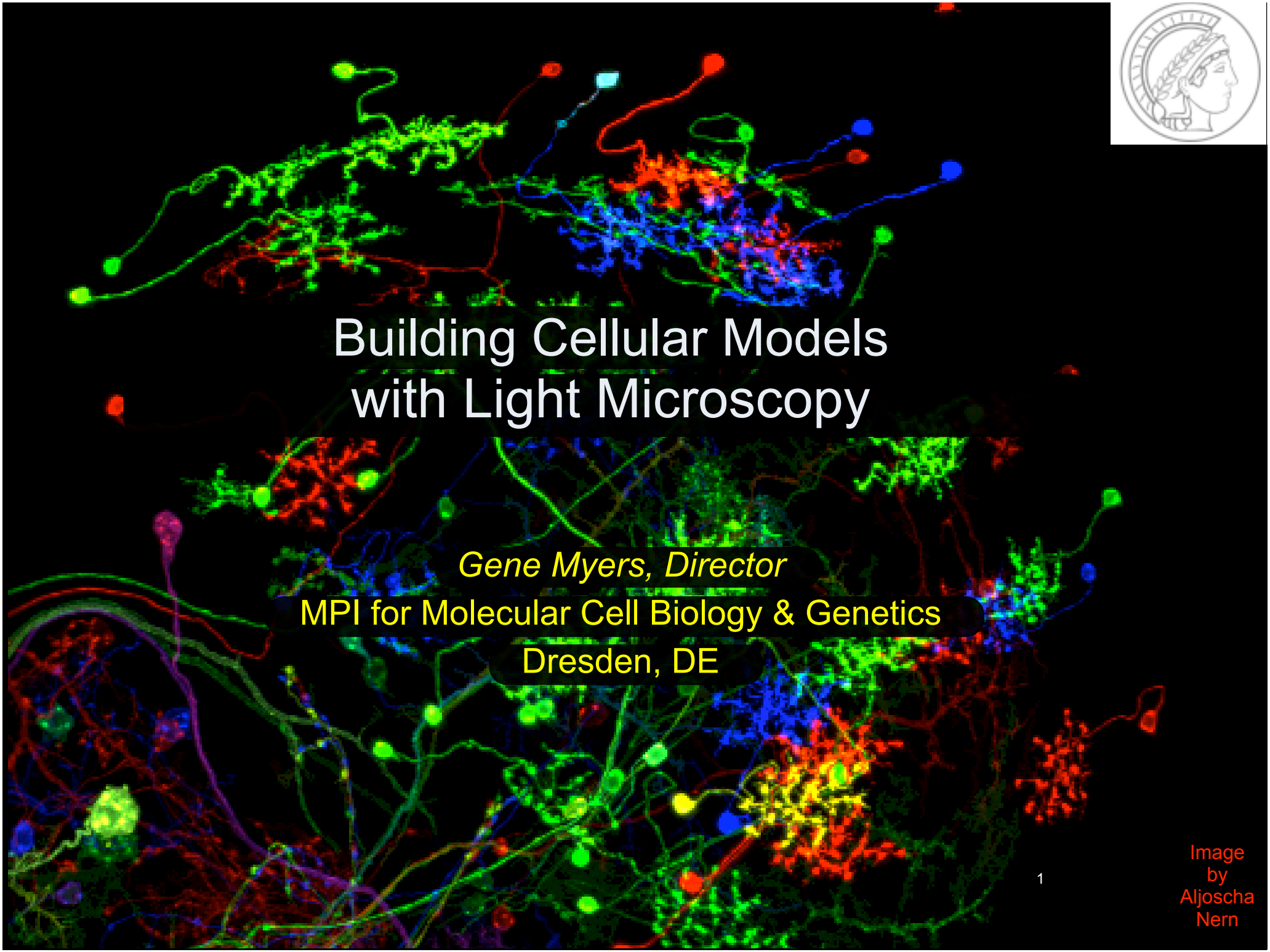


# Building Cellular Models with Light Microscopy

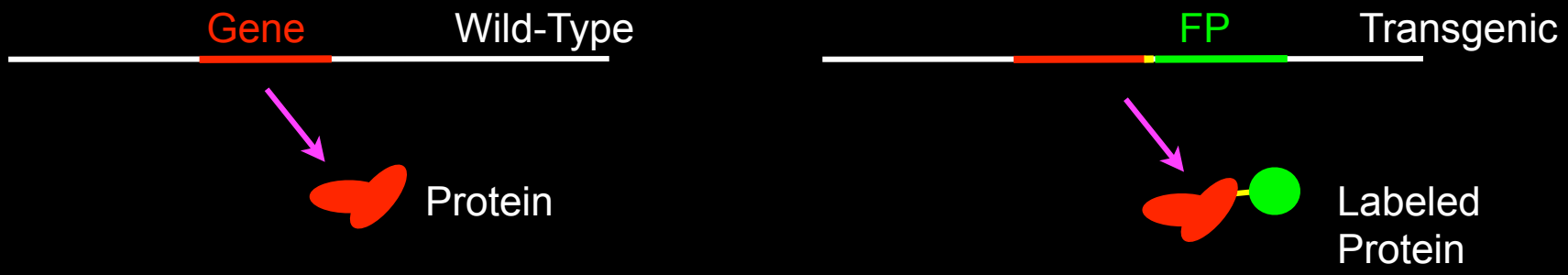
*Gene Myers, Director*
MPI for Molecular Cell Biology & Genetics
Dresden, DE



Image by Aljoscha Nern

# Why BioImage Analysis?

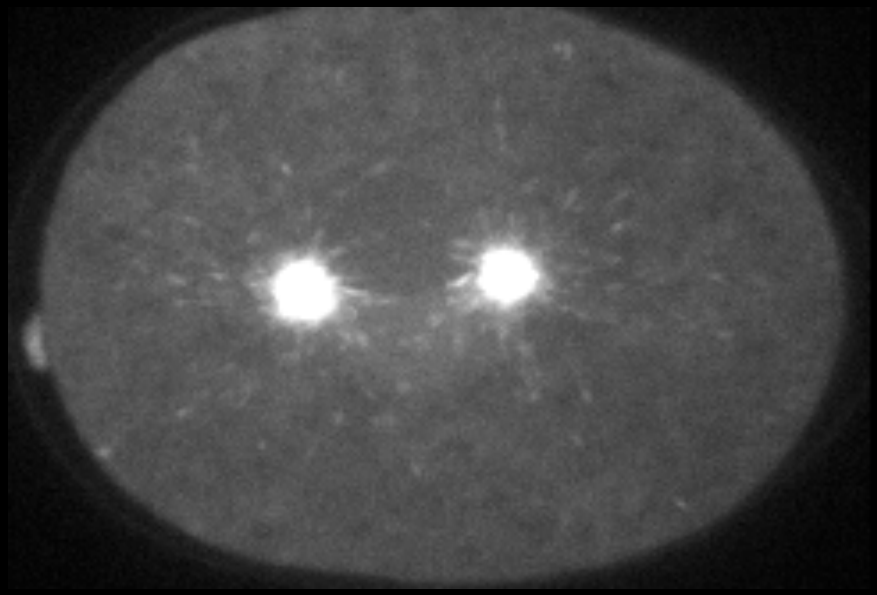
- A very significant outcome of the genome projects is that they permit us, using recombinant genetics, to label any genomic entity of interest.



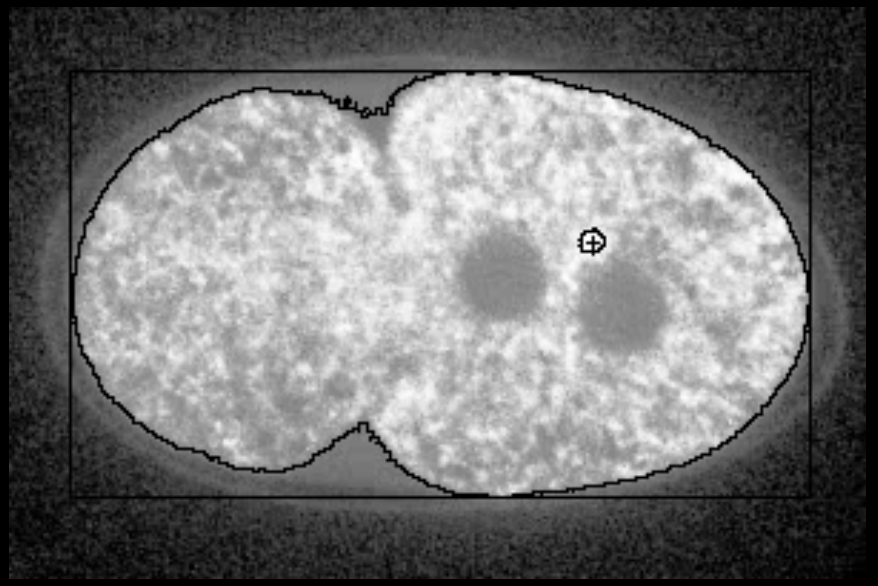

- And we can observe these with current microscopes, to understand function, location, force, signaling, ..
- Interpreting the seas of imagery so produced is computationally hard and increasingly more urgent.

# E.g., First Mitotic Division of C.elegans

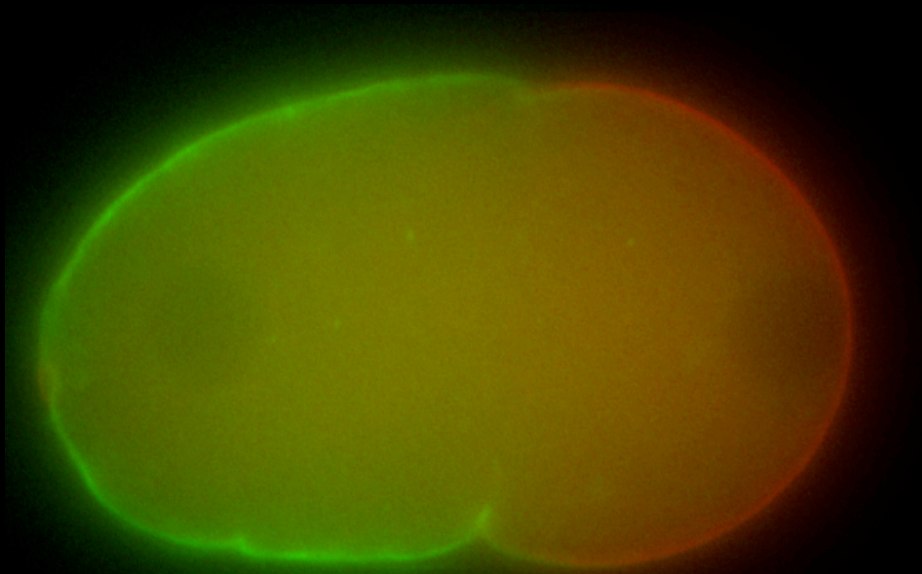
Tony Hyman Lab



EB1 labelled tubulin fibers.



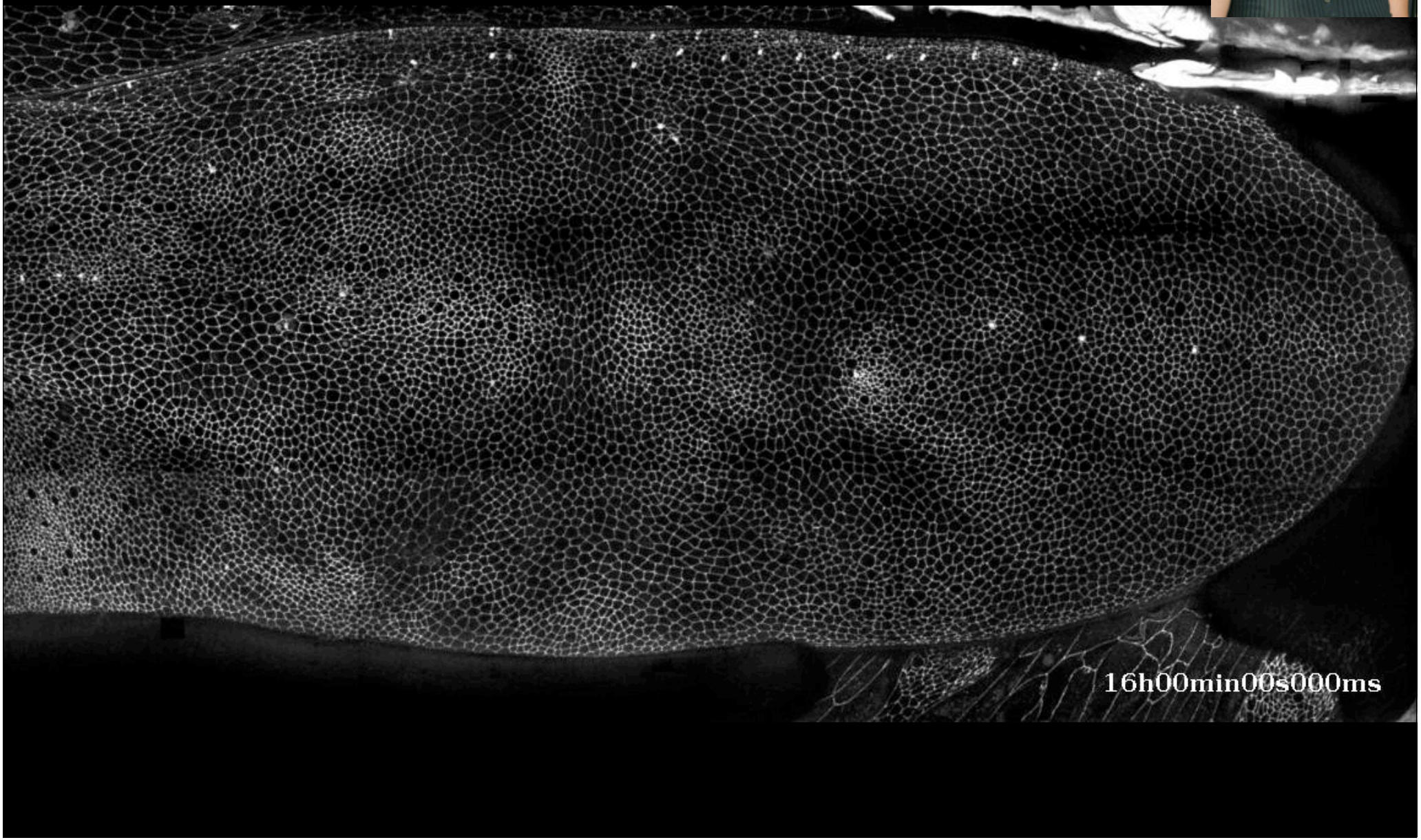
Gamma-tubulin labelled centrosomes



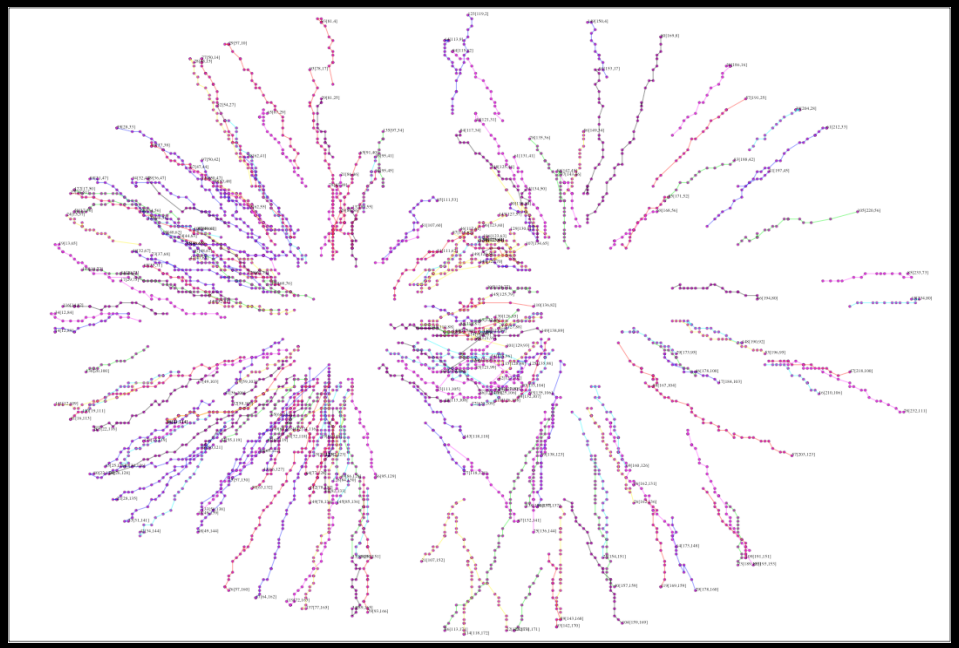
Par2-Par6 labelled membranes

# E.g., Fly Wing Development

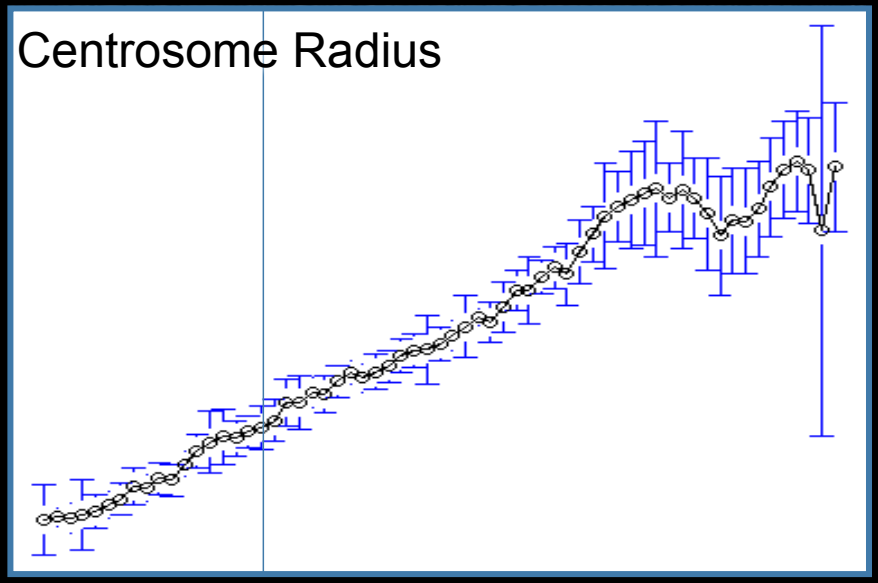
Suzanne
Eaton Lab



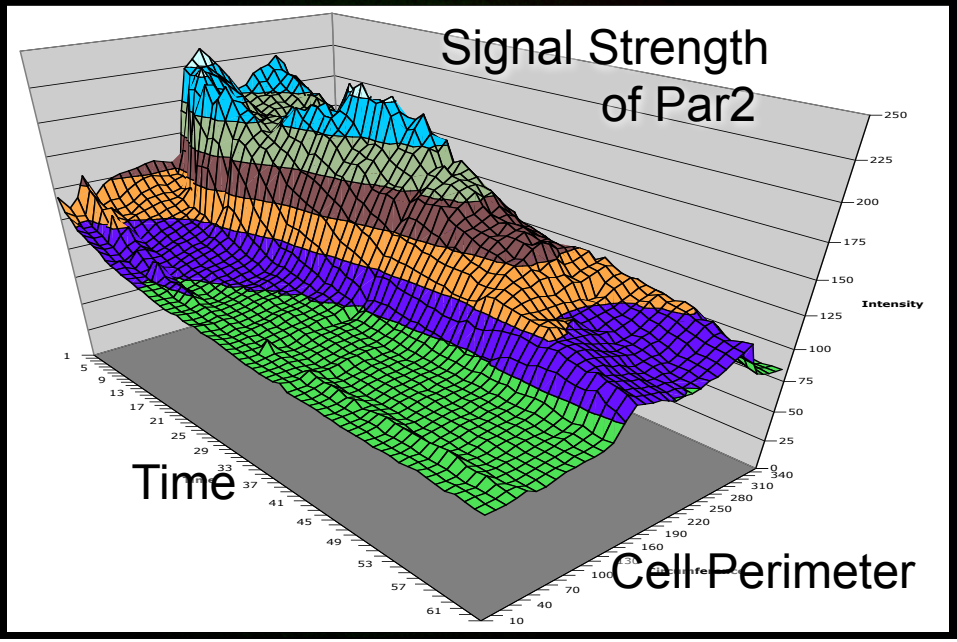
# E.g., First Mitotic Division of C.elegans



EB1 labelled tubulin fibers.




