Calcium Imaging & Analysis

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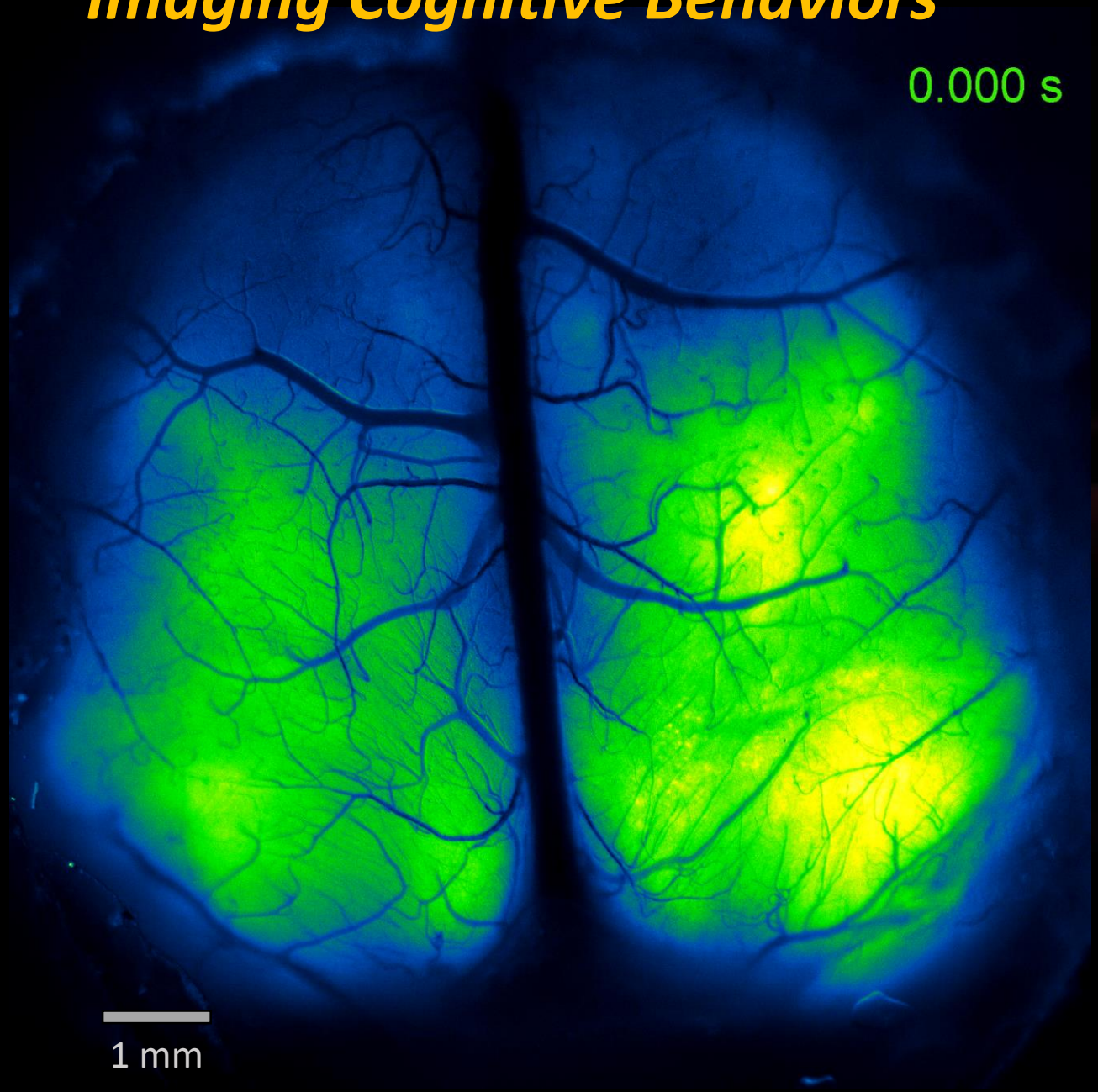
Outline

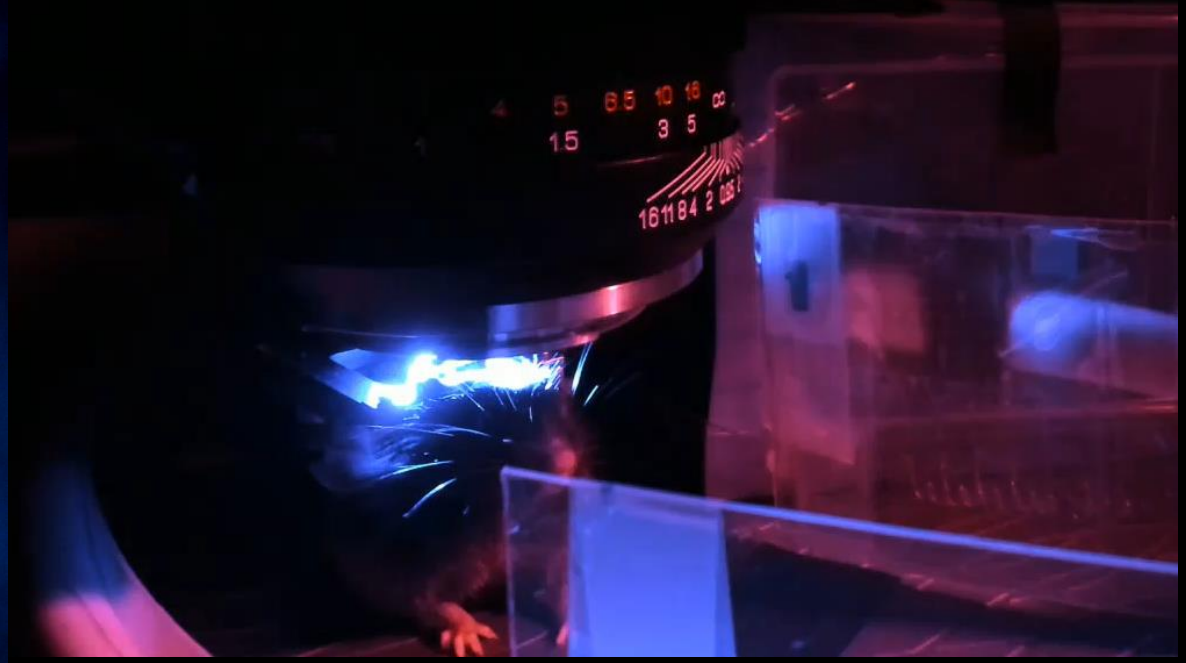
- Basic Principles of Calcium Imaging
  - Fluorescent reporters
  - 2-photon imaging
- Image Processing
  - Motion correction
  - Cell segmentation
  - Spike deconvolution
- Examples of Population Imaging
  - Allen Brain Observatory
  - Cornell OpenCortex Project

