Photonics Technologies for Connectomics Research

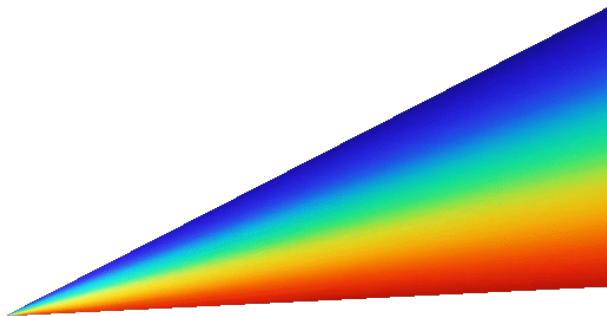
林彥穎 Yen-Yin Lin

Brain Research Center,

Institute of Photonics Technologies,

National Tsing Hua University, Taiwan

2017/May/31st



Neuroscience Inauguration

Observation only

Santiago Ramón y Cajal
(1 May 1852 – 17 October 1934)

The Nobel Prize in Physiology or Medicine 1906

Exposing the Forest: Camillo Golgi and Santiago Ramóny Cajal (1906)

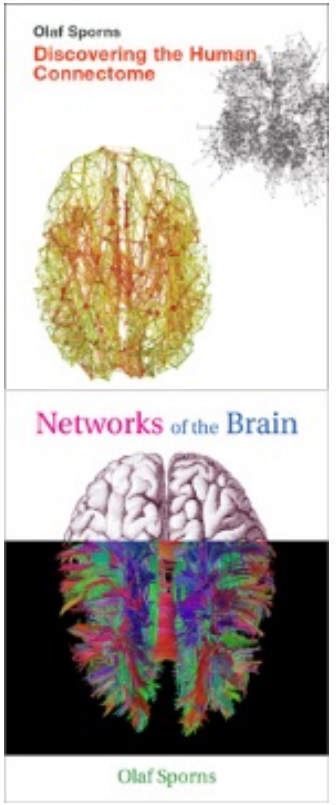
By developing and using methods that stain key components of nerve cells silver, Golgi and Cajal revealed the beauty and complexity of the nervous system.

Camillo Golgi

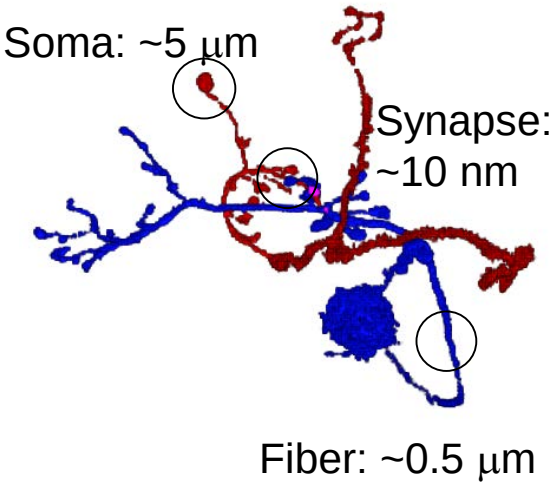
Neuron networks in a real brain

- **What is connectome/connectomics ?**
  The connection matrix of a real brain
- **What do scientists want to do?**
  To understand the human brain &
  To improve the quality of human life

Prof. Olaf Sporns



The basic characteristics of neuron cell



Challenges

- ✓ Field of view
- ✓ Speed
- ✓ Resolution
- ✓ Perturbation & manipulation

Sporns et. al. (2005) *PLOS Comput. Biol.* 1:e42

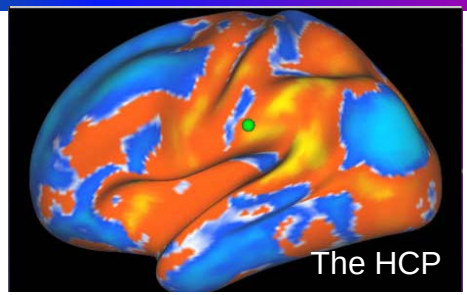
Multi-level Connectomes

According to the adopted technologies

Macroscopic (region) (country & airport)

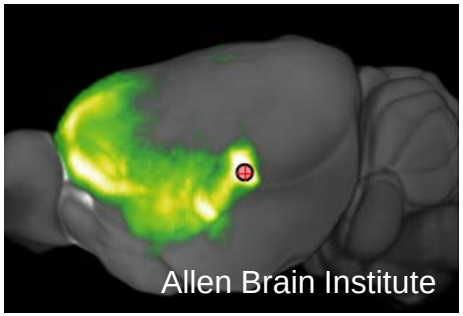
Human fMRI (Human Connectome Project)

Mouse *in situ* hybridization (Allen Brain Institute)



Mesoscopic (tract) (flight path)

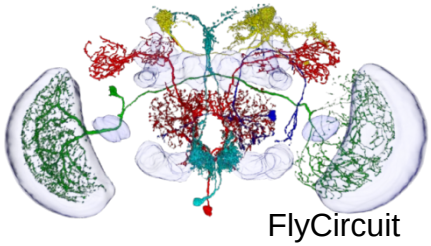
Human BigBrain Project (Human Brain Project)



Microscopic (neuron) (flight)

Drosophila brain (FlyCircuit image database, NTHU)

Mouse (Neuron projections, Allen Brain Institute)

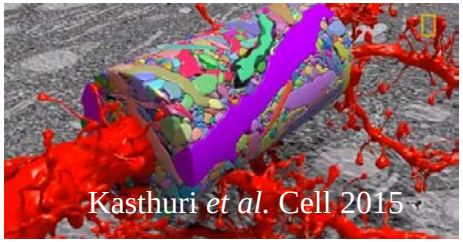


Nanoscopic (synapse) (gate)

C. elegans EM and pathway graph (Albert Einstein)

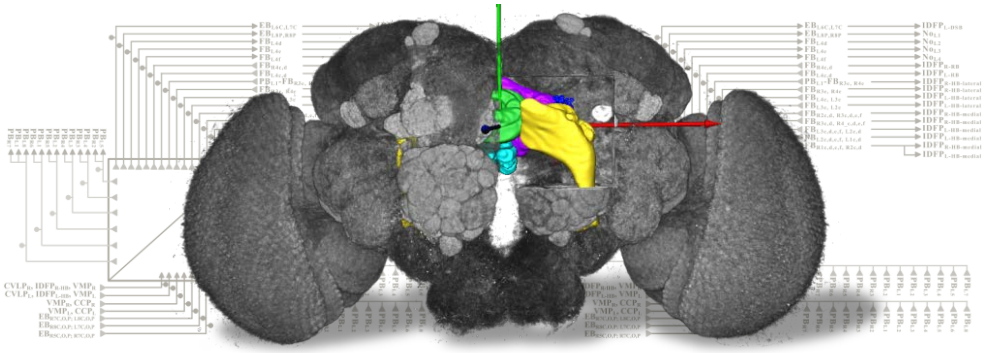
Mouse serial block-face SEM (Harvard)

Drosophila medulla (optical lobe) (Janelia Farm)



Brain Research Center @ NTHU

Ann-Shyn Chiang



Anatomy and function of neural circuits are the foundation of understanding brain's **emergent** properties

Missions:

(i) To construct a complete whole animal functional neural wiring diagram at single neuron resolution;

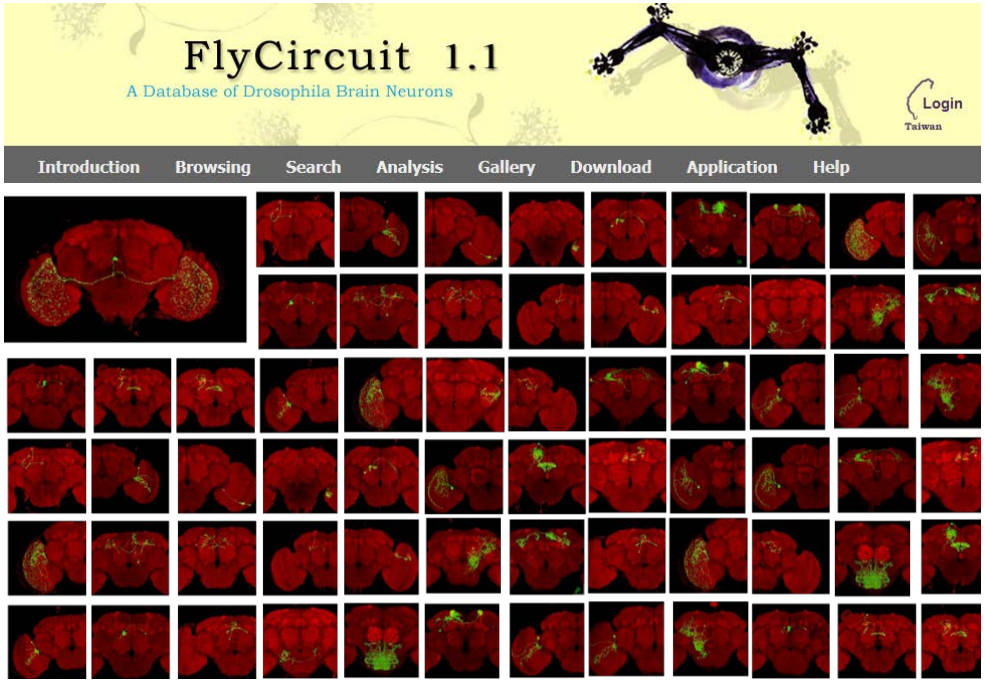
(ii) To formulate and test models of circuit computation in order to find rationales among genes, circuits and behaviors.

(iii) To develop disruptive key technologies for the next generation connectomics mapping.

More descriptions in Lo and Chiang, (2016) The Journal of Neuroscience 36(45):11375–11383.

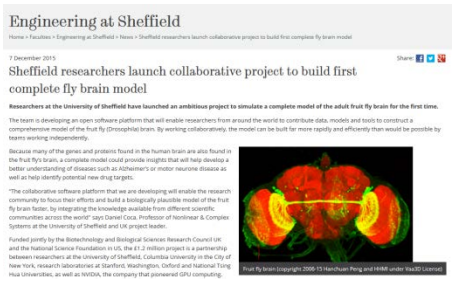
FlyCircuit database

Large-scale 3D Neuron Reconstruction from Optical Microscopic Images



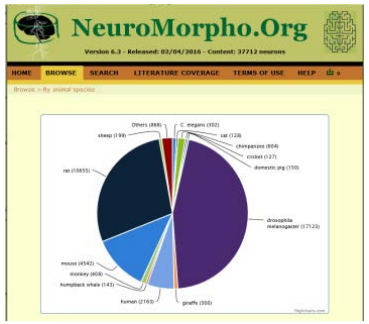
International collaborators Using FlyCircuit data:

Columbia University, University of Sheffield
Allen Institute for Brain Science, Stanford University, Washington University, Oxford University, George Mason University, NVIDIA…etc.



http://www.flycircuit.tw/

- **~23,000 neurons**
- **>130,000 registered users**
- **~90 countries**

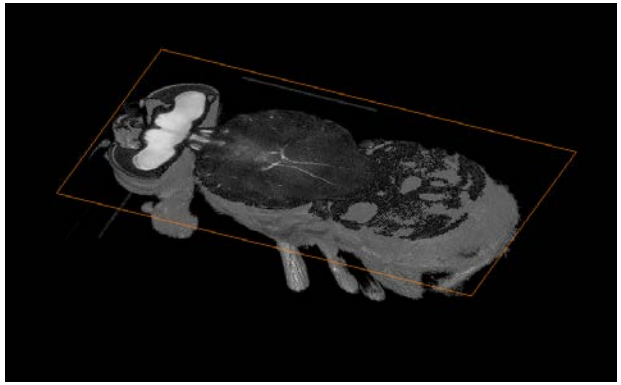


The directions in this webinar

Drosophila is an important exercise.

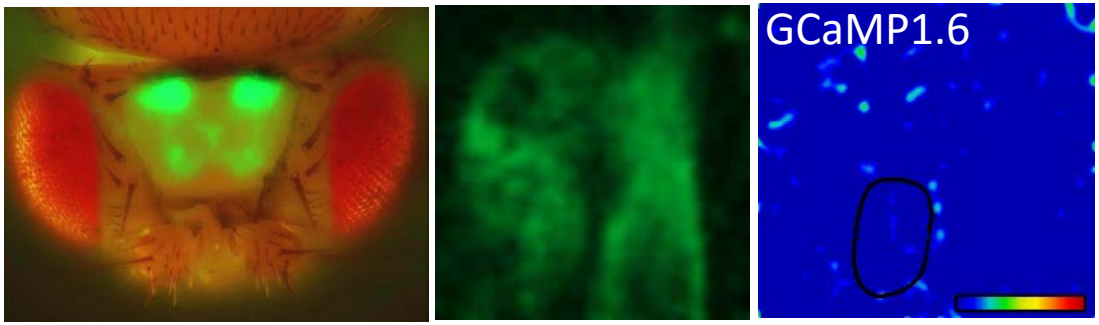
Behavior study

Anatomical Connectome



Neuroscientist's
three "photonics" arrows

Functional Connectome



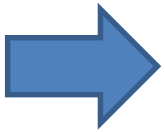
Photonics Technologies

Features

- Non-invasive for samples and the other devices
- Low cost
- Resolution up to ~ 1/10 wavelength

Limitations

- Penetration depth



Photonics systems are friendly integrated to satisfy specific needs.