Gamma-tubulin labelled centrosomes

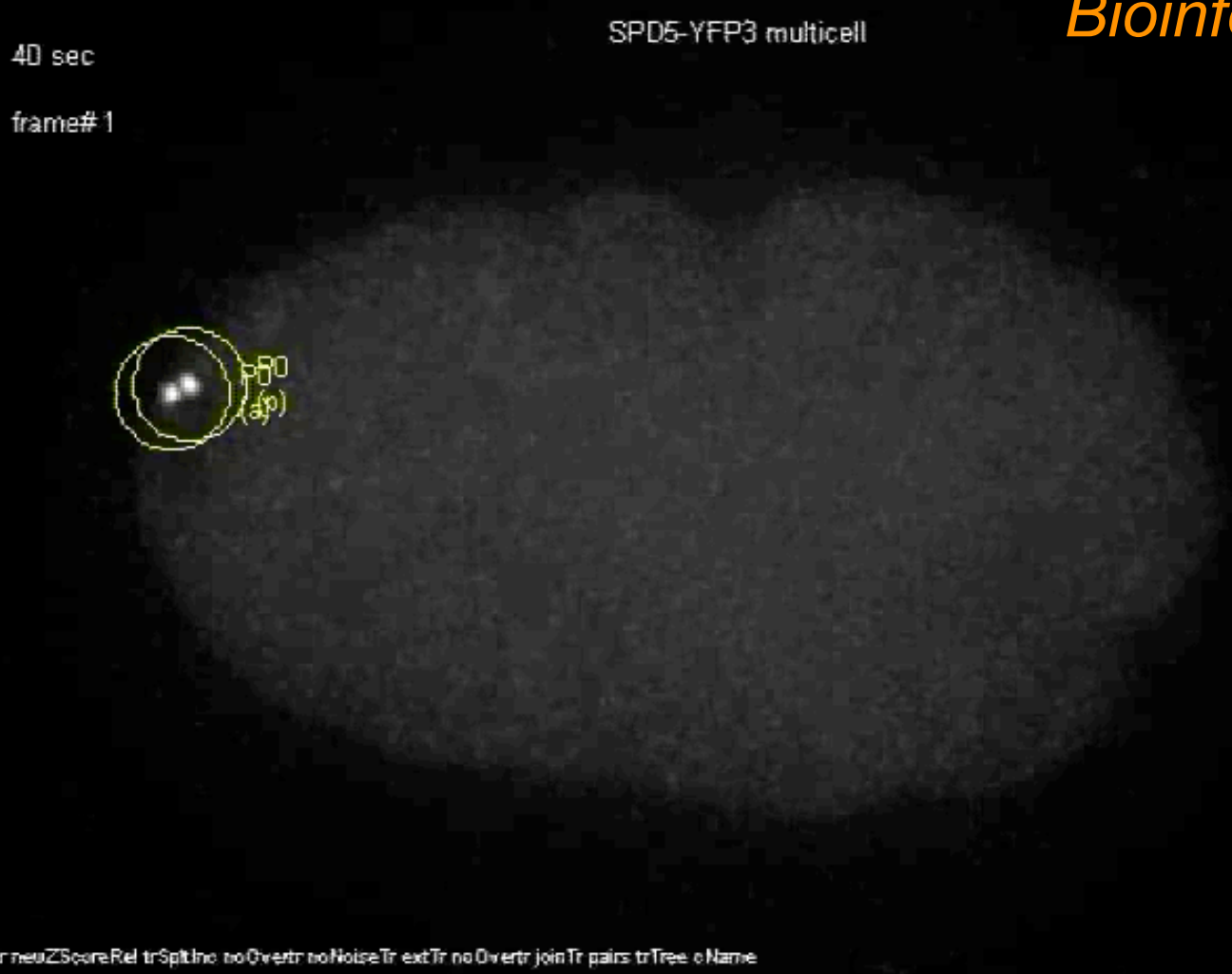



Par2-Par6 labelled membranes

# Tracking Centrosomes During Worm Embryogenesis

w. Steffan Jaench (Hyman & Jülicher)

*Bioinformatics* 26(12) (2010)



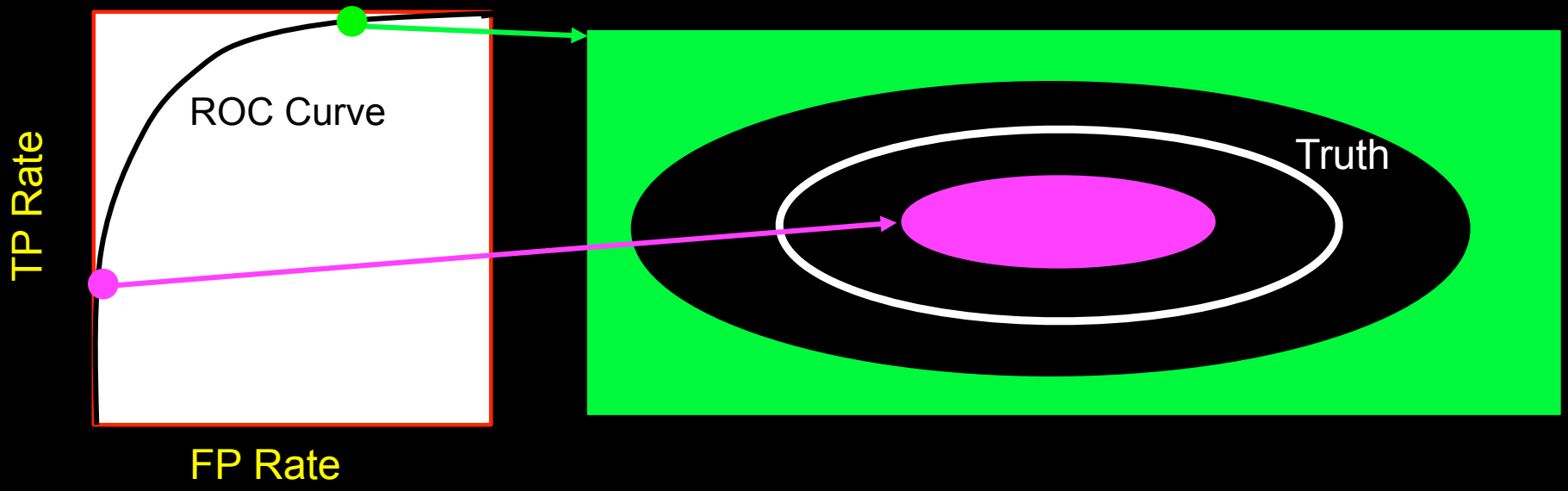

Errors
0% to 8 cells
6.2% to 16 cells



movie

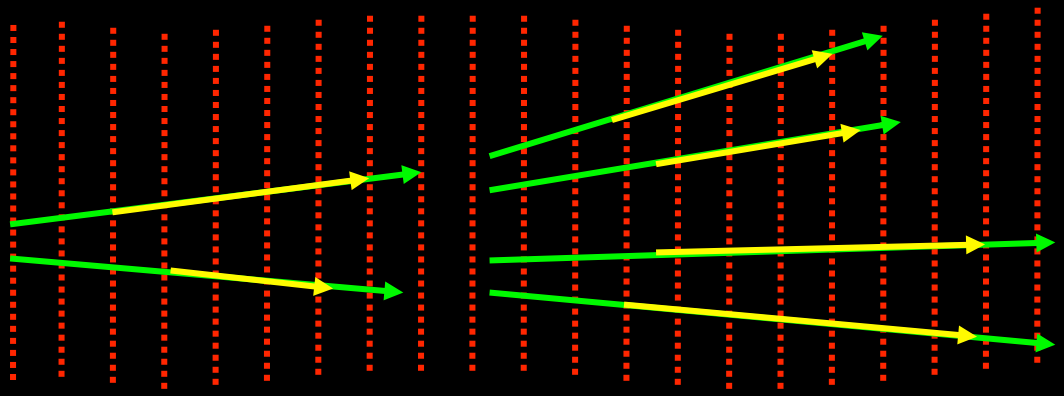
# Layered Inference

- Most heuristics can be tuned to report what is (almost) certainly false and what is (almost) certainly true.

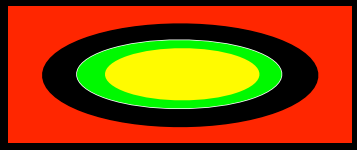



- Using a series of such heuristics, repeatedly enlarge what is true and/or eliminate more of what is false
- Can base inferences on the knowledge within the current state

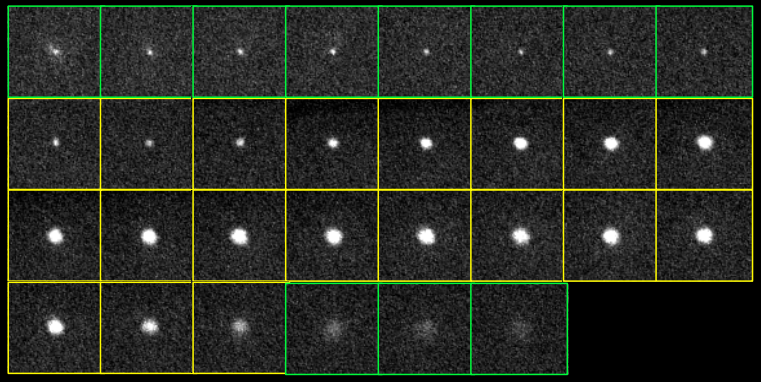
# Tracking Centrosomes



Overdetected spots

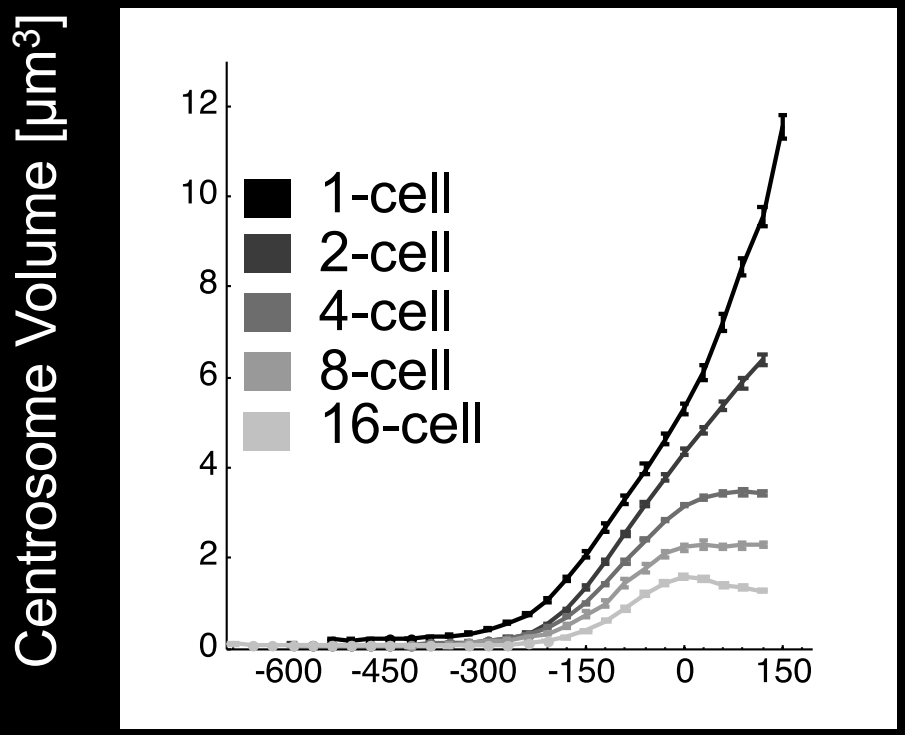


* Find core subtracks you are sure of
  (spatial track = appearance track)
* Learn statistics of true deltas
* Extrapolate using learned statistics

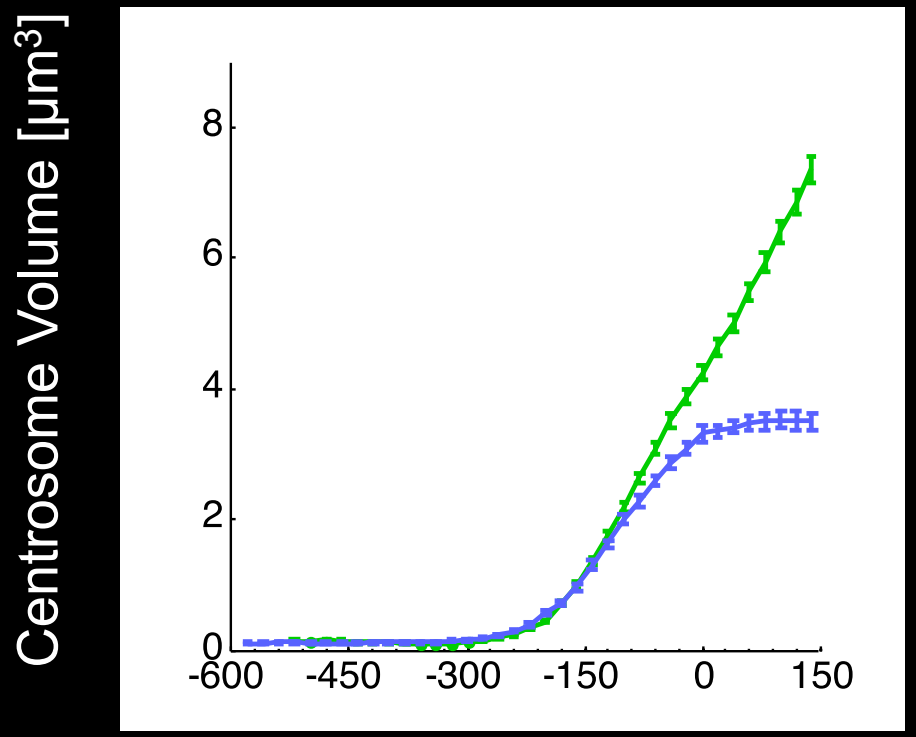


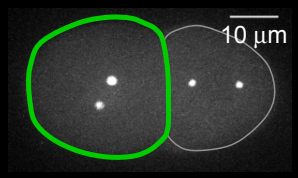
# Centrosome Size over Time

***Centrosome Size through Development***

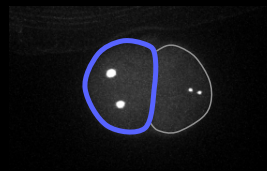



***Centrosome Size in Smaller AB Cells***



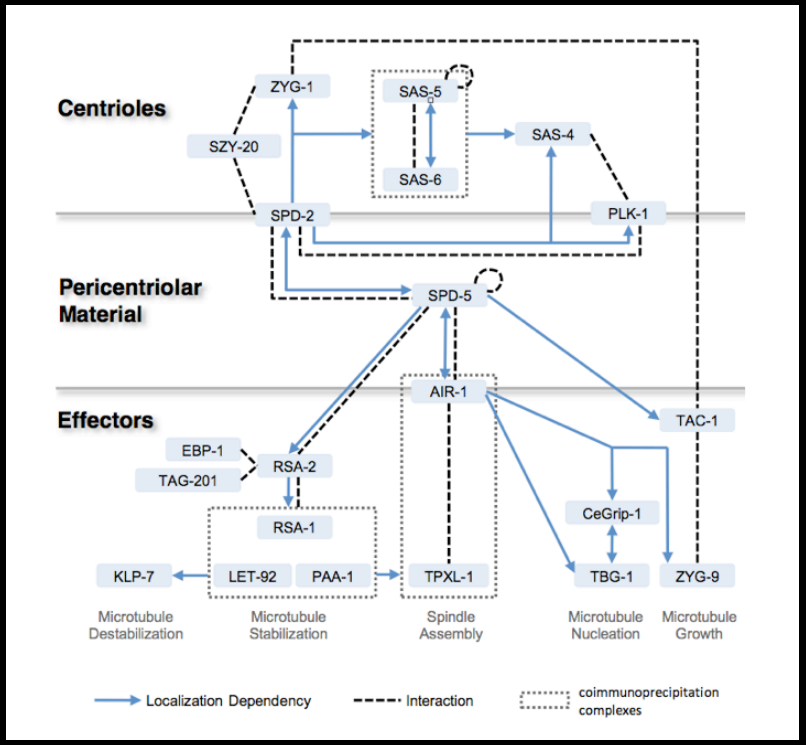




Wild-Type



*ani-2*(RNAi)

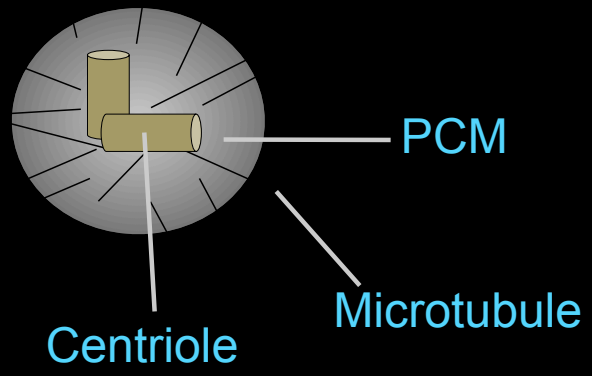
# What sets the size of a centrosome?




