

The landscape of Fly Connectomics data

Exploring the connectome workshop

4th May 2021

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University of Cambridge
Virtual Fly Brain

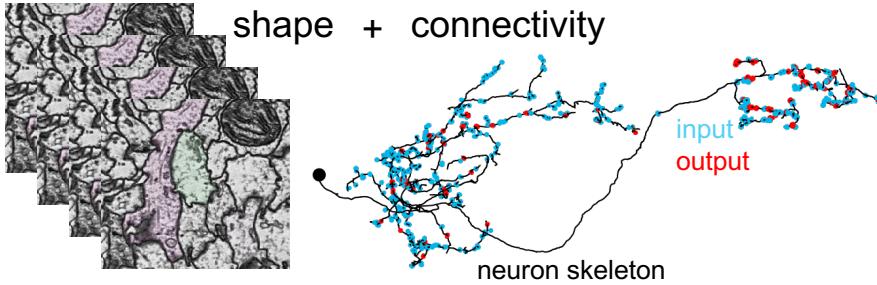
Workshop outline

Workshop structure

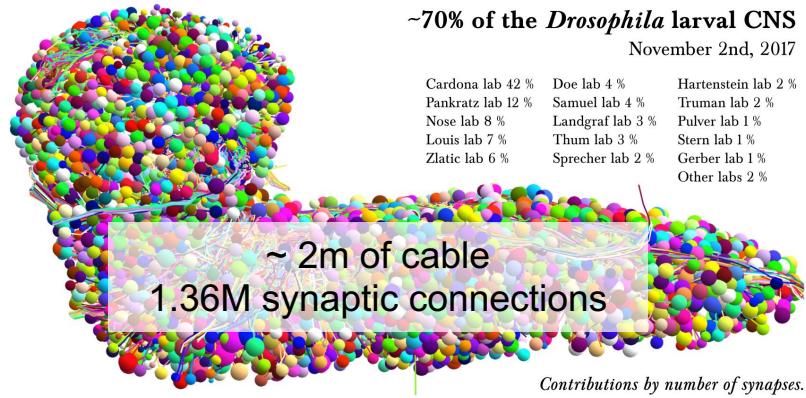
1. Brief presentations:
 - a) available connectomics datasets
 - b) introduction to Virtual Fly Brain (David Osumi-Sutherland)
 - c) tools available to query the data (Philipp Schlegel)
2. Hands on tutorials in groups: R or python notebooks

Available *Drosophila* EM datasets: L1, FAFB, hemibrain

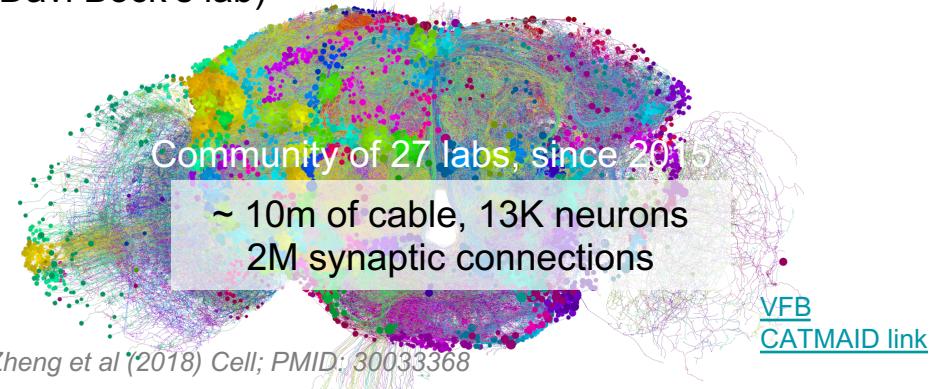
Synaptic resolution



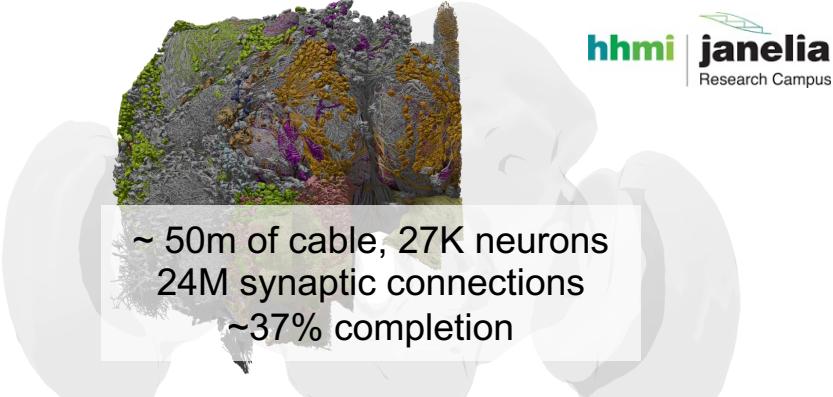
Larval L1 connectome: 10k neurons, ~90% completion
(lead by Albert Cardona's group, since 2013)



FAFB adult female: 100K neurons, ~10% completion
(Davi Bock's lab)