Imaging Cognitive Behaviors

Cornell OpenCortex Project



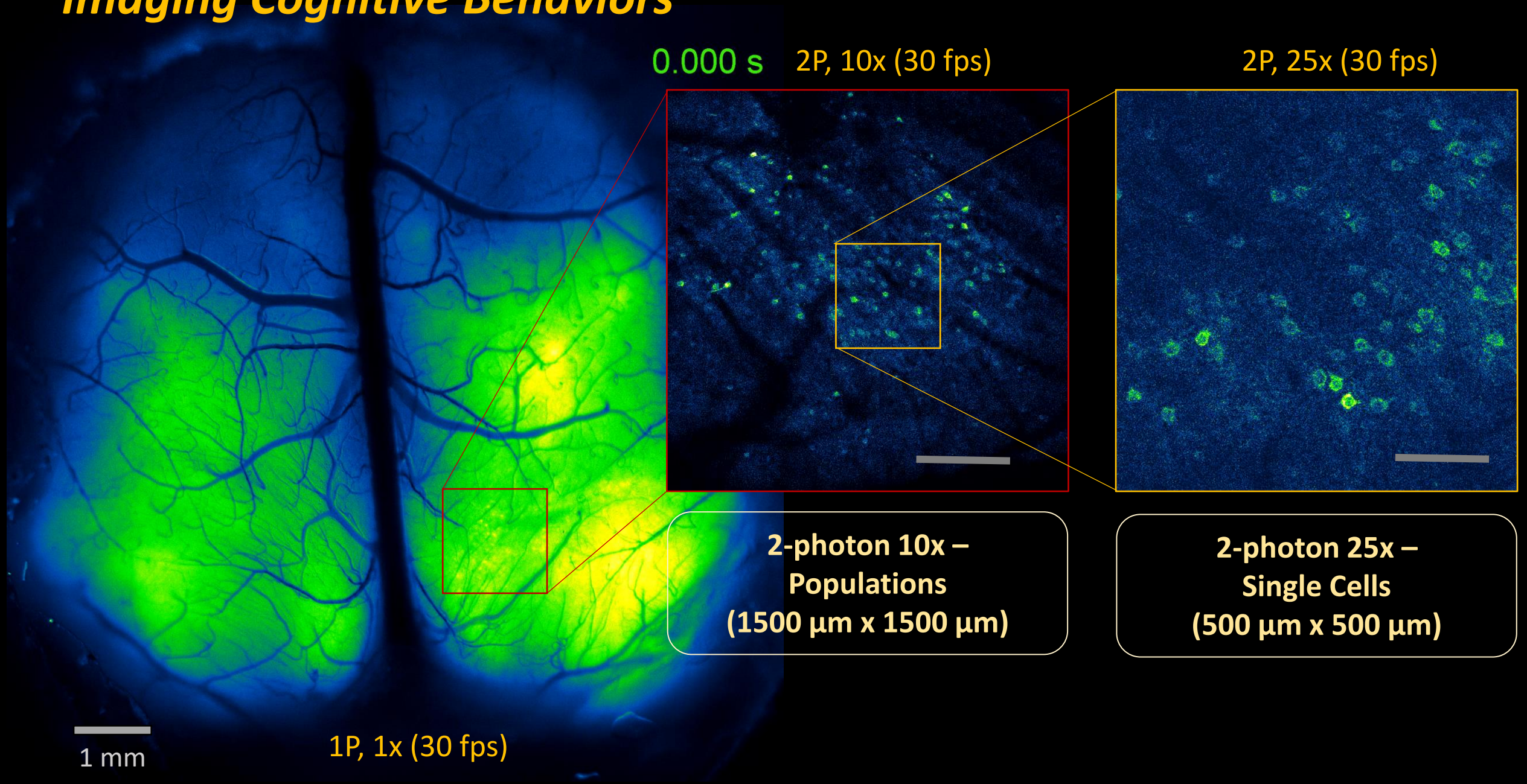
- ***Thy1-GCaMP6s mouse*** **(with 10-mm window)**
- **1-photon widefield imaging**
- **Head-fixed mouse running on a circular maze**



(30 fps, real time)

Imaging Cognitive Behaviors

Cornell OpenCortex Project



2-photon 10x – Populations (1500 µm x 1500 µm)

2-photon 25x – Single Cells (500 µm x 500 µm)

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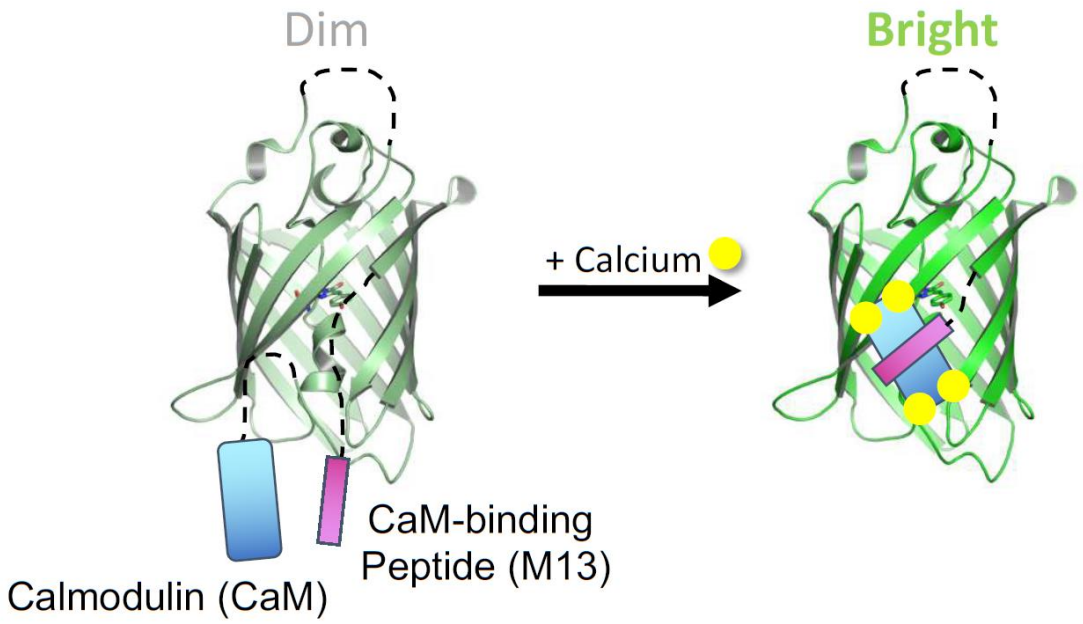
Fluorescent proteins to spy on neuronal function