## Limiting Component Hypothesis:

**The available amount of one or more centrosome components in the cytoplasm determines centrosome size.**

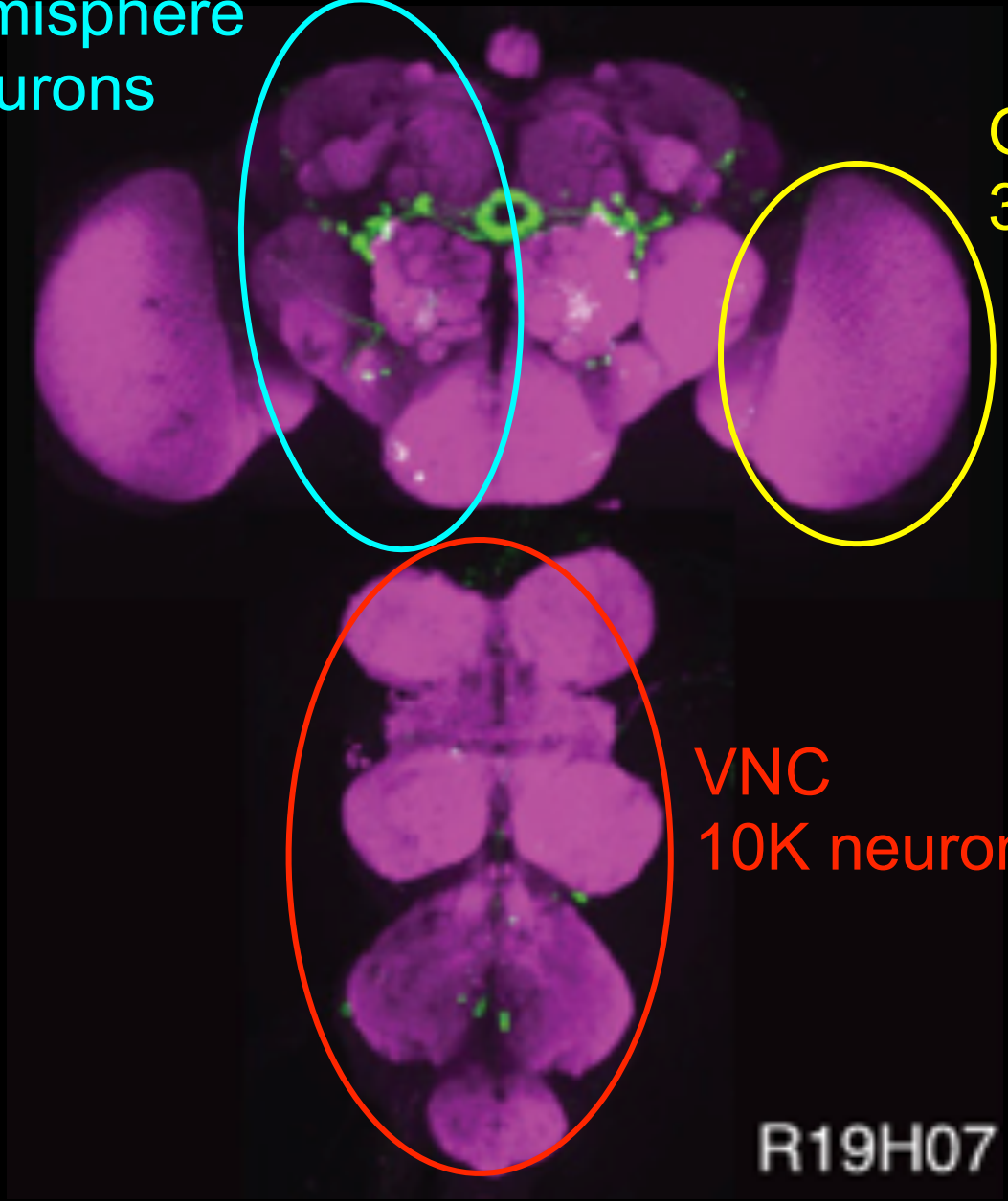
153 conditions
1409 embryos
5.5M images



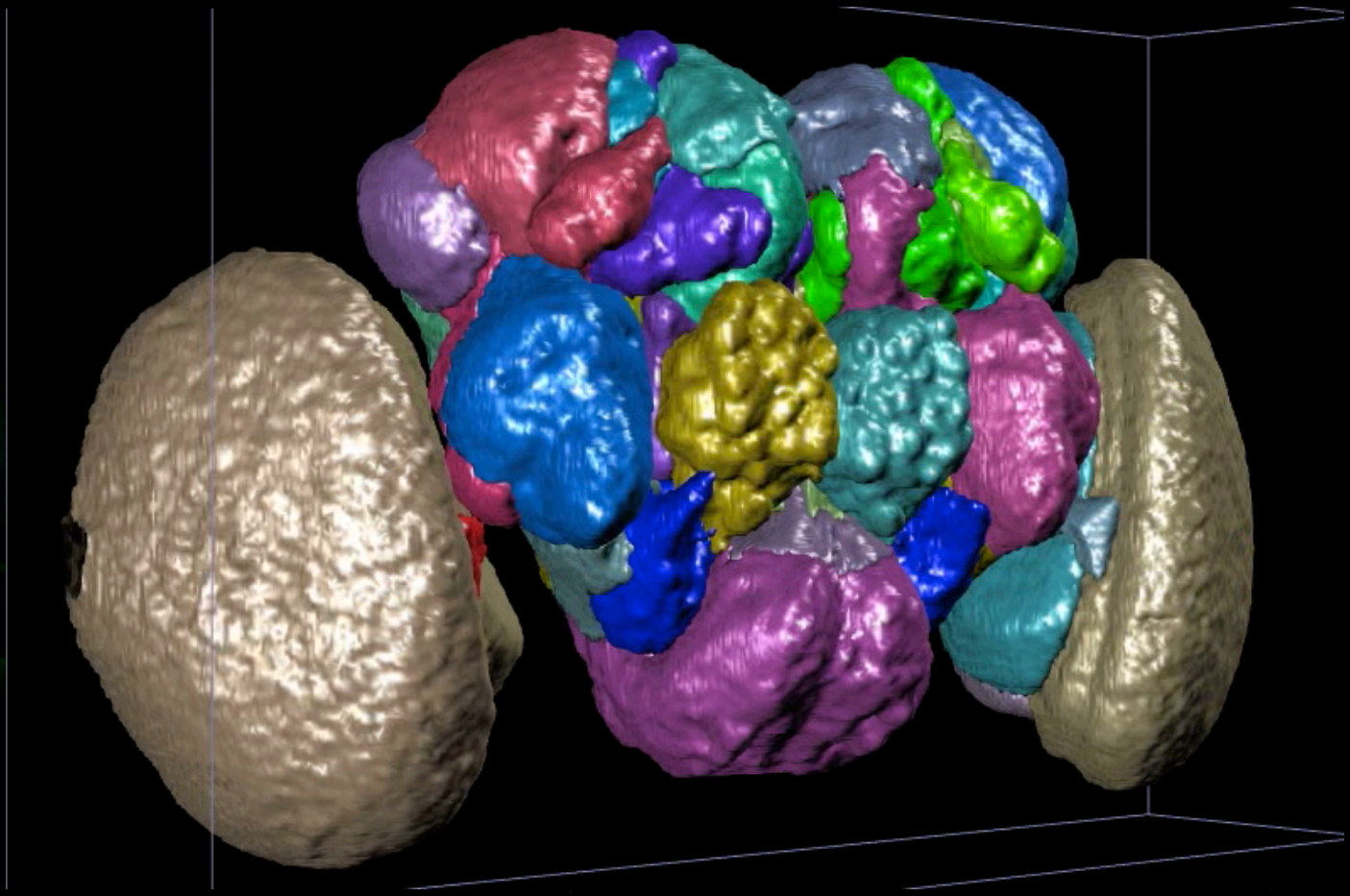

- Size proportional to cell volume
- Size independent of cell type
- Volume independent of # of centrosomes
- Turning off transcription induces no change
- spd-2, spd-5, air-1 all needed
- up/down expression:
  air-1 no, spd-2 yes, spd-5 no

*Currents Biology* 21 (2011)

# The Structure Of A Fly's Brain I



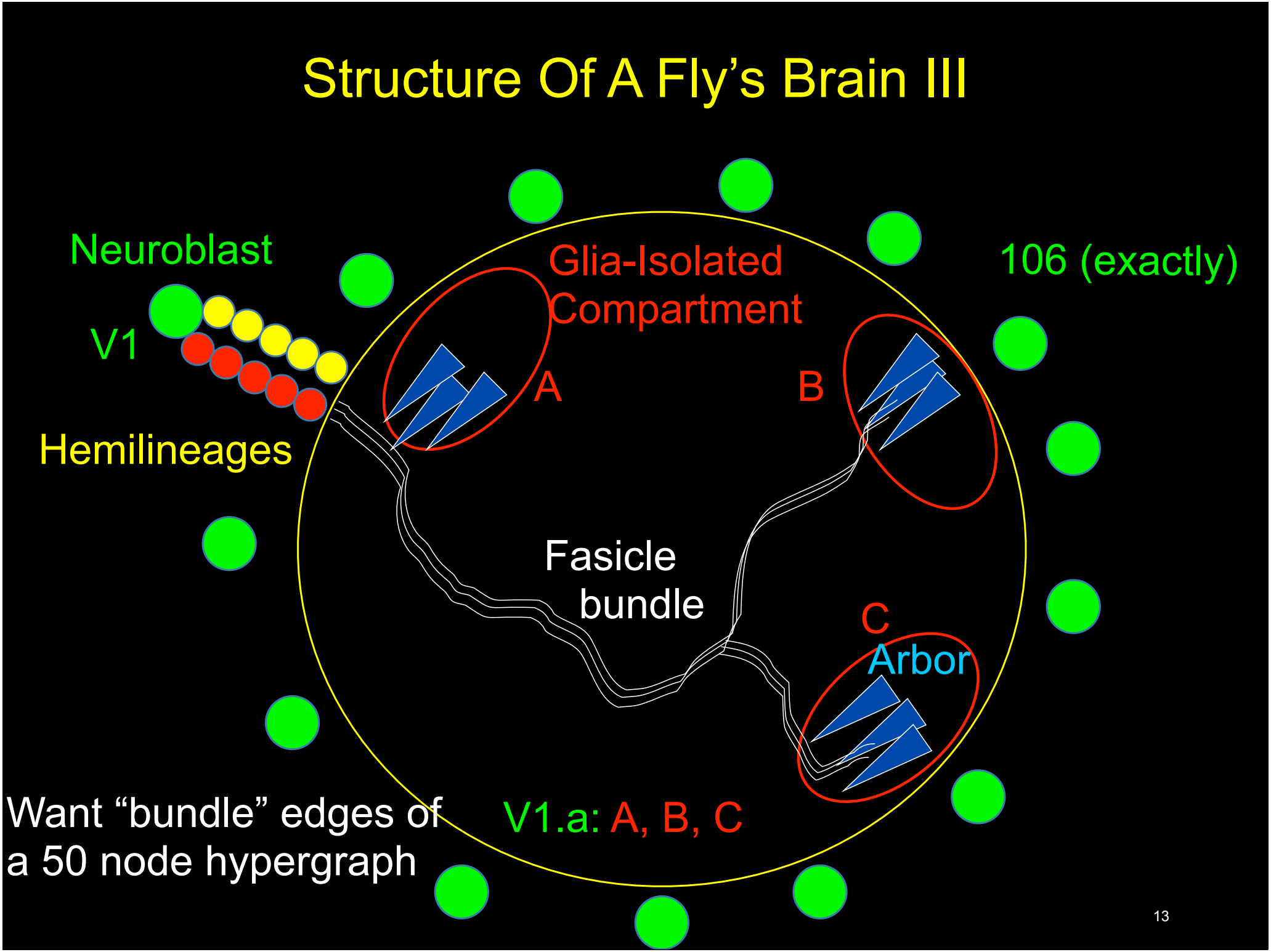
# Structure Of A Fly's Brain II



Vaa3D: w. H. Peng

A hemisphere =
~50 glia-isolated compartments

# Structure Of A Fly's Brain III



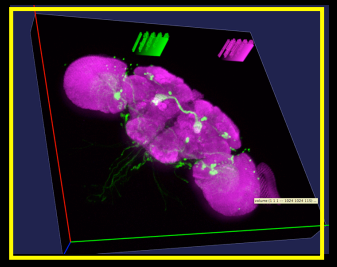
# Anatomy of a Fly Brain *with Light*

What we now know about image analysis for adult fly brain imaging:

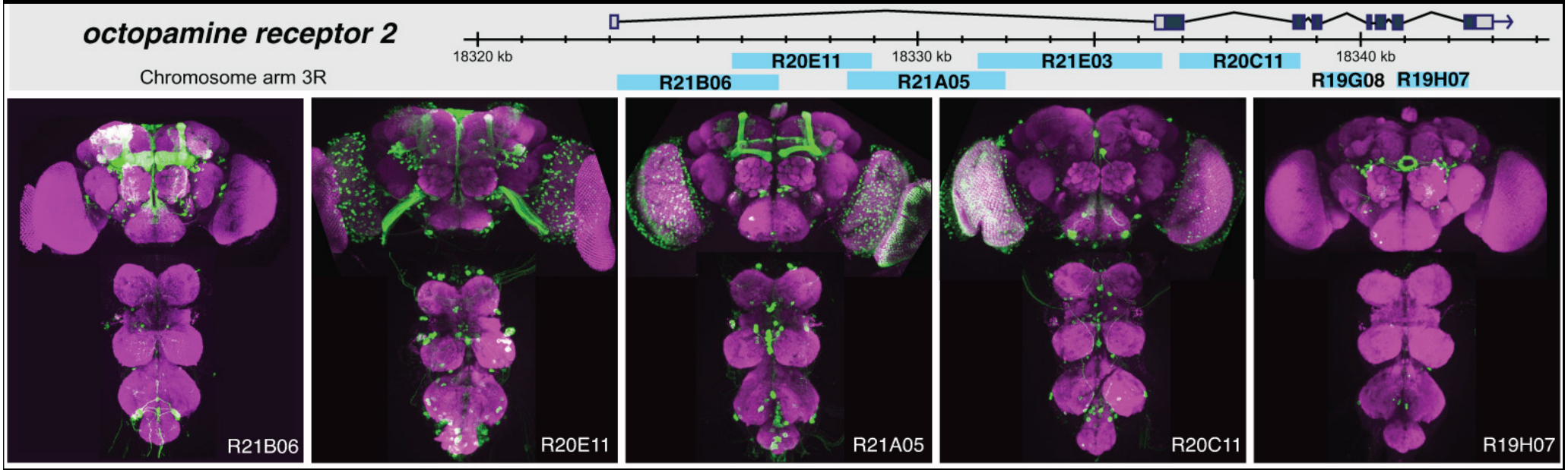
* Can trace individual neurons but not collections thereof
* Can register to 1.3 microns (average) reliably
* The fly brain is highly stereotyped (1-2 micron variance)

## Hierarchical Shotgun Mapping

Promotor Lines: Sample 3Kbp promotor constructs to cover brain sparsely (8K). Select a covering set C (3K).