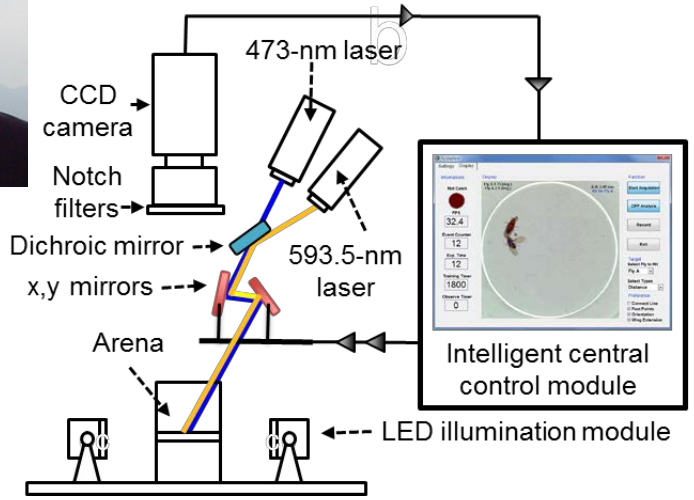
Machine vision + Laser tracking

Linking internal/external worlds

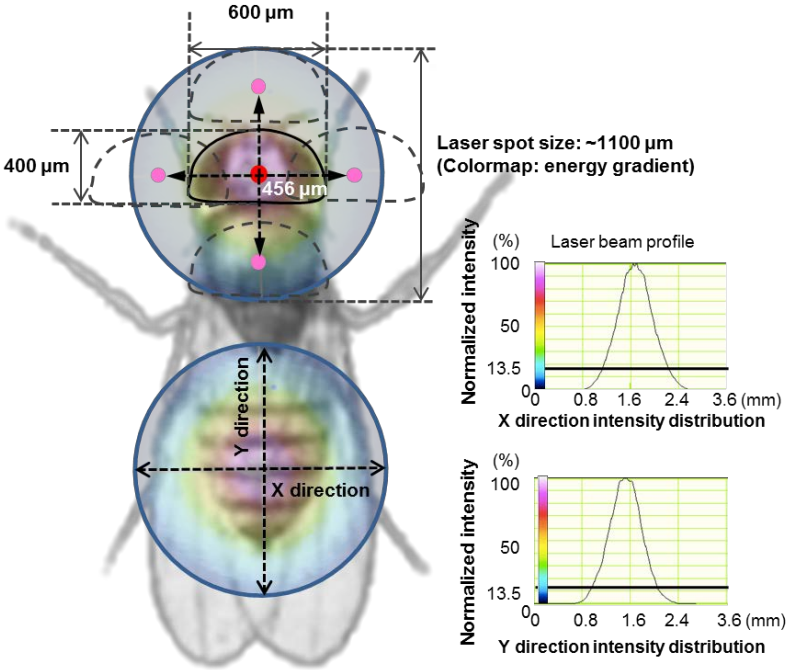
ALTOMS: automated laser tracking and optogenetic manipulation system

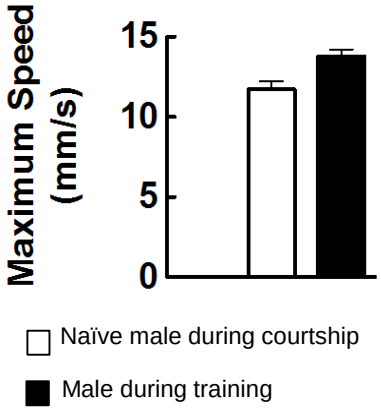
ALTOMS: Automatic Laser Tracking & Optogenetic Manipulation System

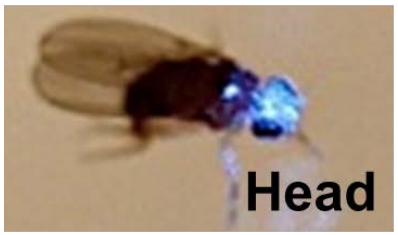
CC Fu



Wu MC et al., (2014) *PNAS* 111,5367-5372







ALTOMS parameters

Laser:
473-nm, 593.5-nm & 1064-nm laser

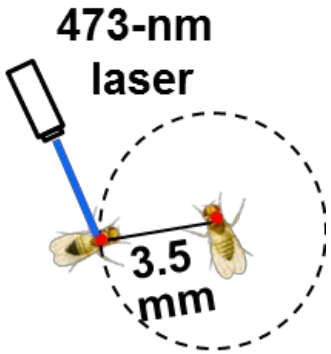
Target:
Head, thorax, and abdomen

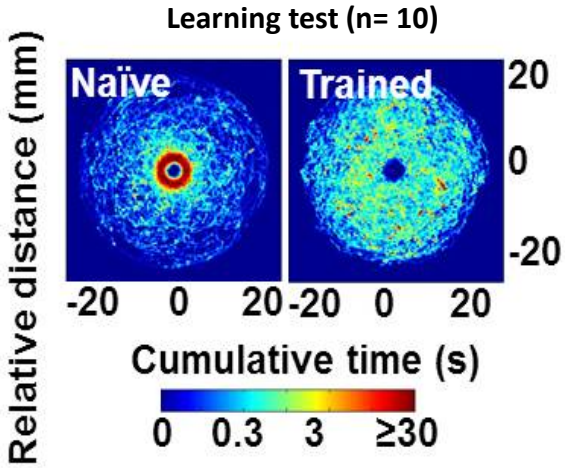
Screening Protocol:
4 cycles (10-s on/20-s off)

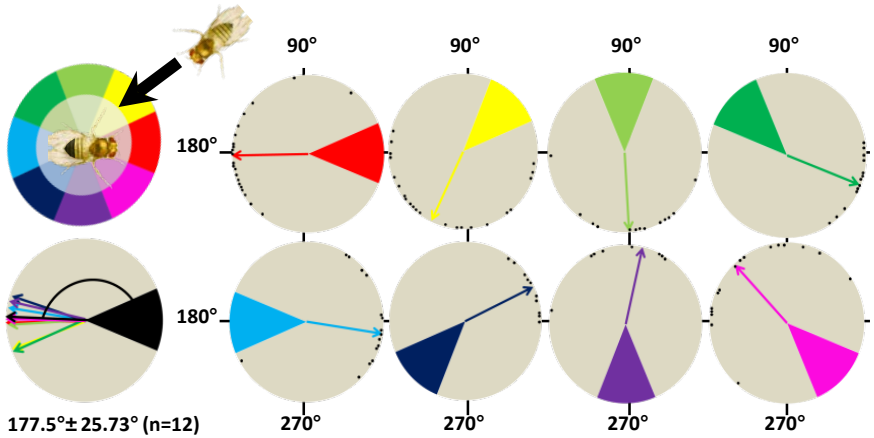
Maximum automated laser tracking time delay: 17 ms

Operant Learning

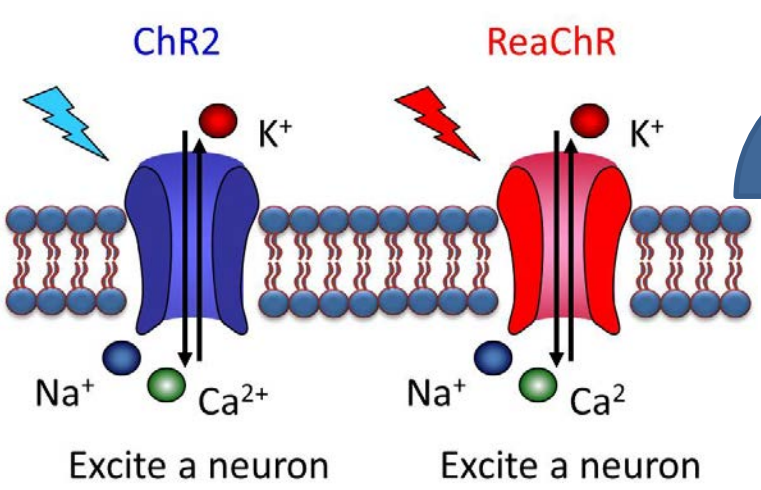
- The male fly learned to stay away from the female after <u>1-hr</u> restraining conditioning. (Conventional assay need 8-hrs)







Optogenetics

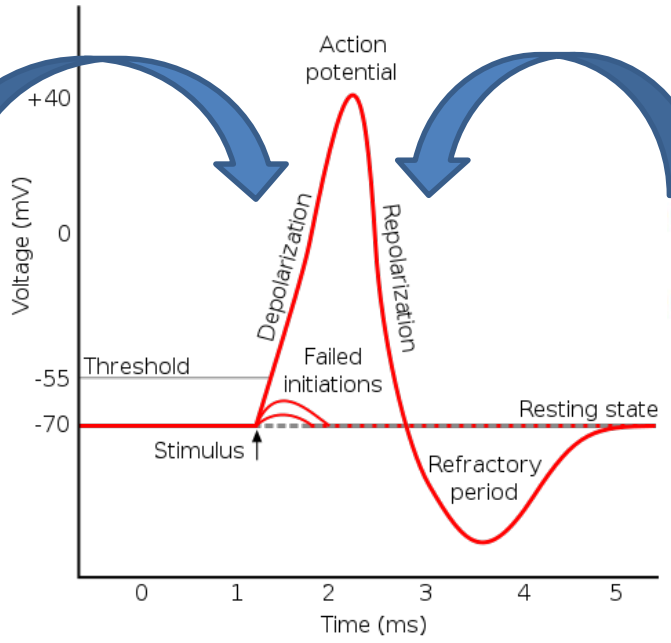


Excite a neuron

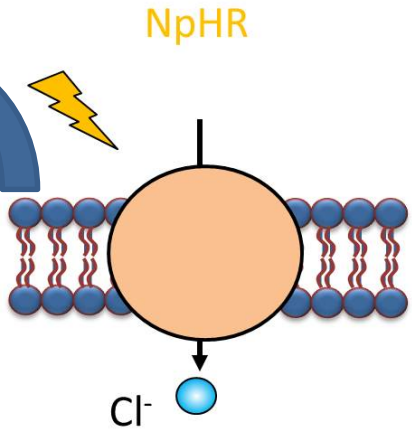
Excite a neuron

Szellas et. al.,(2003)
PNAS 24:13940

Lin et. al.,(2013)
Nat. Neurosci. 10:1499



From Wikipedia



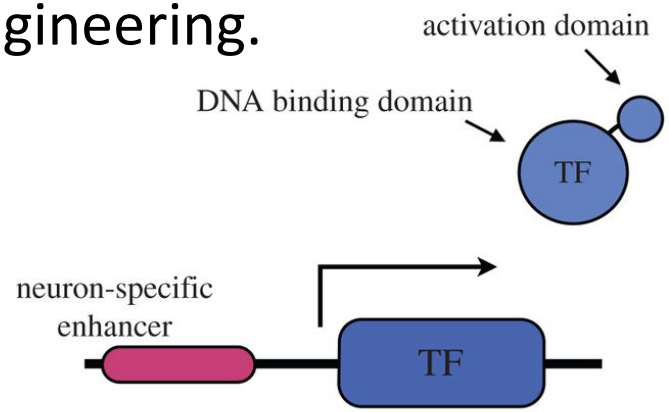
Inhibit a neuron

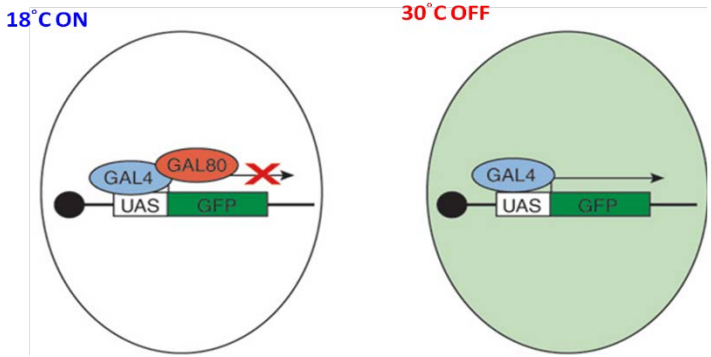
Han et. al.,(2007)
PloS one 2:e299
Zhang et. al.,(2007)
Nature 446:633

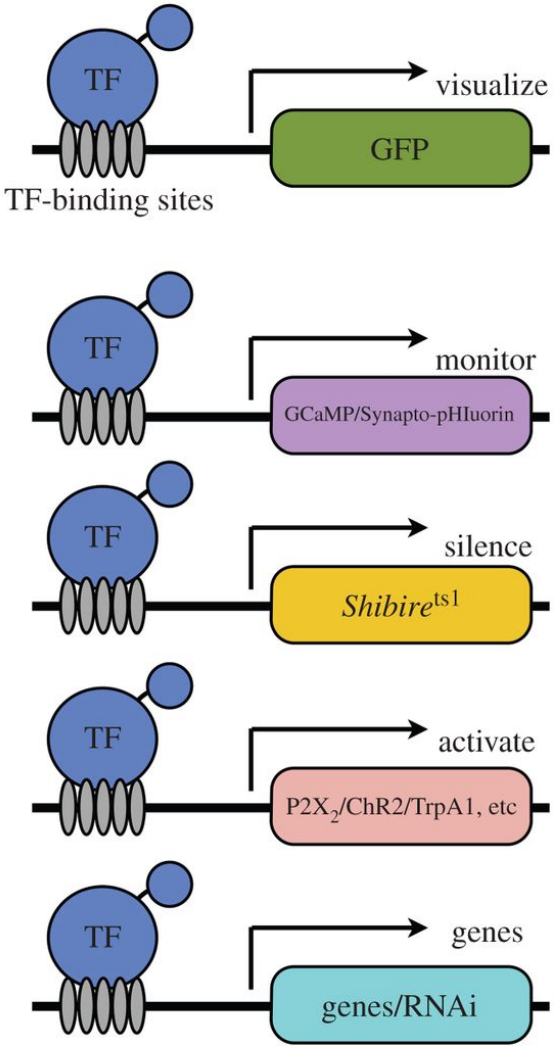
Spatiotemporal Gene Manipulation

Gal4-UAS system

We can manipulate any neuron at anytime by the state of the art genetic engineering.