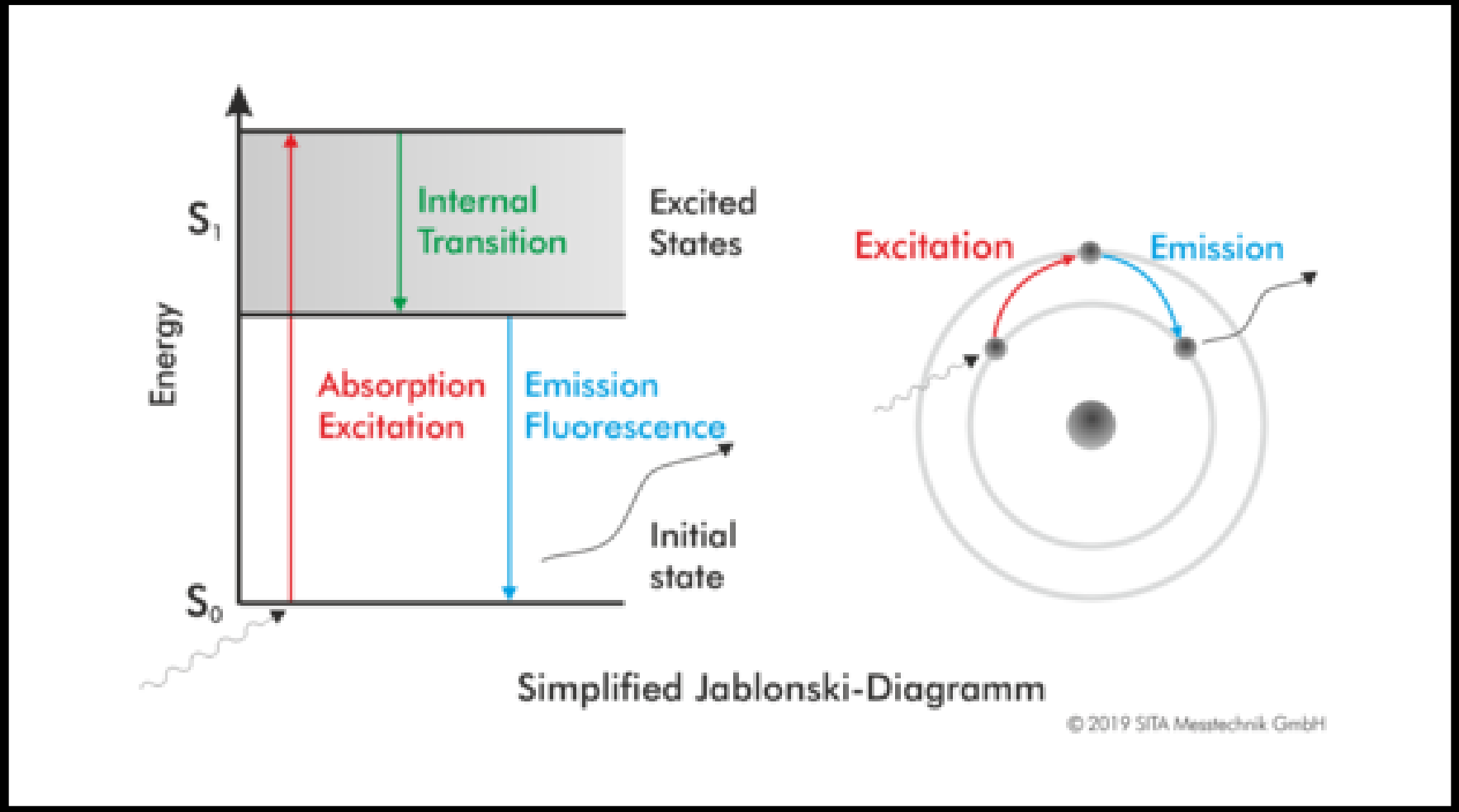


Tsien lab
Miyawaki lab
Nakai lab
Cambell lab
Griesbeck lab

Looger lab
Schreiter lab

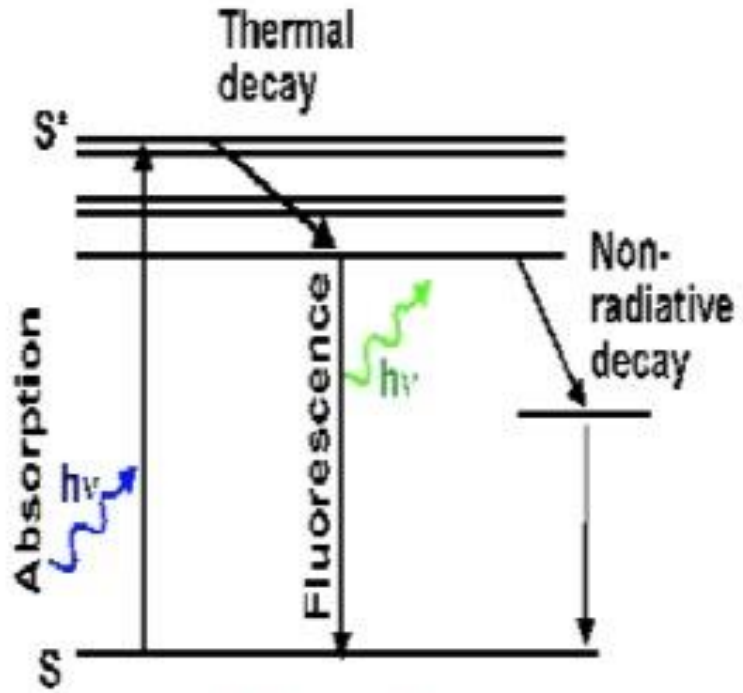
GENIE project

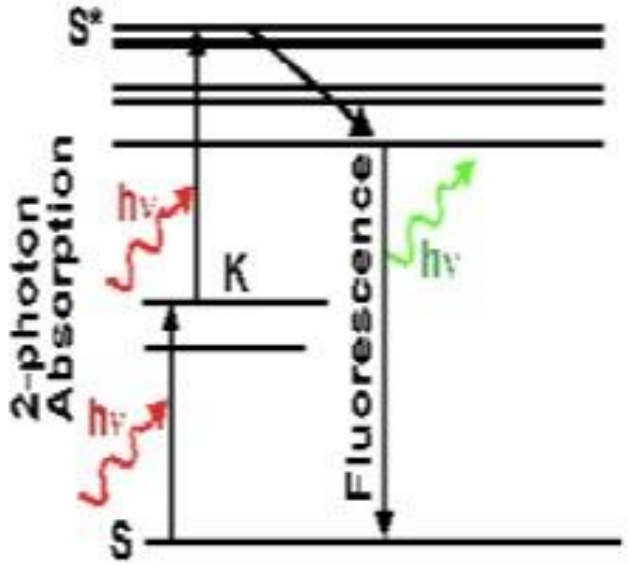
Nakai et al Nat. Biotech 2001; Tian et al Nat. Meth. 2009


Energy
S1
S0
Internal
Transition
Excited
States
Absorption
Excitation
Emission
Fluorescence
Initial
state
Excitation
Emission
Simplified Jablonski-Diagramm
© 2019 SITA Messtechnik GmbH

