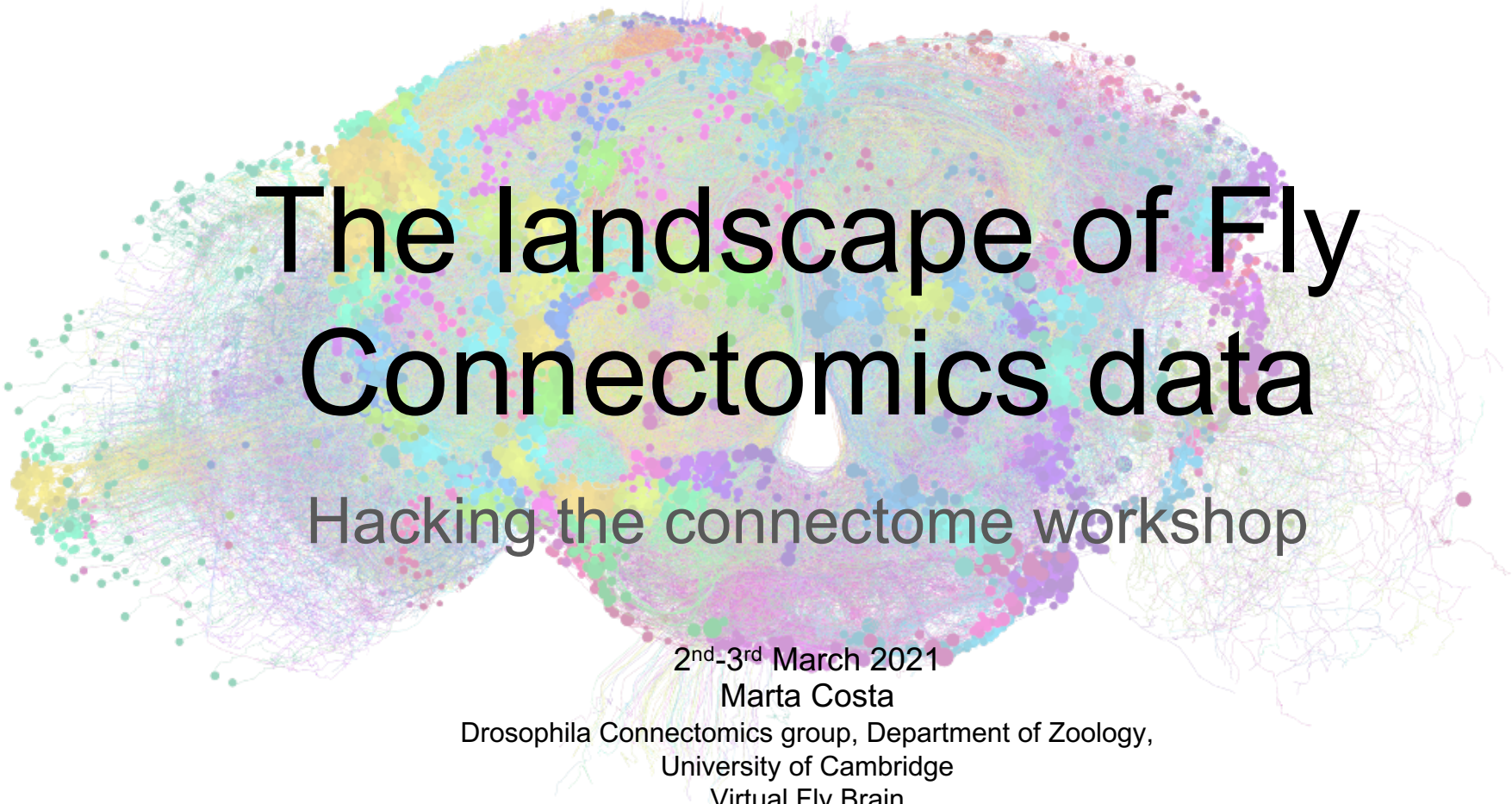


The landscape of Fly Connectomics data

Hacking the connectome workshop

2nd-3rd March 2021

Marta Costa
Drosophila Connectomics group, Department of Zoology,
University of Cambridge
Virtual Fly Brain

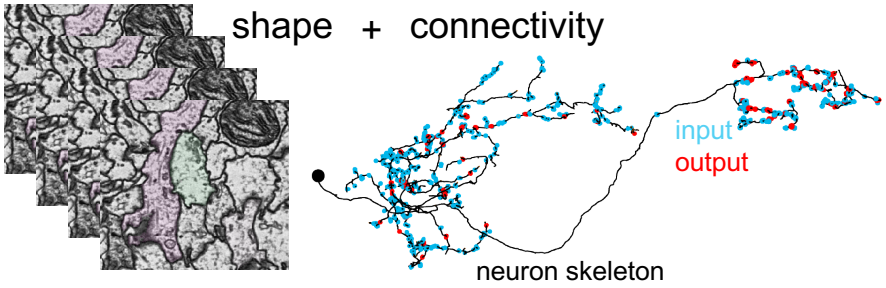
Workshop outline

Workshop structure

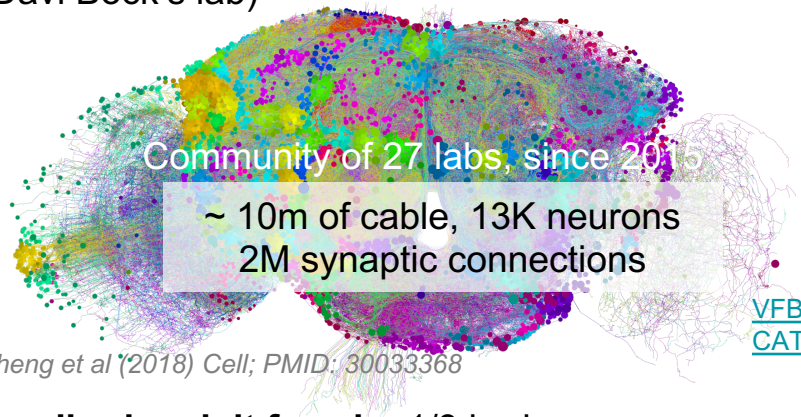
1. Day 1: Brief presentations:
    a) available connectomics datasets
    b) introduction to Virtual Fly Brain (David Osumi-Sutherland)
    c) tools available to query the data (Philipp Schlegel)
2. Day 1: Hands on tutorials in groups: R or python notebooks
3. Day 2: continuing the tutorials, more free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