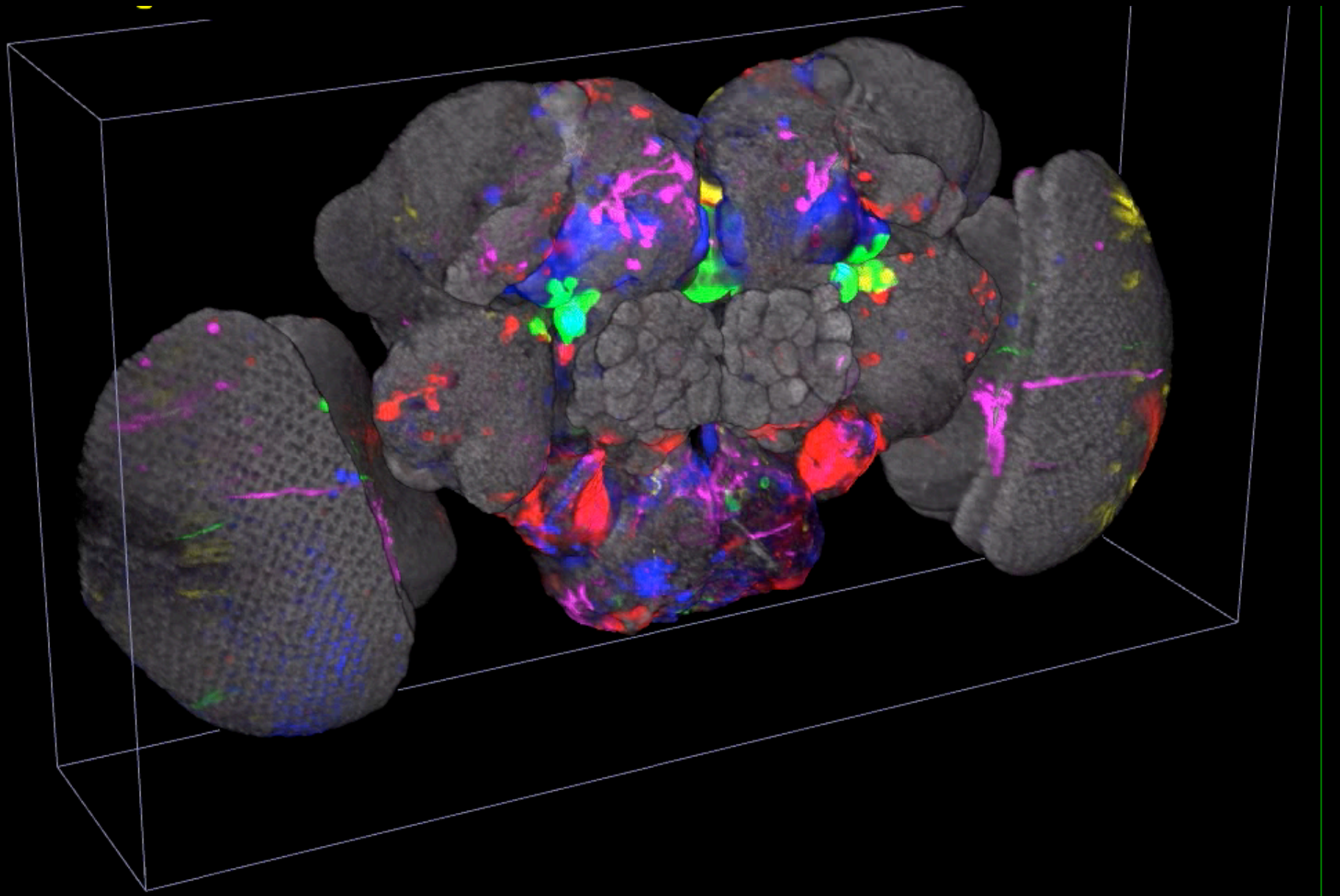


# Examples of Promotor Assay




Pfeiffer *et al. PNAS* (2008)
Released Sept. 2012

# Several Lines In “Atlas”



Vaa3D: hanchuan.peng.googlepages.com

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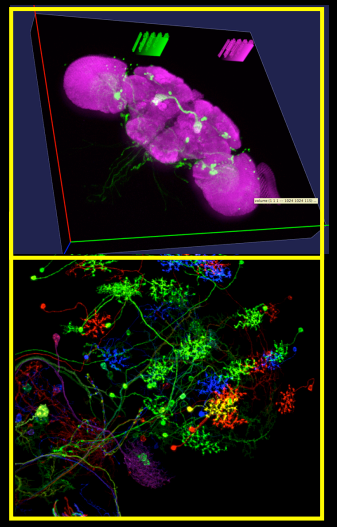
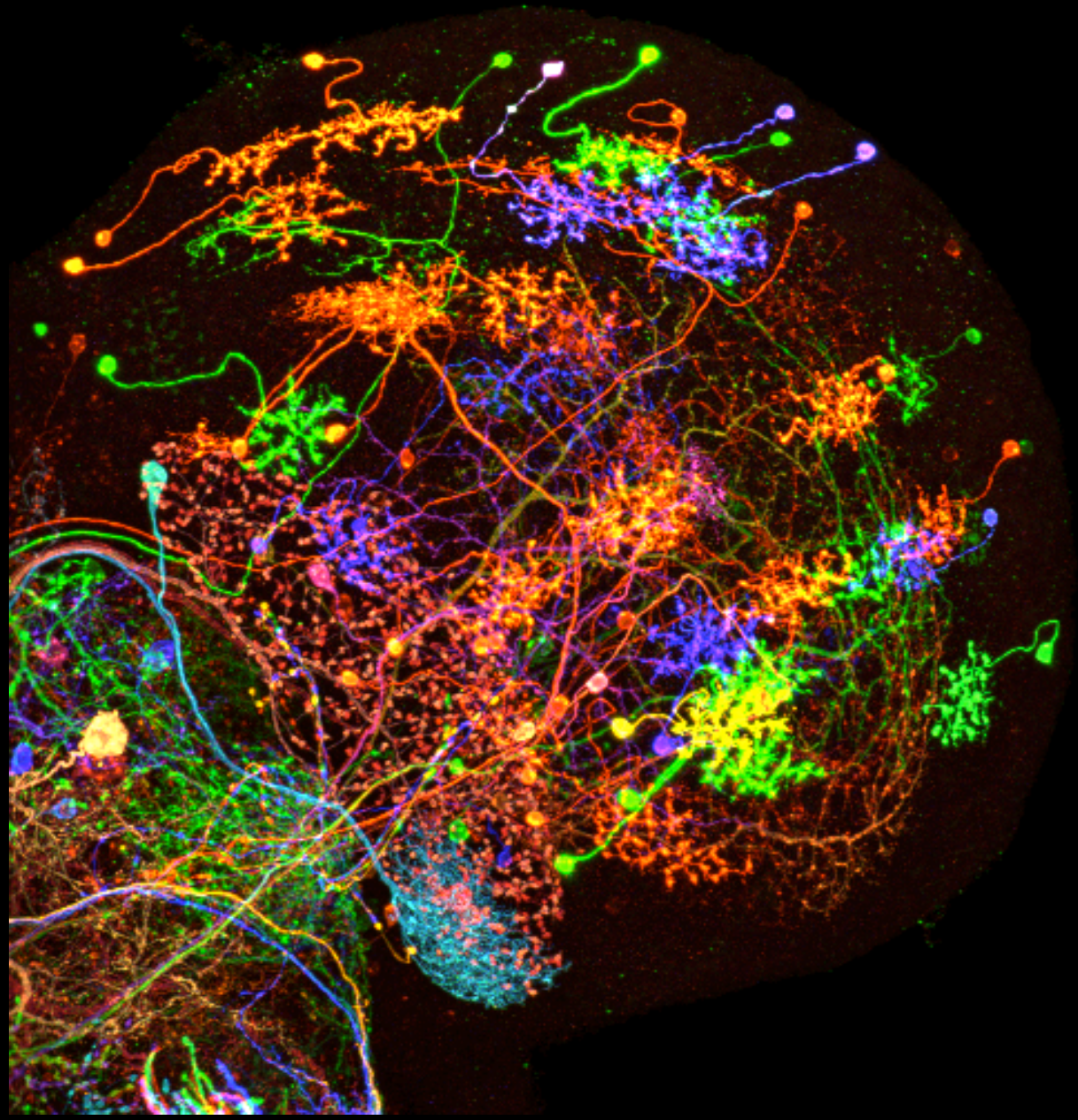
Individual Neurons: Sample 100K neurons within C to cover brain. Use finite color brainbow to enhance throughput.





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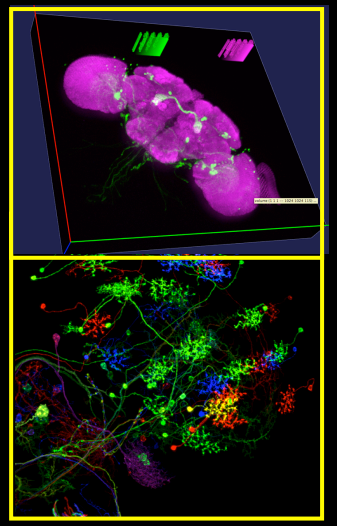
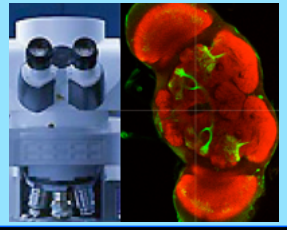


Individual Neurons: Sample 100K neurons within C to cover brain 5X. Use *finite* color brainbow to enhance throughput.

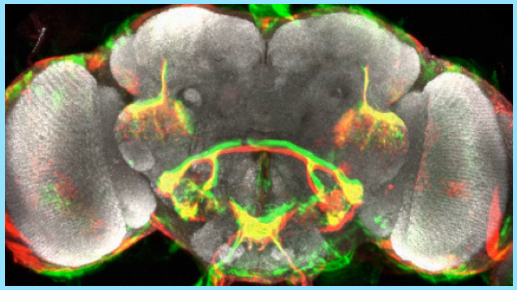
Neuron Interactions?: If possible, test the 300K or so possible interacting pairs resulting from the previous stage?

# Atlas Processing Pipeline

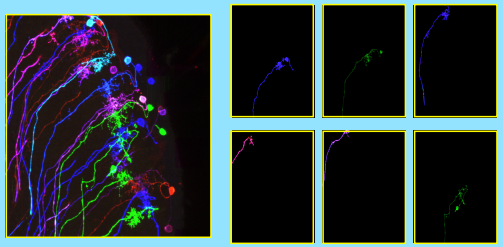
1 Staining & Imaging



2 3D Registration

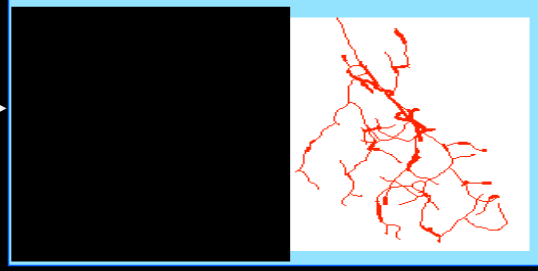


3 Neuron Separation

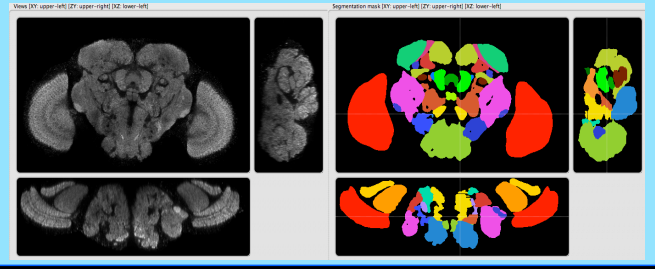


Need to perform:
- Registration
- Neuron Color Separation
- Neuron/Lineage Tracing
- Compartment Analysis

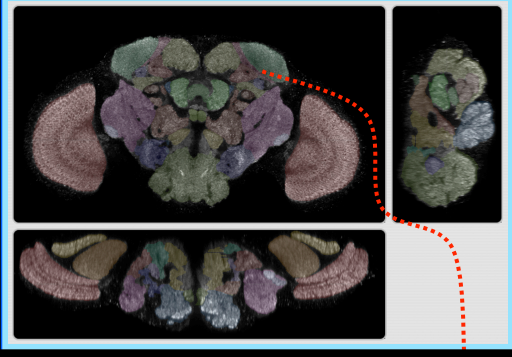
4 Neuron Tracing



5 Compartment Annotation



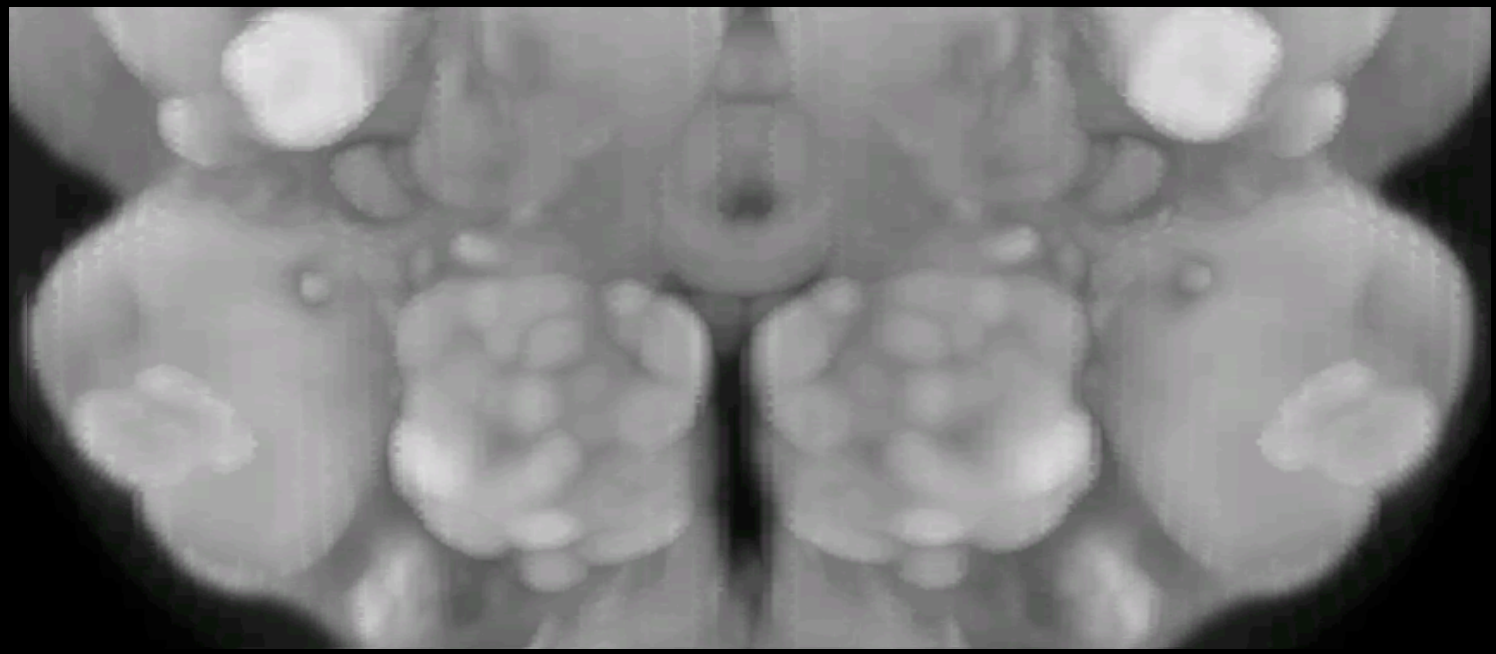
6 Analysis & Modeling



# 3D Registration (w. H.Peng)

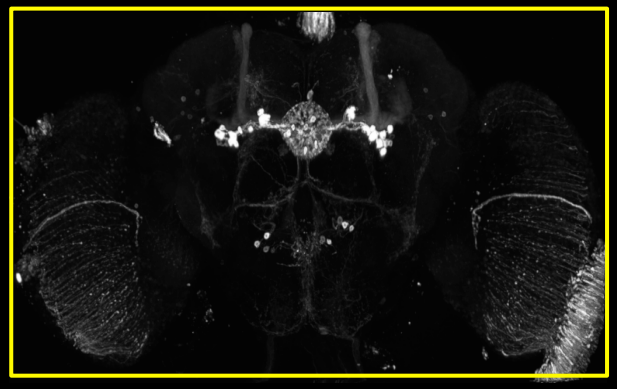
*Nature Methods* 8 (2011)

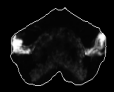
- *Consensus* mapping of landmarks.
- *Figure of Merit* (% of landmarks mapped).
- 95+% of all stacks pass alignment (>85% lm's match)
- Aligned consensus neuropil reveals sub-compartments often not visible in any single stack!