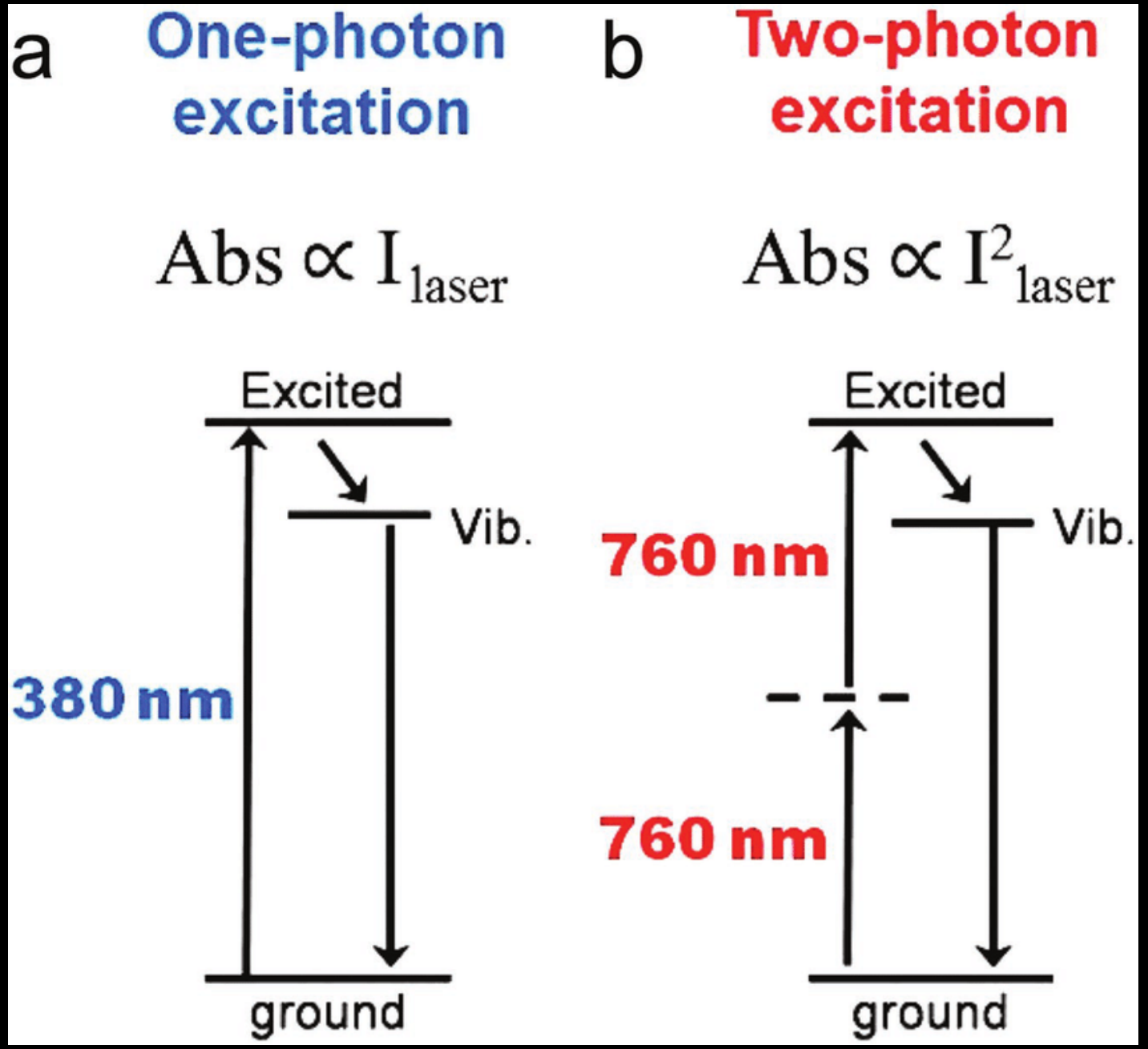
Jablonski Energy Diagram

Two simultaneous lower energy photons can combine and have the same effect as one higher energy photon

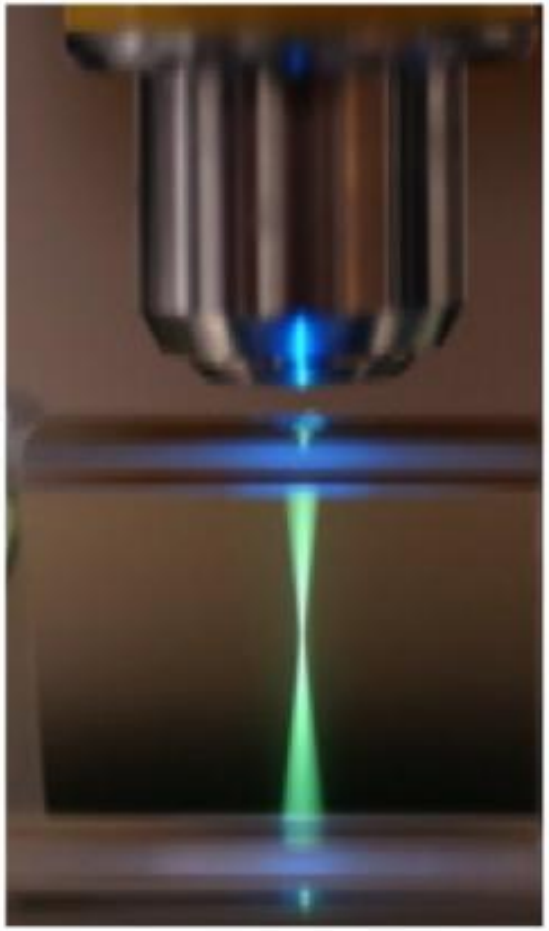


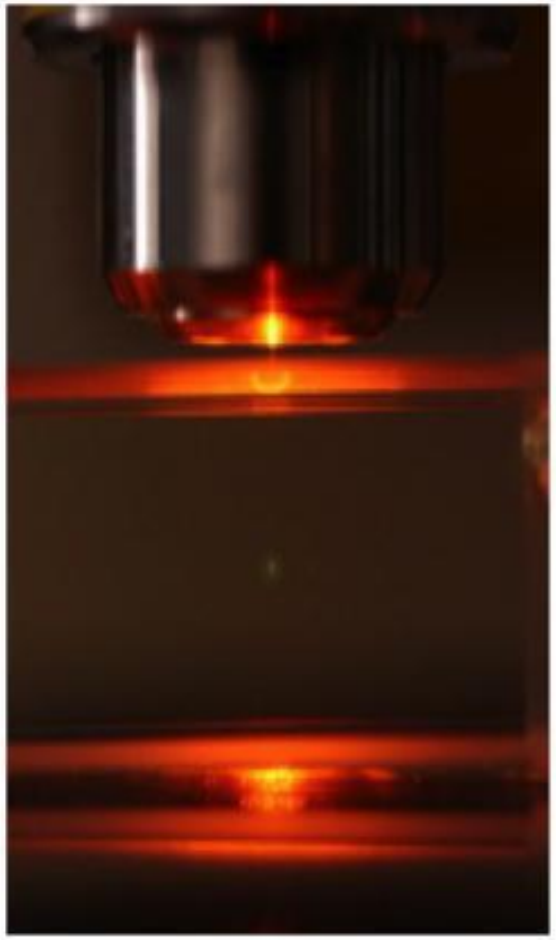


One photon **Two photon**


a
One-photon excitation
Abs ∝ I laser
Excited
Vib.
380 nm
ground
b
Two-photon excitation
Abs ∝ I² laser
Excited
Vib.
760 nm
760 nm
ground