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

Synaptic resolution



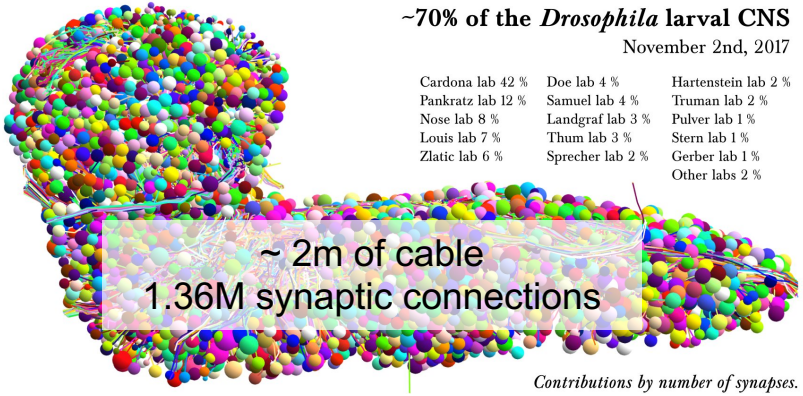
FAFB adult female: 100K neurons, ~10% completion (Davi Bock's lab)



VFB
CATMAID link

Zheng et al (2018) Cell; PMID: 30033368

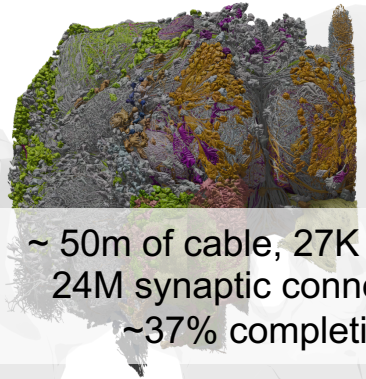
Larval L1 connectome: 10k neurons, ~90% completion (lead by Albert Cardona's group, since 2013)