Hemibrain adult female: 1/3 brain

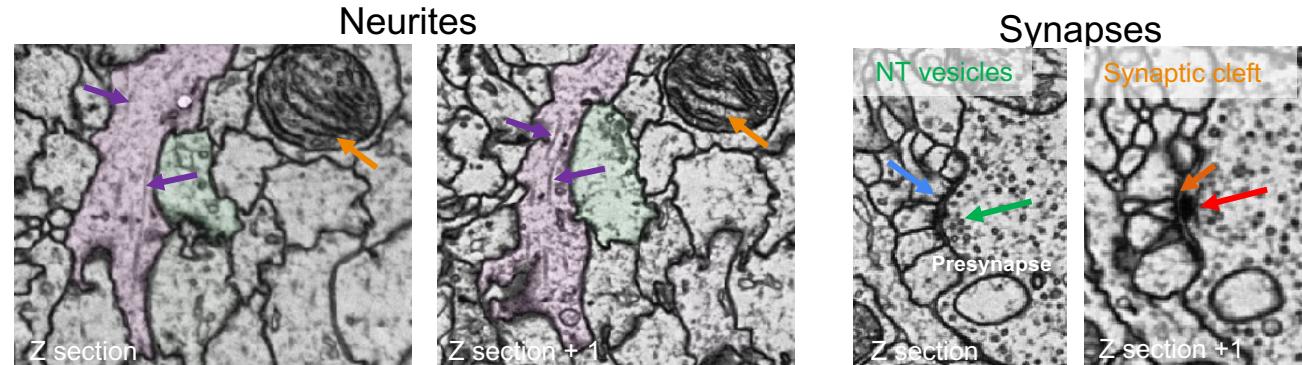


Reconstructing neuronal morphologies

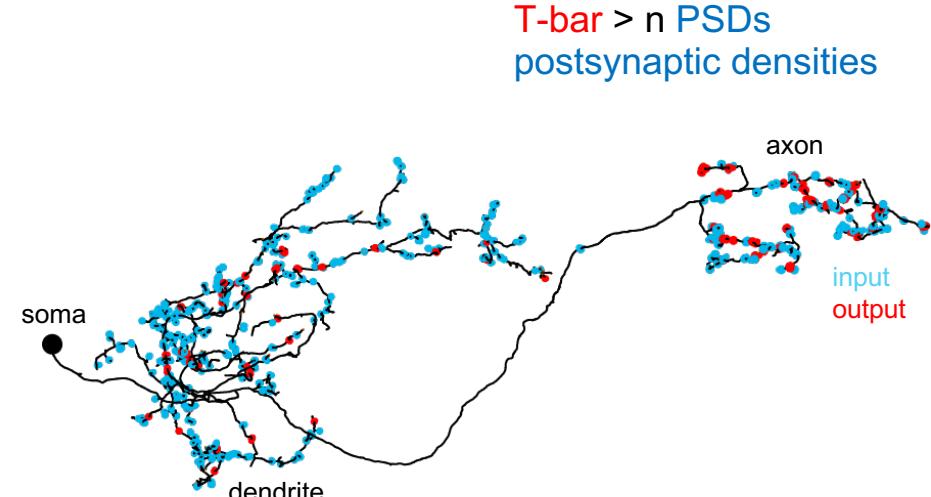
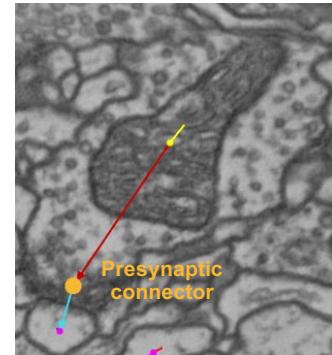
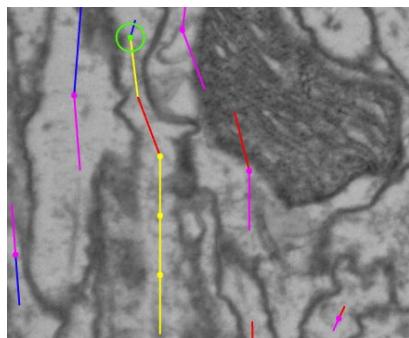
Initially: manual tracing of selected neurons