1000 stacks

# Compartment Analysis of GAL4 Lines

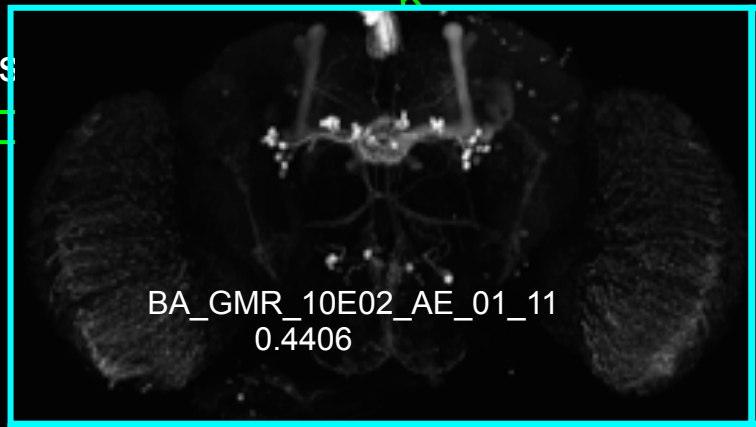


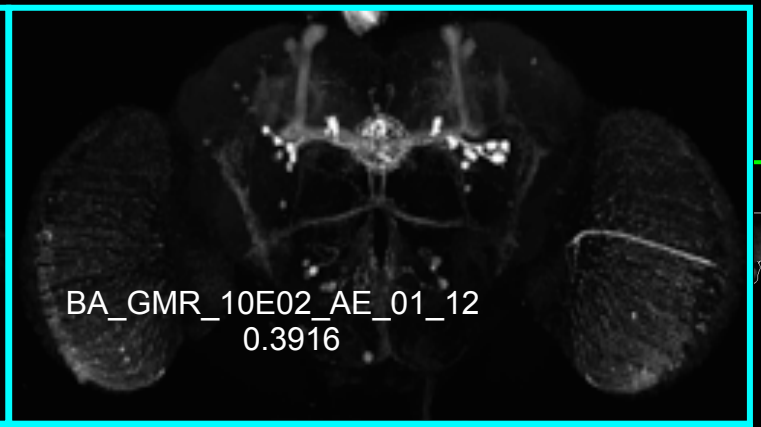


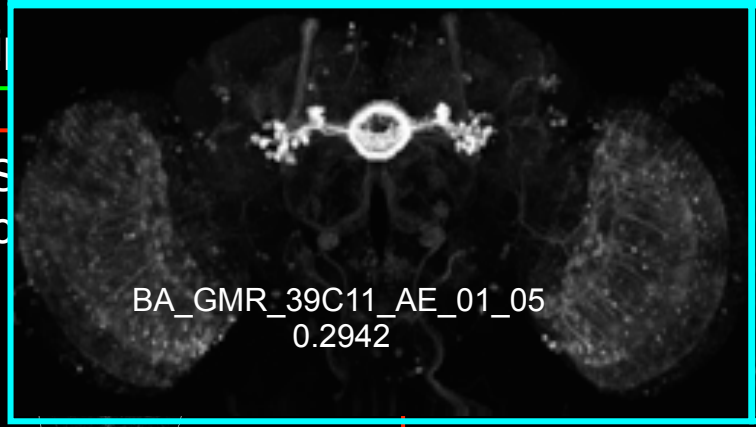
FB (Fan s

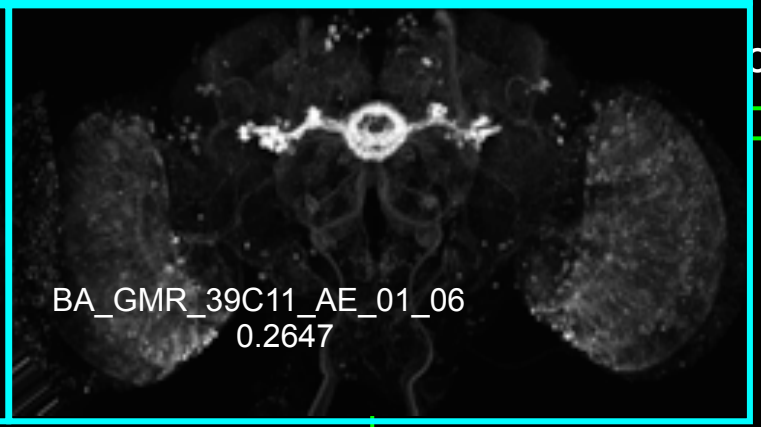
EB (Elli

SOG (S
Ganglio



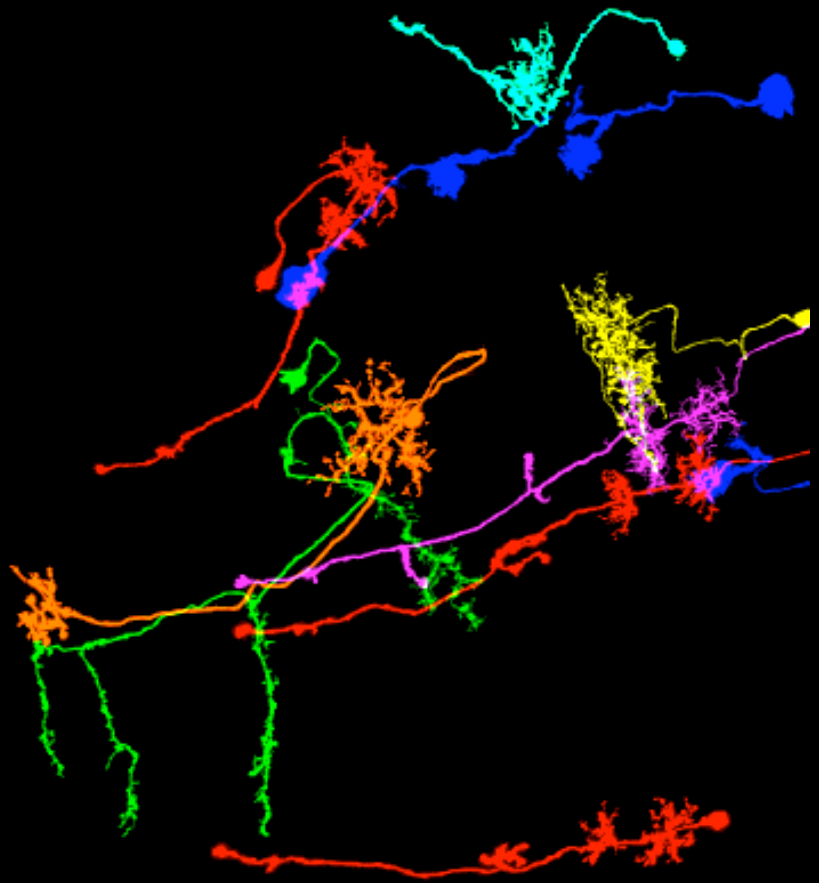



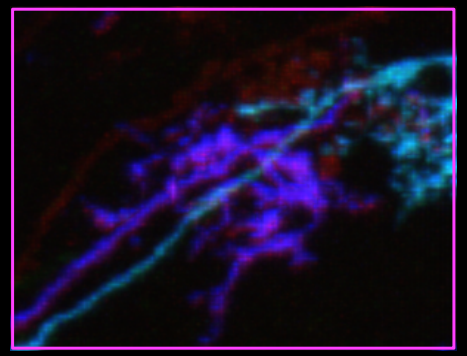

odY), yes

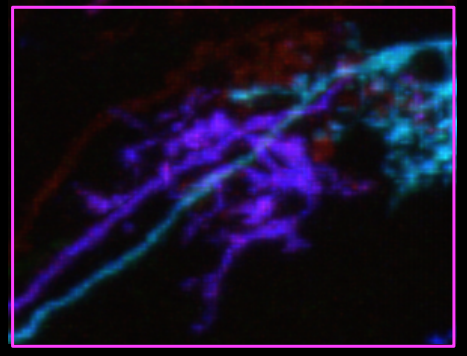
LB (Lateral bulb), yes

# Multi-Color Separation

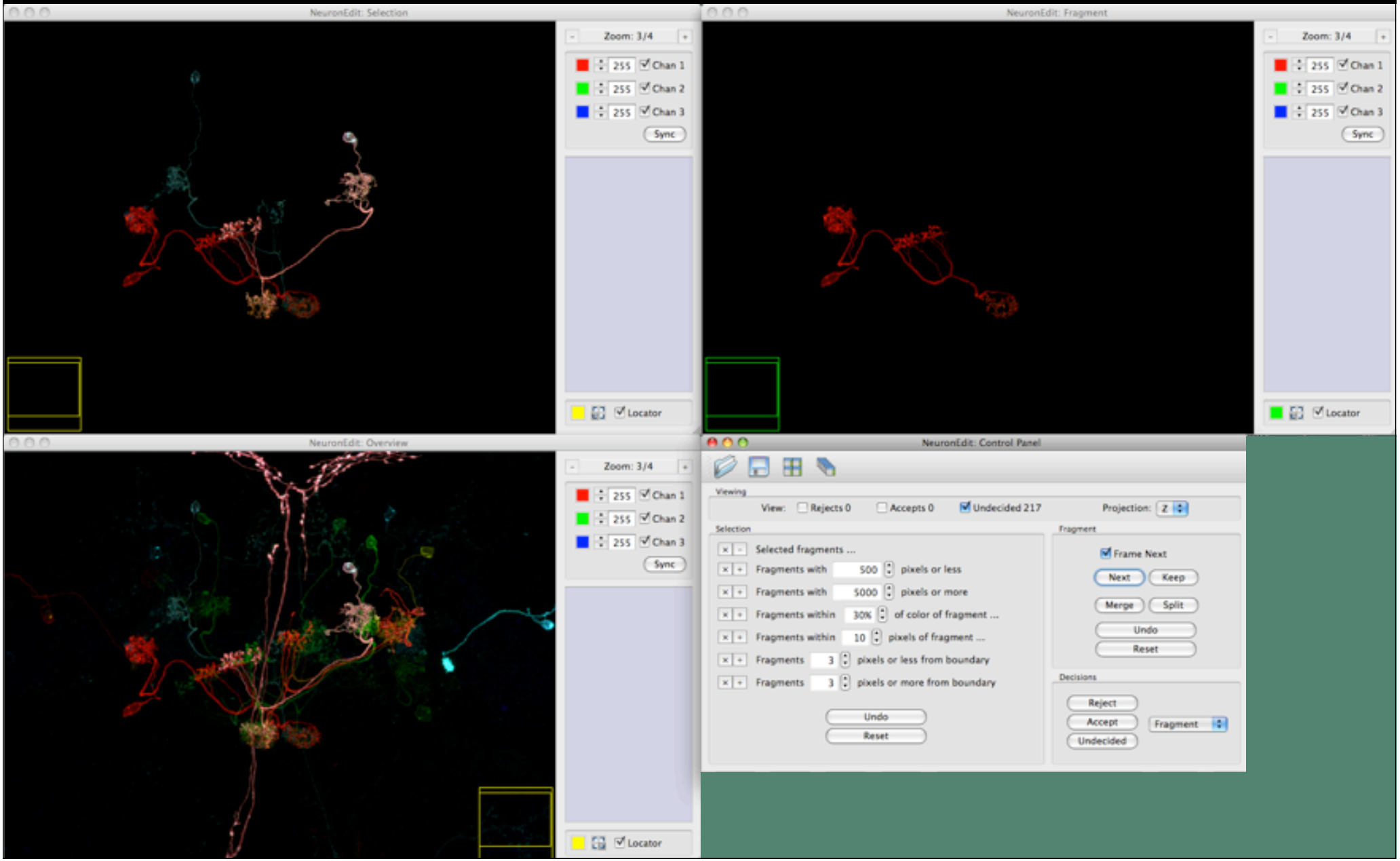


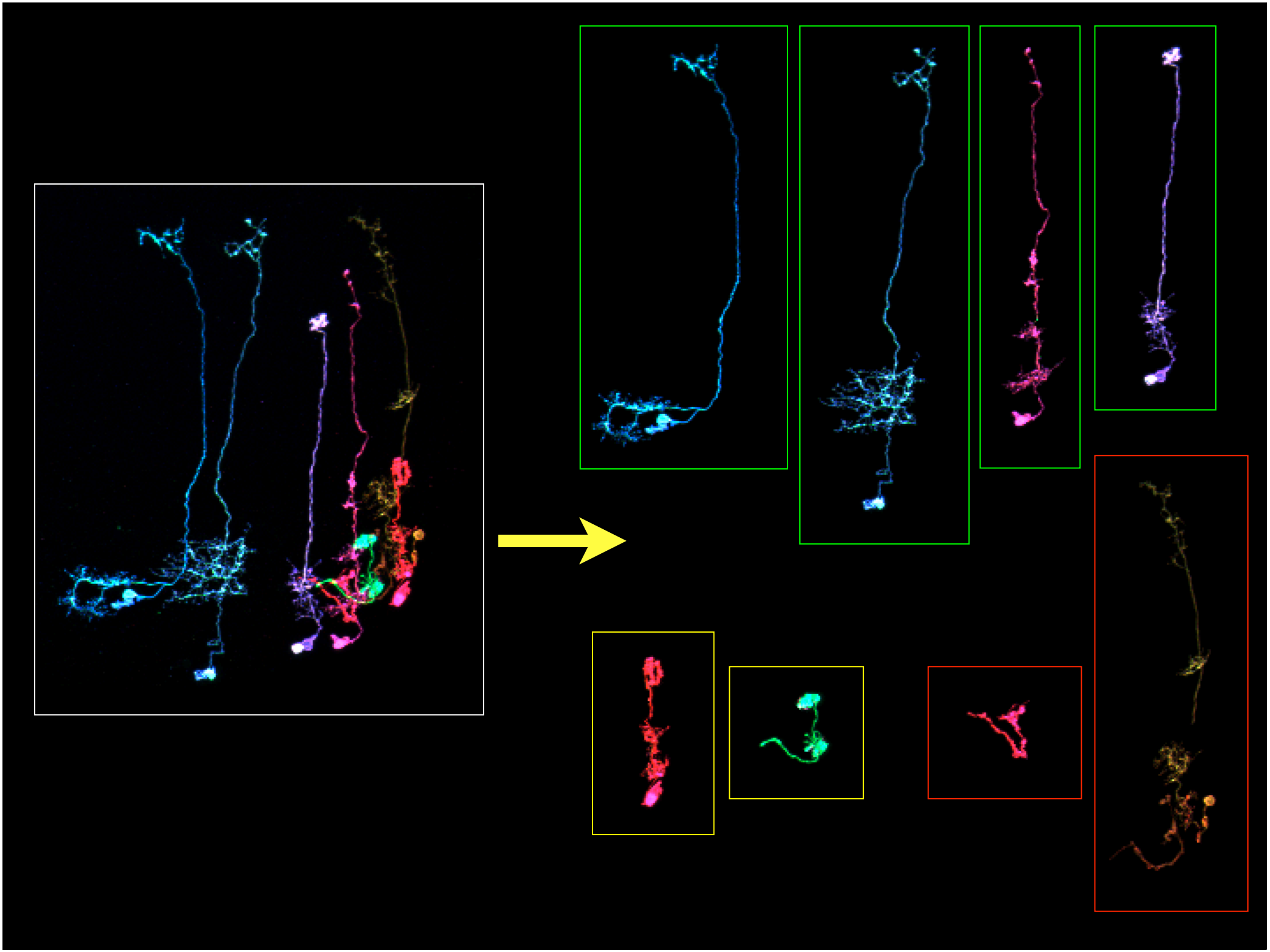
Color shift requires
sub-pixel translations!





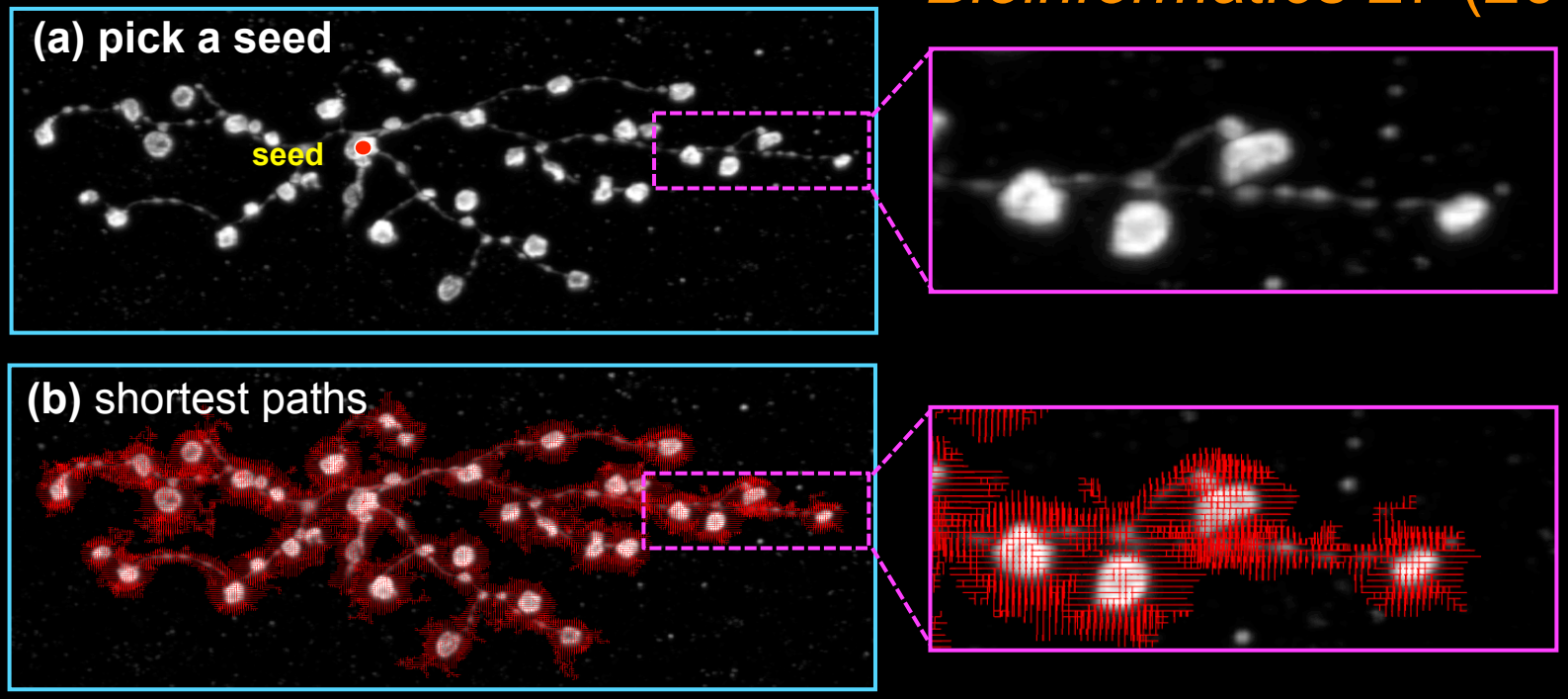
# Multi-Color Neuron Editing & Curation





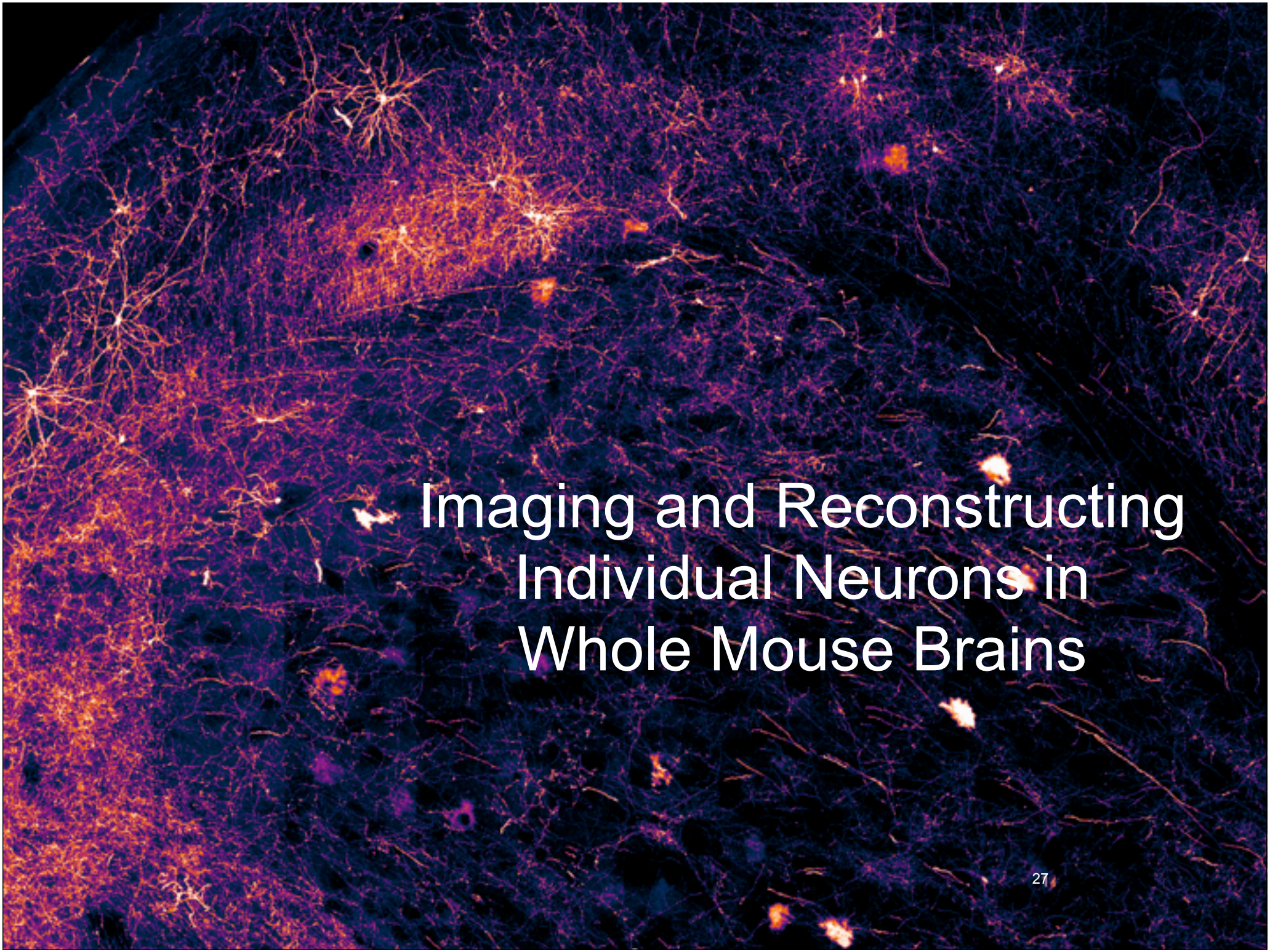
# Neuron Tracing (w. H.Peng)

*Bioinformatics* 27 (2011) ✓




A discrete technique (from 1959), not typically used by CVers.