Ohyama et al. (2015) Nature; PMID: 25896325

VFB CATMAID link

Hemibrain adult female: 1/3 brain



hhmi | janelia Research Campus

~ 50m of cable, 27K neurons
24M synaptic connections
~37% completion

Scheffer et al (2020) eLife; PMID: 32880371

Janelia link

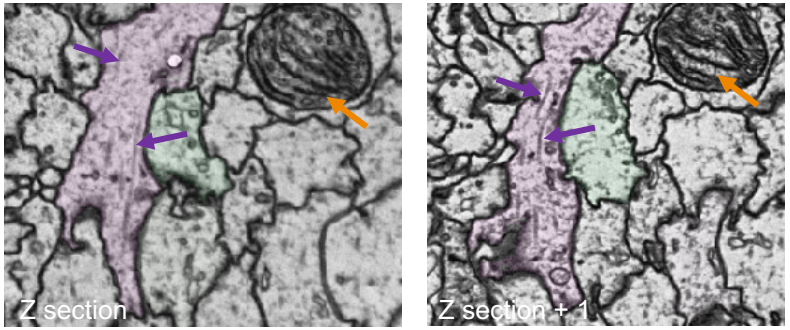
Reconstructing neuronal morphologies

Initially: manual tracing of selected neurons

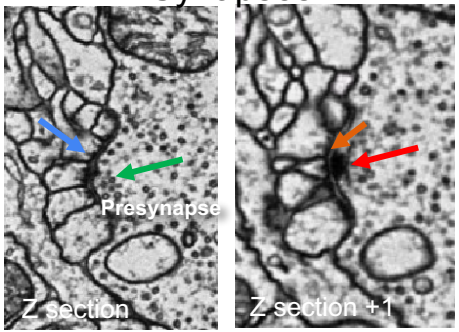
CATMAID

Web-based tracing
and analysis platform

Neurites

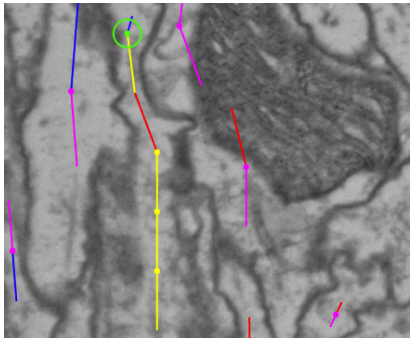


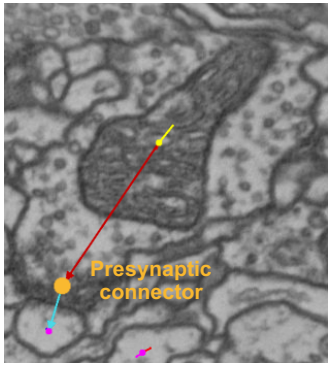
Synapses

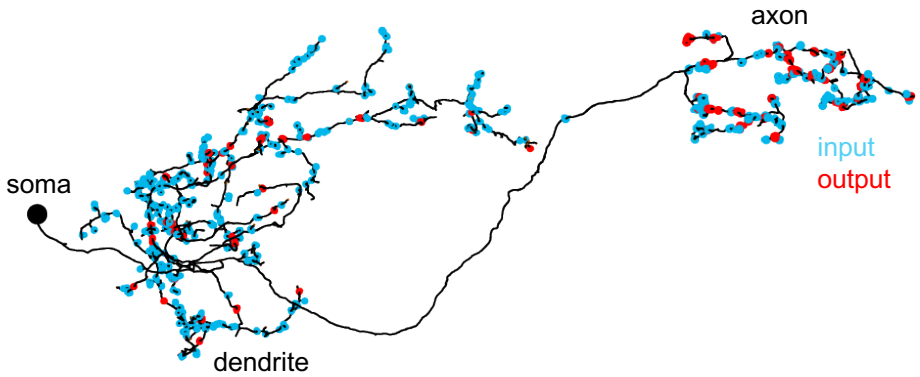


T-bar > n PSDs
postsynaptic densities

Neuron skeletons: manually placed
nodes are connected by lines





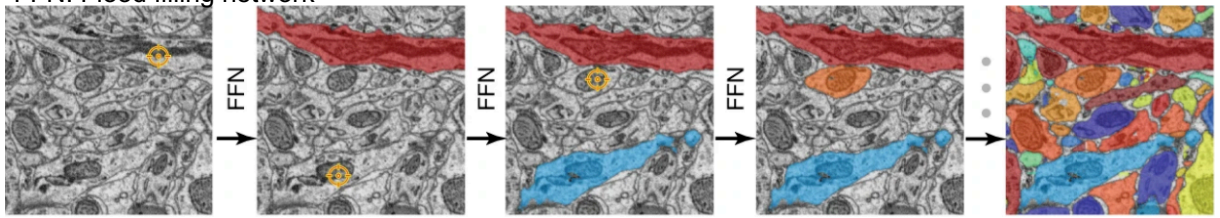


Saalfeld et al. (2009) Bioinf.; PMID: 19376822

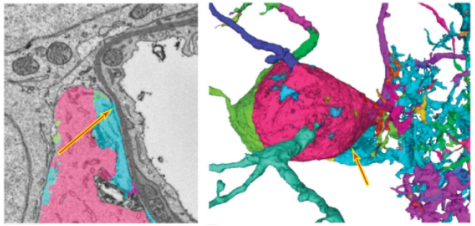
Autosegmentation of neurons

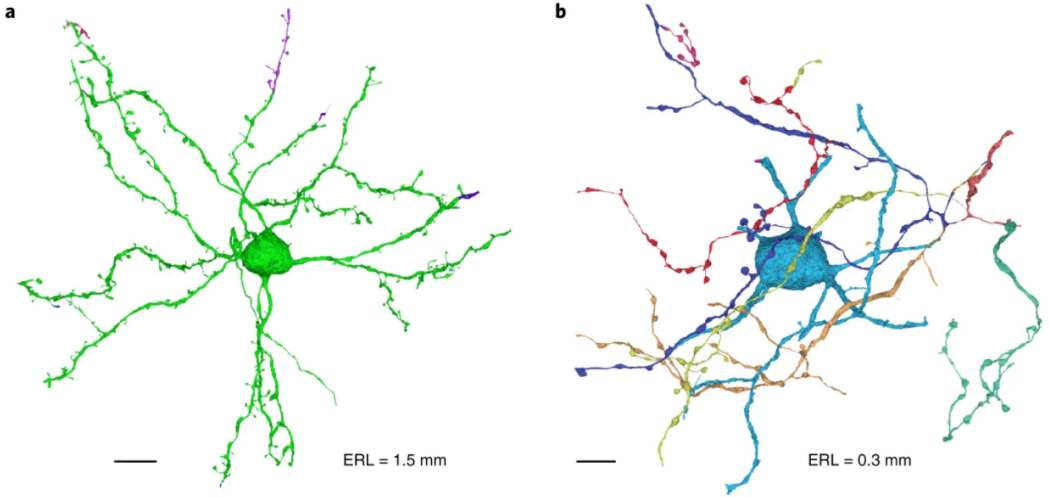
- Different machine learning algorithms
- Iterative process for each voxel, using image features
- Training the algorithm for each volume/region requires ground truth
- Balancing: long cable versus decision confidence <> proofreading effort