Web-based tracing
and analysis platform

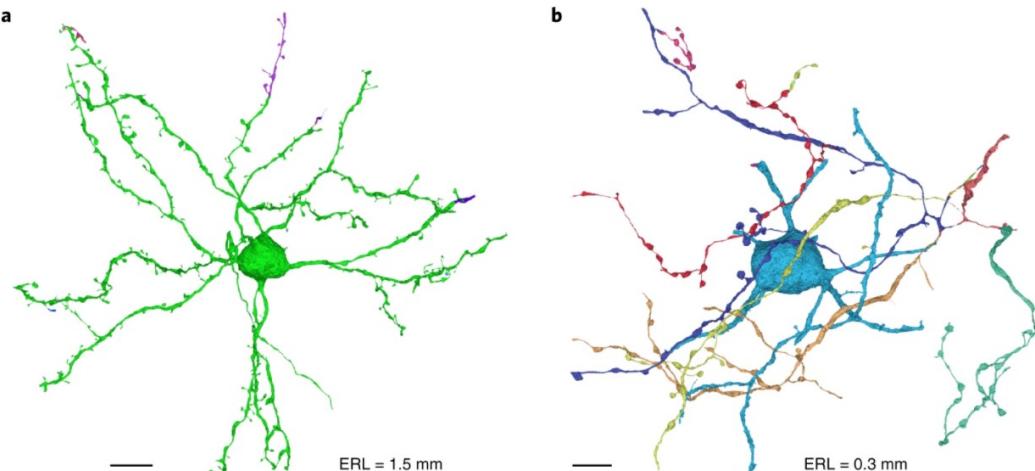
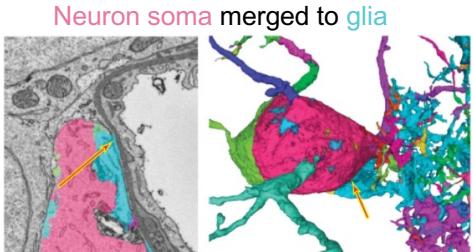
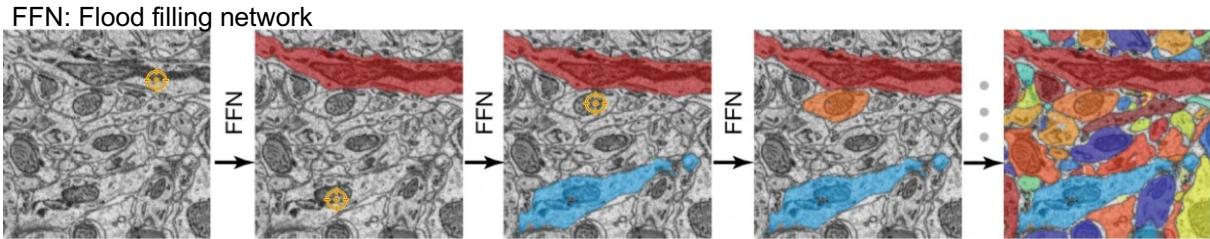


Neuron skeletons: manually placed
nodes are connected by lines

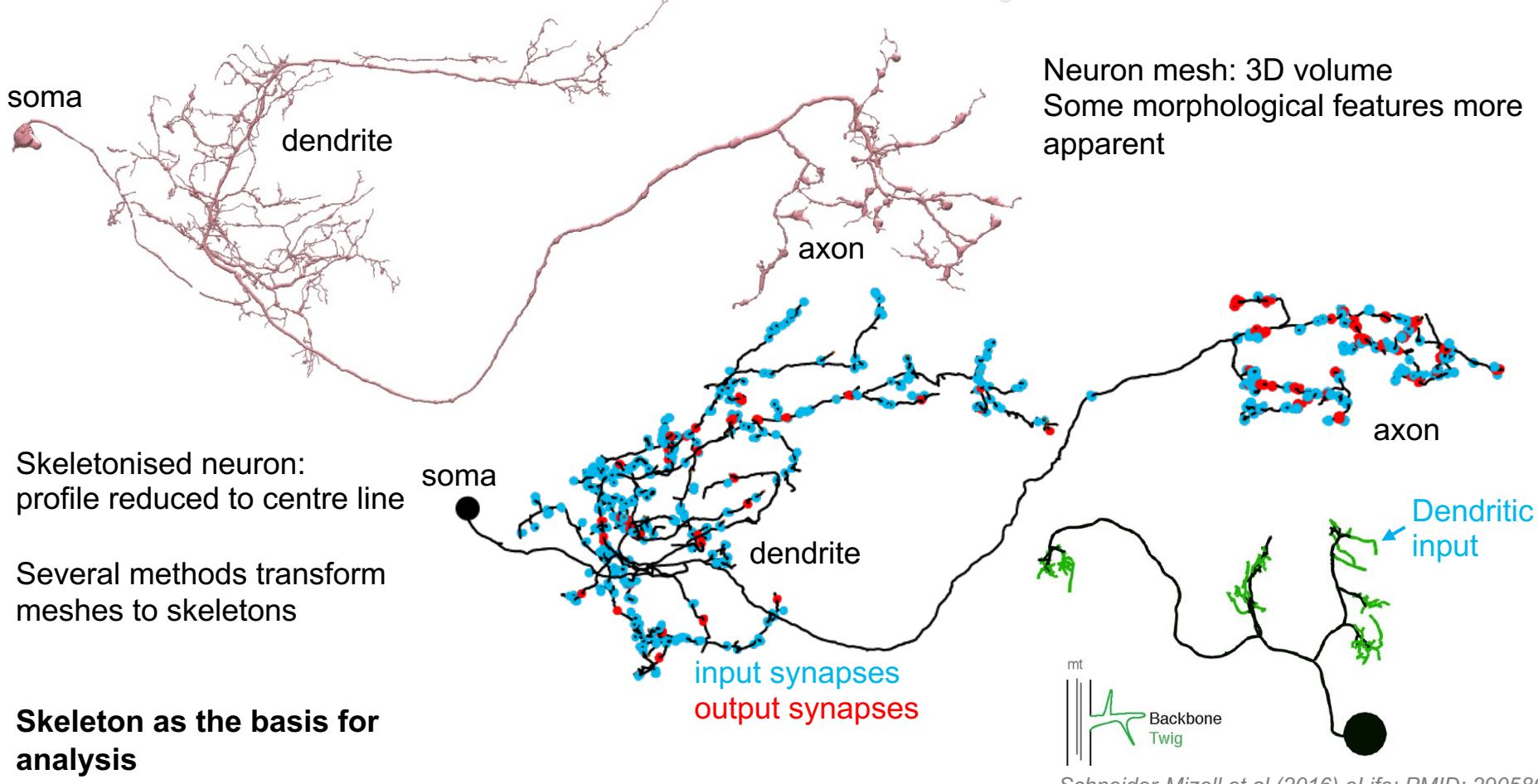


Autosegmentation of neurons

- Different machine learning algorithms
- Iterative process for each voxel, using image features
- Training the algorithm for each volume/region requires ground truth
- Balancing: long cable versus decision confidence <> proofreading effort

