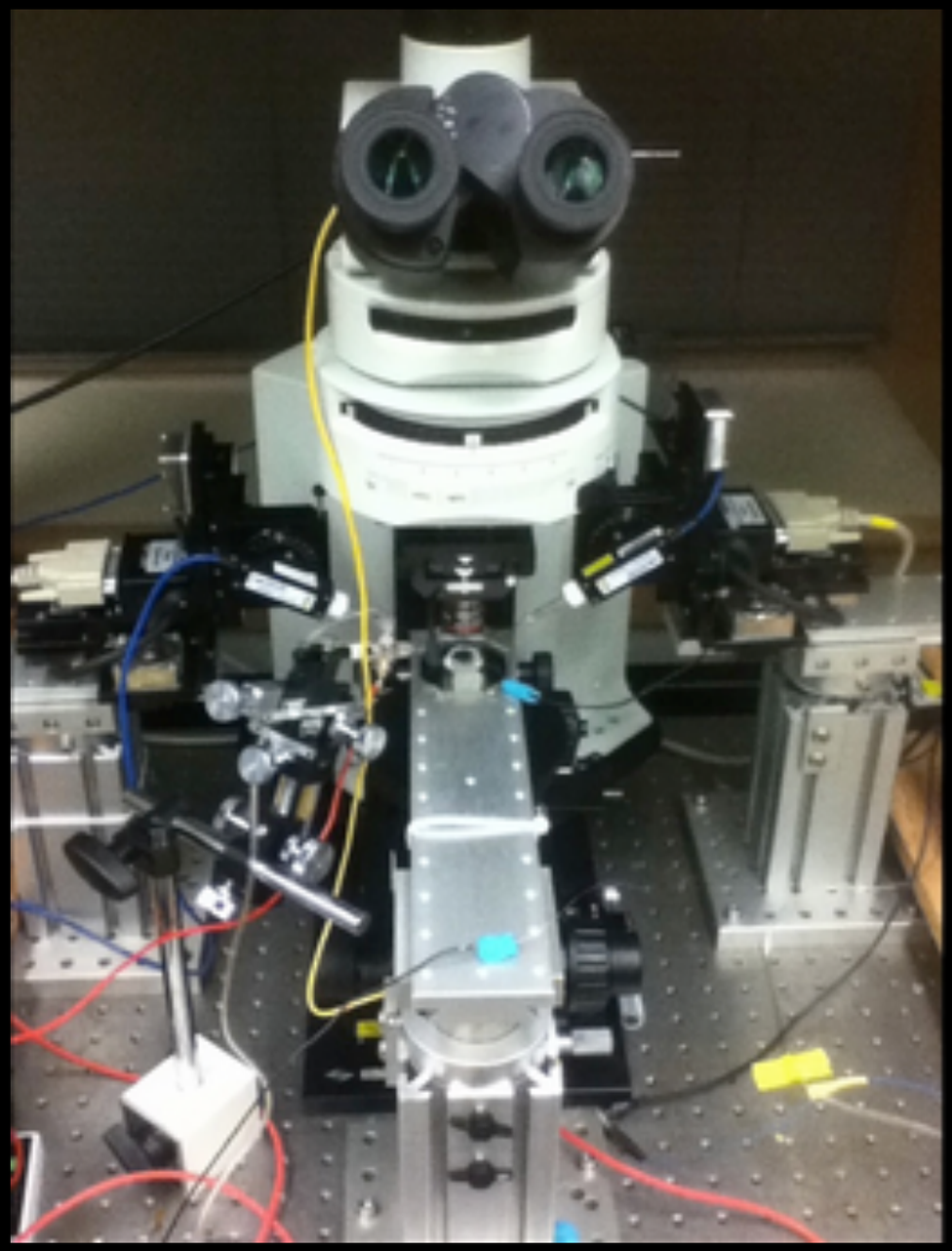
Experimental Manipulation in Microscopy



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EPSRC
Engineering and Physical Sciences Research Council
MRC Medical Research Council