FFN: Flood filling network



Neuron soma merged to glia





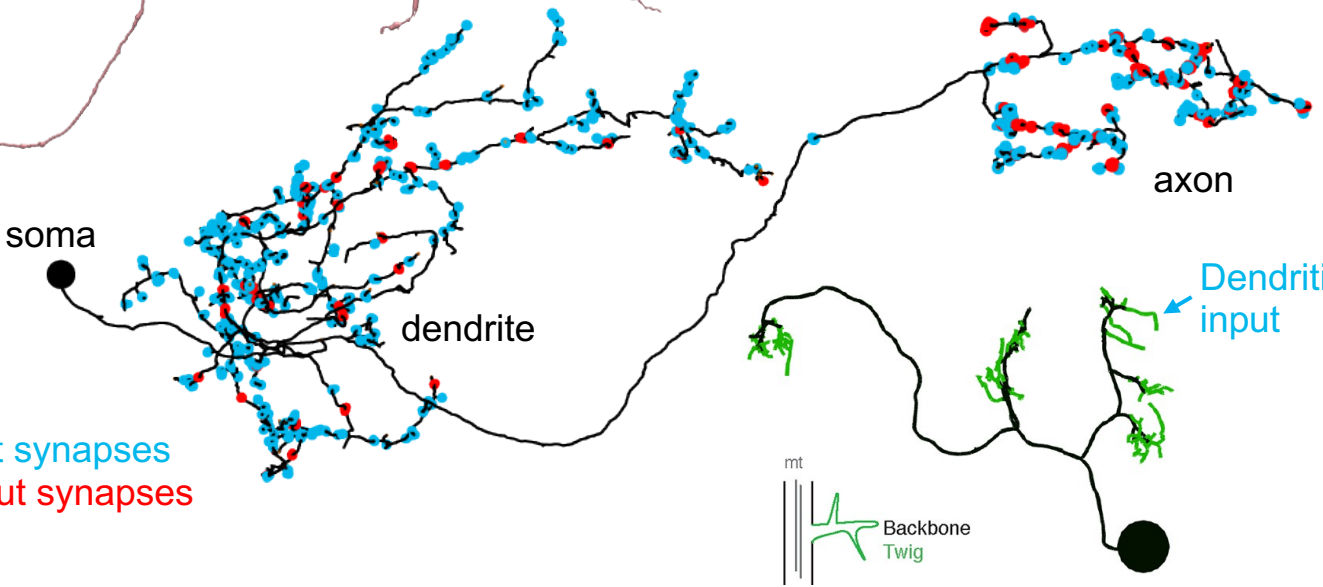
Januszweski et al (2018) Nature Methods; PMID: 30013046
Li et al (2020) bioRxiv; doi: 10.1101/605634

Neuron representations

Neuron mesh: 3D volume
Some morphological features more apparent

Skeletonised neuron:
profile reduced to centre line

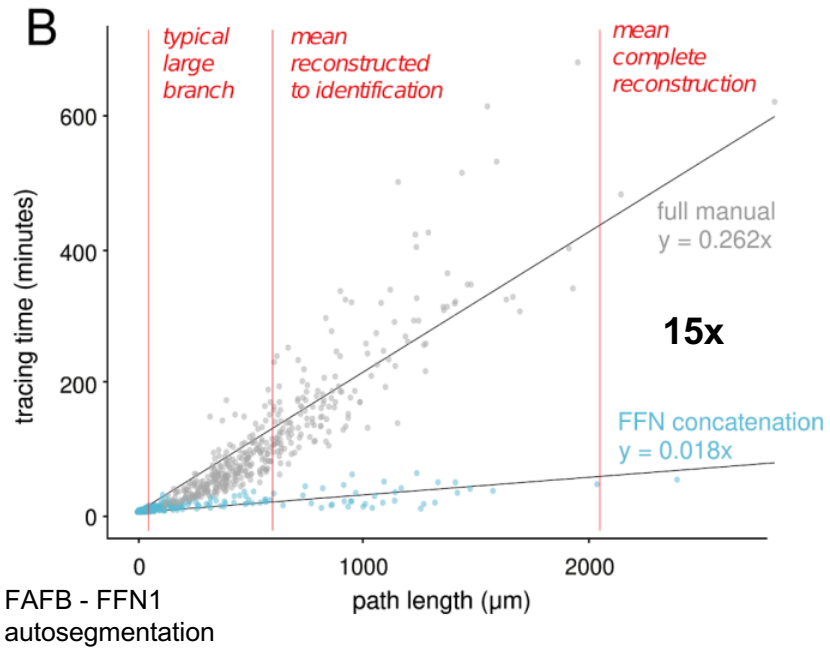
Skeleton as the basis for analysis



Schneider-Mizell et al (2016) eLife; PMID: 29058674

Autosegmentation of neurons

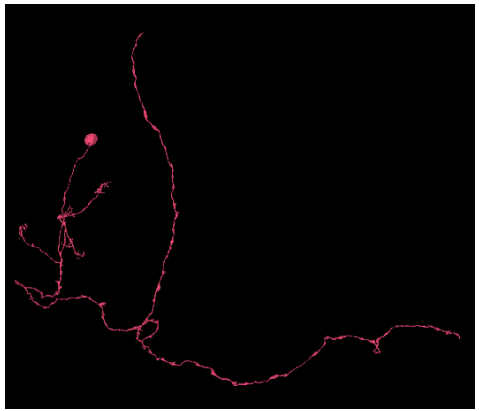
Autosegmentation results in significant speed up in reconstruction.



FAFB - FFN1 autosegmentation

FAFB - FFN1 autosegmentation in CATMAID

FlyWire autosegmentation, after realigning FAFB



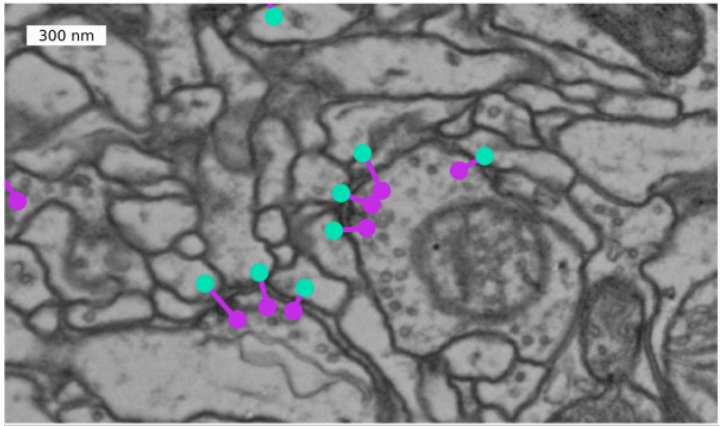
But proofreading is needed to fix errors