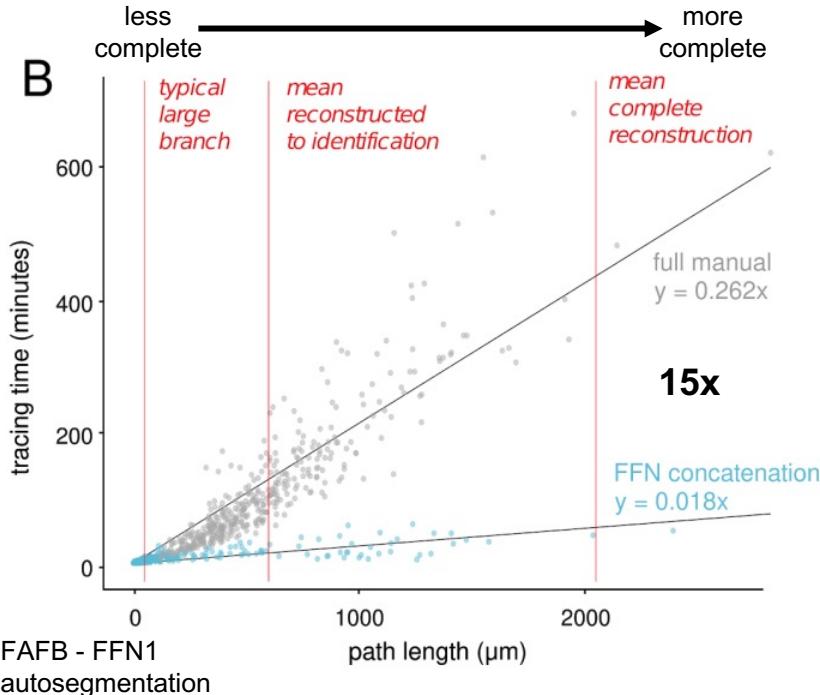


Neuron representations

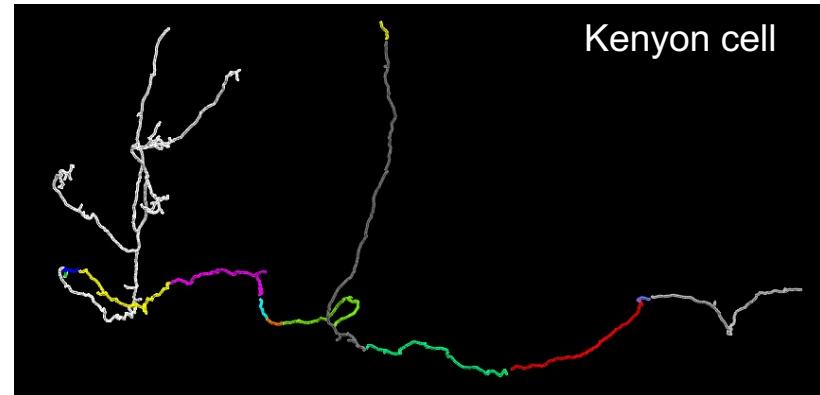


Autosegmentation of neurons

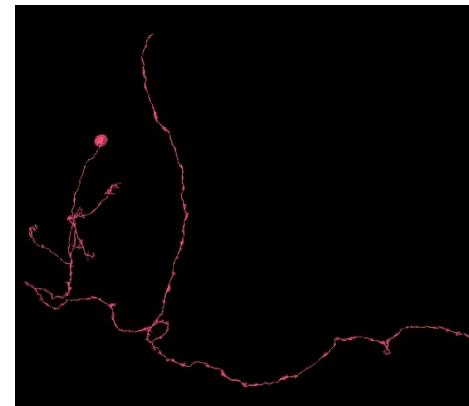
Autosegmentation results in significant speed up in reconstruction.



FAFB - FFN1 autosegmentation in CATMAID



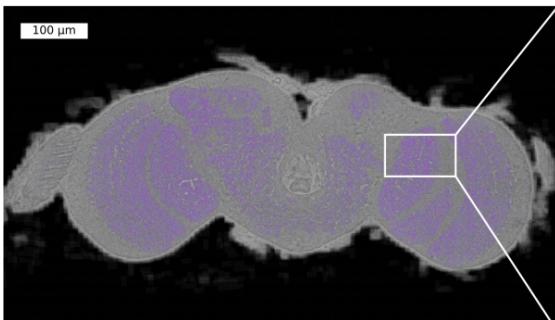
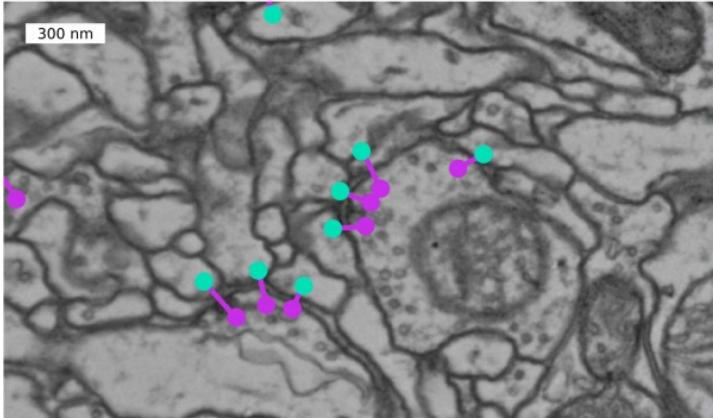
[FlyWire](#) autosegmentation, after realigning FAFB