Another <u>combinatorial</u> idea. Each pixel covers a maximal disk of foreground, find minimum covering tree. Very fast, linear time.


Imaging and Reconstructing
Individual Neurons in
Whole Mouse Brains

# The “Mouse Brain” Scope

Nathan Clack

**Scale:**

15 mm x 7 mm x 5 mm ⇒ 4.2 x $10^{12}$ $0.5^3$µm voxels!

**Specs:**

@ 8.6Mv/sec collect ~13,000 sub-stacks in < 6 days

(86X faster than a conventional 2P scope)

**Scope:**

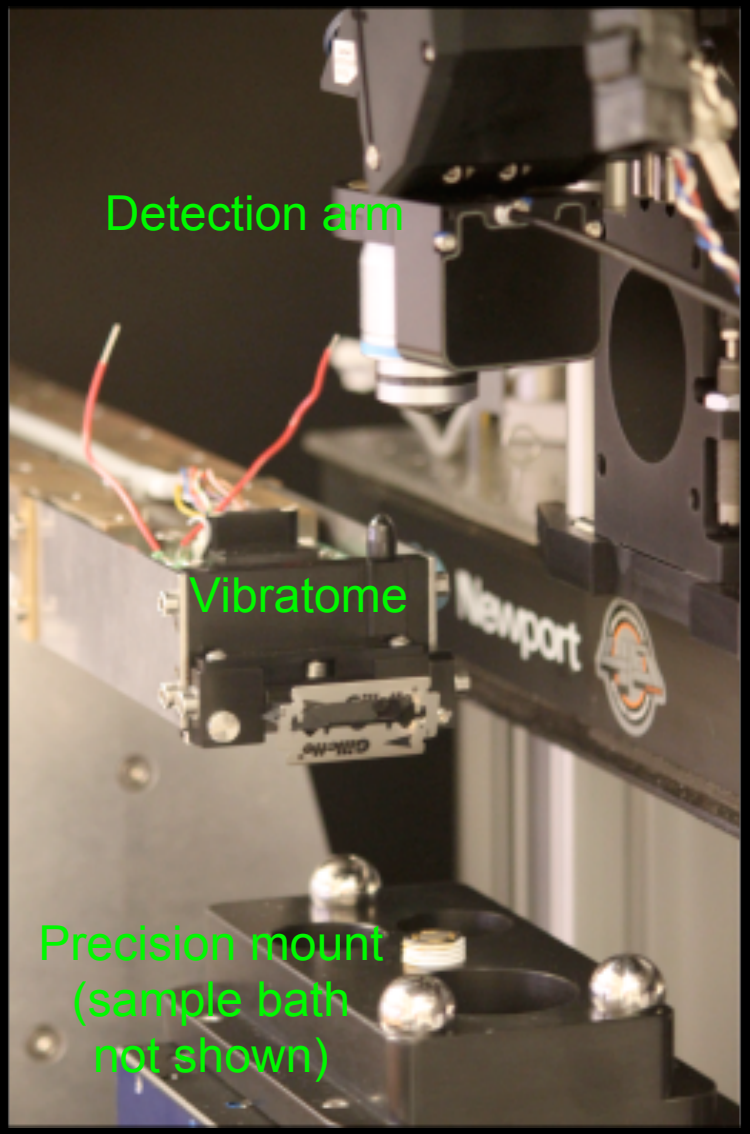
2P, block face, with on-board vibratome and resonant x-mirror automatically executing:

*Repeat until nothing left*

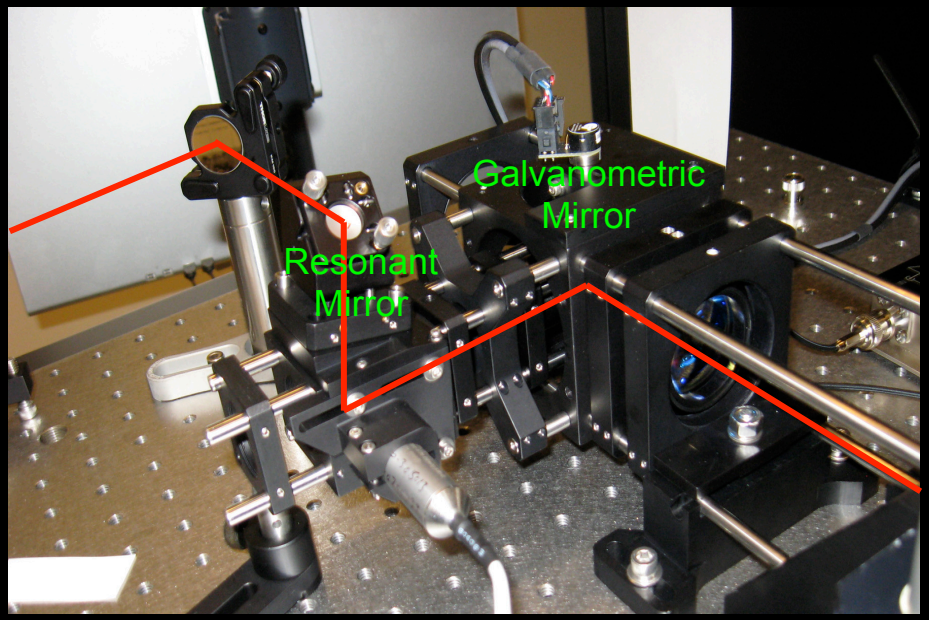
*(1) Image an xy tiling of 200µm deep stacks*

*(2) Cut away top 185µm (implies 15µm overlap)*

# Onboard Vibratome

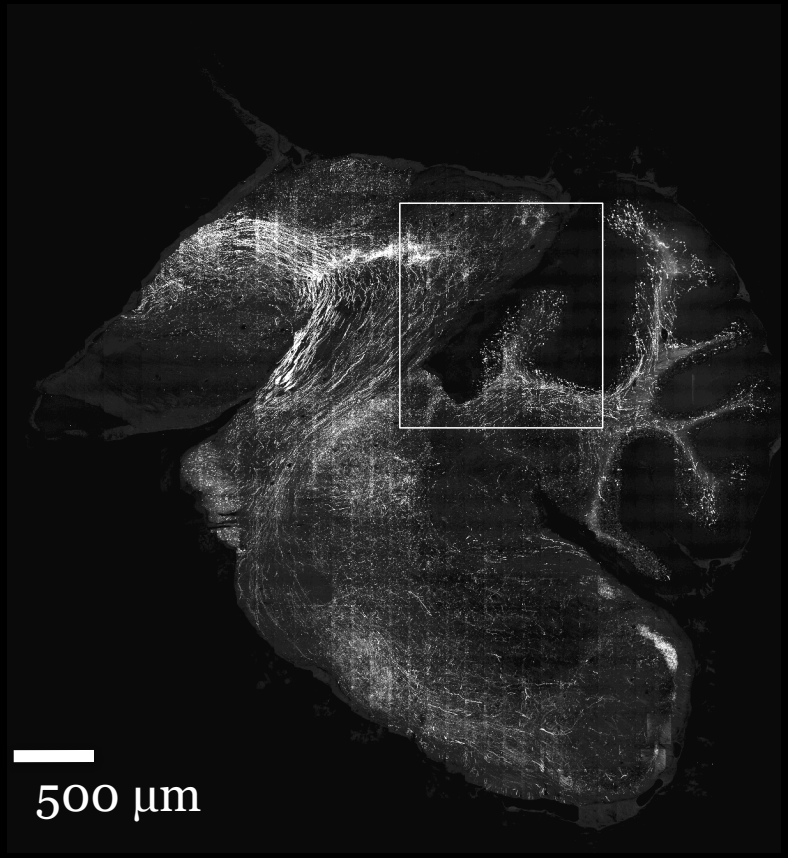



# Resonant X-Mirror



# 30µm Sagittal Slice of Cerebelum (Hantman)

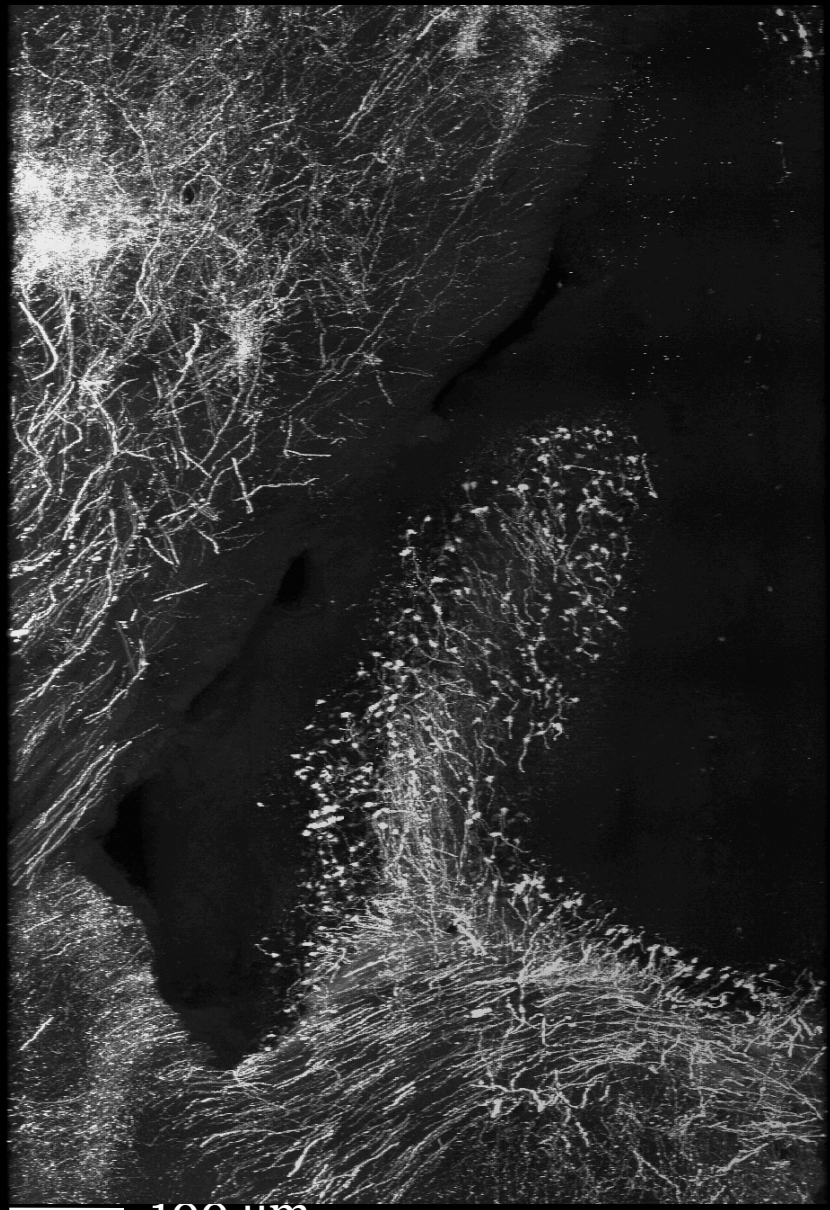
A small set of projecting axons from the central brain are labeled (GFP).



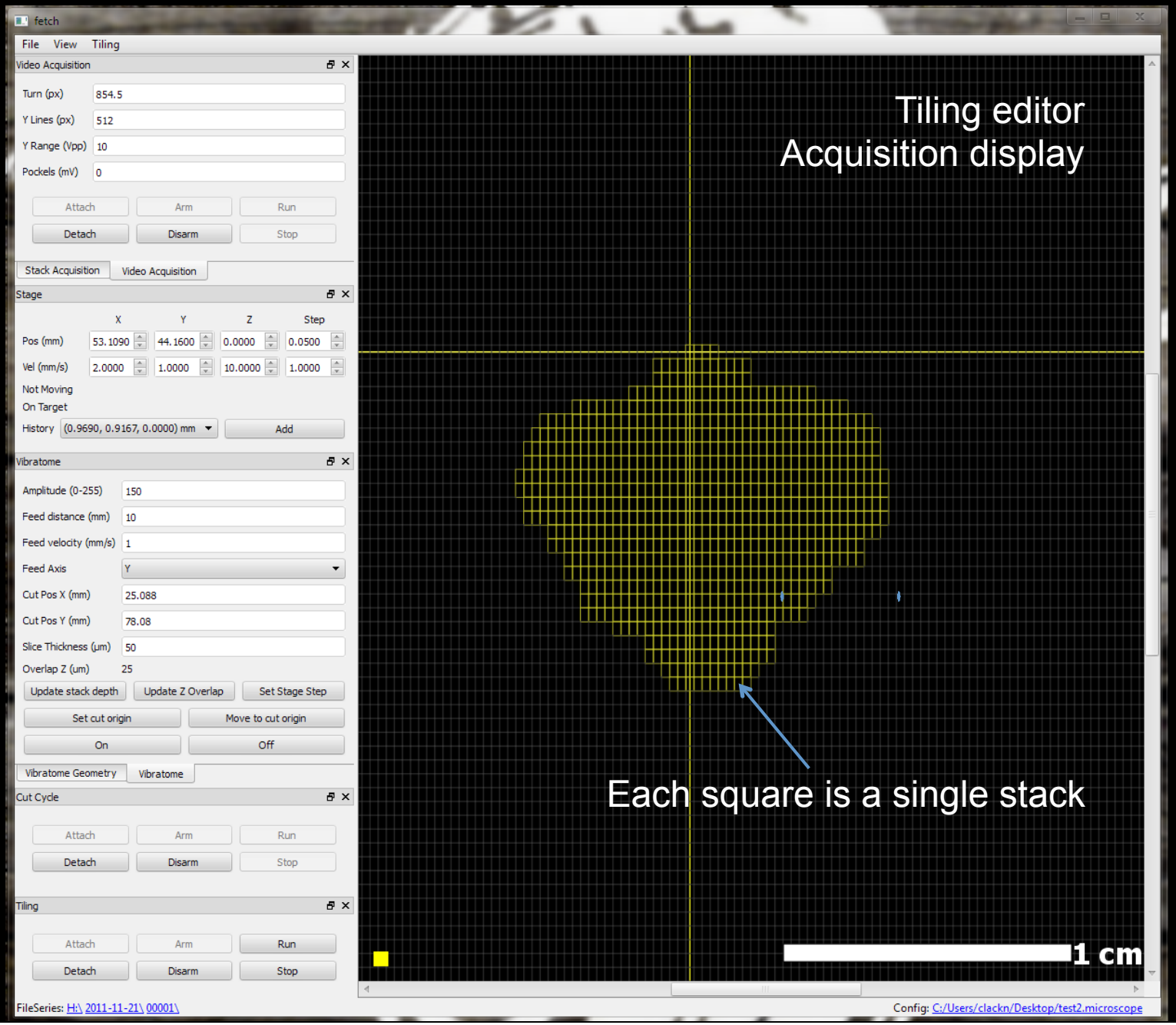

~1000 tiles

Voxel Size:
0.4 x 0.4 x 1 µm

Acquision Time: <1 hr.