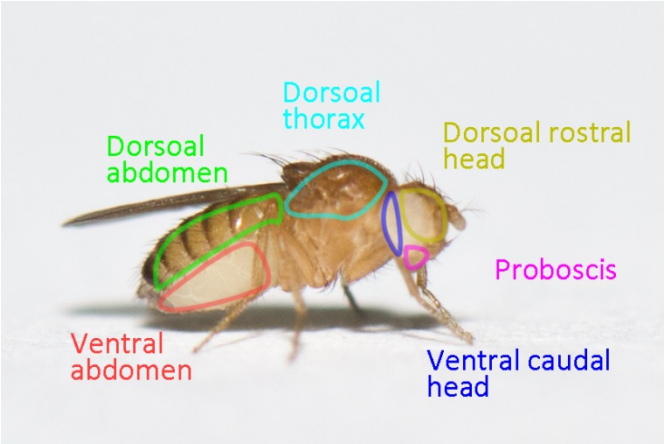


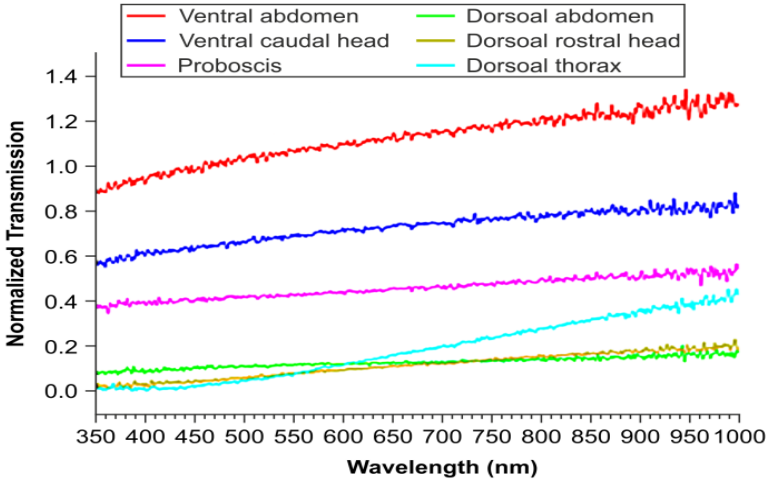


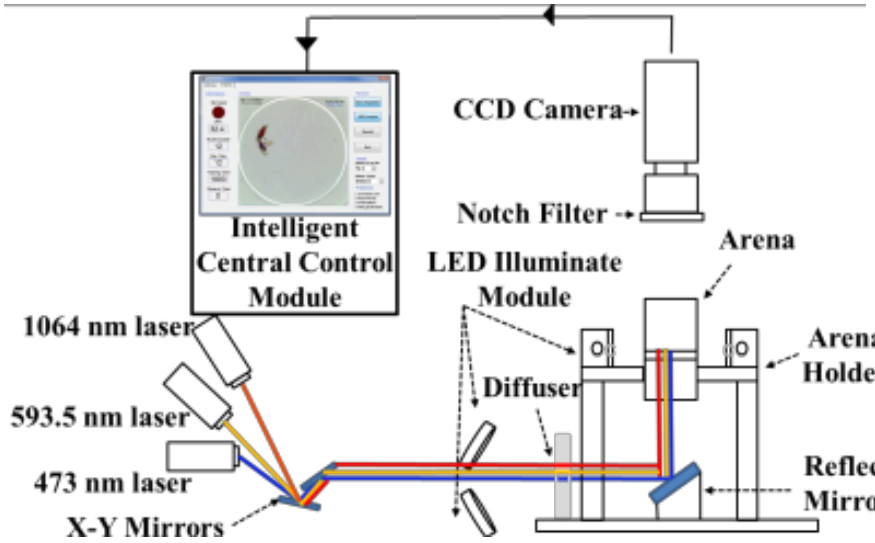
Courtesy to Neuron 72: 202

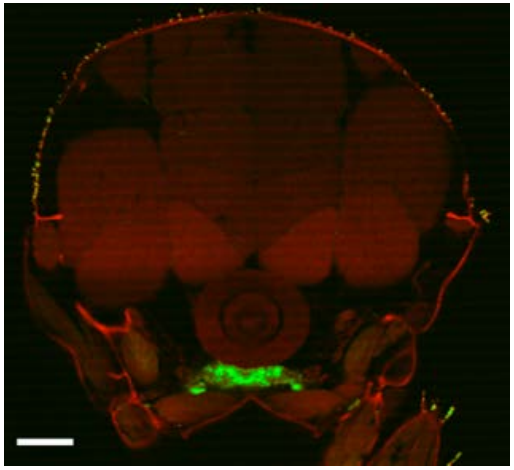
Optogenetic Behavior Manipulation

Ventral irradiation ALTOMS





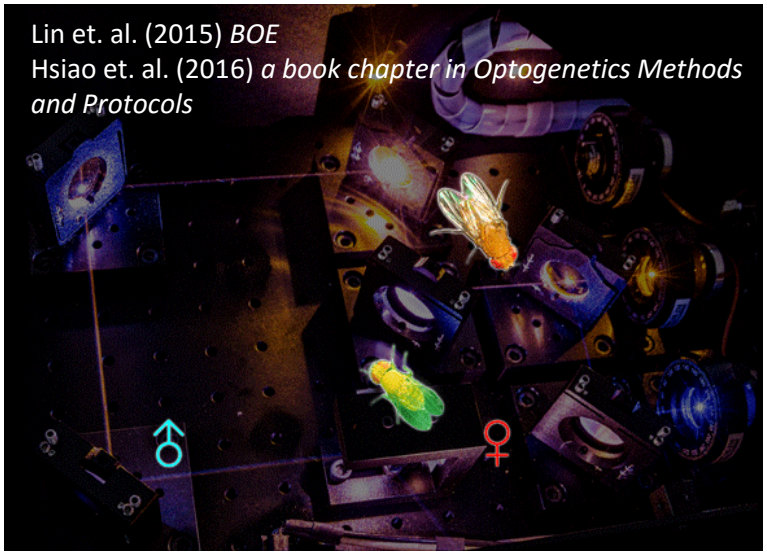


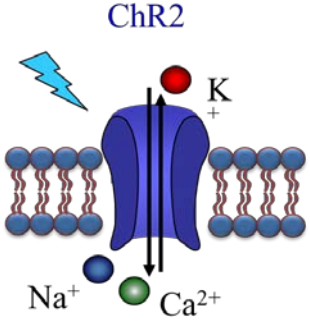


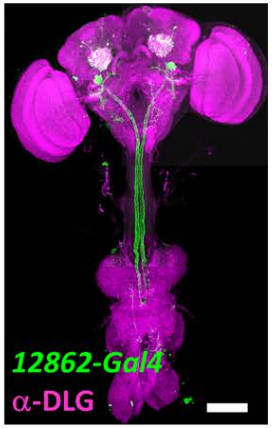
Scale bar 100 μm

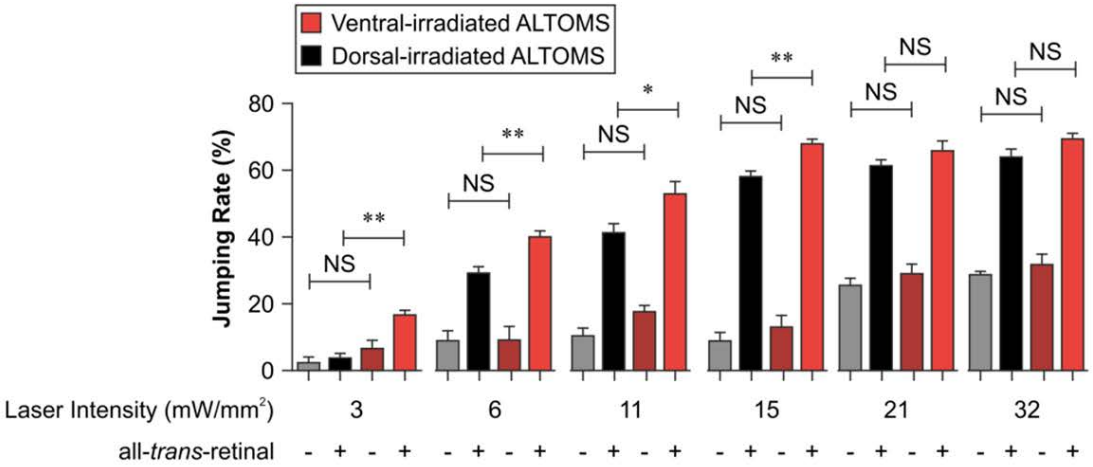
Lin et. al. (2015) *BOE* 6:514

Optogenetic Behavior Manipulation







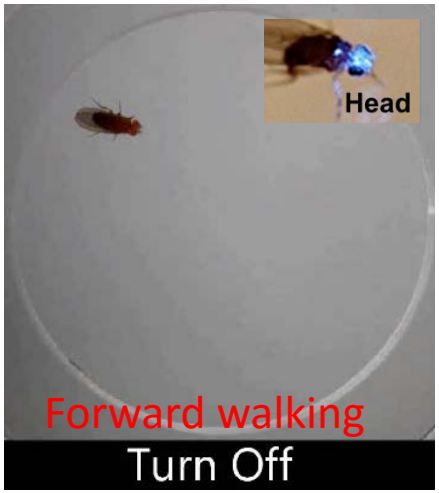


LaserFocusWorld NEWS http://www.laserfocusworld.com/articles/print/volume-51/issue-03/world-news/optogenetics-lasers-control-fruit-flies-for-behavioral-studies.html