But proofreading is needed to fix errors

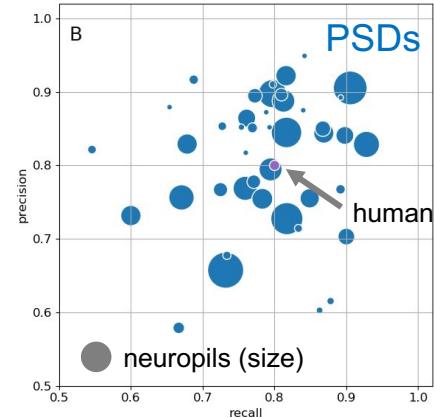
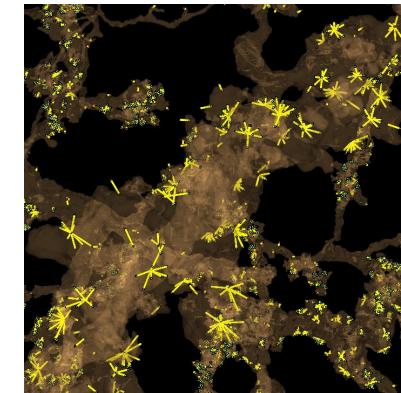
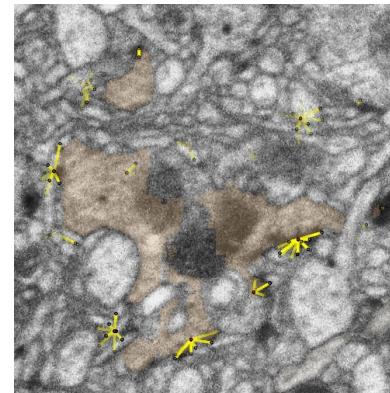
Autosegmentation of synapses

Automatic prediction of synaptic partners in FAFB



244M synaptic partners
Likely ~30% false positives

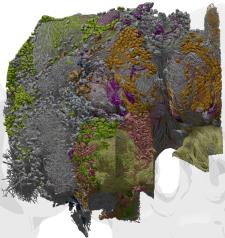
Automatic segmentation of **T-bars** and **PSDs** in the hemibrain:



9M T-bars (>90%)
60M PSDs (20-85%)
Confidence level

Tuning predictions based on regional image quality, training data.

Proofreading neurons

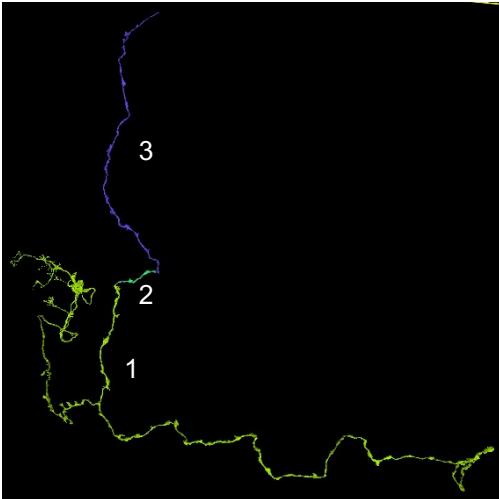


>50 person/years
proofreading time

Effort needed related to
data quality

Incomplete arbours > incomplete connectivity

Neurons with incomplete arbour

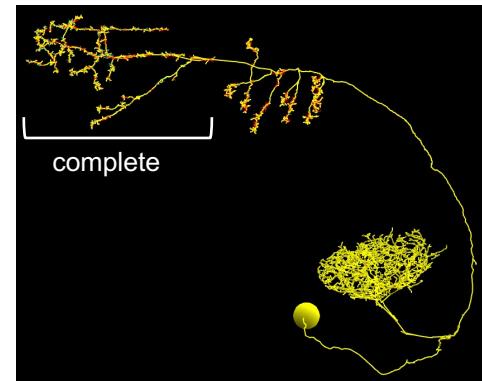
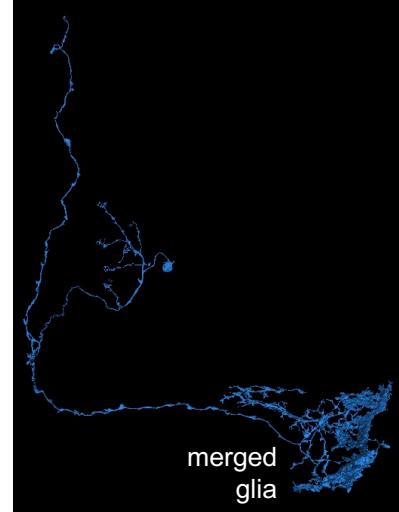
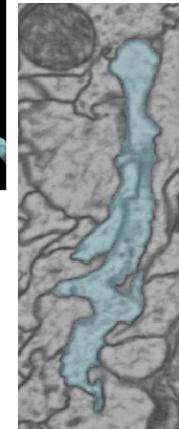
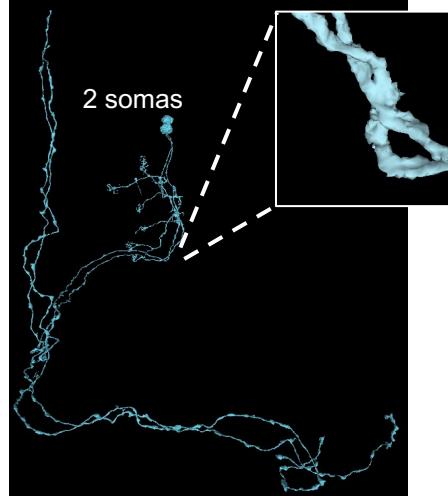