EPSRC & MRC Centre for Doctoral Training in Biomedical Imaging
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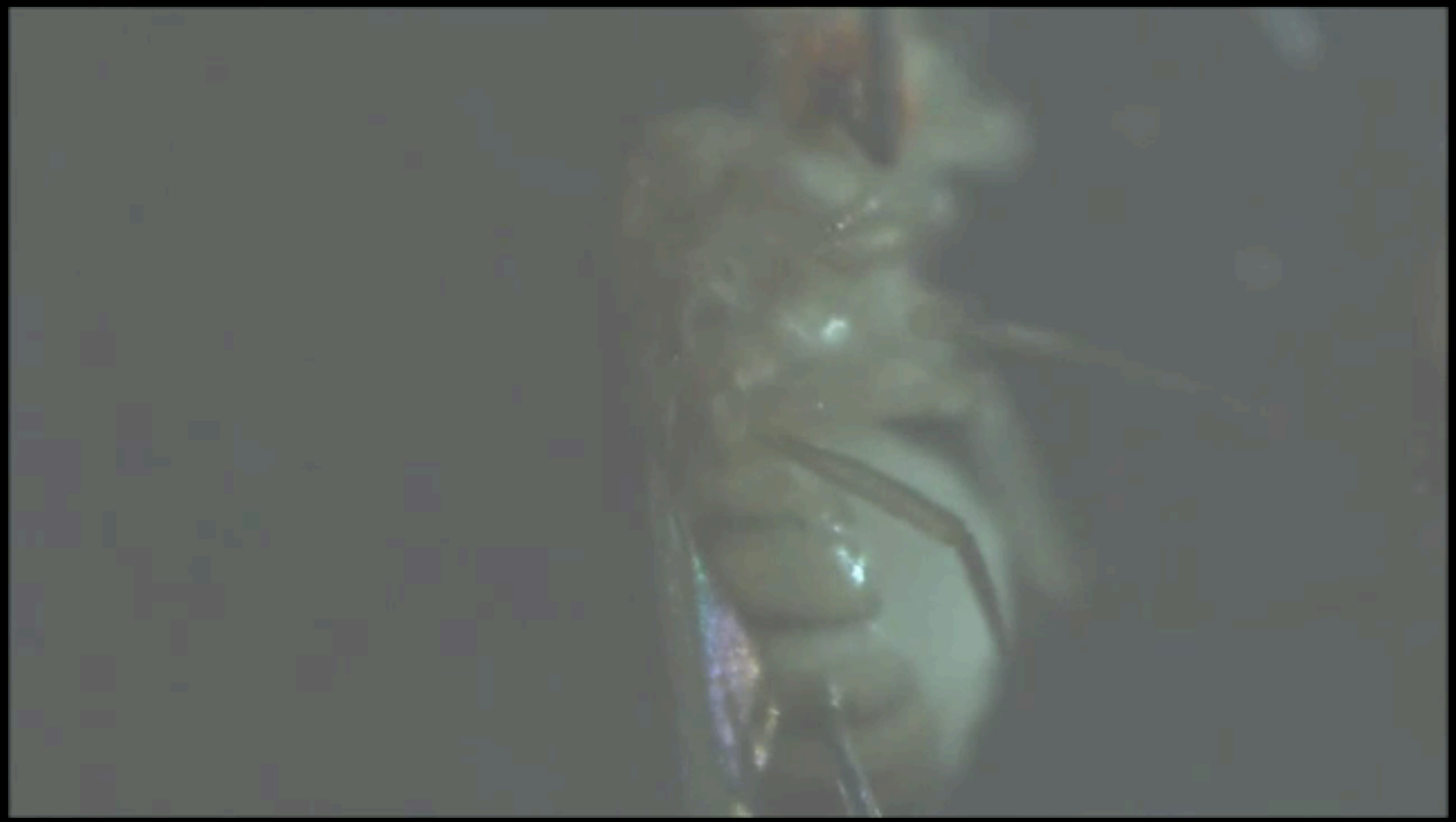
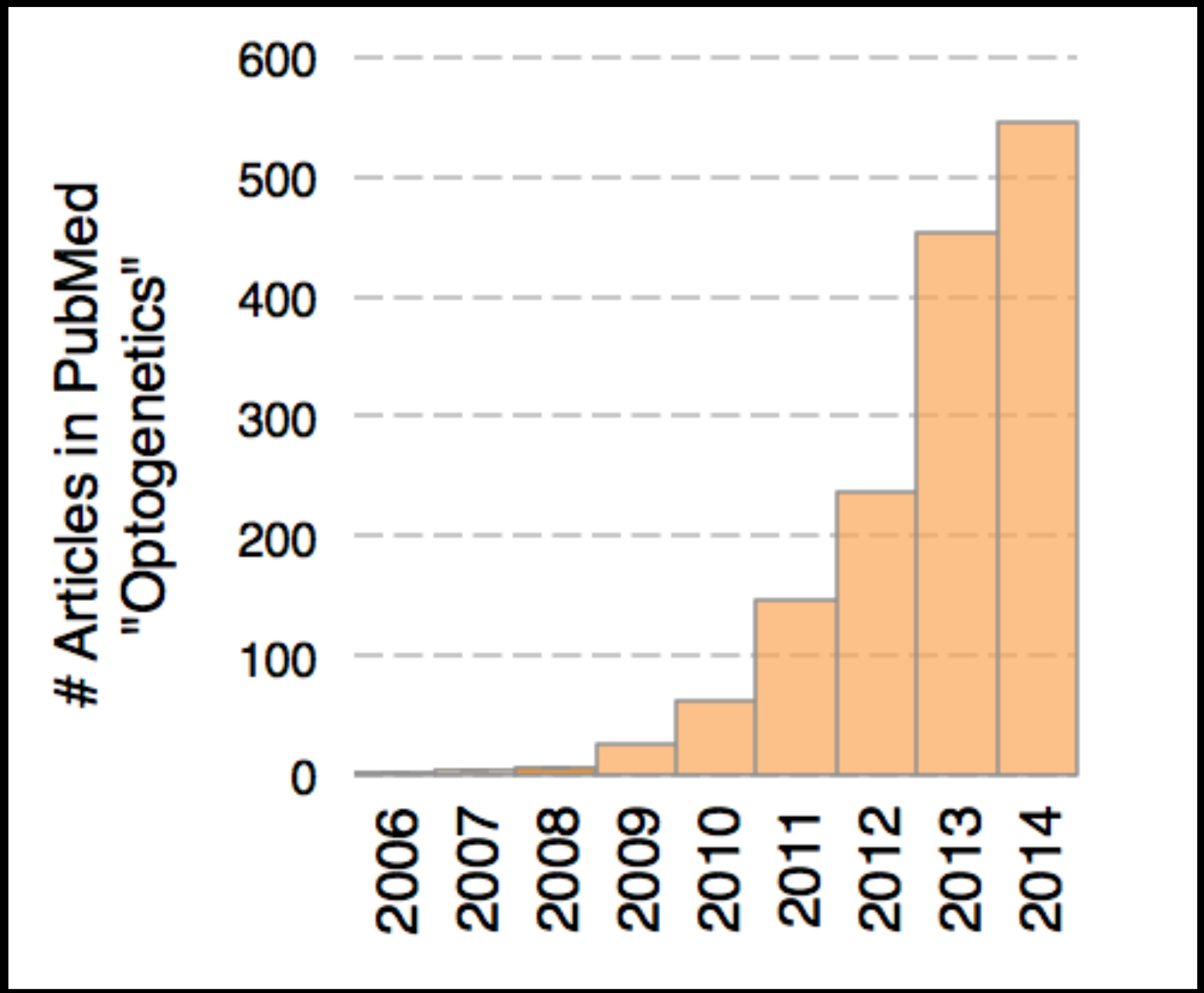
Photo-activation of neurons in *Drosophila*