Position Specific Behaviors and Sexual Dimorphism

Position specific

Line: *VT49842 > UAS-ReaChR*



Sexual Dimorphism

Line: *VT44168 > UAS-ReaChR*

Male thorax Single wing beat

Line: *VT58561 > UAS-ReaChR*

Female thorax Egg laying

Unpublished

Construct a Neuro-Behavior Map

Integrative understanding of how neural circuit control behavior

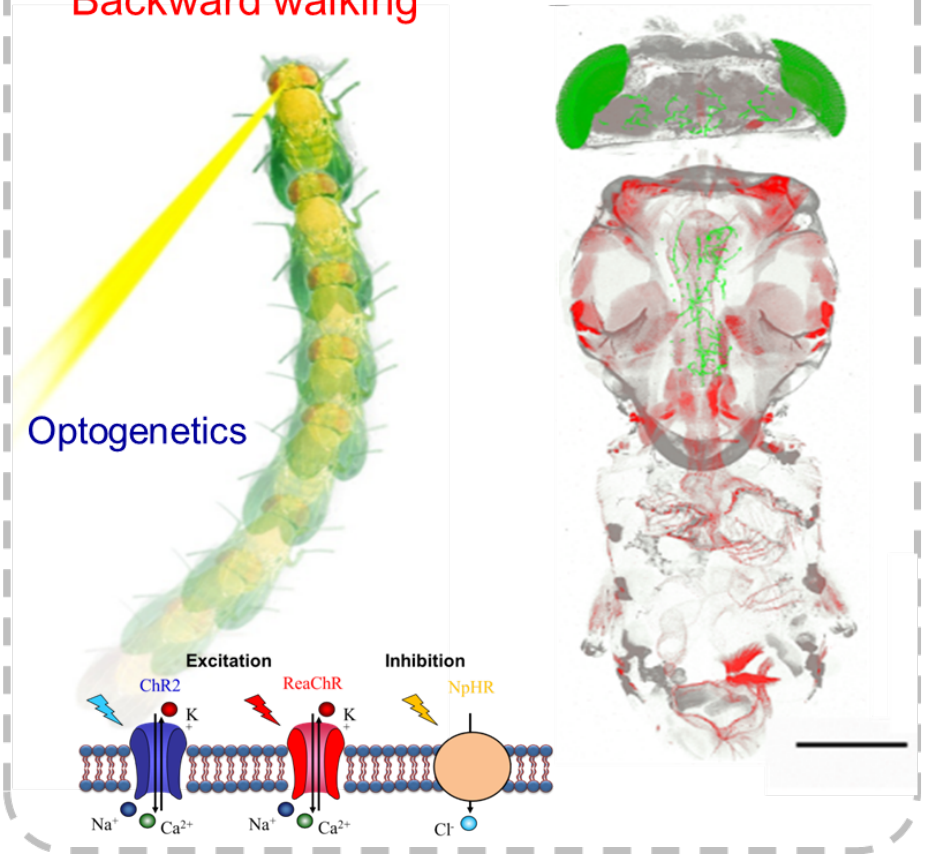
Unpublished

Optogenetic Neuro-Behavior Mapping

Constructing a neuro-behavior map

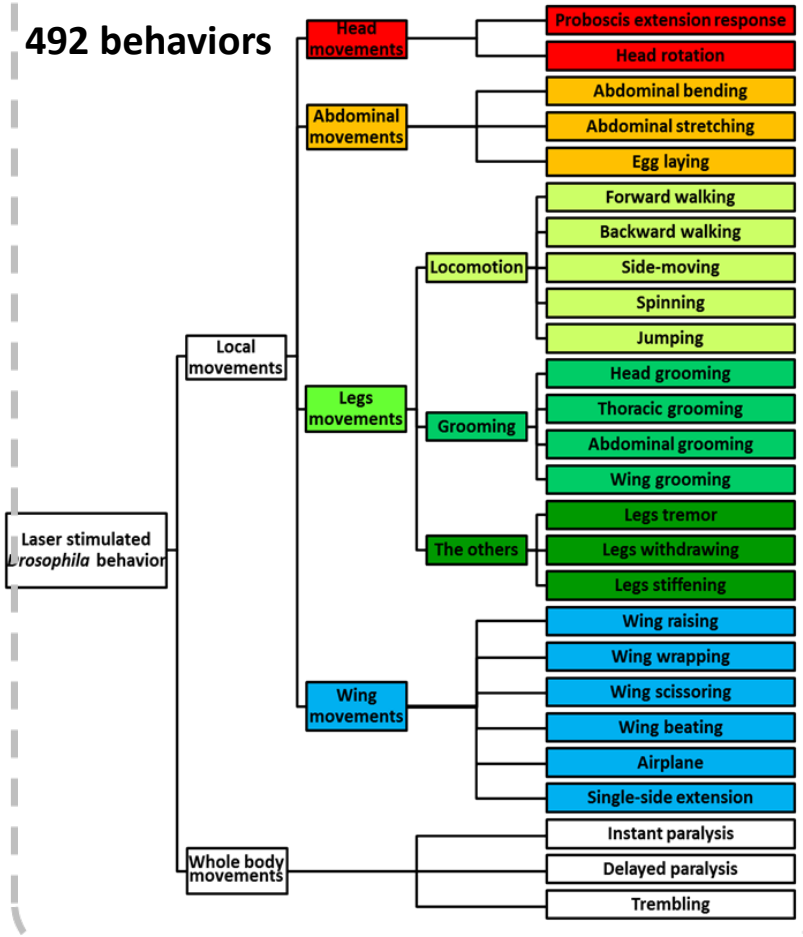
Line: *VT50660 > UAS-ReaChR*

Backward walking



Categorization of motor programs

492 behaviors

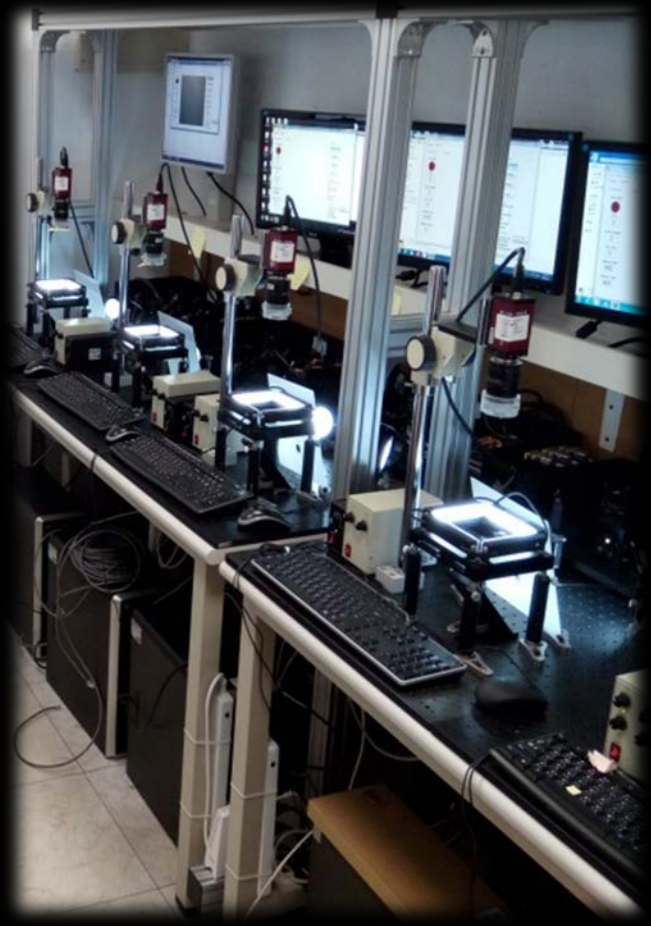


Inspired by Bidaye et al. (2014). Neuronal control of *Drosophila* walking direction. *Science* 6179: 97-101.

Unpublished

Construct a Neuro-Behavior Map

Behavior



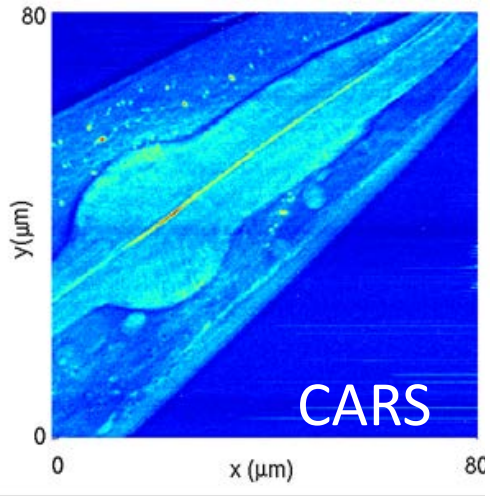
Anatomy

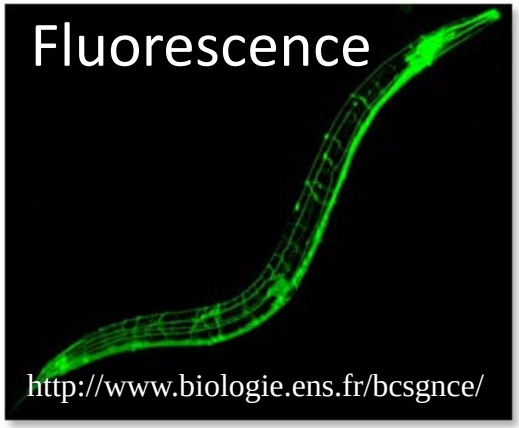
Confocal microscopy + Vibratome

Large field of view imaging system for a neuro-behavior map

Image technologies

- Important issues
  - Contrast
  - Resolution
  - Penetration depth
  - Speed





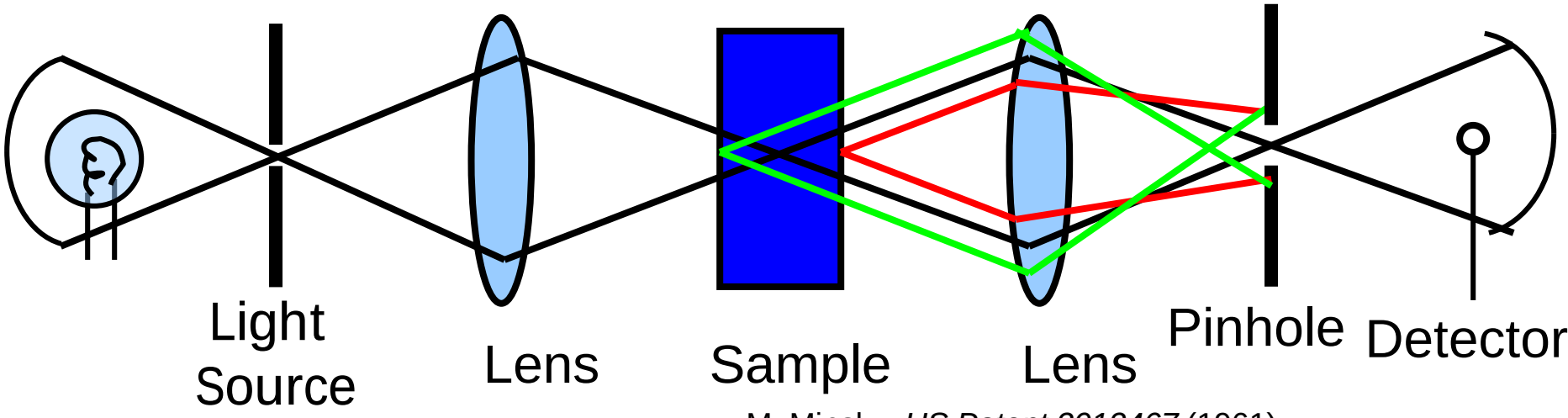
New contrast → New applications

Penetration depth → Applied field

New applications → New discovery

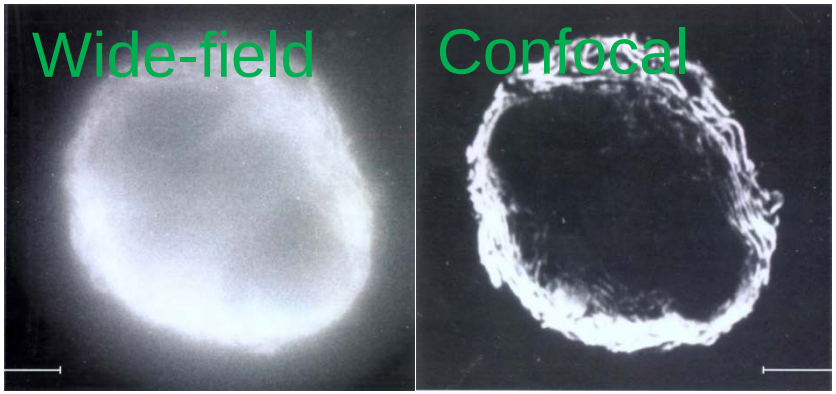
Applied field → When new discovery was disclosed?

Confocal microscopy



M. Minsky, *US Patent 3013467* (1961)

- Contrast enhanced
- Resolution enhanced
  - Due to the rejection of out-of focus light
  - Optical section



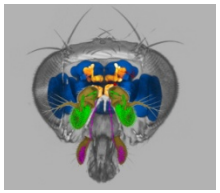
Plasmacytoma cell

J Cell Biology 105, p44 (1987)

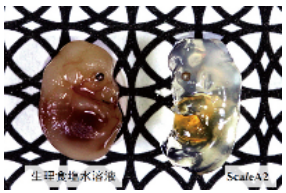
Making the Whole Body Transparent

	Methods	Publications
1	FocusClear	2001 J Comp Neurol
2	Scale	2011 Nature Neuroscience
3	BABB / 3DISCO	2011 Nature Medicine 2012 Nature Protocol
4	Clear T	2013 Development
5	CLARITY	2013 Nature
6	SeeDB	2013 Nature Neuroscience
7	CUBIC	2014 Cell

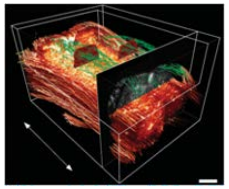
Challenge: high-resolution large volume 3D imaging



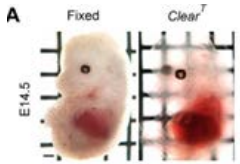
FocusClear



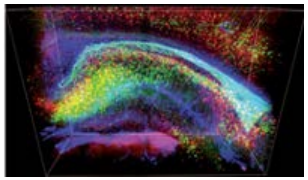
Sca*l*e



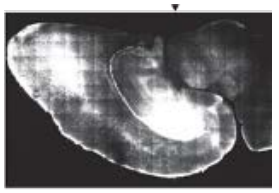
Modified BABB



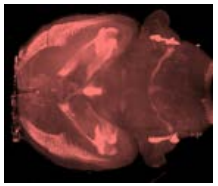
Clear T



CLARITY



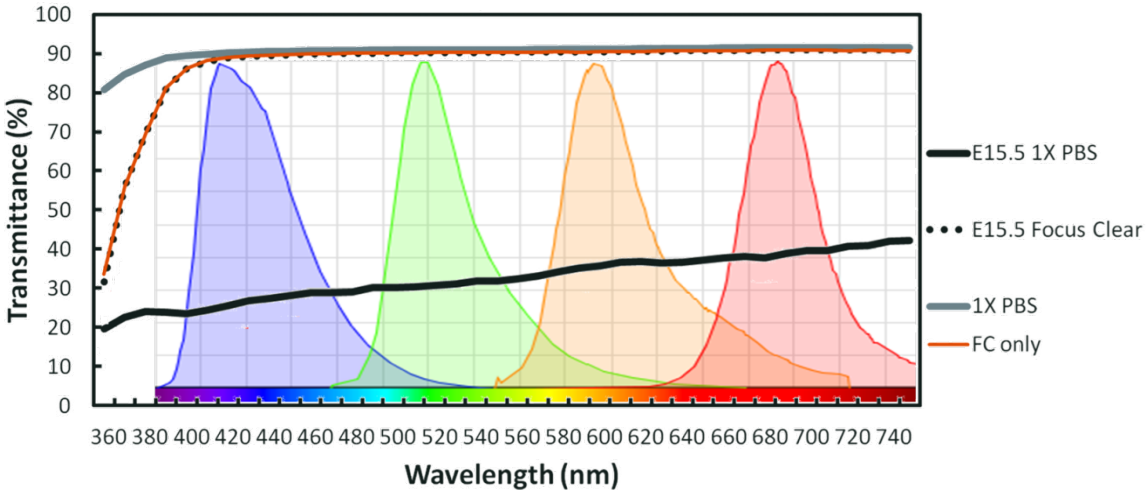
SeeDB



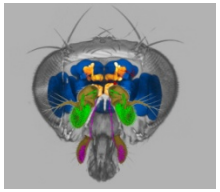
CUBIC

Making the Whole Body Transparent

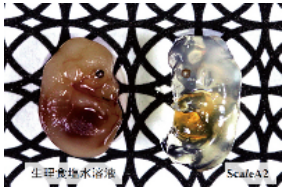
FocusClear



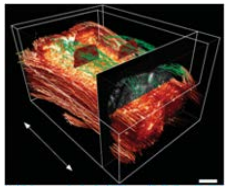
Challenge: high-resolution large volume 3D imaging