1-photon vs. 2-photon

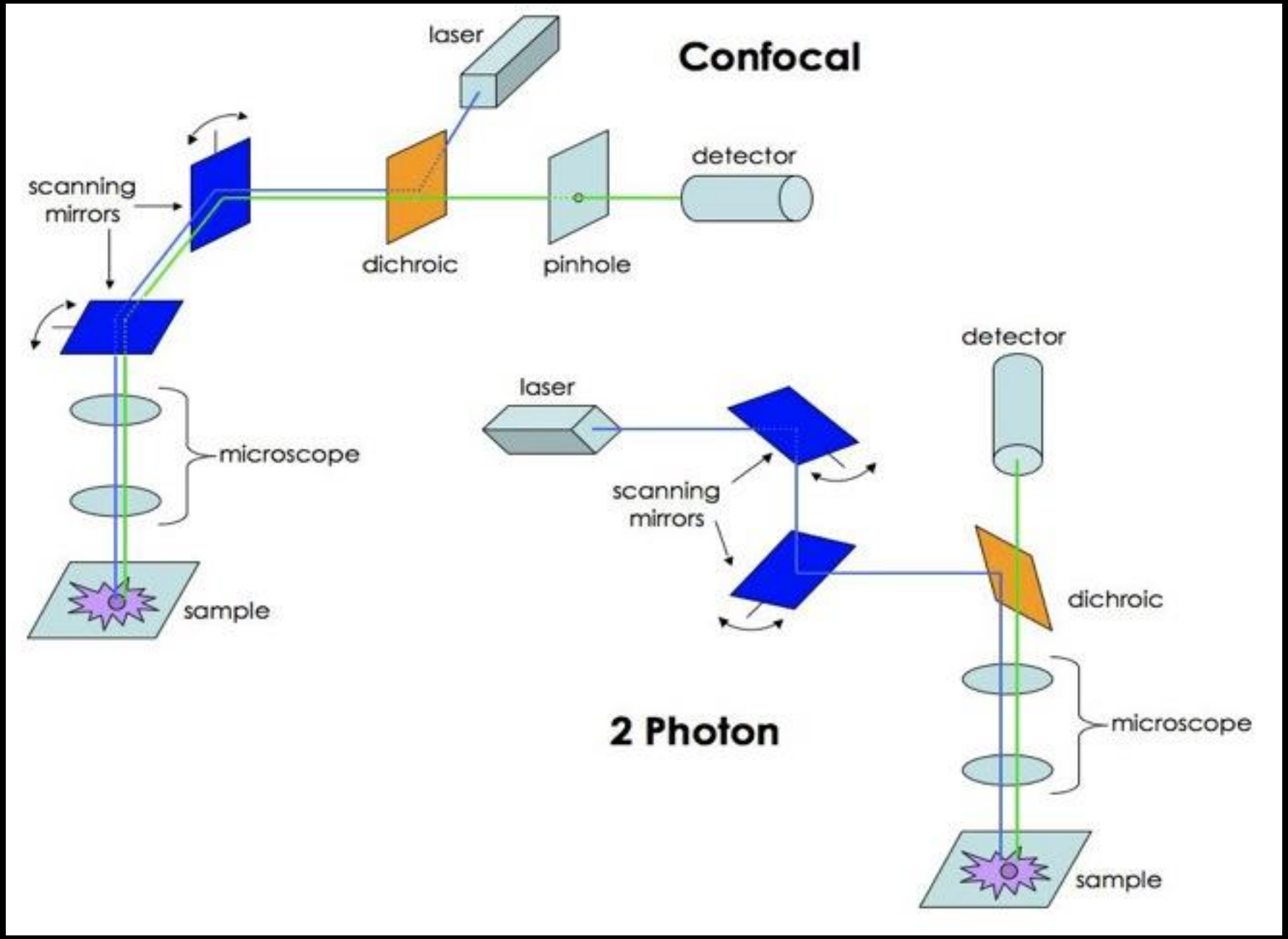


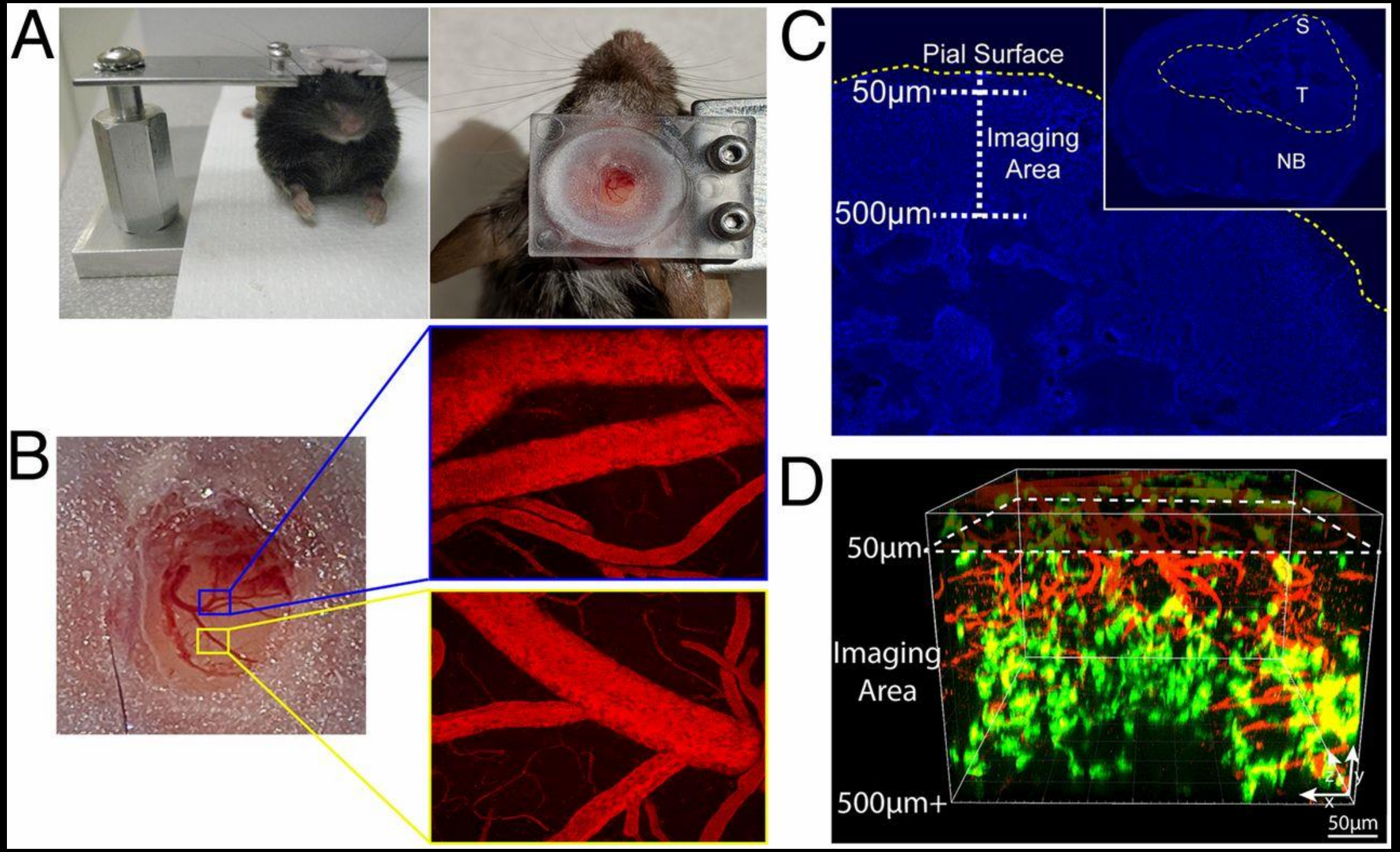


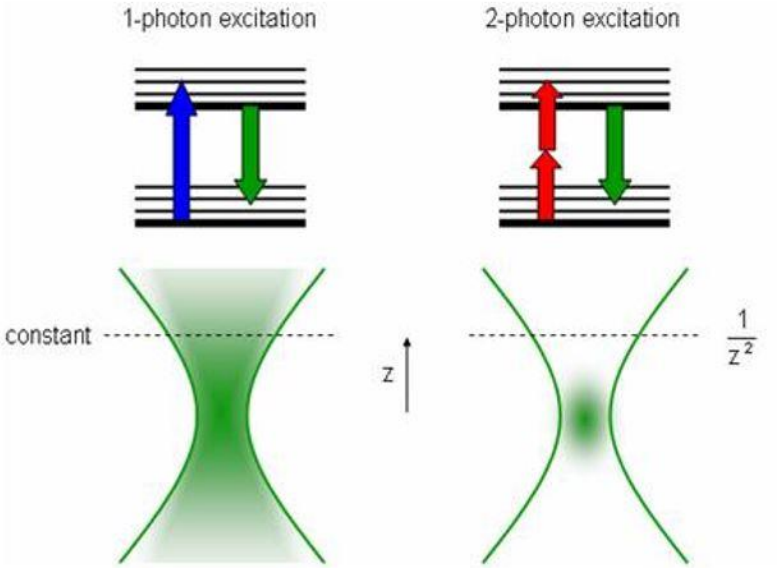
Photos by Steve Ruzin

Fluorescence from out of focus planes

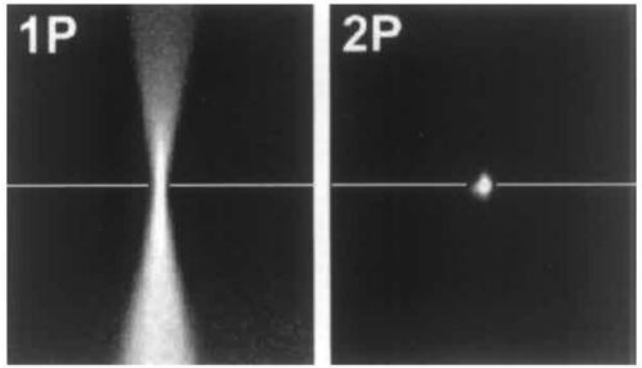
Fluorescence from focal spot only


Confocal
laser
detector
scanning
mirrors
dichroic
pinhole
microscope
sample
2 Photon
laser
detector
scanning
mirrors
dichroic
microscope
sample


A
B
C
Pial Surface
50µm
Imaging
Area
500µm
S
T
NB
D
50µm
Imaging
Area
500µm+