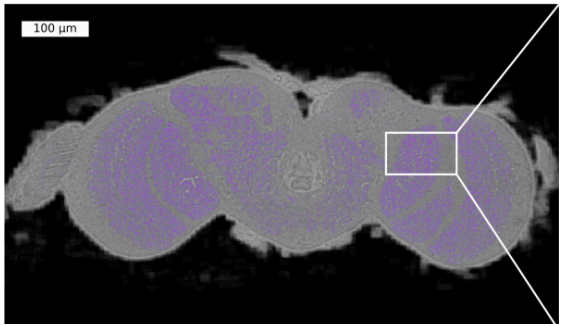
FFN1: Li et al (2020) bioRxiv; doi: 10.1101/605634

FlyWire: Dorkenwald et al (2020) bioRxiv; doi: 10.1101/2020.08.30.274225

Autosegmentation of synapses

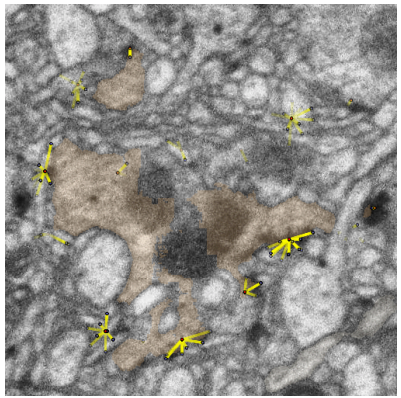
Automatic prediction of synaptic partners in FAFB

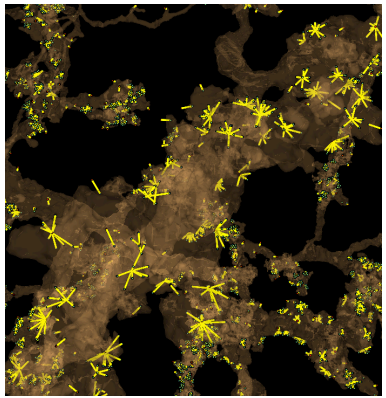


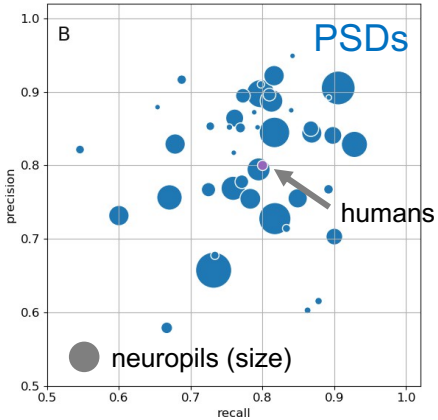


244M synaptic partners
Likely ~30% false positives

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







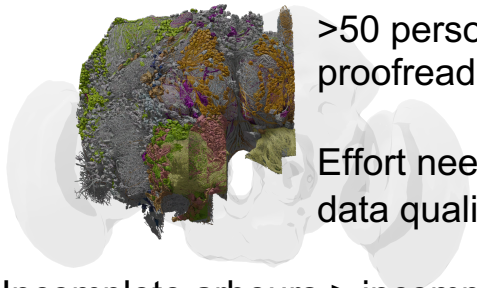
9M T-bars (>90%)
60M PSDs (20-85%)
Confidence level

Tuning predictions based on regional image quality, training data.

Buhmann et al (2020) bioRxiv; doi: 10.1101/2019.12.12.874172

Huang et al (2018) Fr. Neu. Circ.; PMID: 30420797

Proofreading neurons

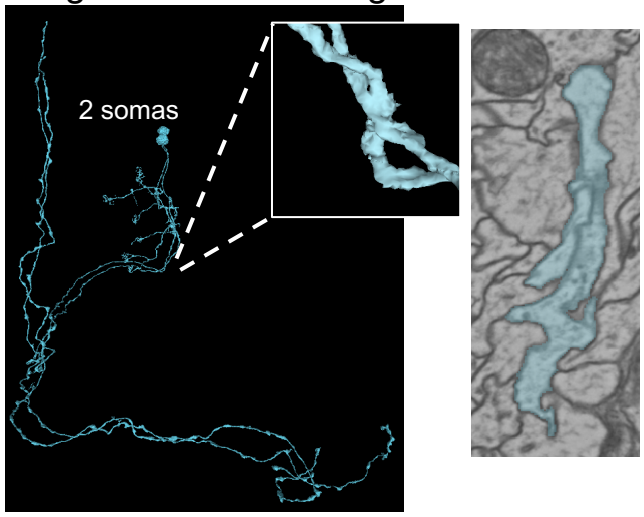


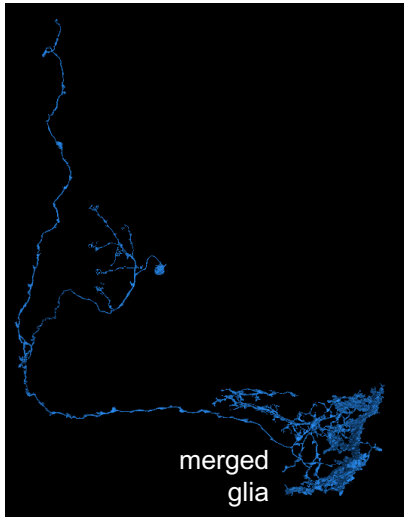
>50 person/years proofreading time

Effort needed related to data quality

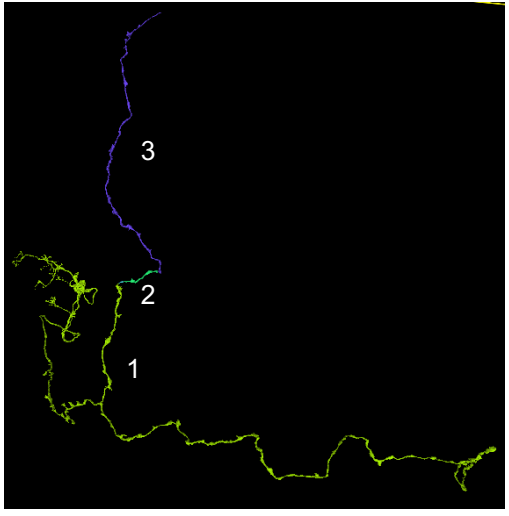
Incomplete arbours > incomplete connectivity