Optogenetics- combination of light microscopy techniques with recombinant photosensitive molecules

Growth of optogenetics in biomedical research



Experimental Manipulation in Microscopy

- Introduction to tools that are combined with light microscopy in live cell biology experiments

- Design a microscopy experiment that uses light to measure and manipulate the function of live cells

Cell physiology tools for microscopy experiments

<u>Manipulating</u> cell physiology (effectors)

Light-gated ion channels

Temperature-sensitive ion channels

Caged molecules

ROS-generating fluorescent proteins

Protein inactivation- FALI

<u>Measuring</u> cell physiology (sensors)

Calcium indicators

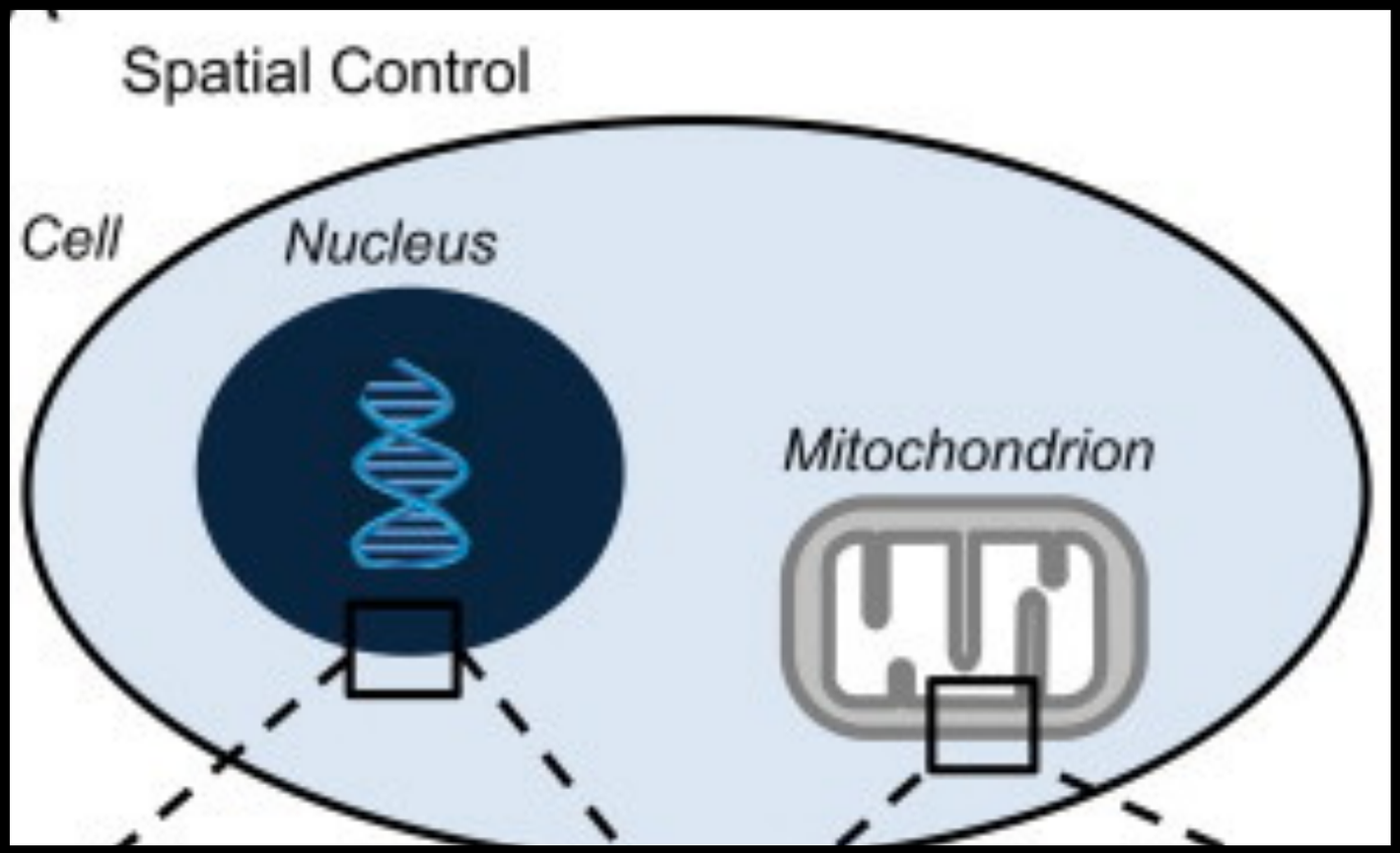
pH indicators

ROS indicators

Reporters (transcription/translation, etc.)

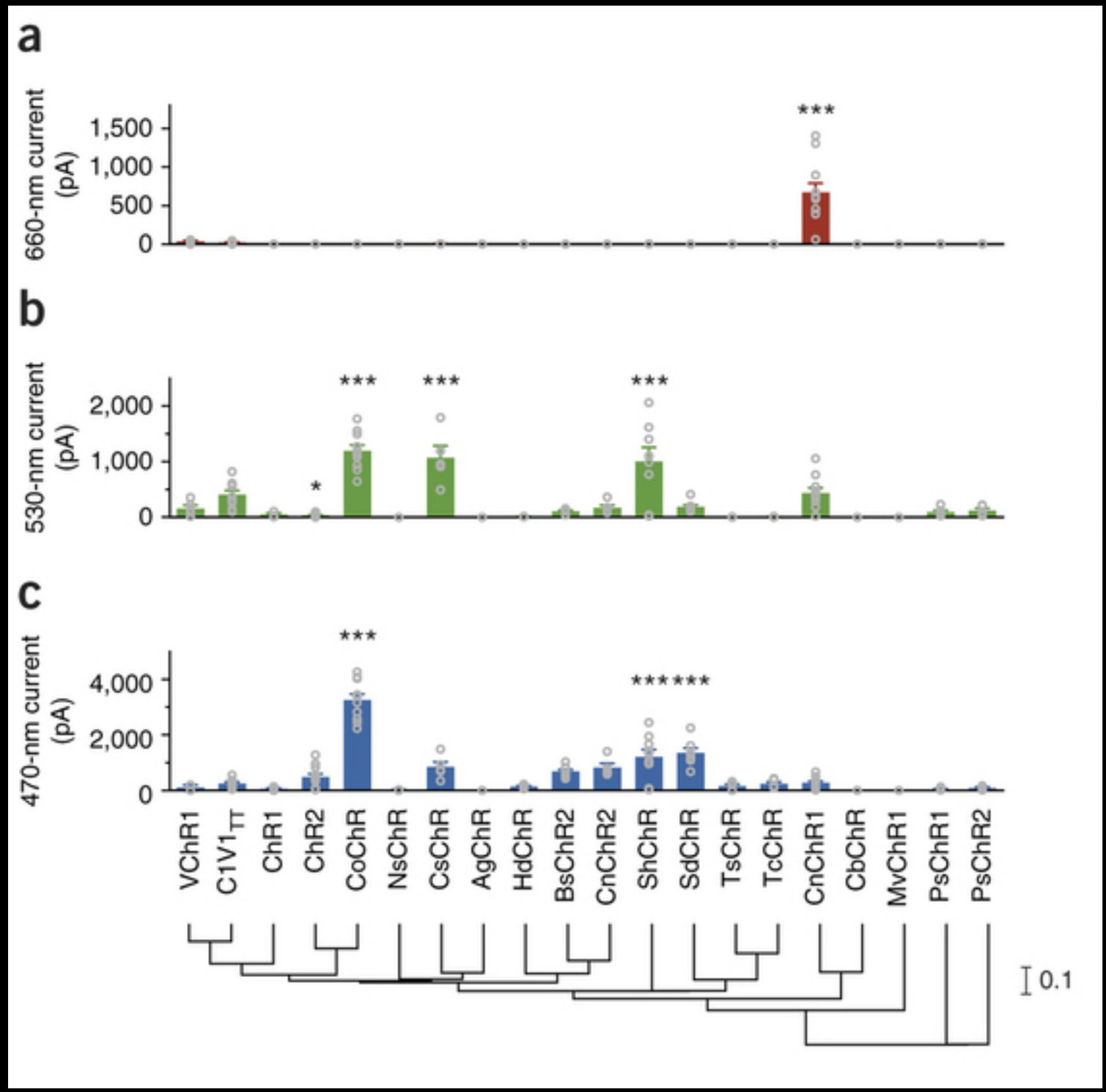
Membrane dynamics (FM dyes)