Necessary level of completeness
depends on data release goals, individual
analysis needs.

Completeness might be regional.

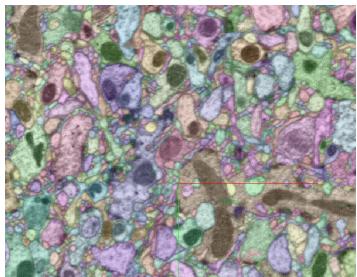
High quality will benefit cell typing efforts

Large fixes: false merges



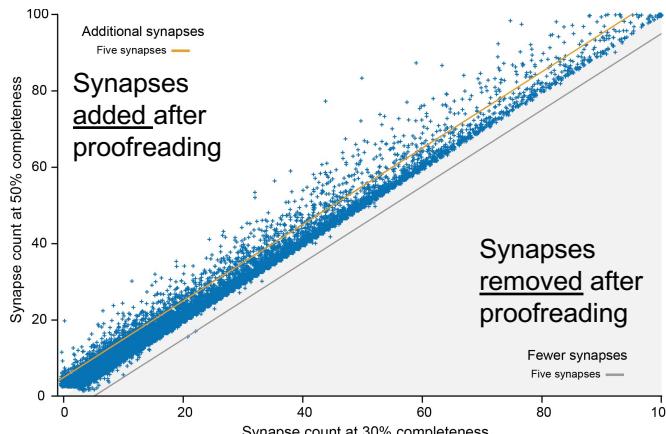
Dense connectomes: what are they

Where ‘every’ neuron in a dataset is reconstructed.
Usually the product of automatic segmentation methods.



Proofreading effort for morphologies and synapses will determine baseline quality of the data and inform completeness.

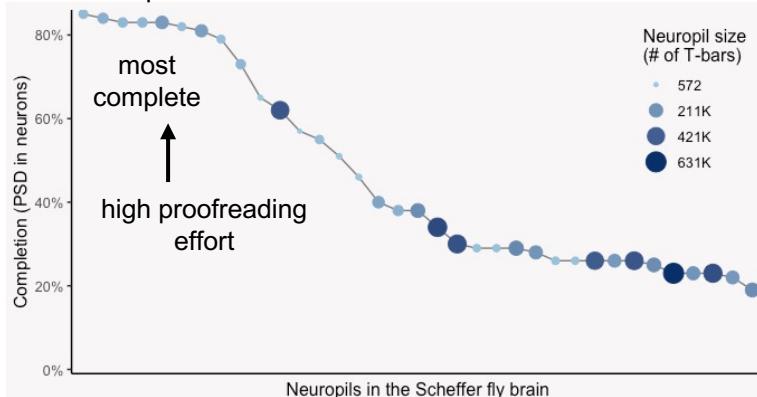
Edited synapses during proofreading



Most connections are weak.

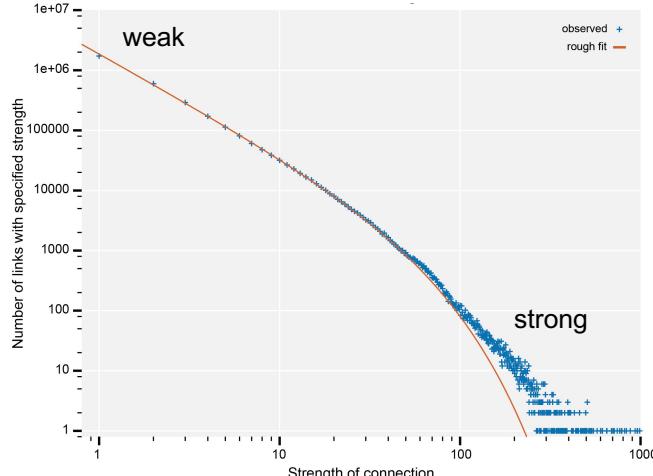
Even at ~40% recovery, strong partners are clear.