# Acquisition Software




Acquisition

Stage

Vibratome

Task control

# The MPI-CBG "Plan"

Technology → Experiment → Analysis → Model

| Optical Eng. | Developmental | Bioimage | BioPhysics |
|---|---|---|---|
| Mol. Reagents | & Cell Biology | Informatics | & "Sys. Bio." |

(a)Meso-scale interpretation & simulation of cellular phenomenon

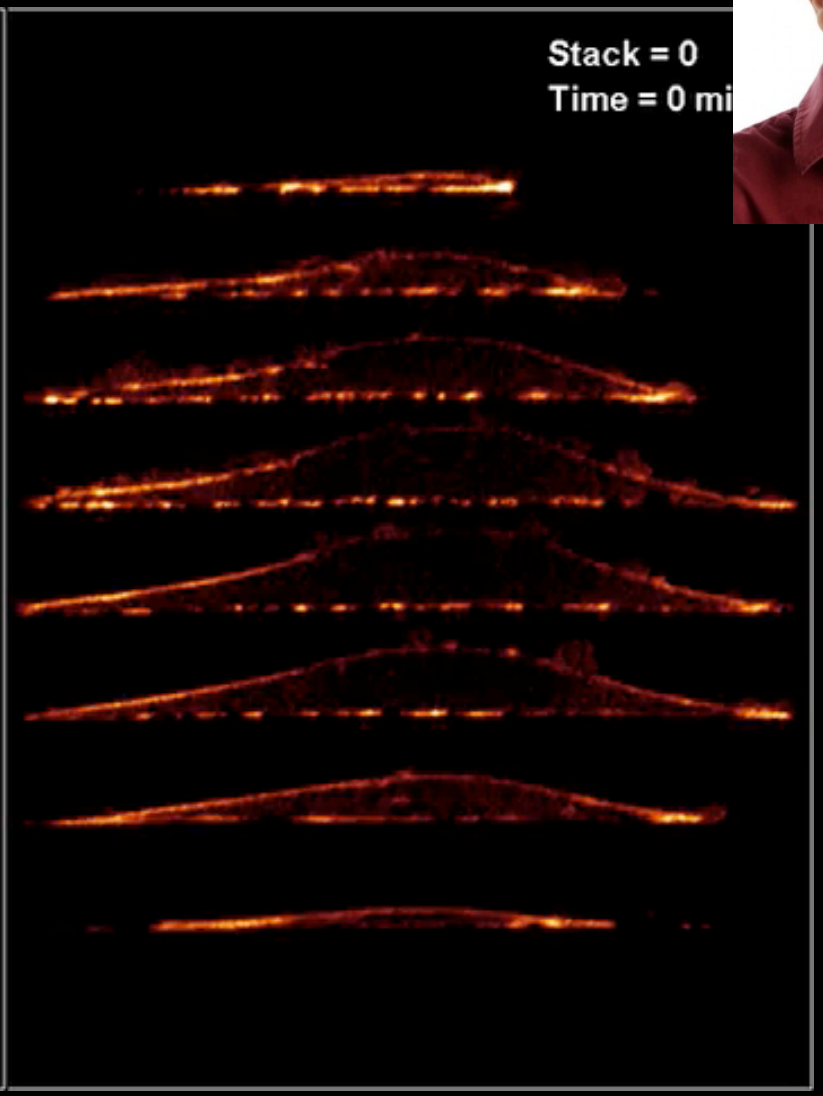
(b)Platform to (perfectly) follow cell lineages through arcs of time
(e.g. worms, fly embryos, fly wing development, z.fish embryos)

(c)Connecting (a) & (b)

We will:

- build (automated) microscopes,
- design algorithms for extracting information and models from images, and
- build computer-assisted interfaces for curation and analysis.

# Optical Engineering

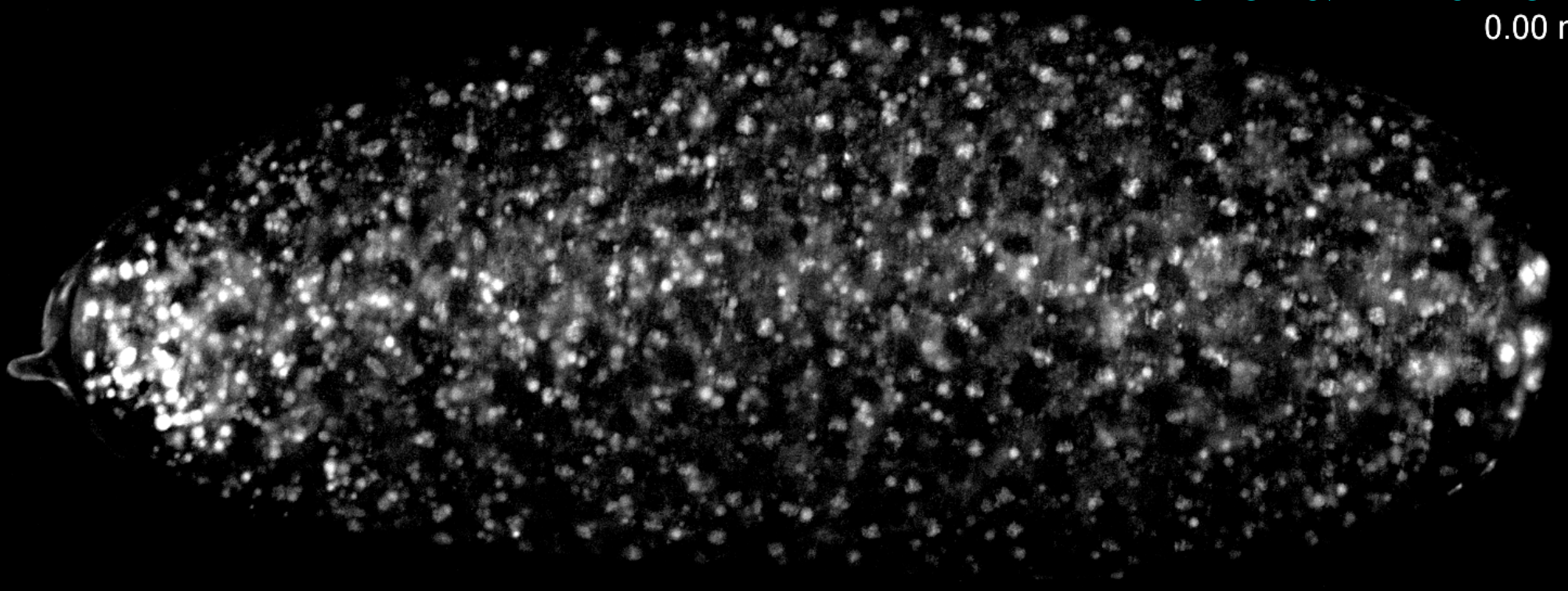

Eric Betzig

2P SI Besel beam scope for observing *in vivo* cell process at λ/4

# Optical Engineering

P. Keller & R. Tomer

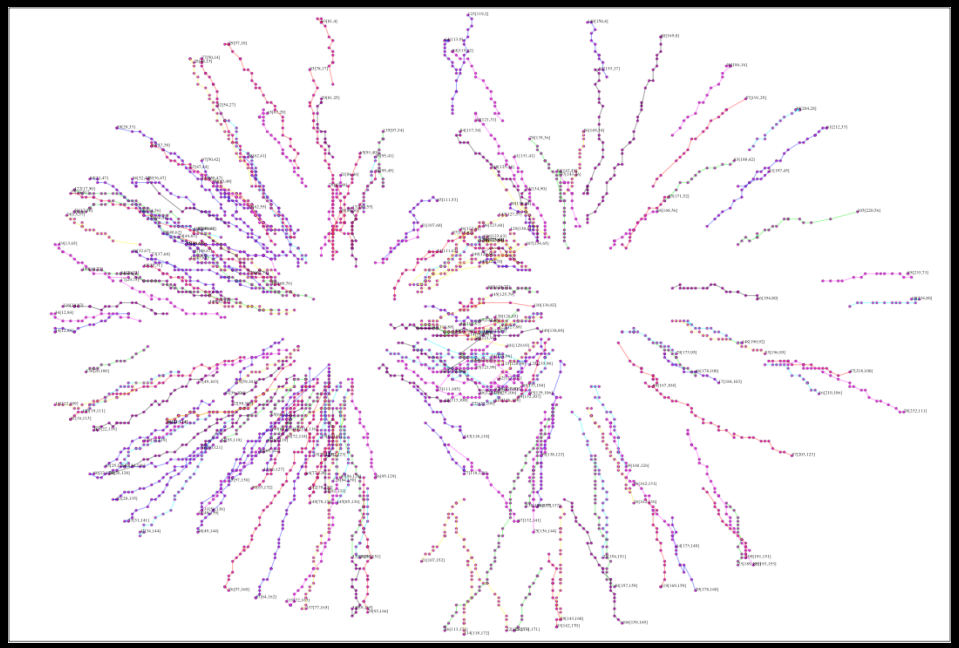
0.00 mi



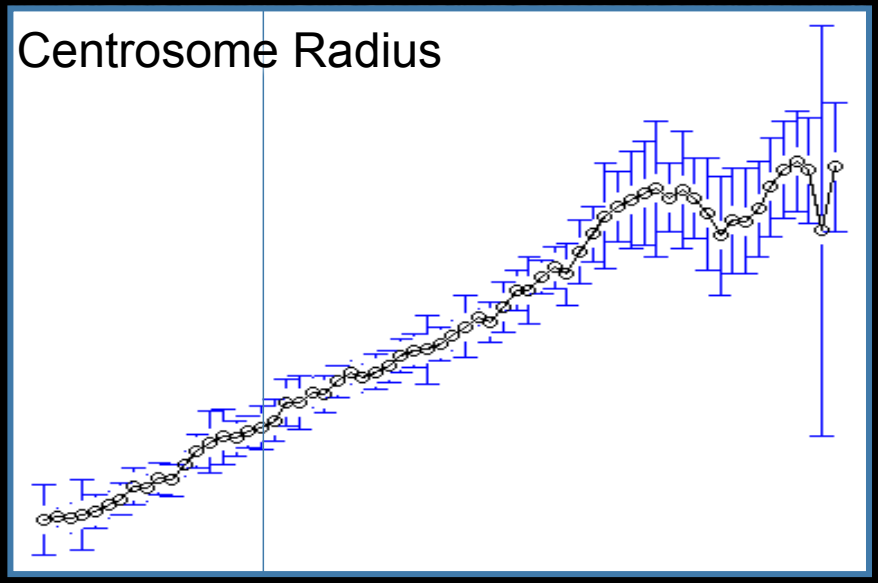
Next gen. DLSM scope to follow 24 hours of fly or fish development

# BioImage Analysis: Intracellular Processes

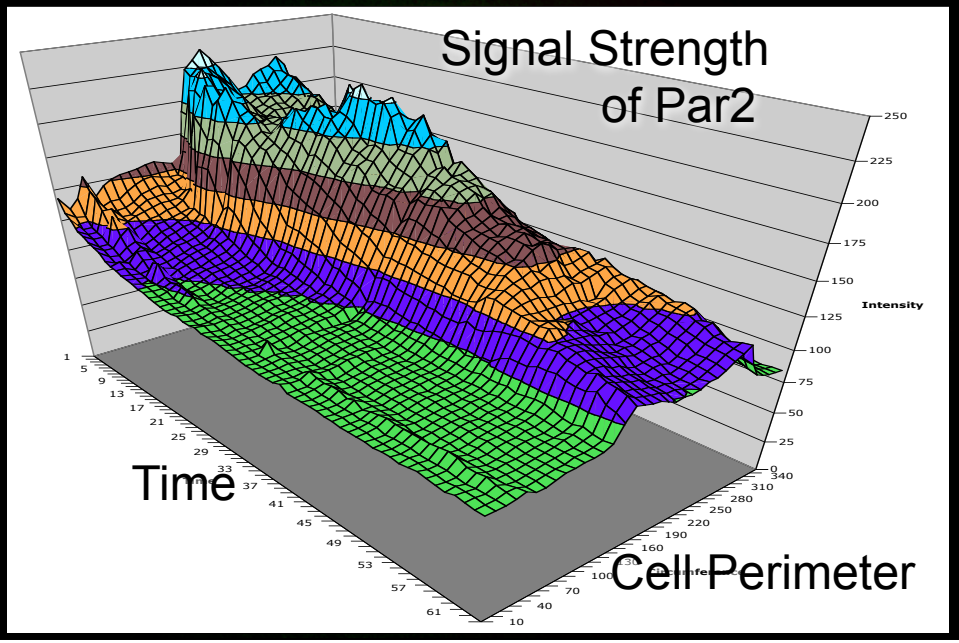
Tony Hyman Lab



EB1 labelled tubulin fibers.