Channelrhodopsin- an effector to activate neurons



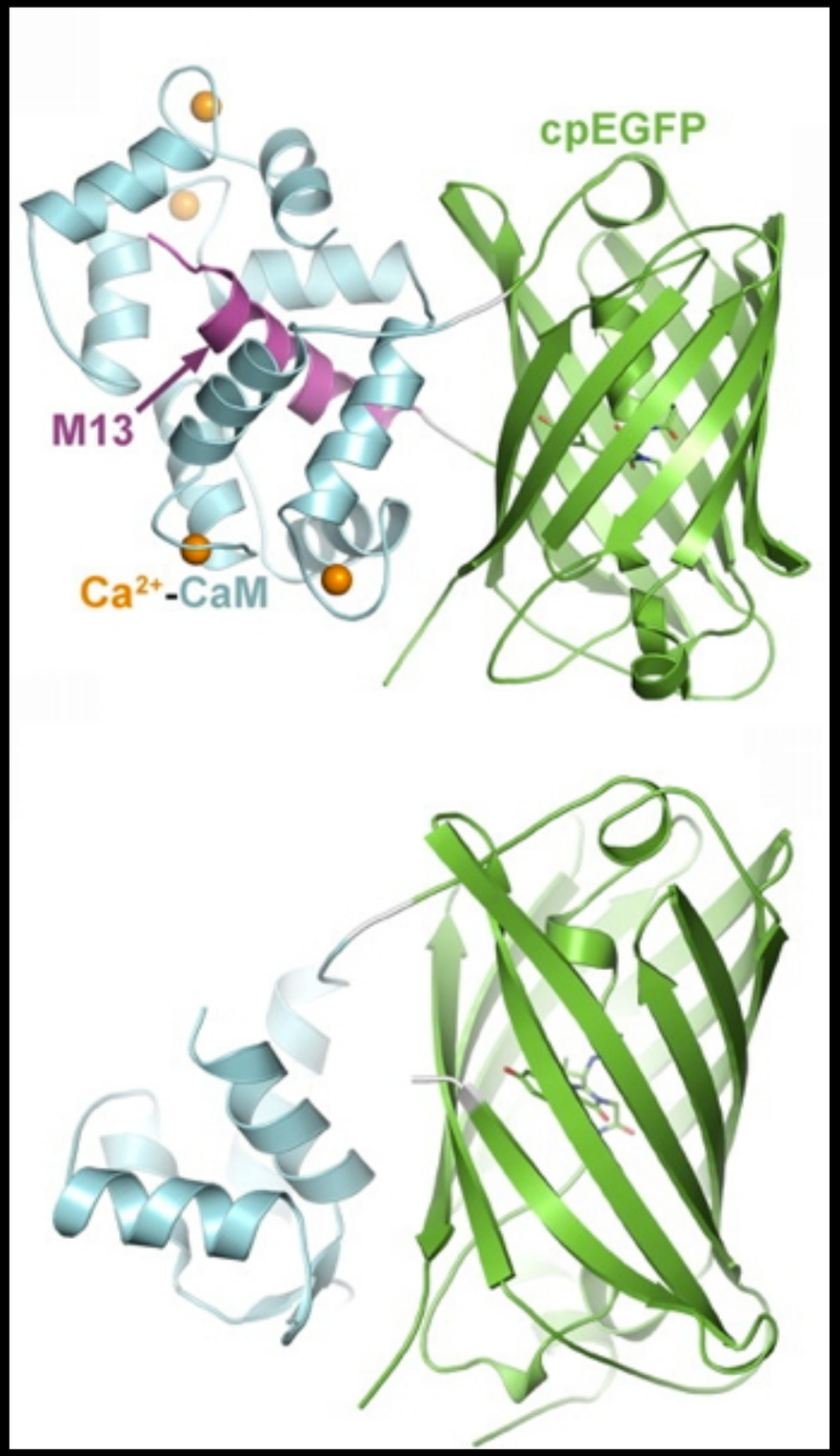
Spectral properties vary in channelrhodopsins from different species