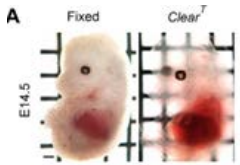
FocusClear



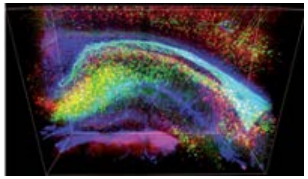
Sca*l*e



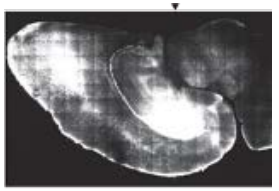
Modified BABB



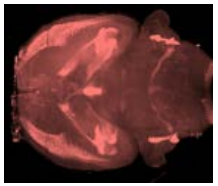
Clear T



CLARITY



SeeDB



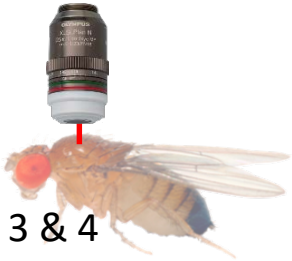
CUBIC

Automated Serial Thick Section (STS) Tomography for large-tissue 3D Imaging

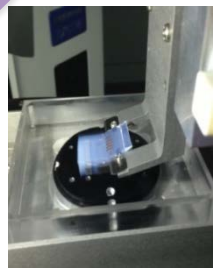
1. Fixation

2. Embedding and tissue clearing

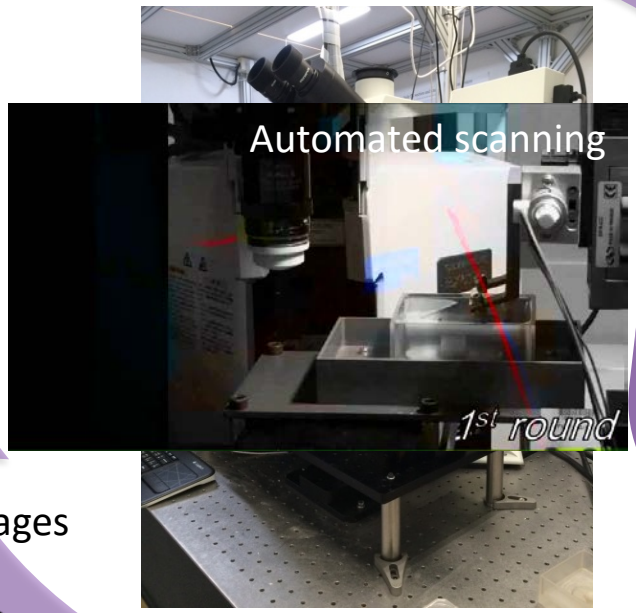
3. Imaging



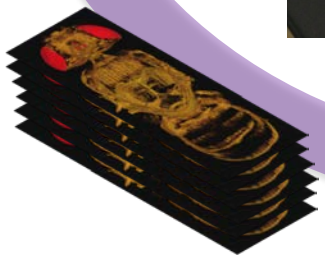
4. Removing imaged regions



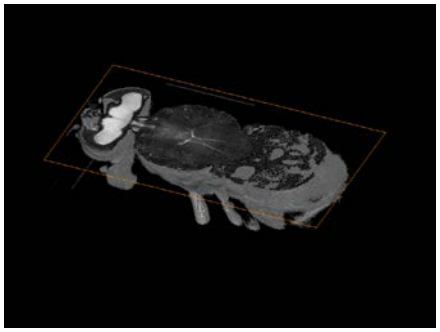
5. Repeating 3 & 4



6. Stitching images



7. Reconstruction & analysis

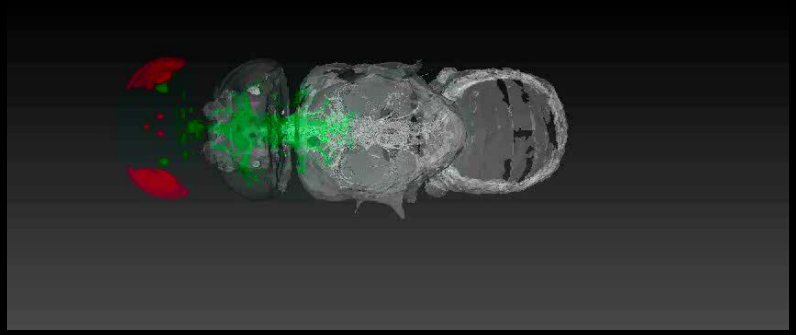


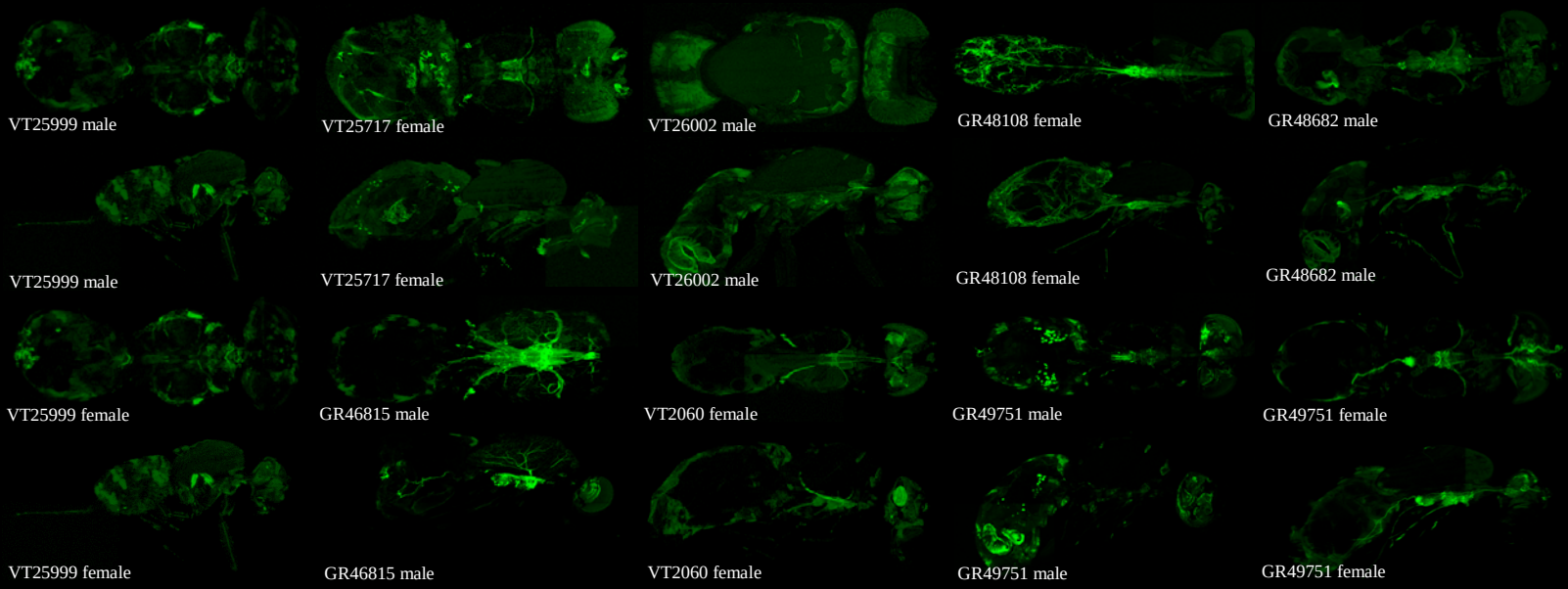
Imaging and sectioning in tissue clearing solution

Mapping Neural Circuits for Behaviors

Line: *VT23830 > UAS-ReaChR*

Dual wing raising



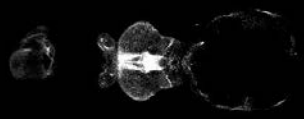


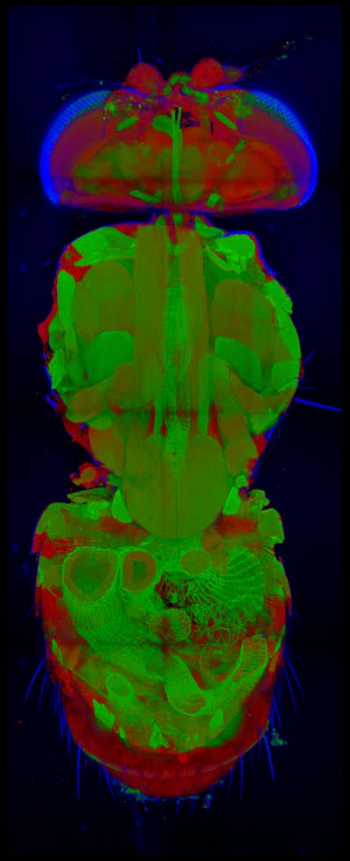
Construct the Whole-Fly Connectome

Cuticle

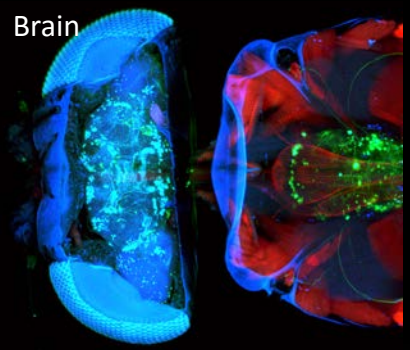
Neuron

Muscle

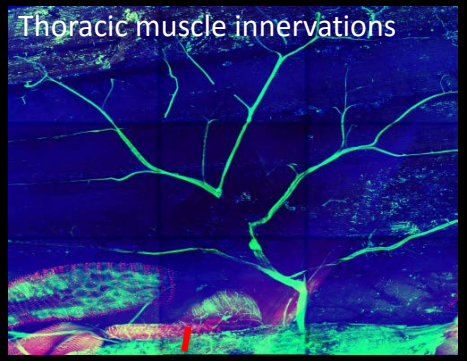




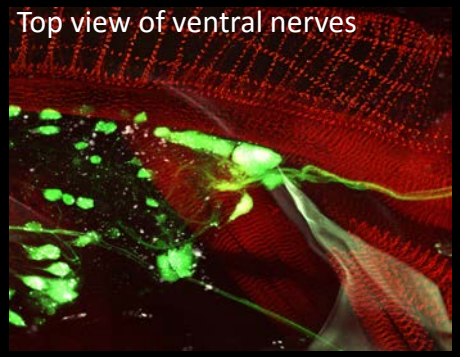
Brain

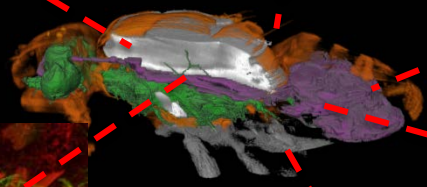


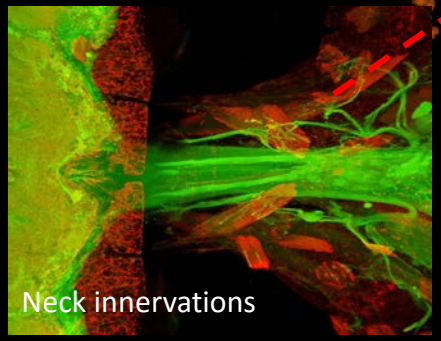
Thoracic muscle innervations



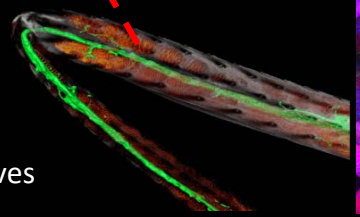
Top view of ventral nerves



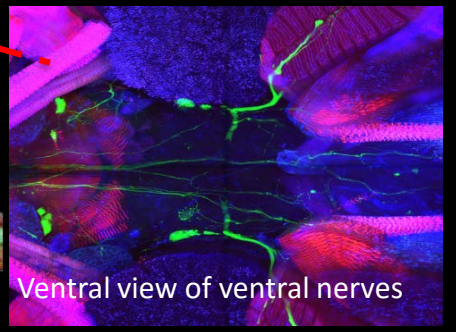




Neck innervations



Leg nerves



Ventral view of ventral nerves

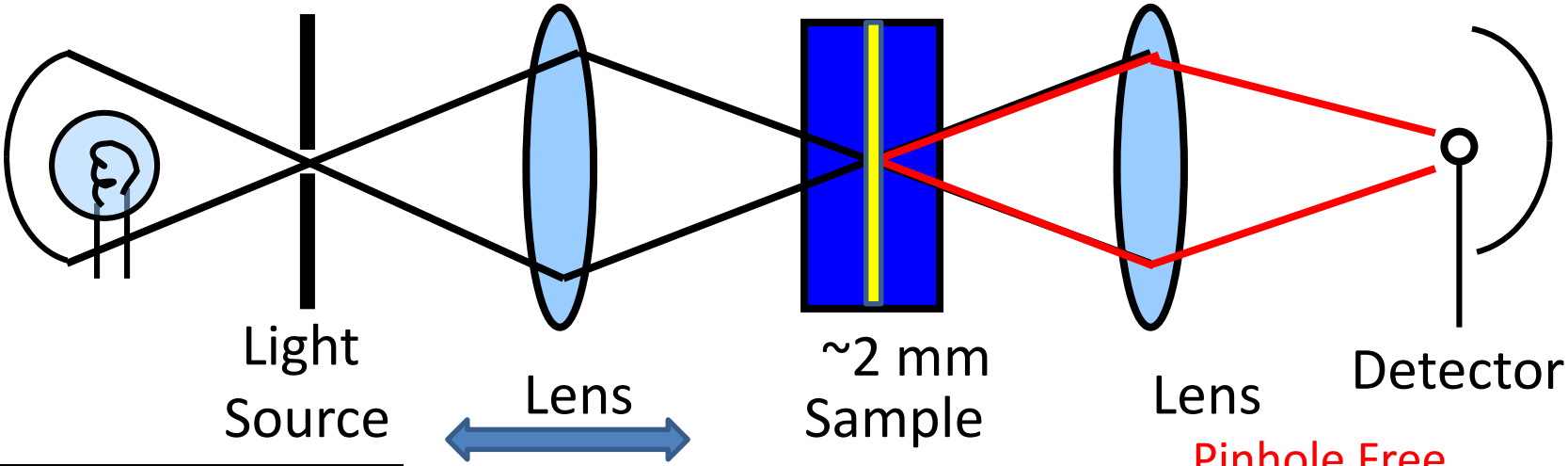
Two-photon excitation microscopy +
New devices & Signal processing

3D Dynamic functional images

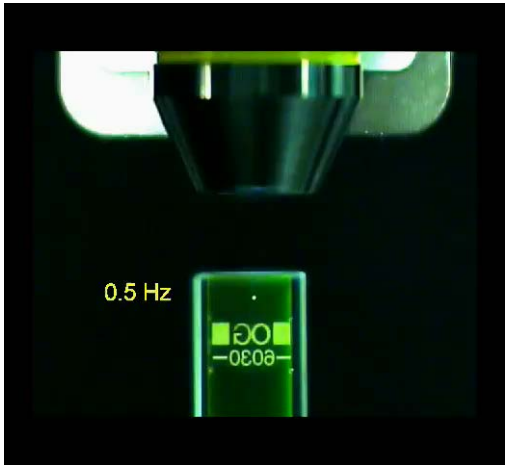
Two-photon excitation microscopy

Longer excitation wavelength → less scattering

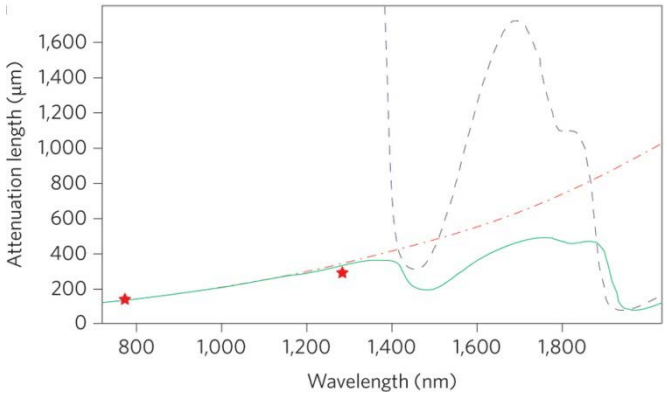
Can we extend ~ 2 μm to ~ 100 μm thick with good resolution?