Large fixes: false merges





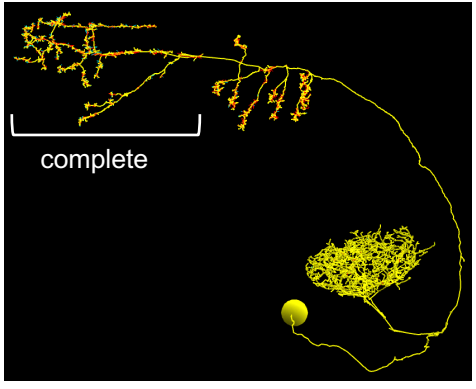
Neurons with incomplete arbour



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

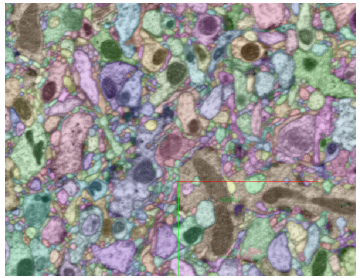
Completeness might be regional.

High quality will benefit cell typing efforts



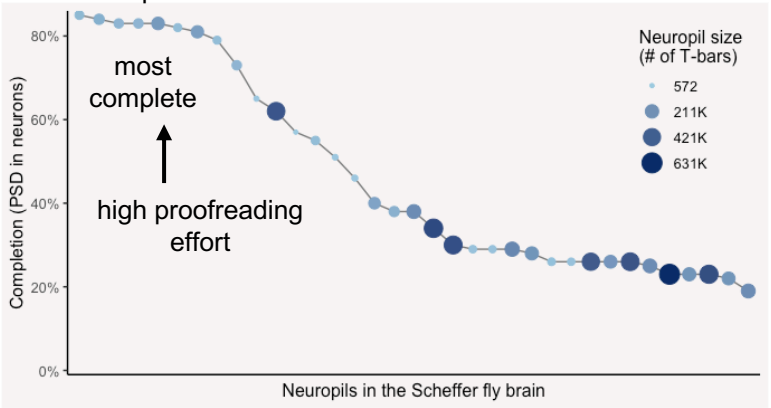
Dense connectomes: what are they

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



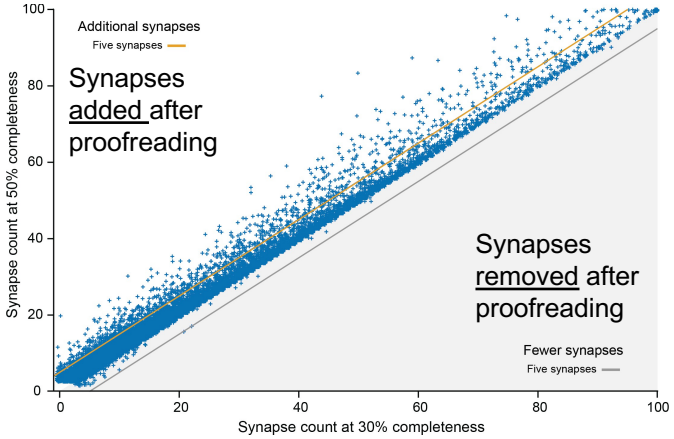
Proofreading effort for morphologies and synapses will determine baseline quality of the data and inform completeness.

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



Data from Scheffer et al (2020)

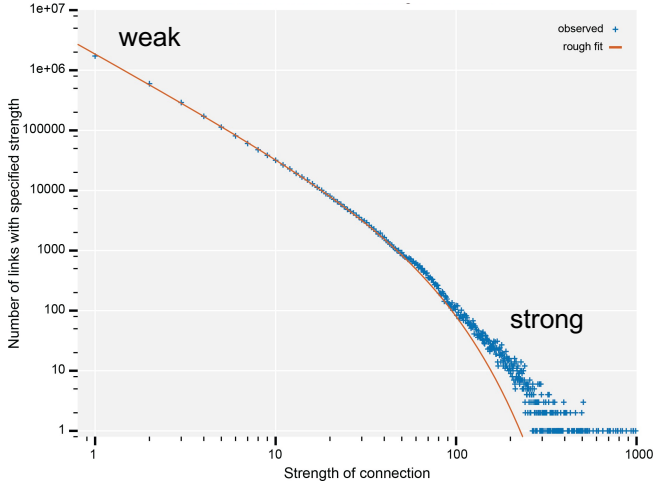
Edited synapses during proofreading



Most connections are weak.