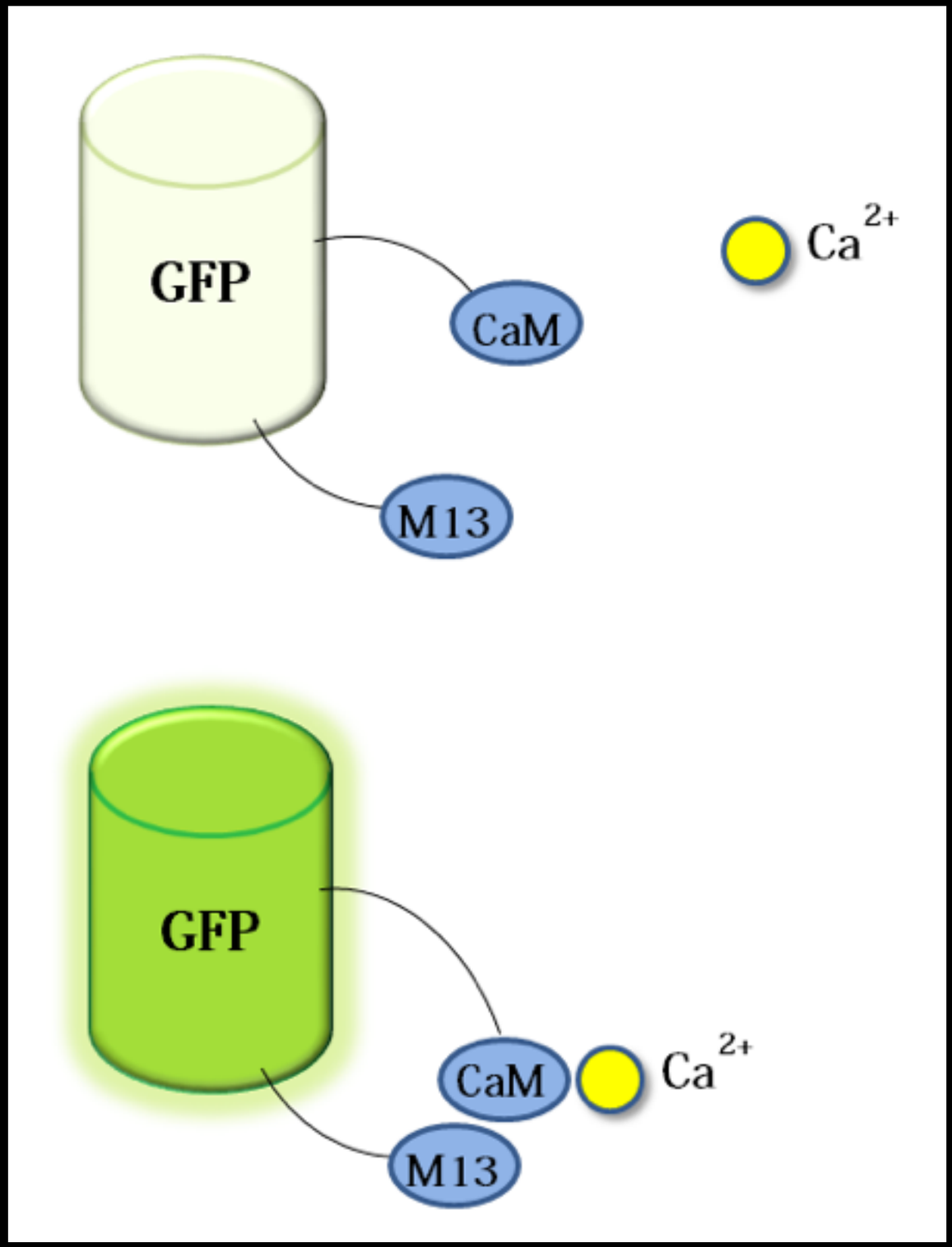
-Evolution has produced important variants for optogenetics



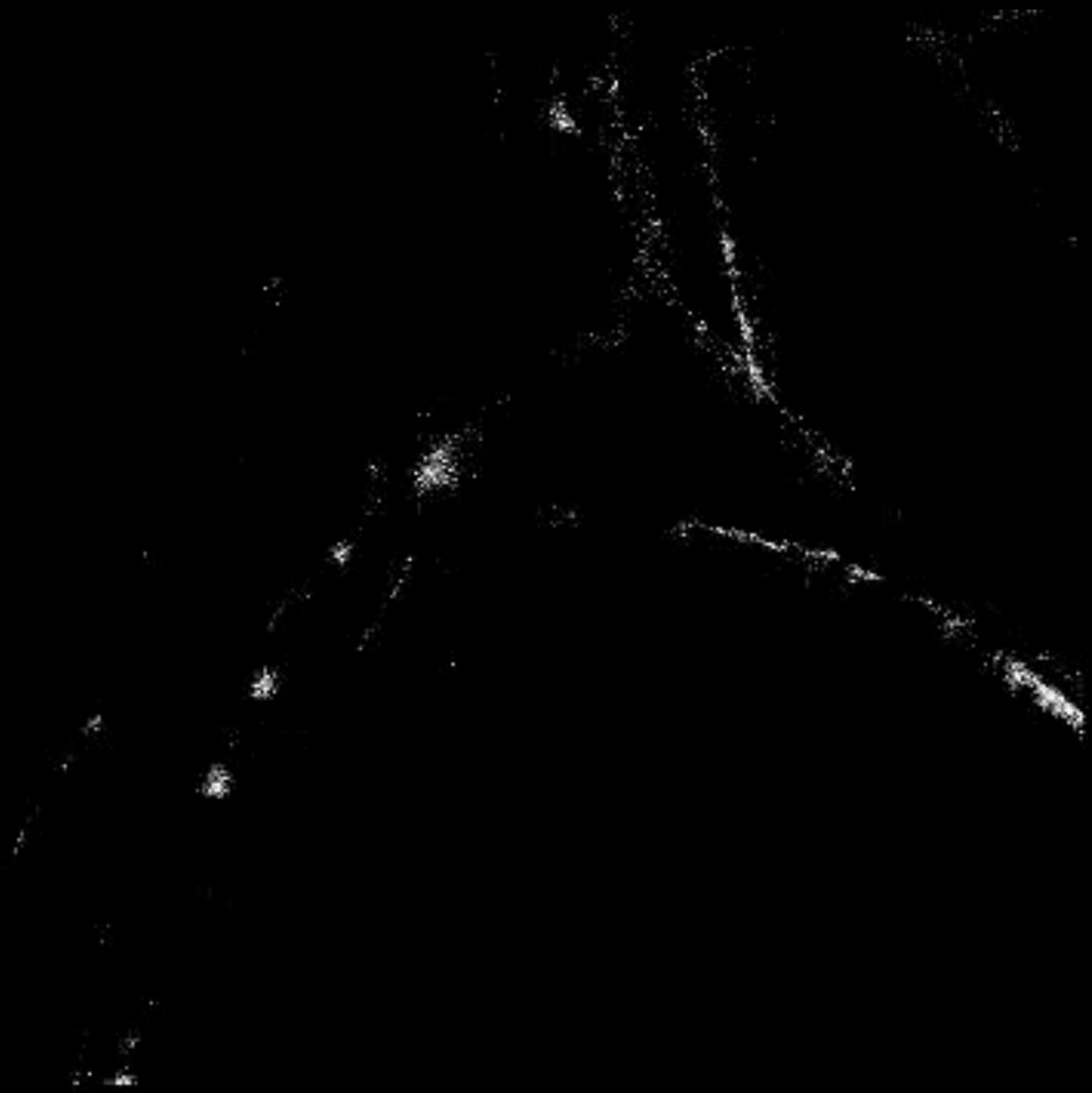
Klapoetke et al., 2014- *Nature Methods*