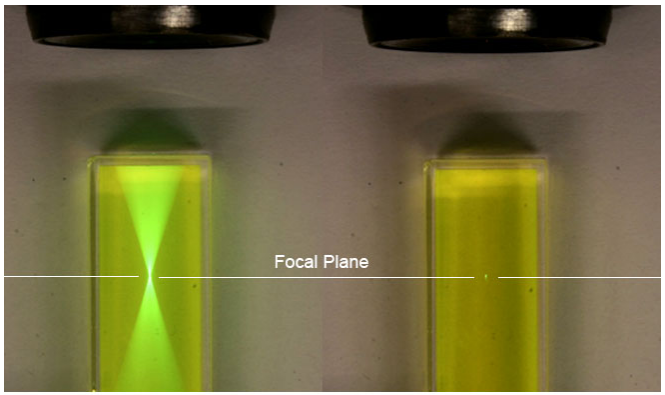
Pinhole Free



W. Göbel *Nature Methods* - **4**, 73 - 79 (2007)



Horton et. al. (2013)
Nature Photonics 7, 205–209

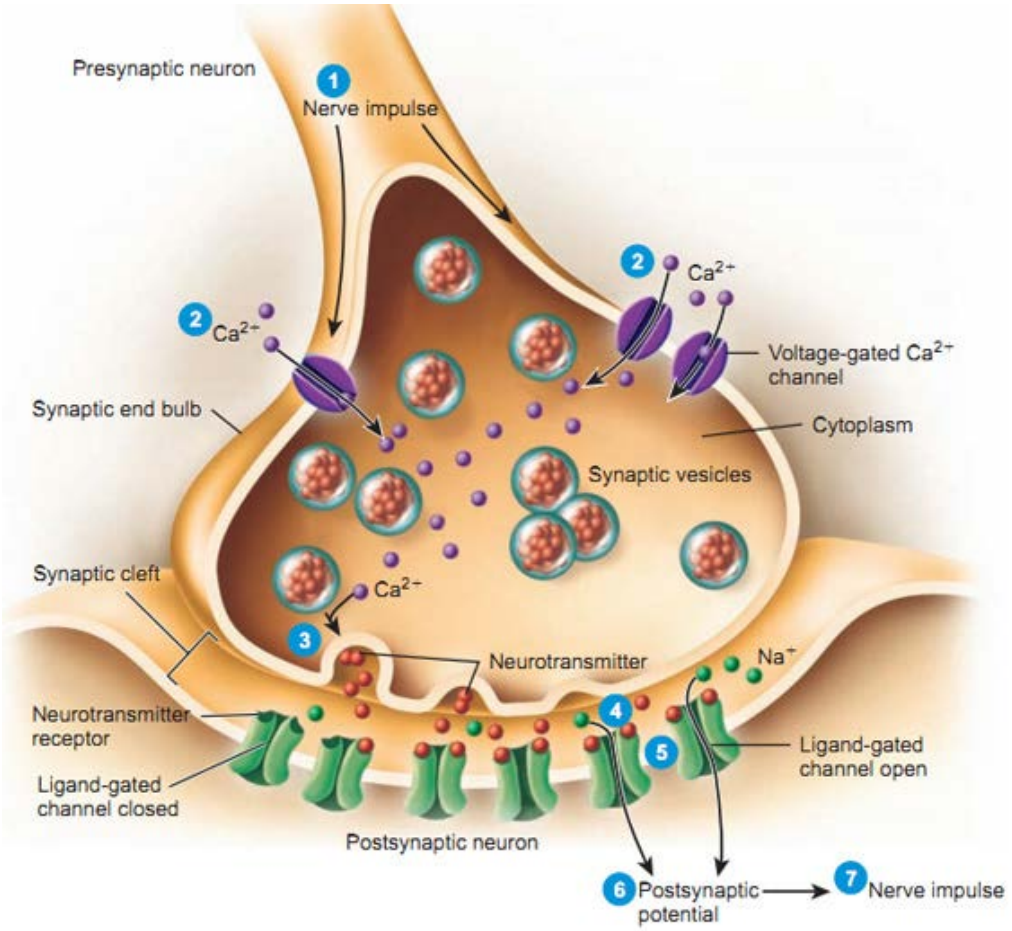


One Photon $Signal \propto I$

Two Photon $Signal \propto I^2$

http://xu.research.engineering.cornell.edu/research

Contrast



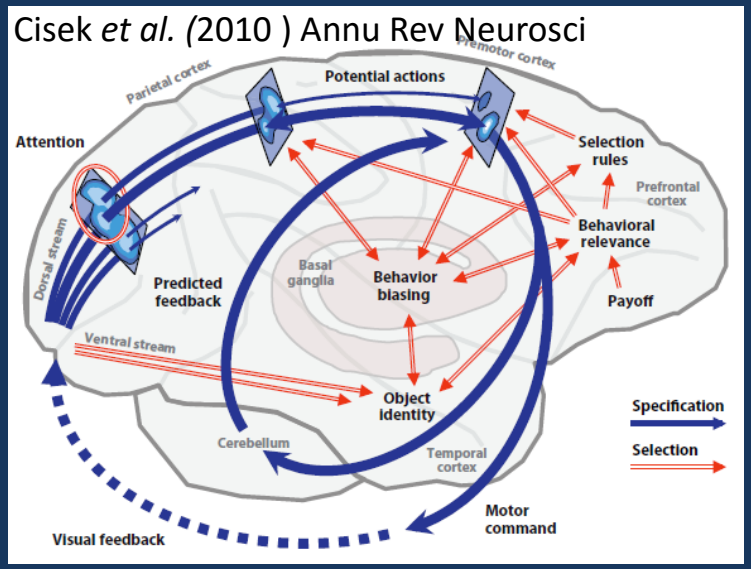
- Action potential
- Ca^{2+} concentration
- Metabolism

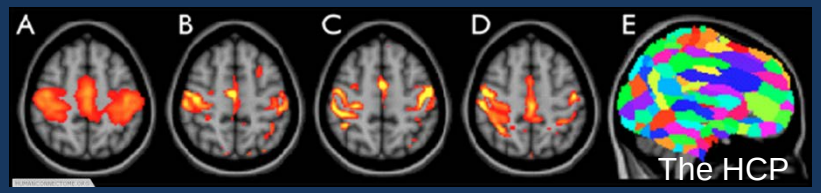
http://antranik.org/synaptic-transmission-by-somatic-motorneurons/

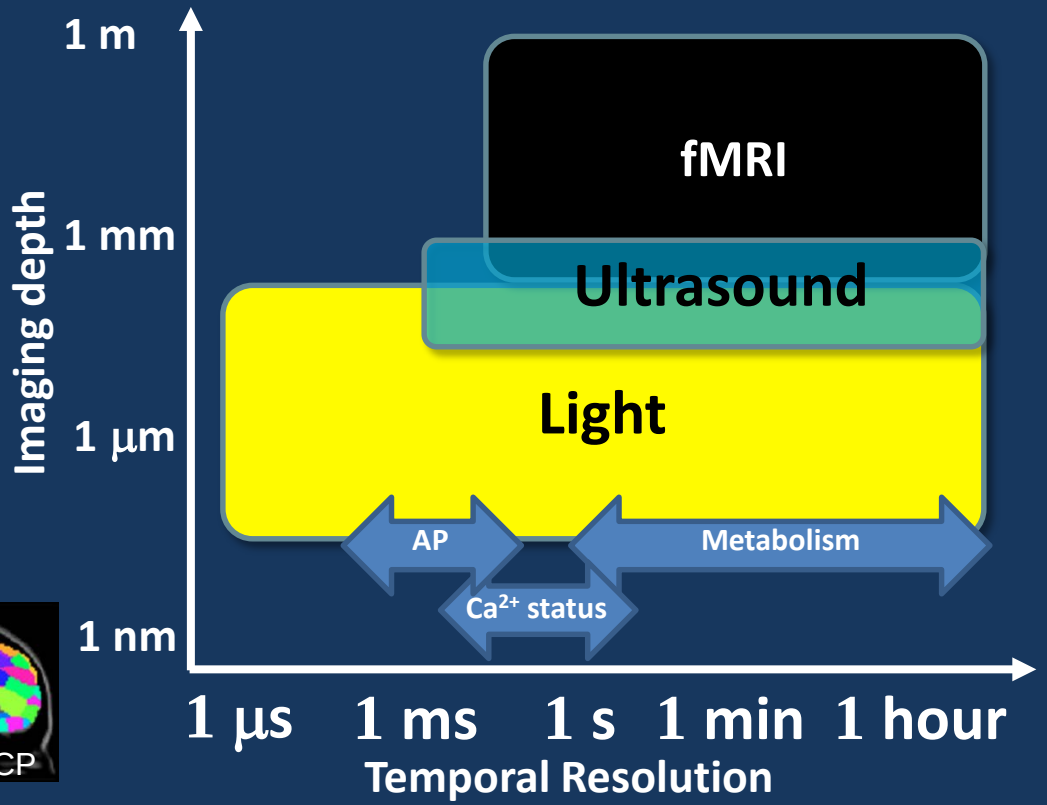
Information flows in Life

The first Goal: imaging whole brain dynamics

Deep and fast imaging of dynamics



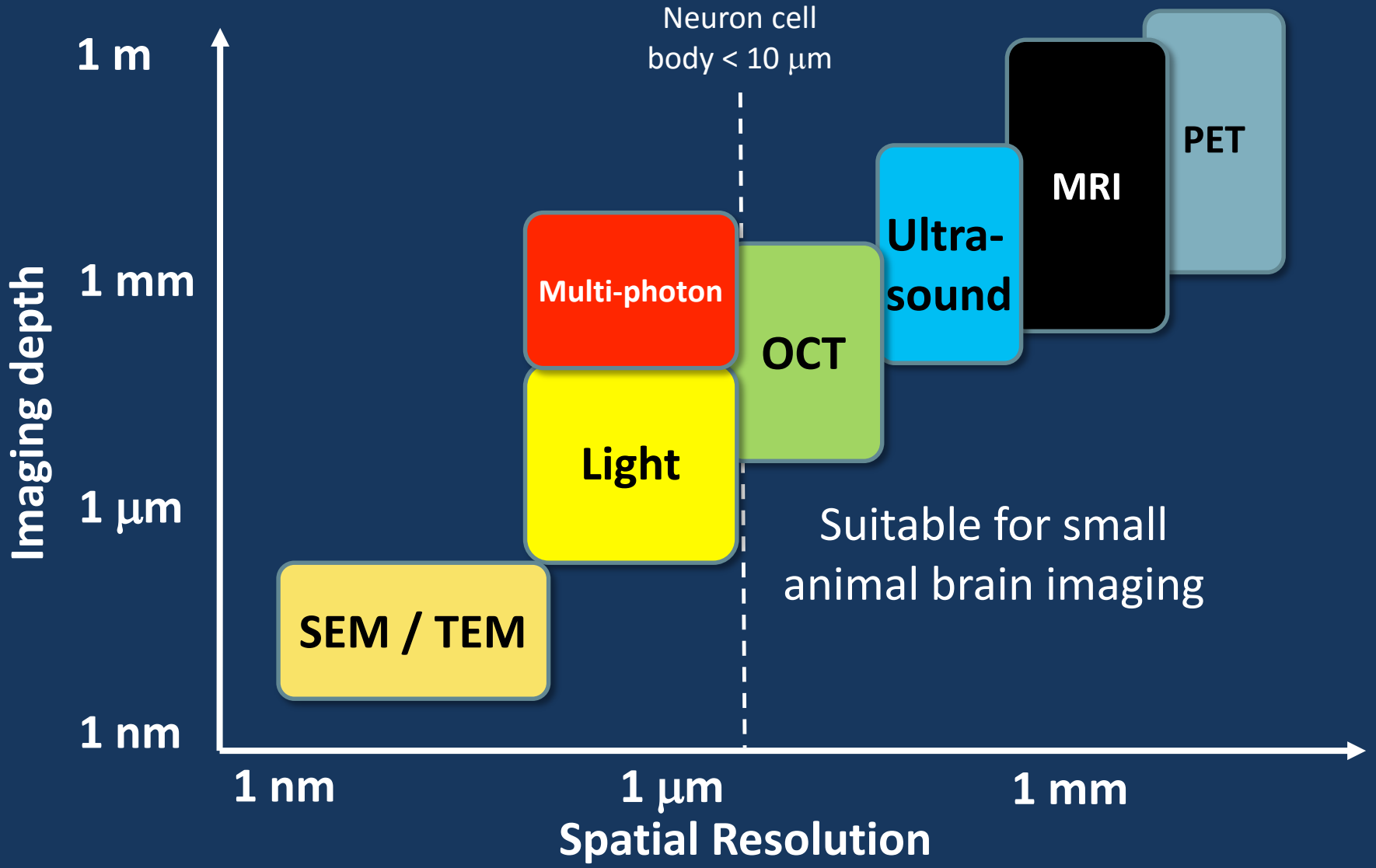




For understanding how the brain controls behavior, we need to know how each responsive neuron works and how they communicate with each others in a large 3D space.