50µm



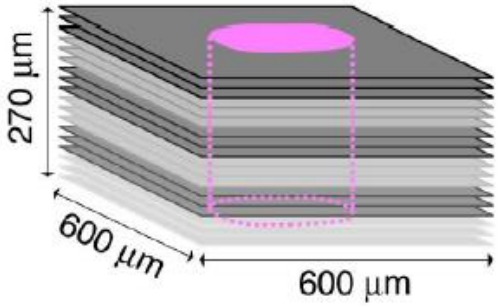
http://biomicroscopy.bu.edu/research/nonlinear-microscopy



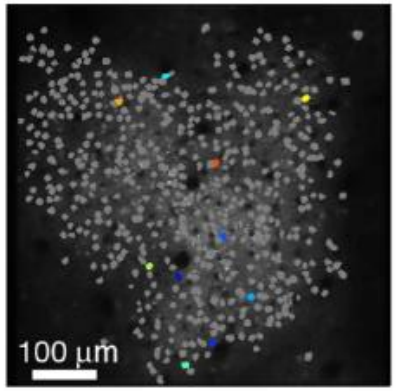
M. Rubart (2004) Two-Photon Microscopy of Cells and Tissue. Circulation Research 95:1154-1166

Comprehensive mapping of neural activity

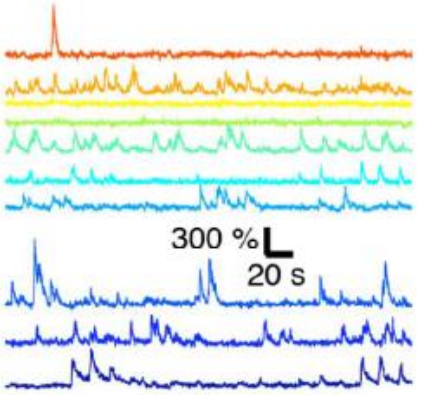
Cortical 'barrel' column



Imaging plane



Example neuron fluorescence



Peron et al 2015

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See separate slide deck on the topic