G-CaMP- A sensor to measure cellular activity





Designing an optogenetics experiment



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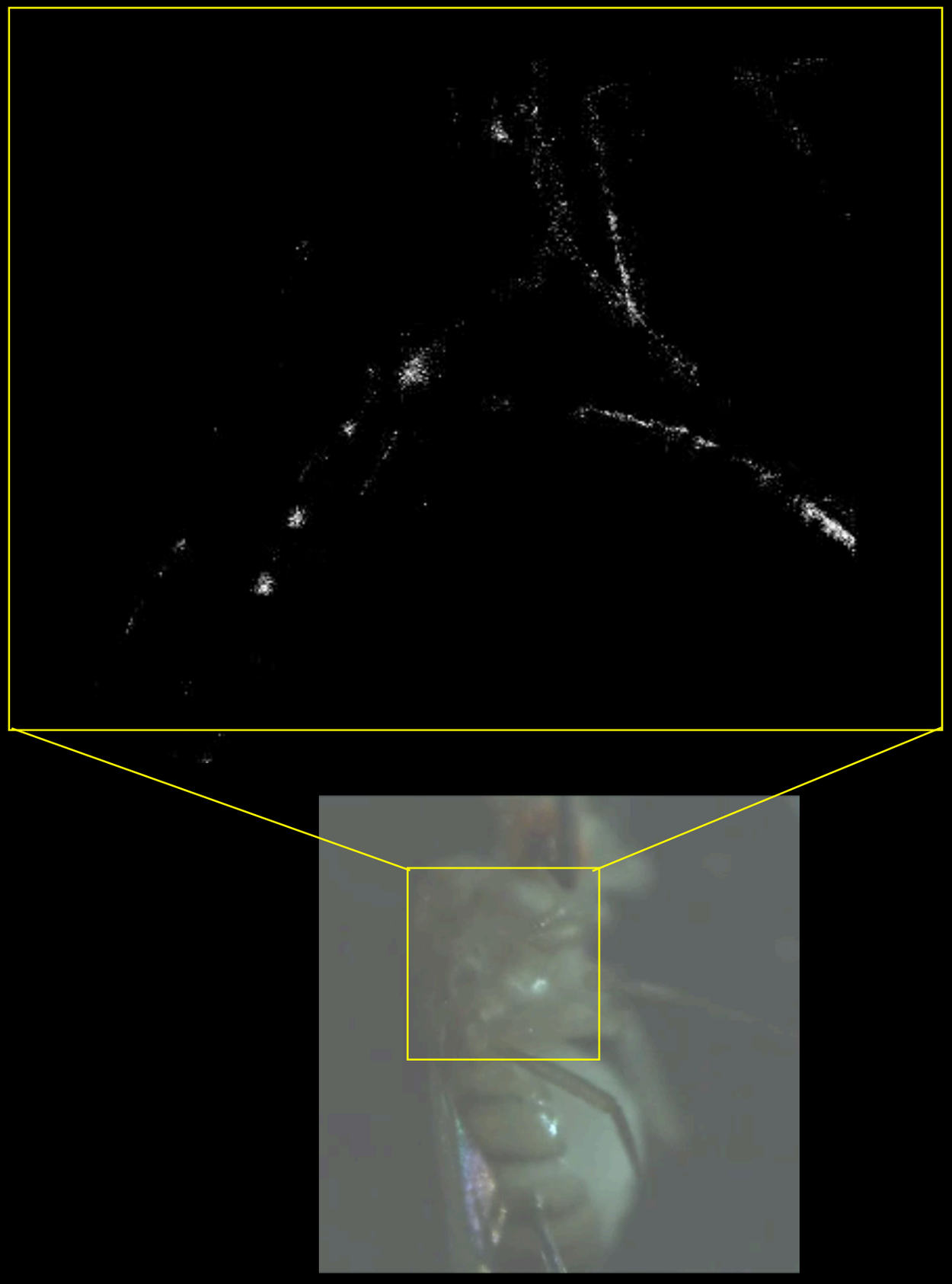