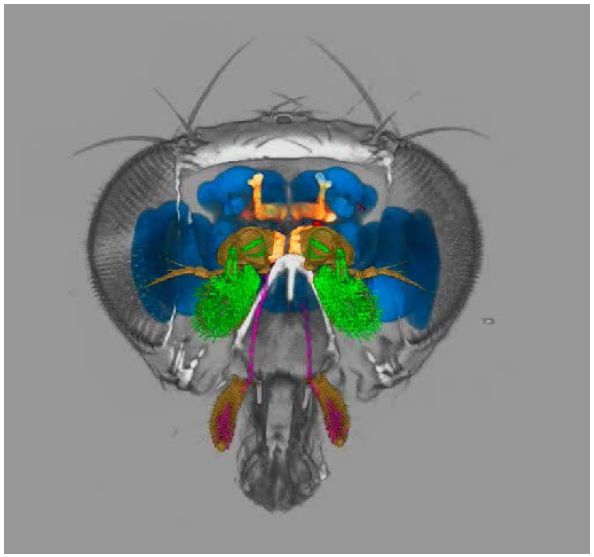
Information flows in Life

The second Goal: imaging every spike from every neuron



Speed

- Size of fly brain



450 μm × 250 μm X 150 μm

At cell body resolution (5 μm)

90 × 50 × 30 = 135,000 voxels

At axon resolution (1 μm)

450 × 250 × 150 = 16.9×10^6 voxels

At terminal resolution (0.2 μm)

2250 × 1250 × 750 = 2.1×10^9 voxels

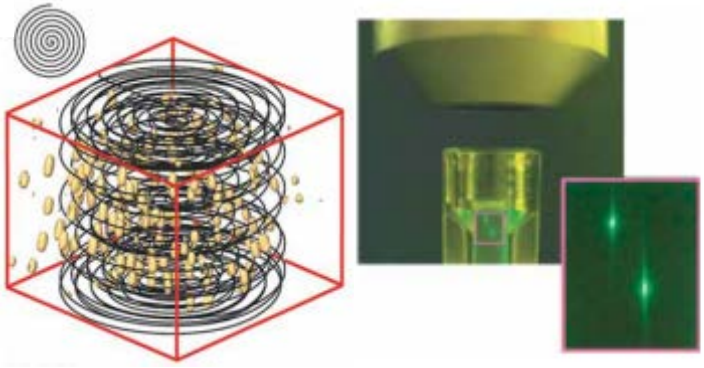
Desired frame rate:
Action potential ~ ms
Calcium changes ~ sub-second
Metabolism ~ few

→ Detection scheme
- Point detection
- 1D array detection
- 2D array detection

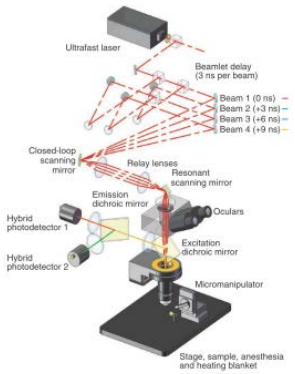
Methods review

	Imaging	Multiphoton excitation	
Characteristic			
Pros	Compatible with commercial microscope		
Spatial resolution			
Temporal resolution*			
Cons			

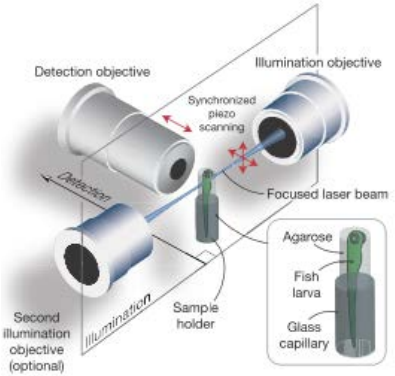
*Defined by the time required to complete one volume image by 256×256×100 pixels



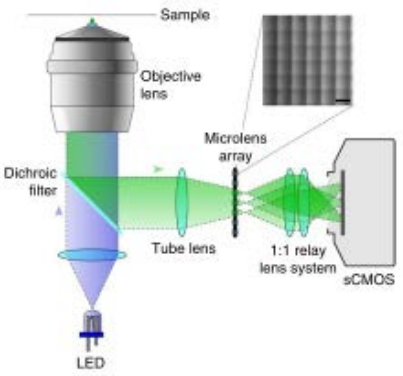
G. D. Reddy, Nat. Methods (2008)



W. Gobel, Nat. Methods (2007)



M. B. Ahrens, Nat. Methods (2013)



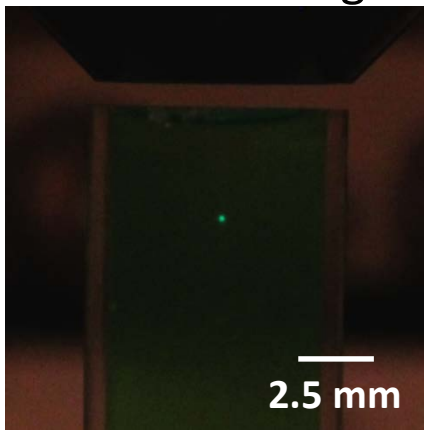
R. Prevedel, Nat. Methods (2014)

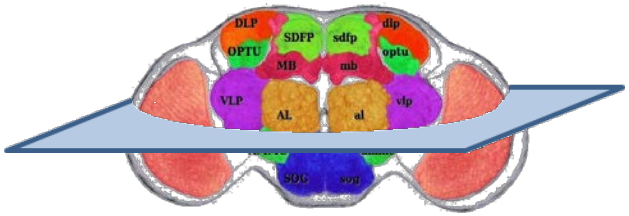
A. Cheng, Nat. Methods (2011)

All-in-focus Microscopy

Nonlinear optic effects provide axial resolution

Conventional two-photon functional image



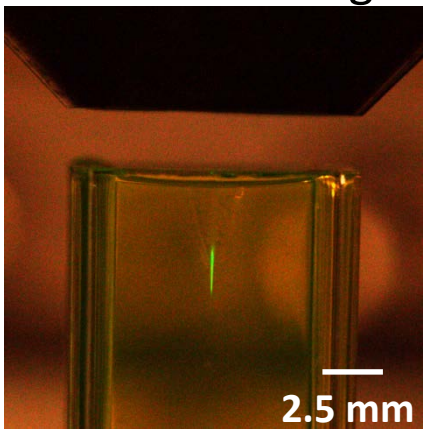


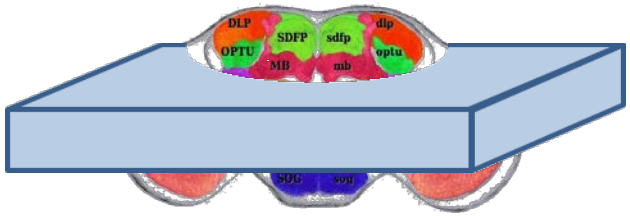
Thin section slide

350 μm × 350 μm × 2 μm

Rapidly focal point scanning can extend axial range.

All-in-focus whole brain functional image

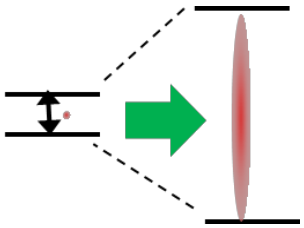




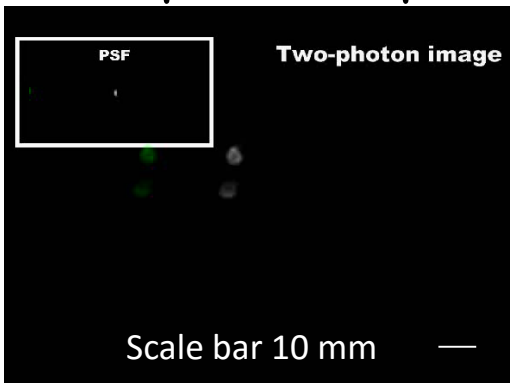
Thin section slide

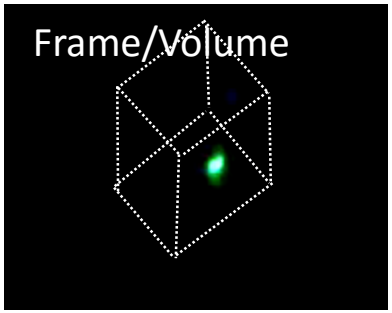
350 μm × 350 μm × 200 μm

Extended PSF



2 μm → 200 μm

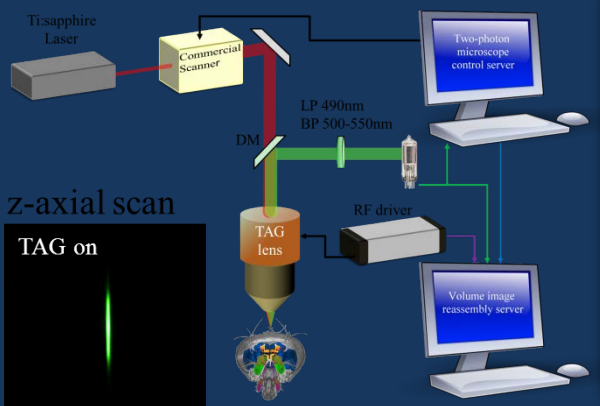


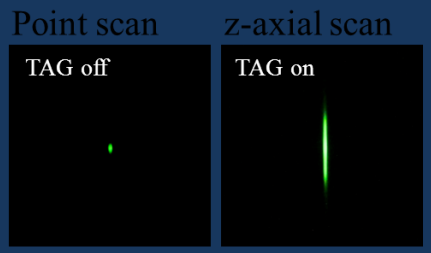


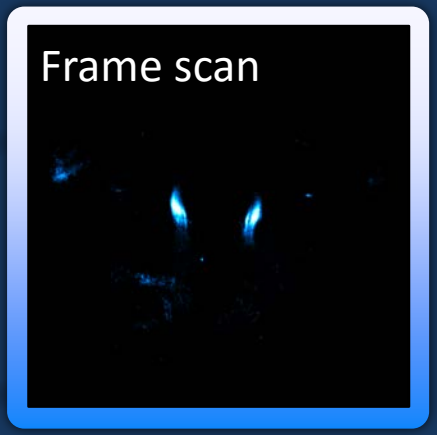
Unpublished

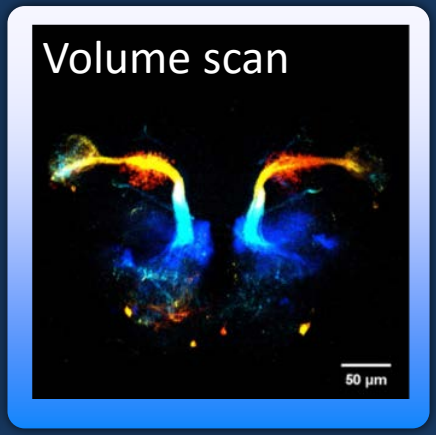
Depth-resolvable all-in-focus microscopy

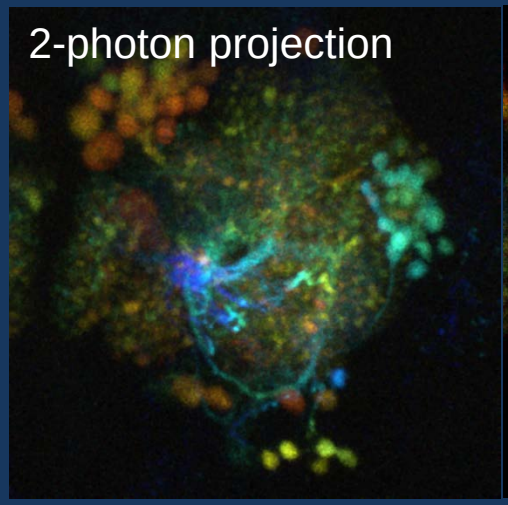
Compatible with commercial microscope can be integrated into any two-photon microscopy in the world.