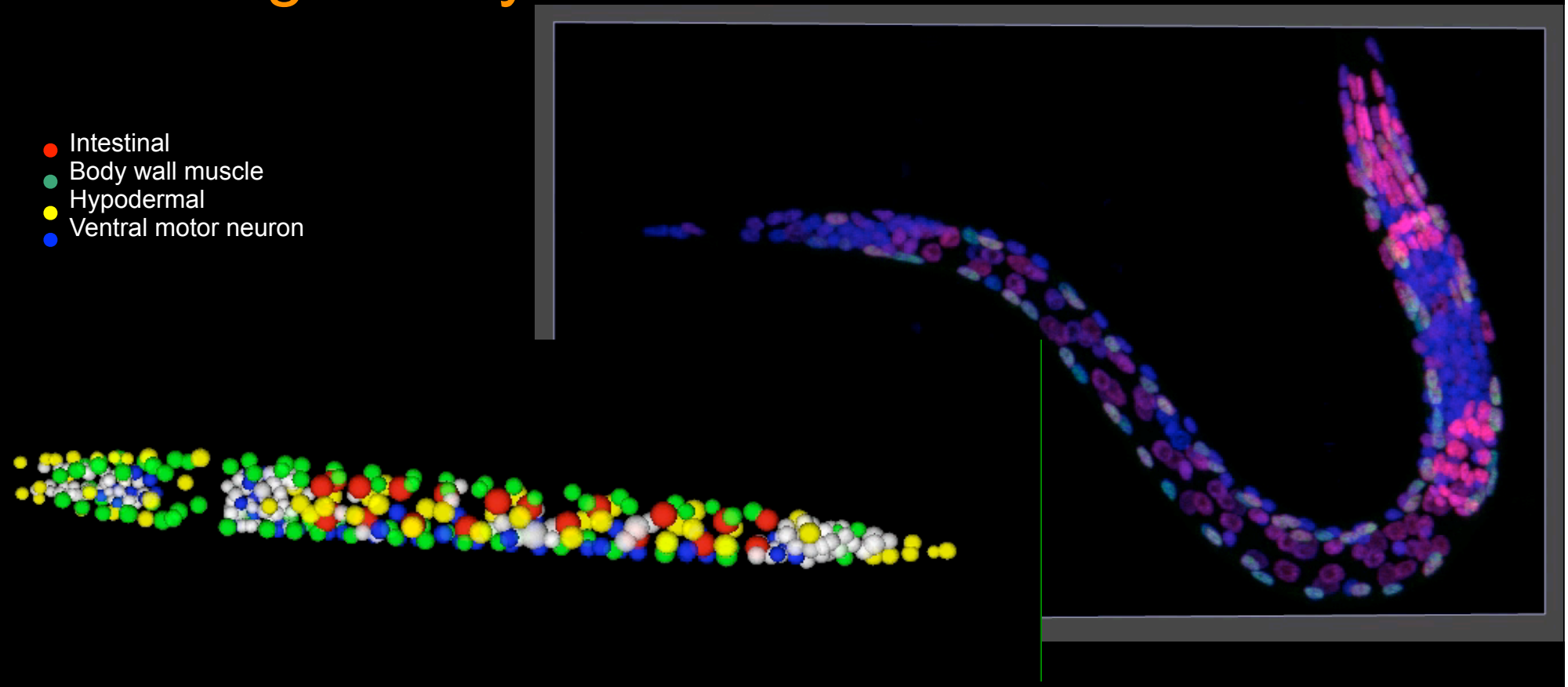



Gamma-tubulin labelled centrosomes




Par2-Par6 labelled membranes

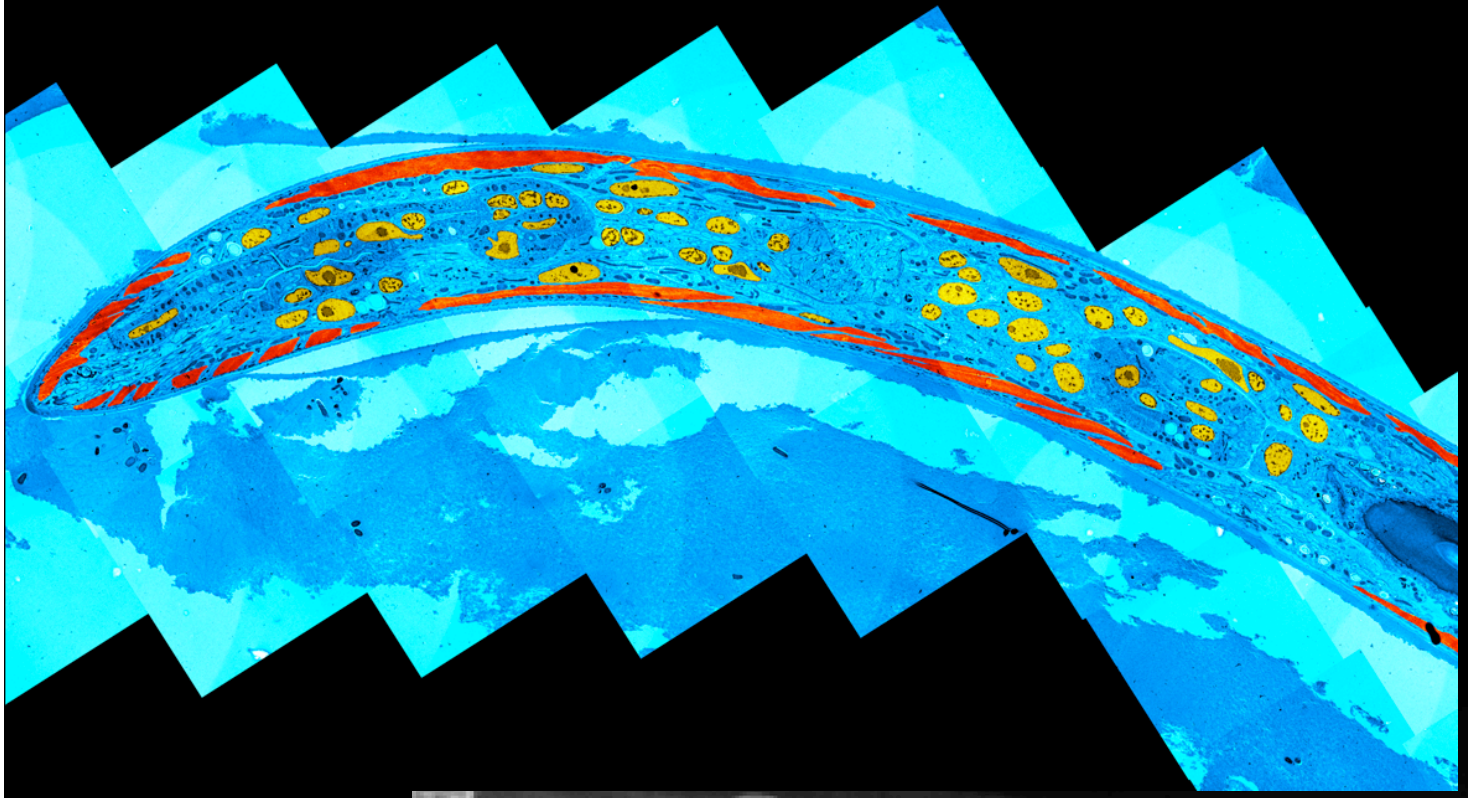
# BioImage Analysis: Worm Atlas & SCE




Fuhui Long

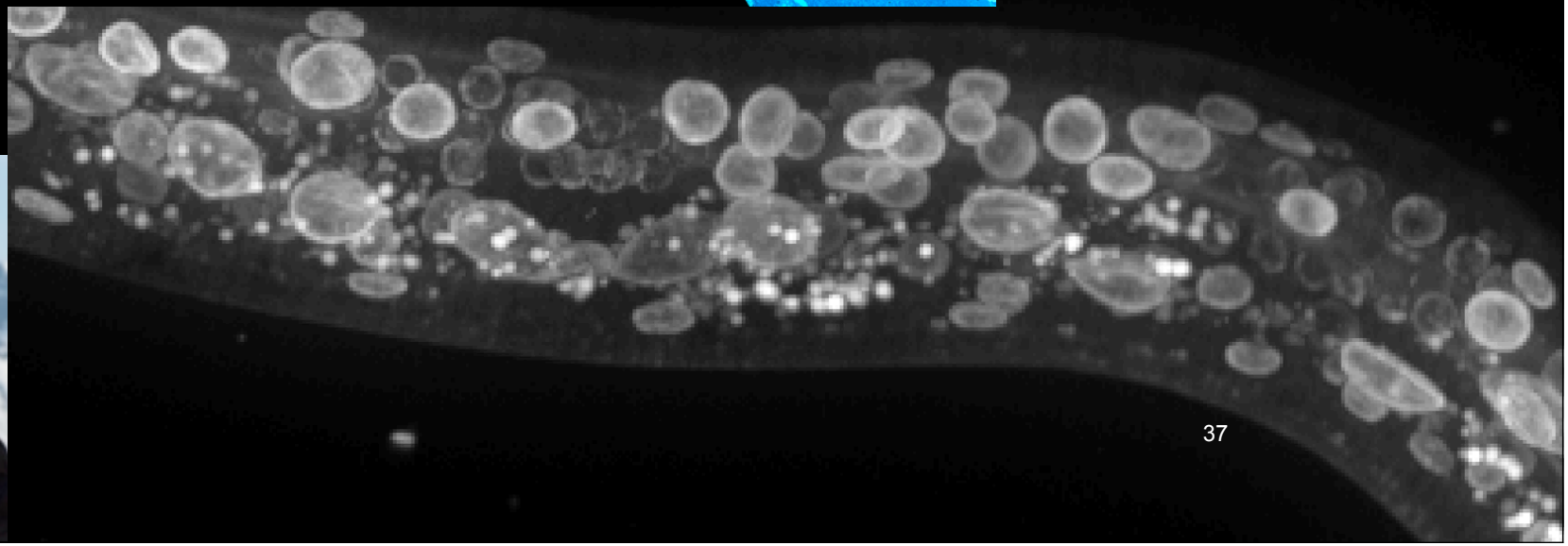
| Ord | Cell | Mean | Neighboring | Spacing | Size | Shape | … |
|---|---|---|---|---|---|---|---|
| 332 | INDR1 | X: 81.5257± 2.0397<br>Y: 2.6659± 0.4637<br>Z: 1.8412 ± 0.3335 | HYP7ABPRAAPPPP, INDL1, BWMDR11, HYP7ABARPAAPPP, BWMDL10 | 3.8246 ±1.1421<br>3.8407 ±0.9327<br>4.3572 ±0.7644<br>4.5065 ±1.2958 | 9005 ± 993 (px)<br>1.5005 ±0.7195 (μm) | Aspect ratio 1.5243 ±0.2069<br>… | … |
| … | … | … | … | … | … | … | … |

# BioImage Analysis: Worm Development



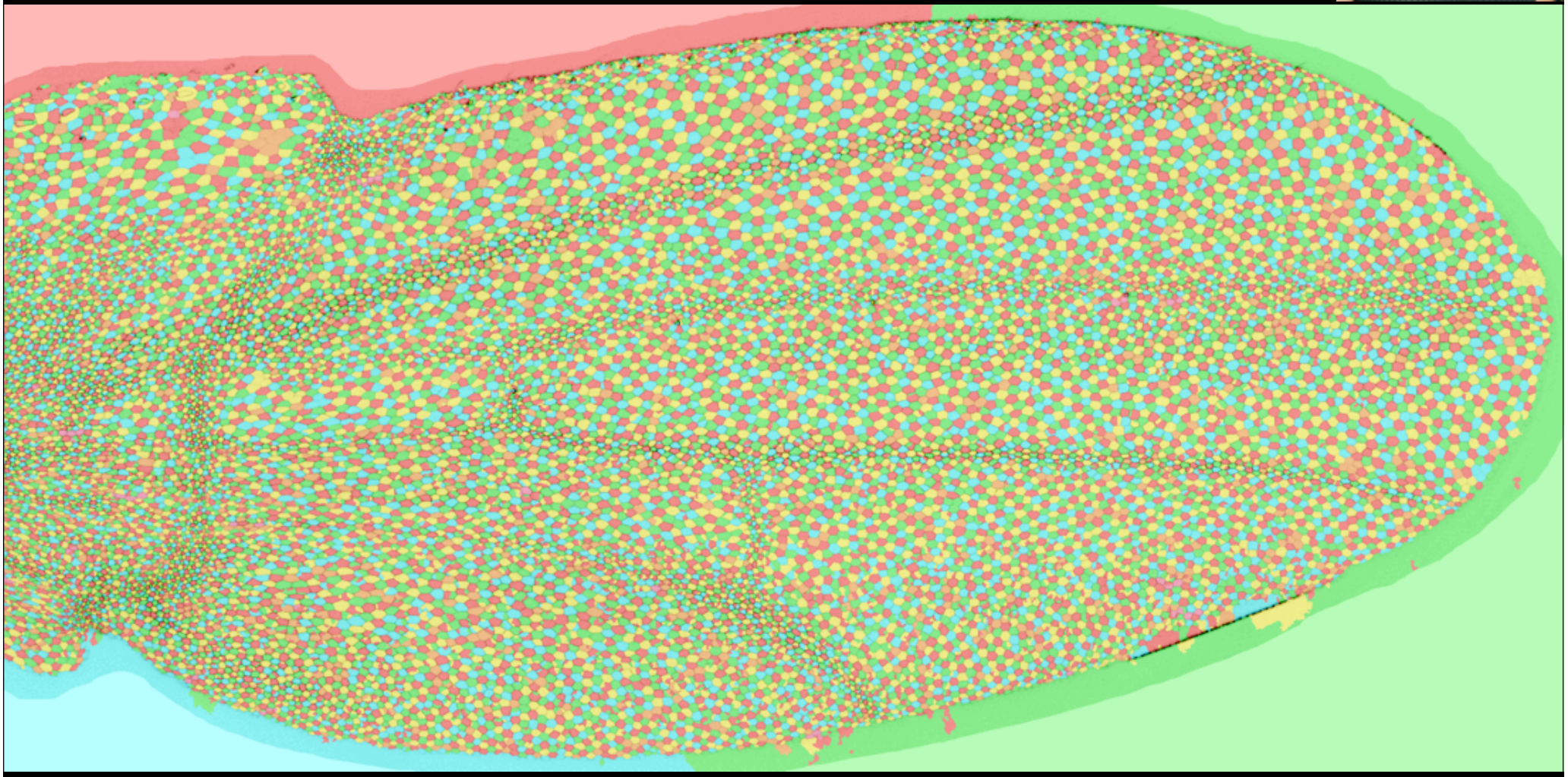
Stephen Preibisch

Mihail Sarov



# BioImage Analysis: Fly Wing

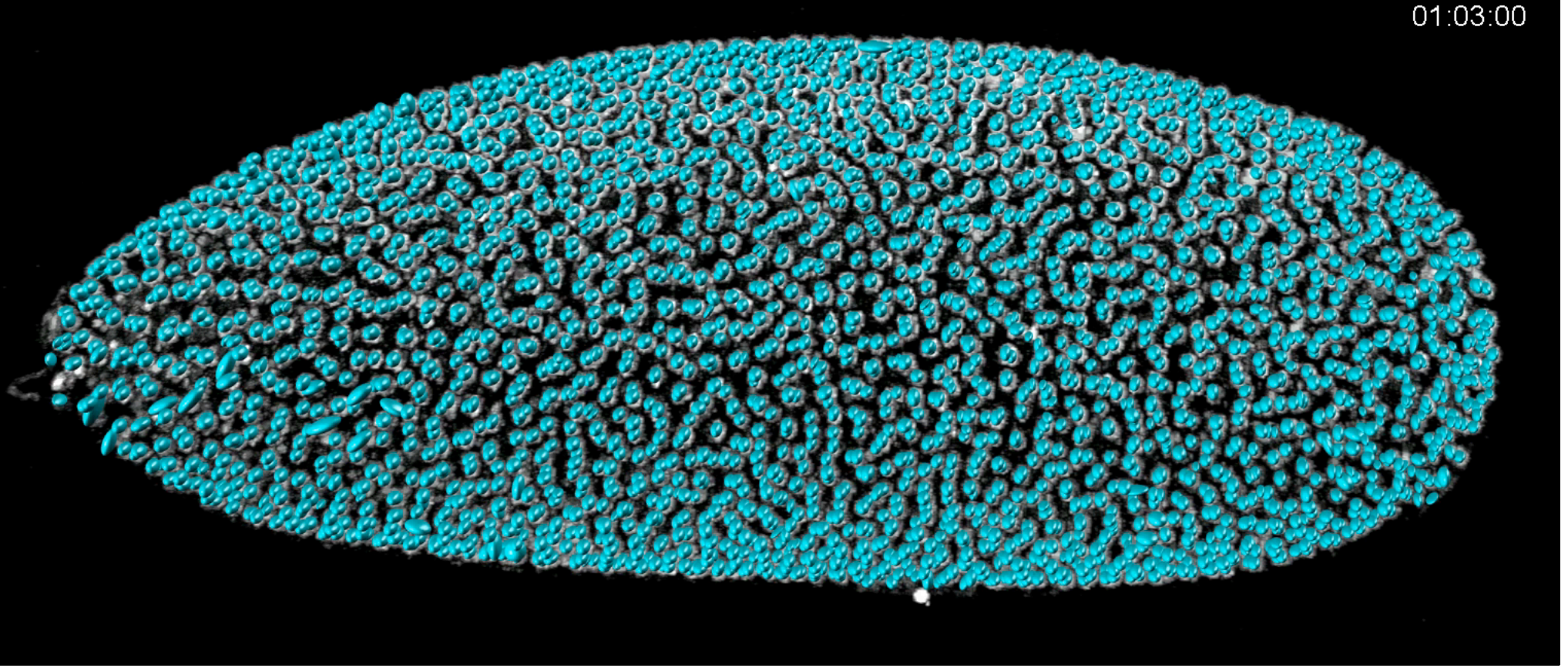
Suzanne
Eaton Lab



# Tracking Lineages During Fly Embryogenesis

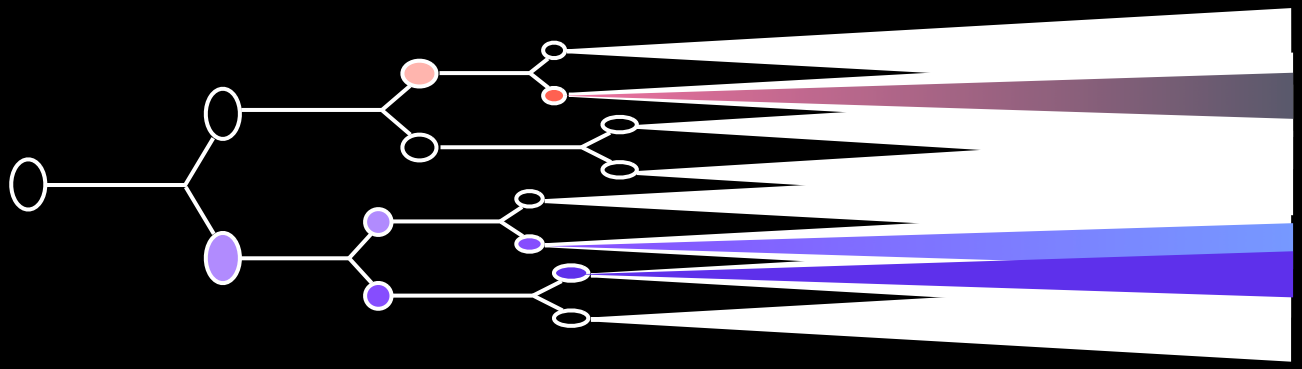
- 96 time points; 35secs/time point (TP)
- ~4800-9600 cells/TP
- CPU+GPU: 110 secs/TP

Fernando Amat



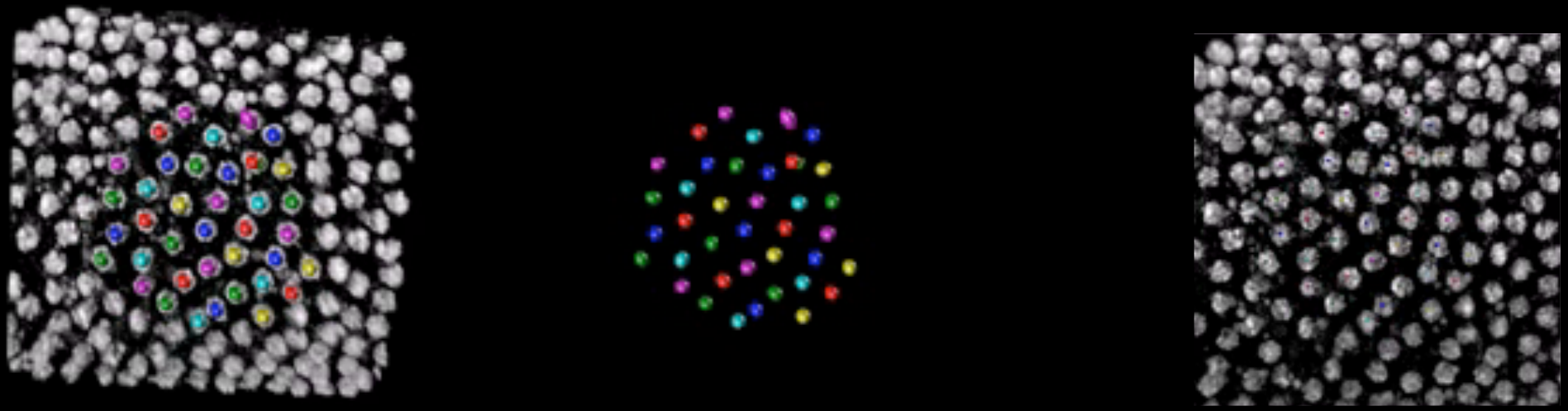
# Tracking Lineages During Fly Embryogenesis

- Lineage tree with arc-lengths, shape(t), and expression(t) of 1 or more agents.



- If can attain this goal then can go from developed structures backward in time to founder cells, and
- Can throw away Tb's of "raw data". The "lineage" can be encoded in a few Mb's.

# Tracking Detail View



- Segmentation accuracy: 96.7% ± 1.0%
- Simple linkage accuracy: 99.2% ± 0.6%
- Cell division linkage accuracy*: 94.8% ± 2.6%
- Error-free lineages (approx): 74.5% (1502 trees)

# How Hard is the Problem?

- At 99.9% tracking step accuracy and 2,880 steps (1 stack/30 sec.s), 5.6% of lineages will be correct.

  At 99.99%, 75%.

  At 99.999%, 97%.

- We need better scopes (even 50% more rez will help), we need software that is 3 orders of magnitude more accurate than current solutions.

- We will always need to curate, so need computer-assisted GUI's that make that as efficient as possible.

# So ....

I believe that direct *in-vivo* and *in-situ* observations of cells and cellular systems is going to lead to the most exciting and meaningful discoveries in molecular biology in the next decade. This belief is founded on two facts.

1. We can now label any protein, RNA, or other genetic element of interest within a model organism as we now have their DNA sequences.
2. Advances in microscopy enable us to view these labeled agents with greater fidelity, speed, and duration then every before.


# "Gene Myers" Group

A Technology Group: Microscopy & Bioimage Analysis

Concept: 25% optics / 75% informatics

The team so far:

Stephan Preibisch

Nicola Maghelli

Florian Jug

David Richmond

Laurent Abouchar

Loic Royer

Corinna Blase

Dagmar Kainmueller

Martin Weigert

**You ?**

**& 2 of your friends?**

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(neuron tracing)

Keller Group
Raju Tomer

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MPI-CBG Dresden
Steffen Jaensch*
Martin Decker


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Arnim Jenett (fly brain images)
Aljoscha Nern (flip-outs)

Svoboda, Hartman Groups (mouse imaging)

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MPI-CBG