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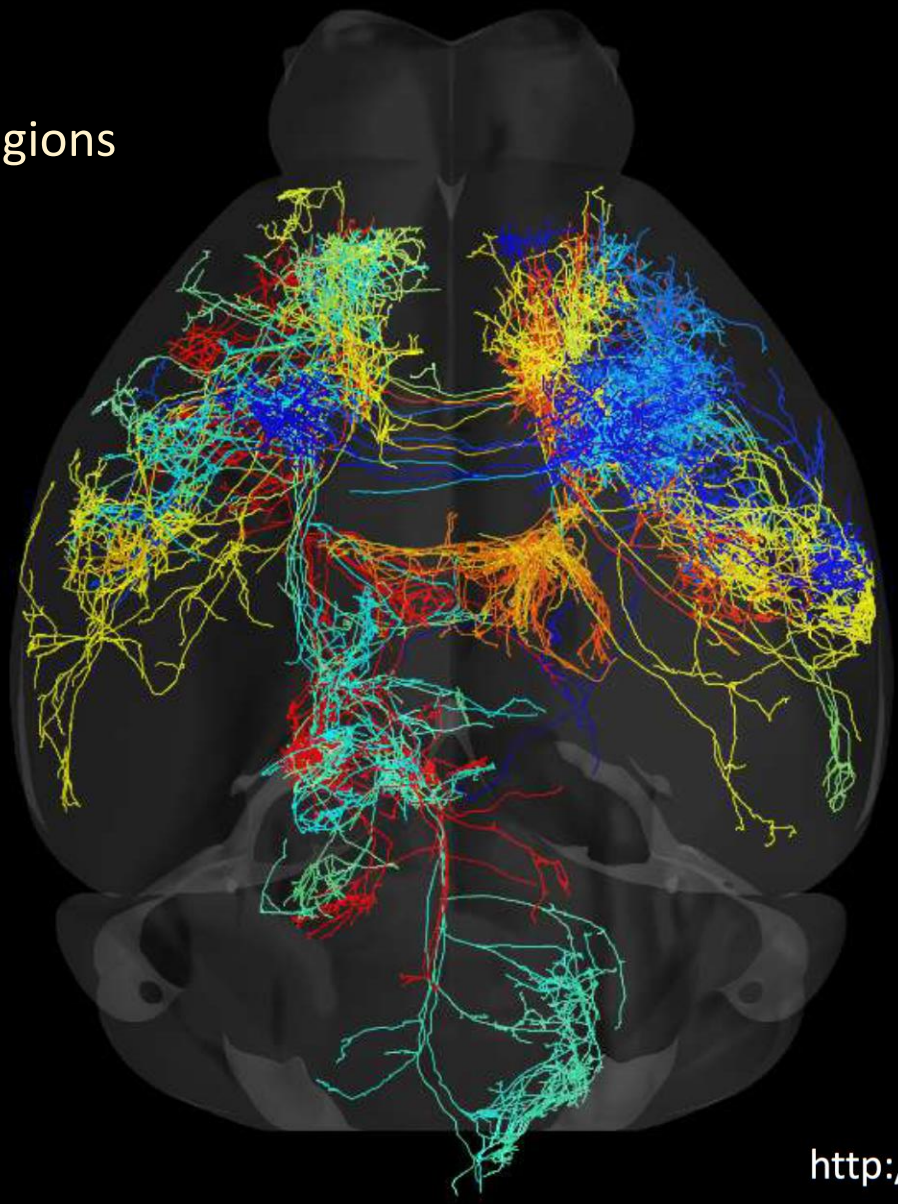
  - Cornell OpenCortex Project (*led by Ben Huang*)