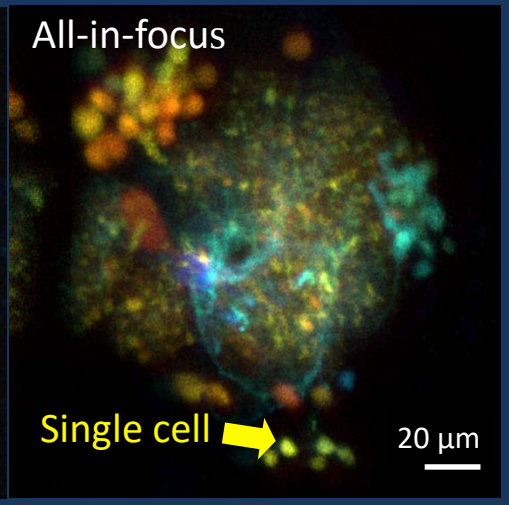








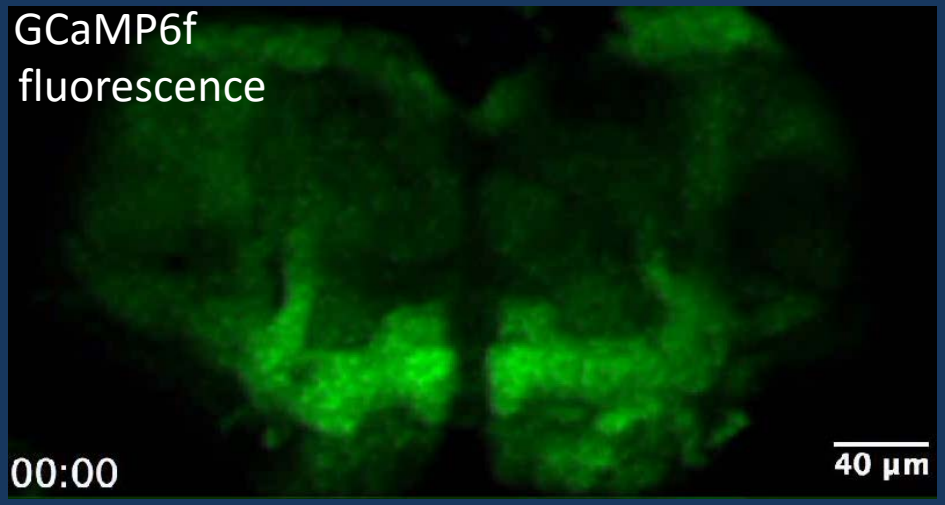


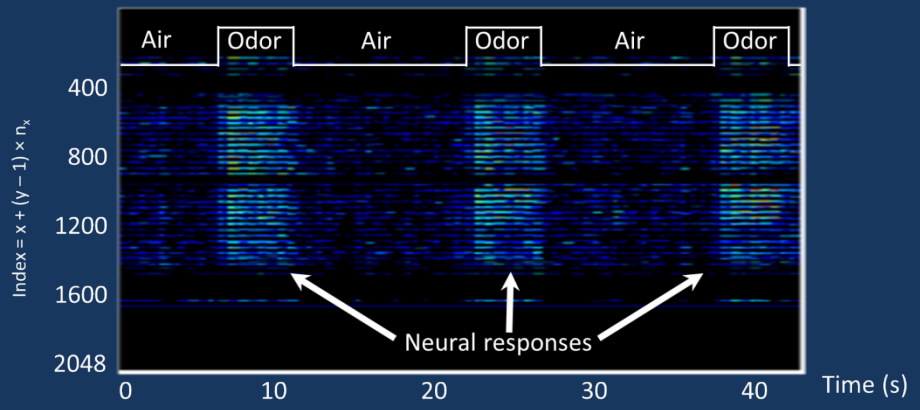


Unpublished

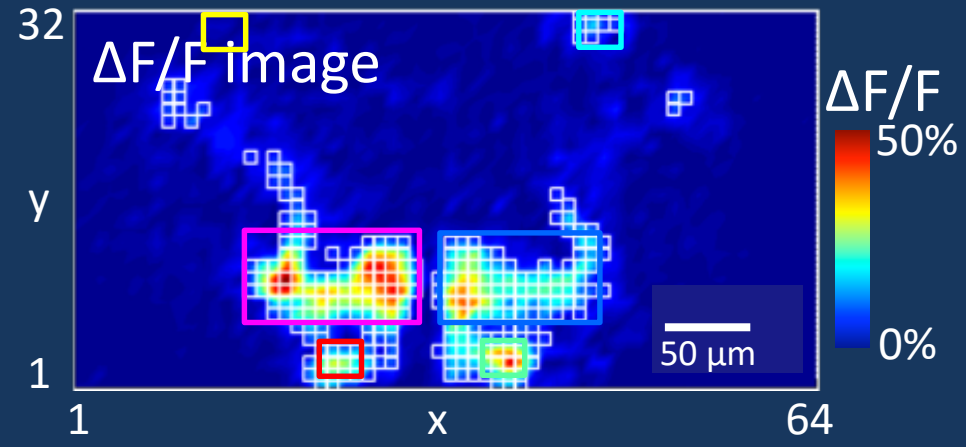
All-in-focus Whole-brain Imaging

Volume scan





Correlation between response and stimuli

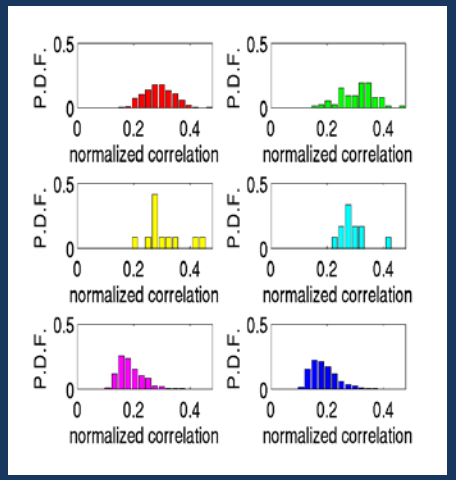


AL→ Kenyon cells→ MB

Antenna lobes

Kenyon cells

Mushroom body



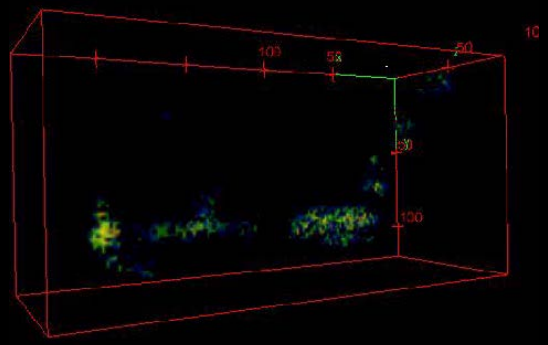
Unpublished

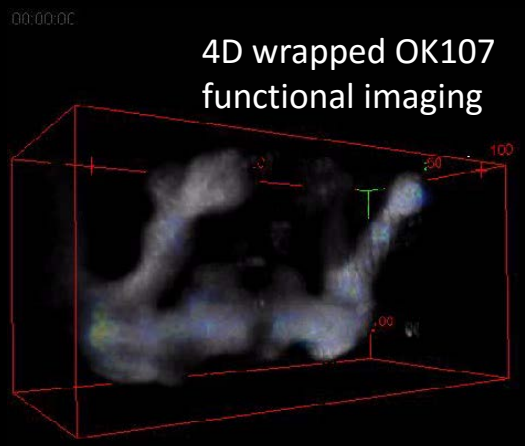
All-in-focus Whole-brain Imaging

4D Functional Imaging

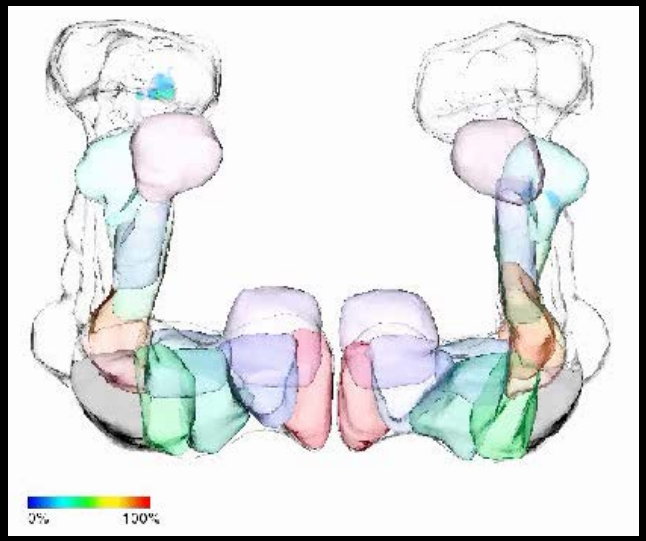
OK107-Gal4/UAS-GCaMP6f

All-in-focus 4D OK107 functional imaging





4D wrapped OK107 functional imaging

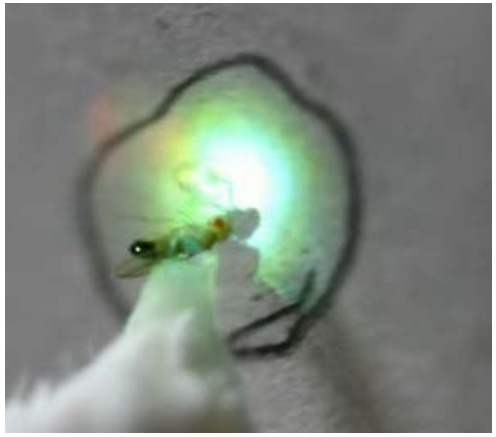


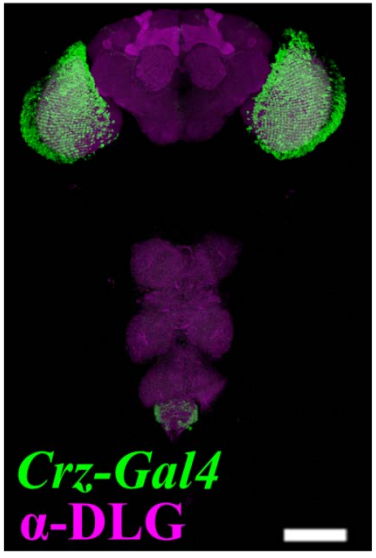
Deposit to Database

Unpublished

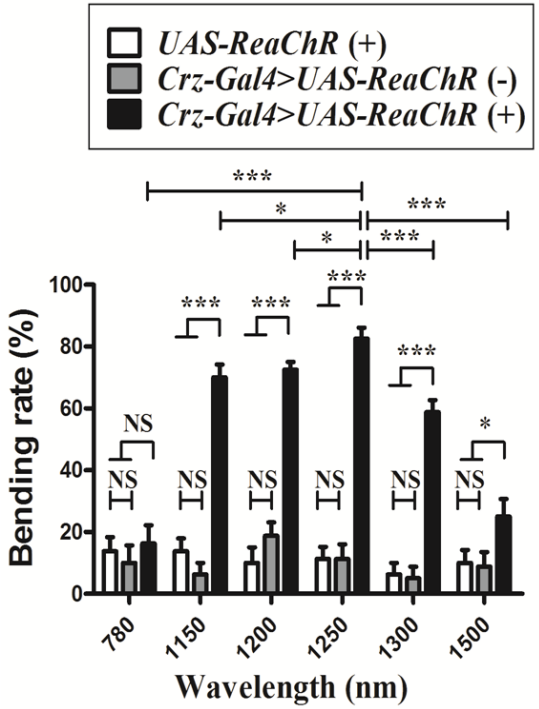
Two-photon excitation ReaChR

100 μJ , ~1.2 μm

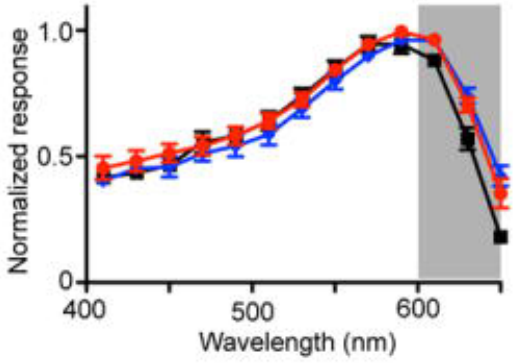


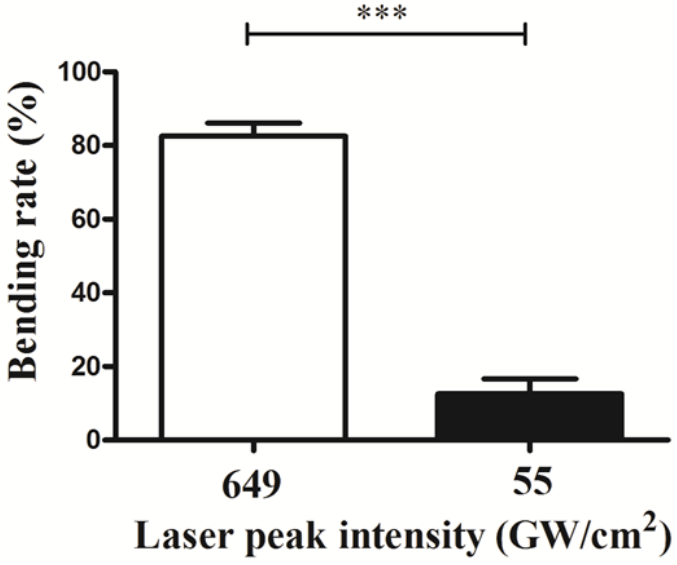


Excite a neuron



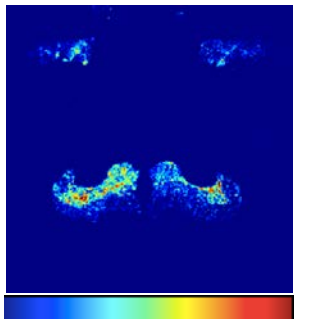
Nat Neurosci. 2013 Oct;16(10)



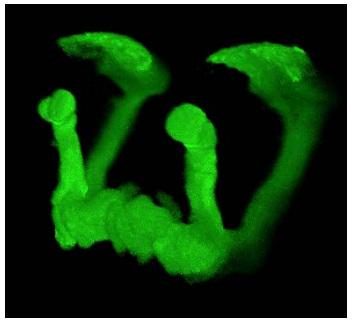


Toward Integrative Connectome

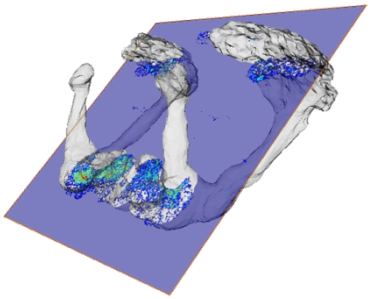
Functional Integration



Functional response



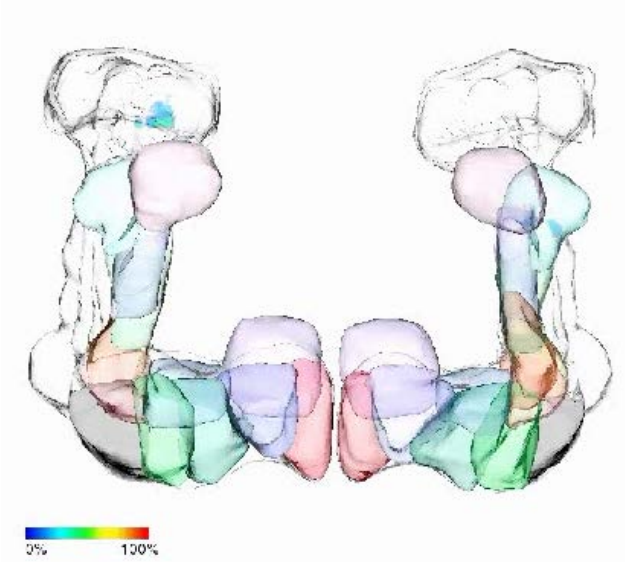
In situ structure



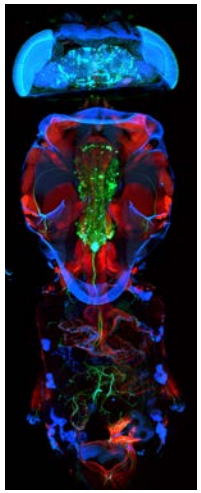
Integration into *in situ* brain model

Lin et al. (2015) *J Neurogenet*

All-in-focus 4D Functional Imaging



Behavior and Whole fly anatomy



Summary:

- 492 locomotion neuro-behavior map.
- Automated imaging *in situ* whole-body neuron circuits.
- Whole Brain-wide functional analysis
- Open resources http://brc.life.nthu.edu.tw/
- Automated whole-body mapping of neuro-behavior circuits.
- Nonlinear excitation can image and trigger neurons.

Acknowledgement

- Ministry of Science and Technology, Taiwan
- Ministry of Education, Taiwan

- NTHU
  - Prof. Ann-Shyn Chiang
  - Prof. Chien-Chung Fu
  - Prof. Shang-Da Yang
  - Prof. Ming-Chang Chen
  - Prof. Shun-Chi Wu
  - Prof. I-Chin Wang
- NTU
  - Prof. Shi-Wei Chu
- NSYSU
  - Prof. Yuan-Yao Lin

More information for public

Nature http://www.nature.com/nature/journal/v541/n7638/full/541559a.html#references

Nature blogs http://blogs.nature.com/naturejobs/2017/01/26/new-neuroscience-tools-for-team-science-in-big-data-era/

Scientific American https://www.scientificamerican.com/article/neuroscience-big-brain-big-data/

TECHNOLOGY FEATURE

BIG BRAIN, BIG DATA

Neuroscientists are starting to share and integrate data — but shifting to a team approach isn't easy.

Yen-Yin Lin
Brain Research Center,
National Tsing Hua University
Taiwan
yylin@life.nthu.edu.tw