Completion status (# PSDs in neurons) of neuropils in hemibrain



Data from Scheffer et al (2020)

Synaptic strength



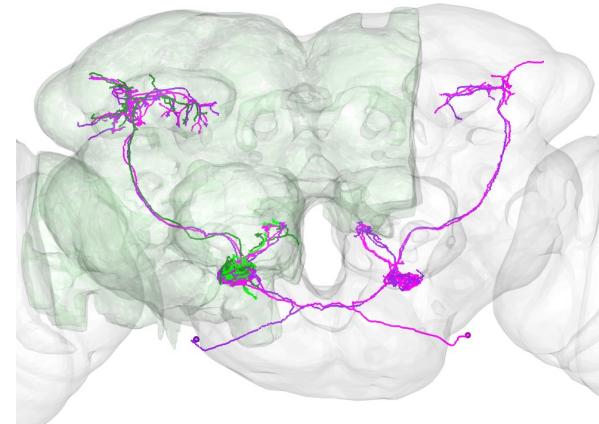
What is connectome completeness

1. Completeness of the volume: whole or partial region/brain

Neuronal arbours and connectivity missing
Cell typing can be difficult

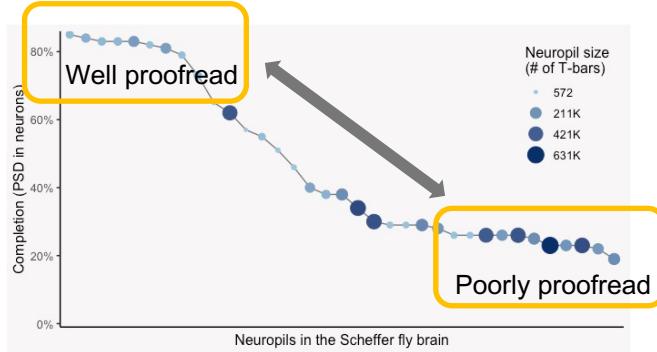
hemibrain and 2 truncated neurons
(same type)

2 whole FAFB neurons (same type)

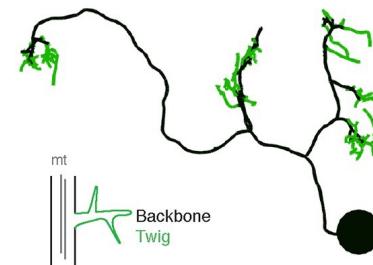


2. Completeness of morphologies: all neurons reconstructed or only some

3. Completeness of proofreading



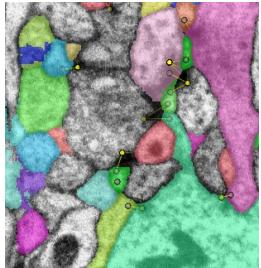
4. Completeness of synaptic connectivity



In the hemibrain:
>90% of T-bars recovered
20-85% of PSDs recovered

Limitations of dense vs sparse connectomes

Sparse

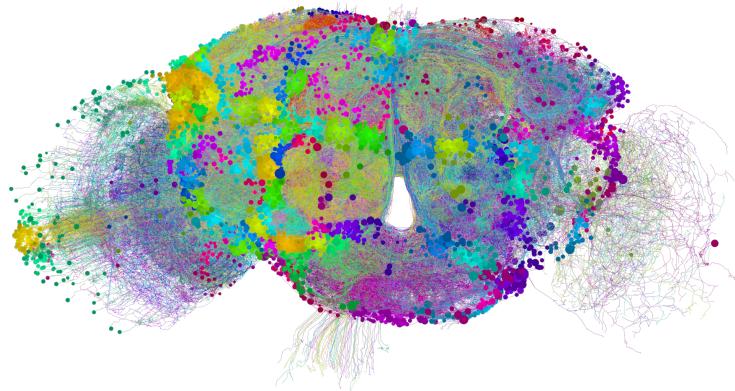


Manual or semi-manual reconstruction

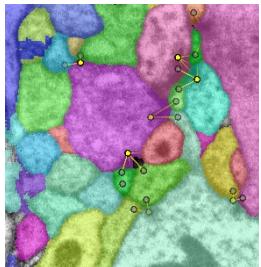
Smaller number of neurons

Selected neurons reconstructed and completed

Validated synaptic connectivity



Dense



Automatic reconstruction or intense proofreading effort

Quality can differ by region

Most neurons reconstructed (fragments present) to a certain level of completeness

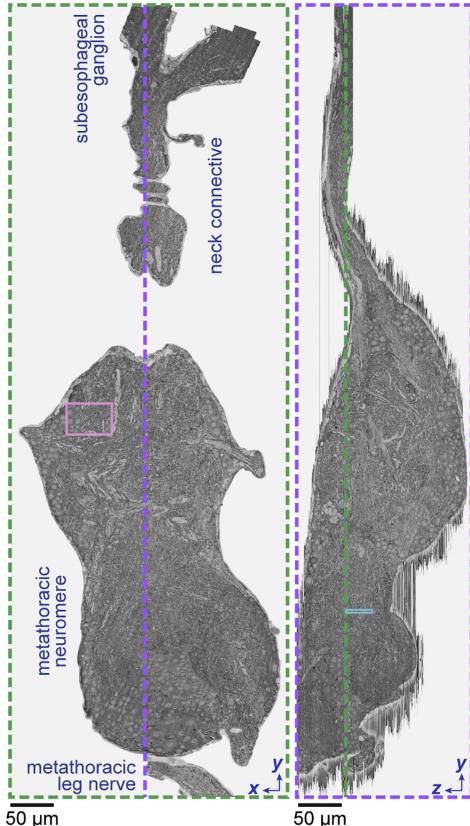
Baseline level of proofreading: major errors fixed

Synaptic connectivity can be validated



Additional datasets

Female VNC (Wei Lee's group)