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

Synaptic strength



Scheffer et al (2020) eLife; PMID: 32880371

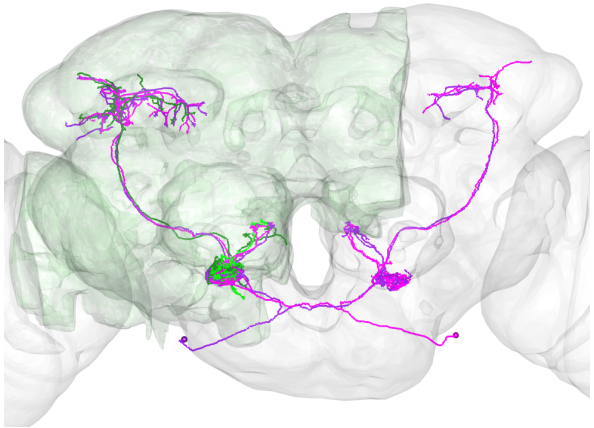
What is connectome completeness

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

   Neuronal arbours and connectivity missing
   Cell typing can be difficult

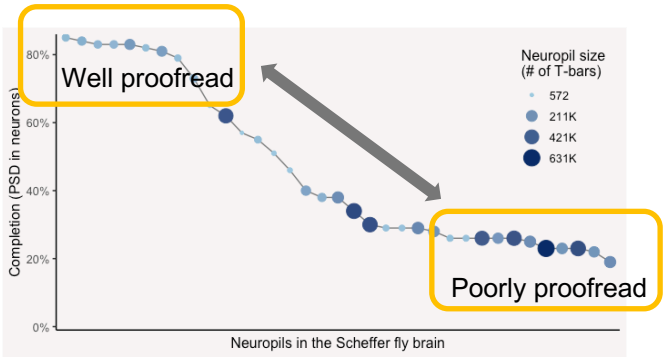
hemibrain and 2 truncated neurons (same type)

2 whole FAFB neurons (same type)

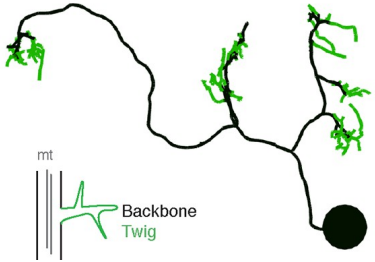


2. Completeness of morphologies: all neurons reconstructed or only some

3. Completeness of proofreading



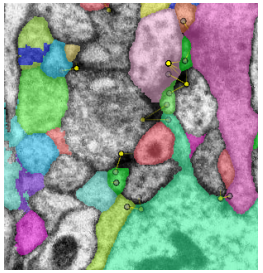
4. Completeness of synaptic connectivity



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

Limitations of dense vs sparse connectomes

Sparse

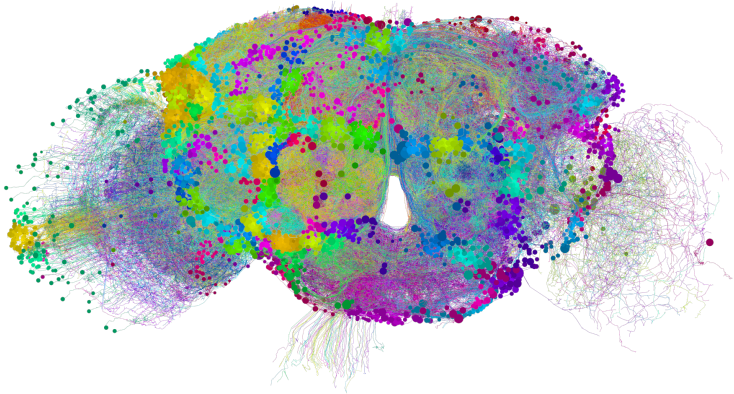


Manual or semi-manual reconstruction

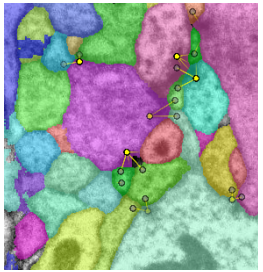
Smaller number of neurons

Selected neurons reconstructed and completed

Validated synaptic connectivity



Dense



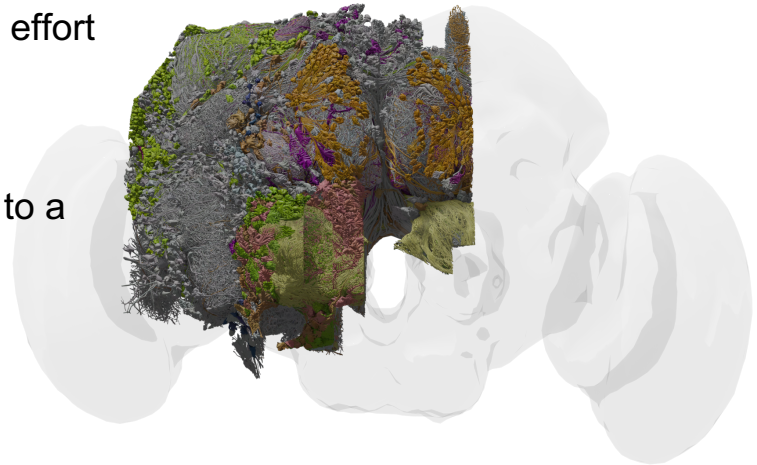
Automatic reconstruction or intense proofreading effort

Quality can differ by region

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

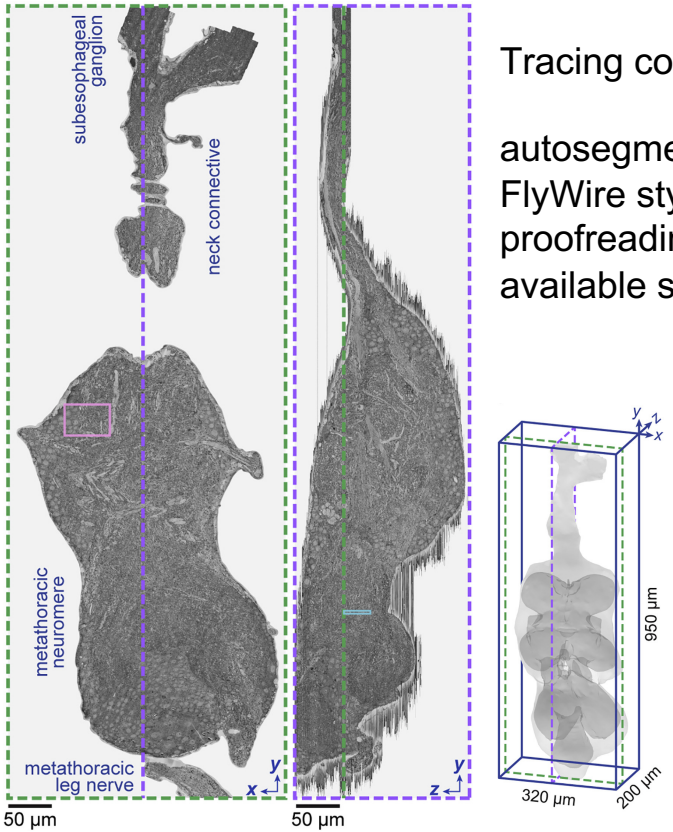
Baseline level of proofreading: major errors fixed