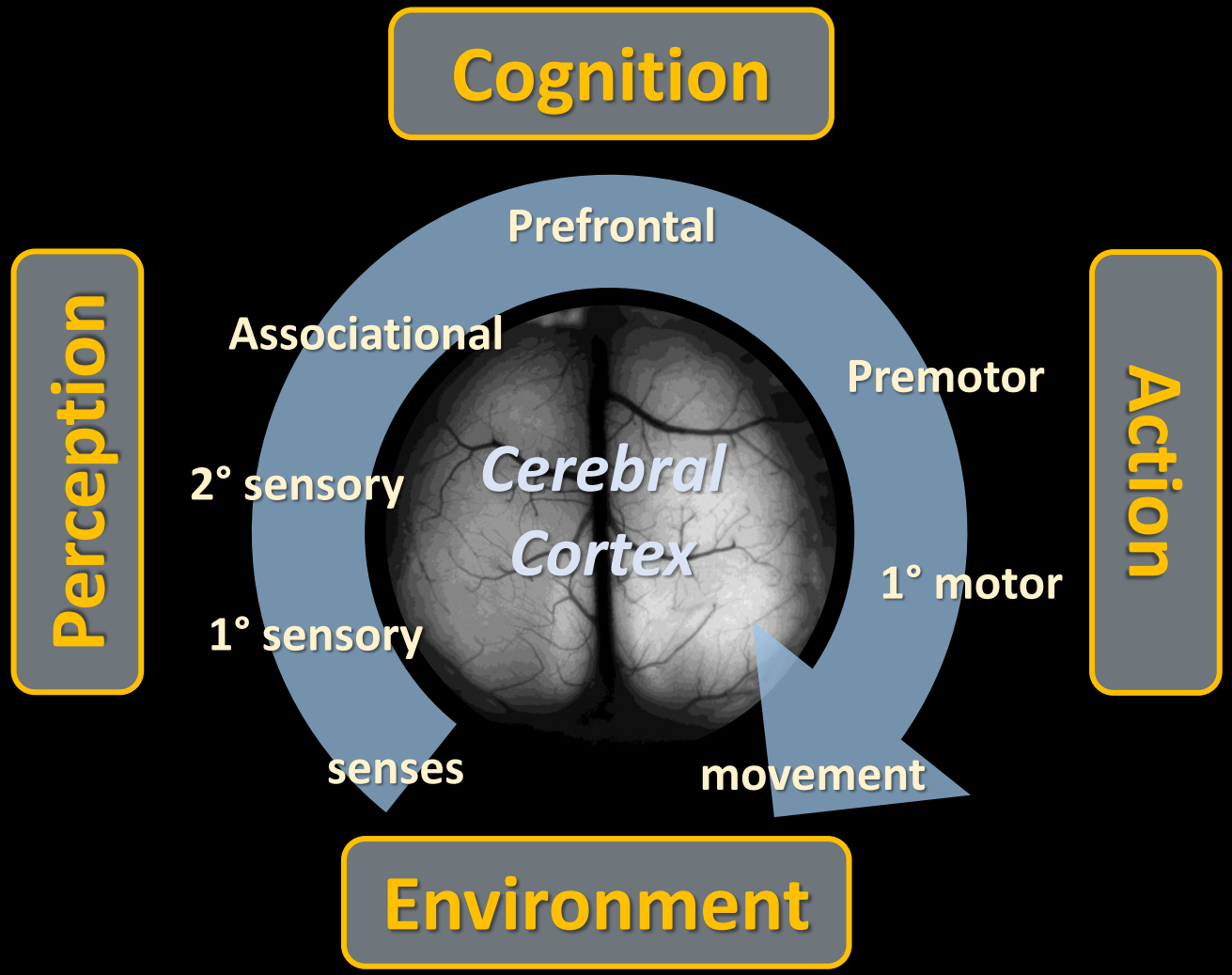
The Need for Cortex-wide Imaging

- neurons extend across multiple regions



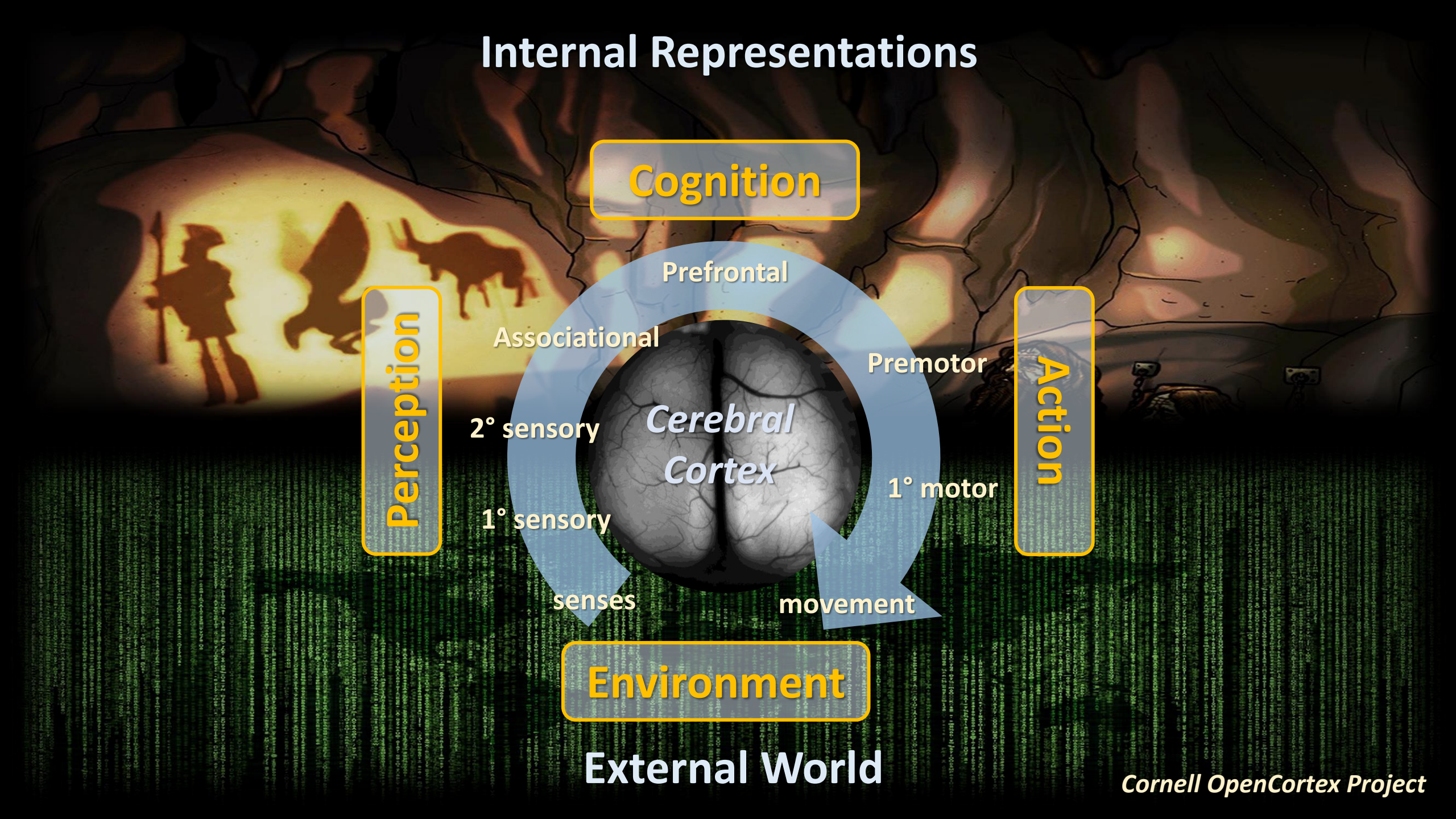
http://mouselight.janelia.org/

Towards deconstructing cortex-wide cognitive networks



Cornell OpenCortex Project

Internal Representations

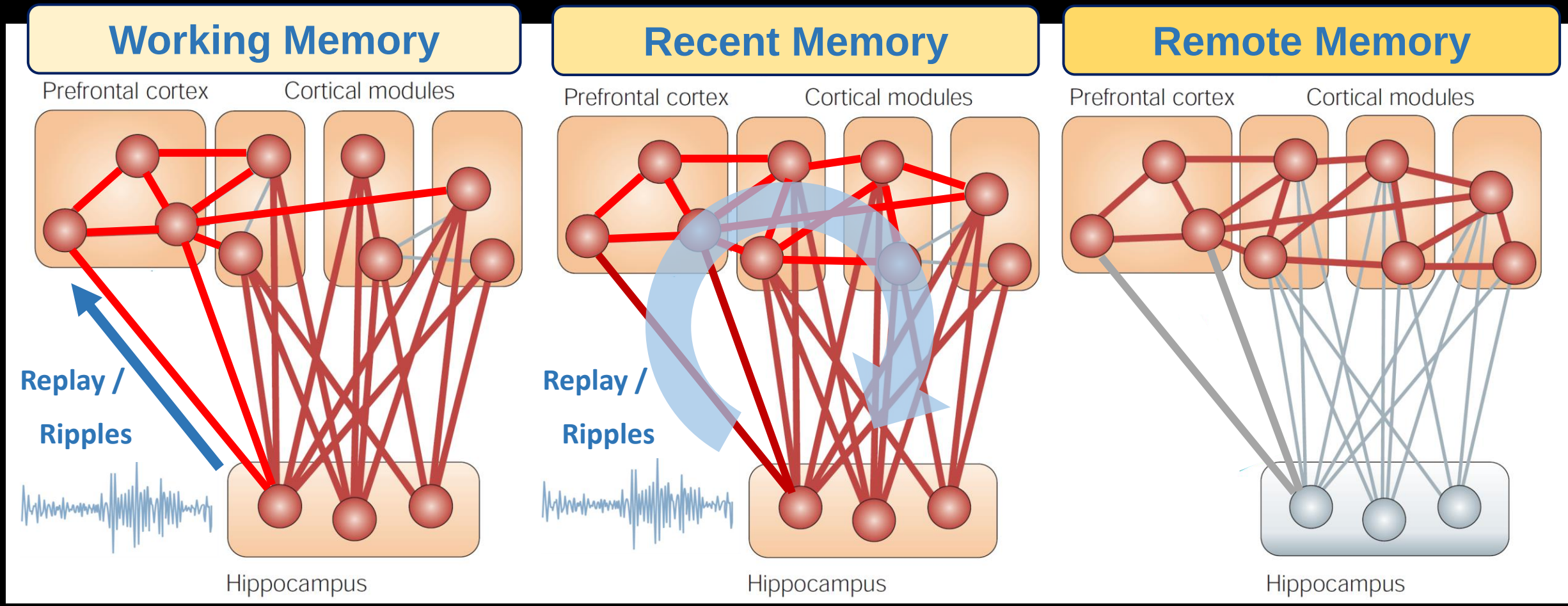


External World

Cornell OpenCortex Project

QUESTION:

How are internal representations (*i.e.* memories) formed and maintained ?



time

Cornell OpenCortex Project

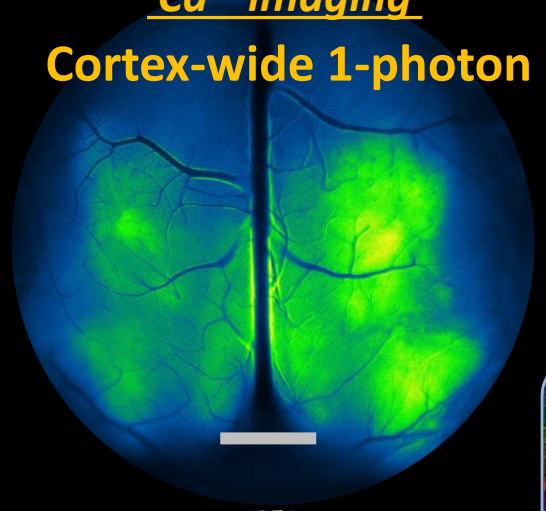
OpenCortex project

Opening access into investigating cortex-wide network function

Ben S. Huang *et al.*
Weill Cornell Medicine

Ca^{2+} *imaging*

Cortex-wide 1-photon



Cortex-wide 2-photon