Building Cellular Models with Light Microscopy

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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

EB1 labelled tubulin fibers.

Gamma-tubulin labelled centrosomes

Par2-Par6 labelled membranes
E.g., Fly Wing Development
E.g., First Mitotic Division of C.elegans

EB1 labelled tubulin fibers.

Gamma-tubulin labelled centrosomes

Par2-Par6 labelled membranes

Signal Strength of Par2

Centrosome Radius

Time

Cell-Perimeter
Tracking Centrosomes During Worm Embryogenesis

w. Steffan Jaench (Hyman & Jülicher)

Bioinformatics 26(12) (2010)

Errors
0% to 8 cells
6.2% to 16 cells

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6.2% to 16 cells
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

* 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**

<table>
<thead>
<tr>
<th>Time relative to NEBD [sec]</th>
<th>Centrosome Volume [µm$^3$]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-cell</td>
<td>12</td>
</tr>
<tr>
<td>2-cell</td>
<td>10</td>
</tr>
<tr>
<td>4-cell</td>
<td>8</td>
</tr>
<tr>
<td>8-cell</td>
<td>6</td>
</tr>
<tr>
<td>16-cell</td>
<td>4</td>
</tr>
</tbody>
</table>

**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.

- 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

153 conditions
1409 embryos
5.5M images

Current Biology 21 (2011)
The Structure Of A Fly’s Brain I

- Core Hemisphere
  - 20K neurons

- Optic Lobe
  - 30K neurons

- VNC
  - 10K neurons
A hemisphere = 
~50 glia-isolated compartments
Want “bundle” edges of a 50 node hypergraph
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: Sample 3Kbp promoter constructs to cover brain sparsely (8K). Select a covering set C (3K).
Examples of Promotor Assay

Released Sept. 2012
Several Lines In "Atlas"

Vaa3D: hanchuan.peng.googlepages.com
Anatomy of a Fly Brain *with Light*

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Hierarchical Shotgun Mapping

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

**Individual:** Sample 100K neurons within $C$ to cover brain. Use finite color brainbow to enhance throughput.
Anatomy of a Fly Brain with Light

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

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Hierarchical Shotgun Mapping

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

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

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

1. Staining & Imaging
2. 3D Registration
3. Neuron Separation
4. Neuron Tracing
5. Compartment Annotation
6. Analysis & Modeling

Need to perform:
- Registration
- Neuron Color Separation
- Neuron/Lineage Tracing
- Compartment Analysis
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!
Compartment Analysis of GAL4 Lines

- FB (Fan shaped body), yes
- MB (Mushroom body), yes
- EB (Ellipsoid body), yes
- LB (Lateral bulb), yes
- SOG (Suboesophageal Ganglion), weak pattern

BA_GMR_10E02_AE_01_11
0.4406

BA_GMR_39C11_AE_01_05
0.2942

BA_GMR_10E02_AE_01_12
0.3916

BA_GMR_39C11_AE_01_06
0.2647

✓
Multi-Color Separation

Color shift requires sub-pixel translations!
Multi-Color Neuron Editing & Curation
Neuron Tracing (w. H. Peng)

Bioinformatics 27 (2011)

(a) pick a seed

(b) shortest paths

(c) pruned & smoothed

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

Another combinatorial 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

Scale:
15 mm x 7 mm x 5 mm ⇒ 4.2 x 10^{12} 0.5^3\mu m 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 200\mu m deep stacks
2. Cut away top 185\mu m (implies 15\mu m overlap)
Onboard Vibratome

Detection arm

Vibratome

Precision mount
(sample bath not shown)

Resonant X-Mirror

Galvanometric Mirror

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

Voxel Size: 0.4 x 0.4 x 1 µm

Acquisition Time: <1 hr.
Acquisition Software

Acquisition

Stage

Vibratome

Task control

Tiling editor
 Acquisition display

Each square is a single stack
The MPI-CBG “Plan”

(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.
2P SI Besel beam scope for observing *in vivo* cell process at $\lambda/4$
Next gen. DLSM scope to follow 24 hours of fly or fish development
BioImage Analysis: Intracellular Processes

EB1 labelled tubulin fibers.

Gamma-tubulin labelled centrosomes

Par2-Par6 labelled membranes

Signal Strength of Par2

Centrosome Radius

Tony Hyman Lab
### BioImage Analysis: Worm Atlas & SCE

#### Fuhui Long

<table>
<thead>
<tr>
<th>Ord</th>
<th>Cell Name</th>
<th>Mean Location</th>
<th>Neighboring Nuclei</th>
<th>Spacing</th>
<th>Size</th>
<th>Shape</th>
</tr>
</thead>
<tbody>
<tr>
<td>332</td>
<td>INDR1</td>
<td>X: 81.5257 ± 2.0397</td>
<td>HYP7ABPRAAPP PP, INDL1, BWMDR11, HYP7ABARPAAP PP, BWMDL10</td>
<td>3.8246 ±1.1421</td>
<td>9005 ± 993 (px)</td>
<td>Aspect ratio 1.5243 ±0.2069</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Y: 2.6659 ± 0.4637</td>
<td></td>
<td>3.8407 ±0.9327</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Z: 1.8412 ± 0.3335</td>
<td></td>
<td>4.3572 ±0.7644</td>
<td>1.5005 ±0.7195 (µm)</td>
<td></td>
</tr>
<tr>
<td></td>
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<td></td>
<td>4.5065 ±1.2958</td>
<td></td>
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</tr>
<tr>
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<td></td>
<td></td>
<td></td>
<td>4.6709 ±1.0913</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- Intestinal
- Body wall muscle
- Hypodermal
- Ventral motor neuron
BioImage Analysis: Fly Wing

Suzanne Eaton Lab
Tracking Lineages During Fly Embryogenesis

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

You?
& 2 of your friends?
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