Synaptic connectivity can be validated



Additional datasets

Female VNC (Wei Lee's group)



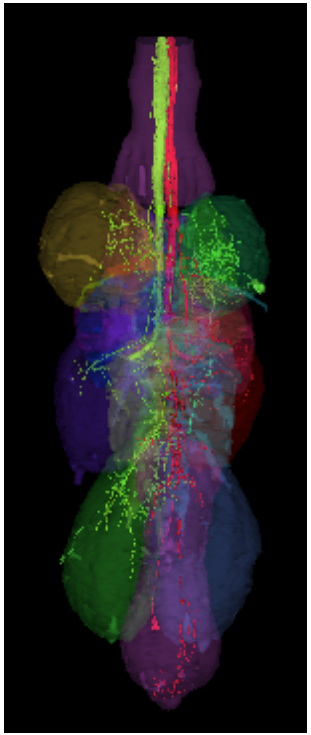
Tracing community

autosegmentation and FlyWire style proofreading of neurons available soon

Phelps et al (2020) Cell; PMID: 33400916 VFB link

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

Male VNC



Connectome expected late 2021

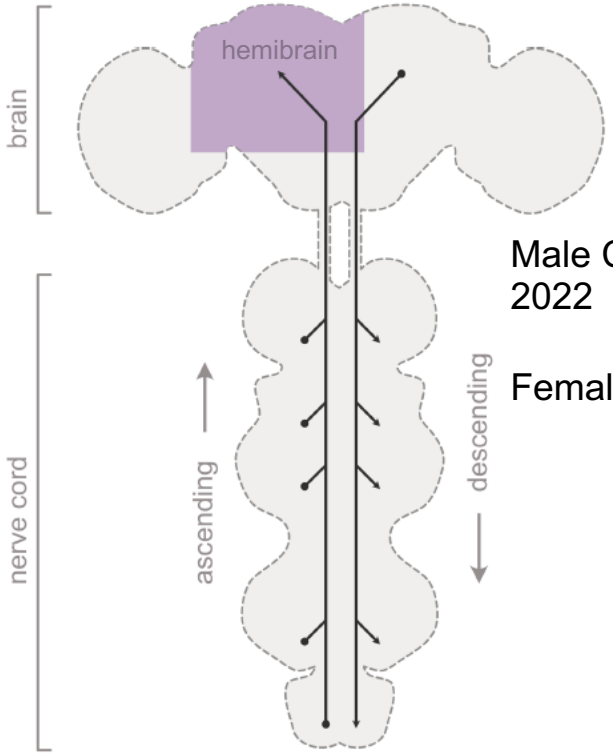
Via neuPrint+

neuPRINT+
exploring inter and intra cellular interactions

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

Future datasets and other advances

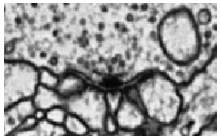
Wellcome collaboration: Jefferis, Landgraf (Cambridge)
Waddell (Oxford), Rubin, Card (Janelia)

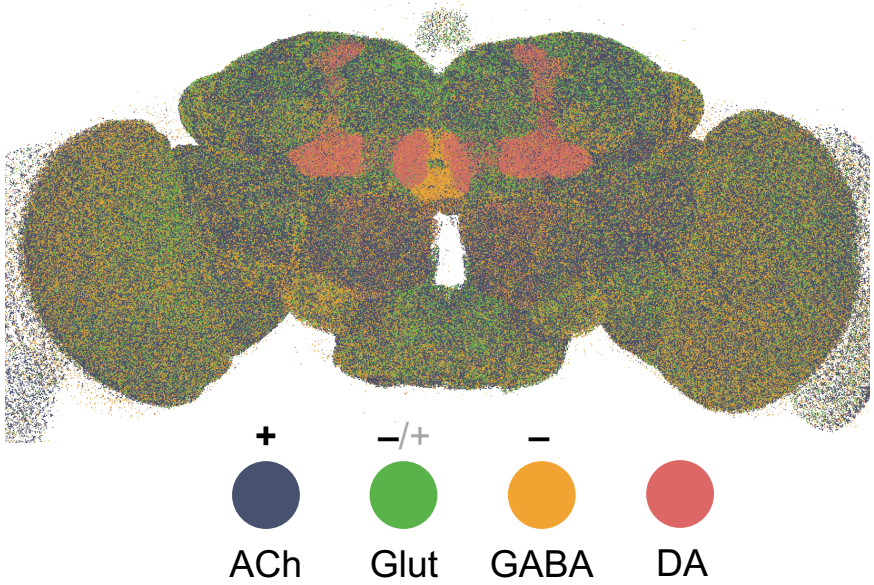


Male CNS: mid-late 2022

Female CNS: 2024

Neurotransmitter prediction, from EM synapses





Eckstein et al (2020) bioRxiv; doi: 10.1101/2020.06.12.148775

Workshop outline

Workshop structure

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