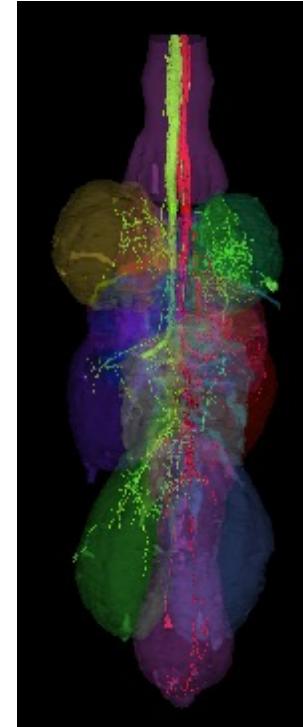


Tracing community

Autosegmentation and
FlyWire style
proofreading of neurons
available for the tracing
community

Wellcome collaboration: Jefferis, Landgraf (Cambridge)
Waddell (Oxford), Rubin, Card (Janelia)

Male VNC



Connectome expected
late 2021

Via [neuPrint+](#)

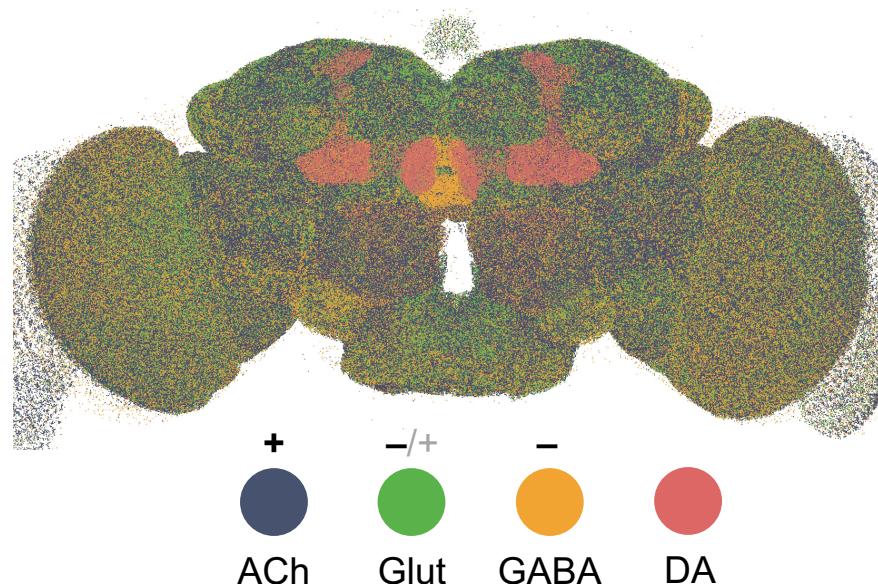
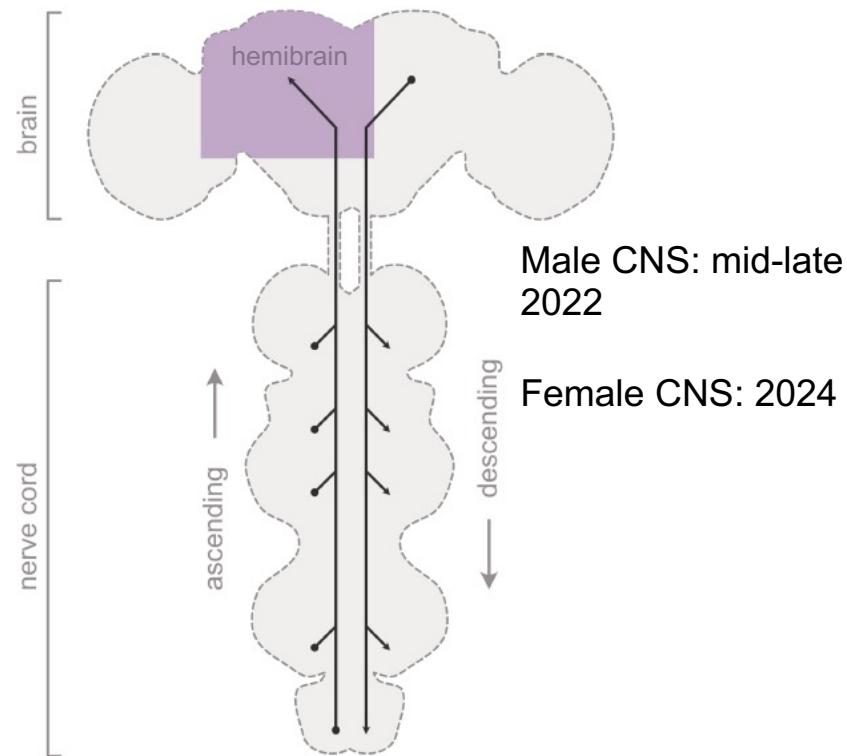
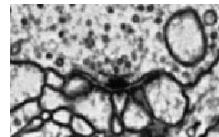
neuPRINT+
exploring inter and intra cellular interactions

Clements et al (2020) *bioRxiv*;
doi: 10.1101/2020.01.16.909465

Future datasets and other advances

Wellcome collaboration: Jefferis, Landgraf (Cambridge)
Waddell (Oxford), Rubin, Card (Janelia)

Neurotransmitter prediction, from EM synapses



Workshop outline

Workshop structure

1. Brief presentations:
 - a) available connectomics datasets
 - b) **introduction to Virtual Fly Brain (David Osumi-Sutherland)**
 - c) tools available to query the data (Philipp Schlegel)
2. Hands on tutorials in groups: R or python notebooks