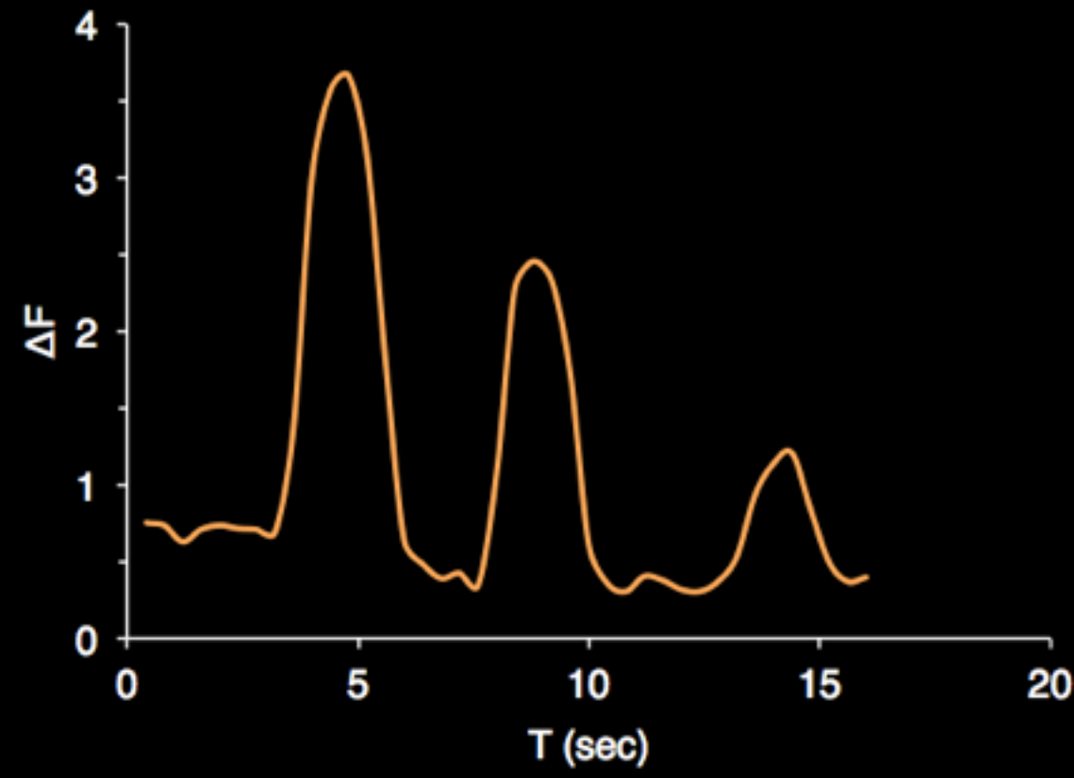
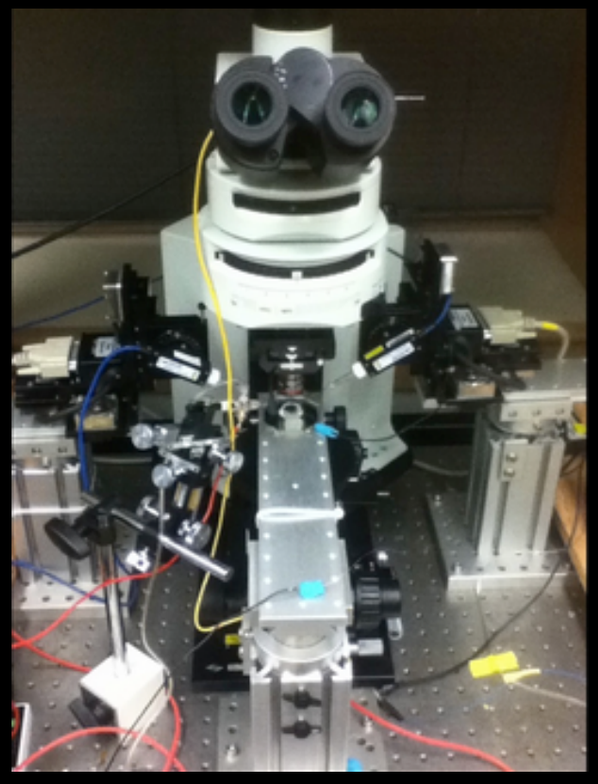
Designing an optogenetics experiment







Microscopy setup for experiments on live tissue

i. Light sources (stimulus & record)

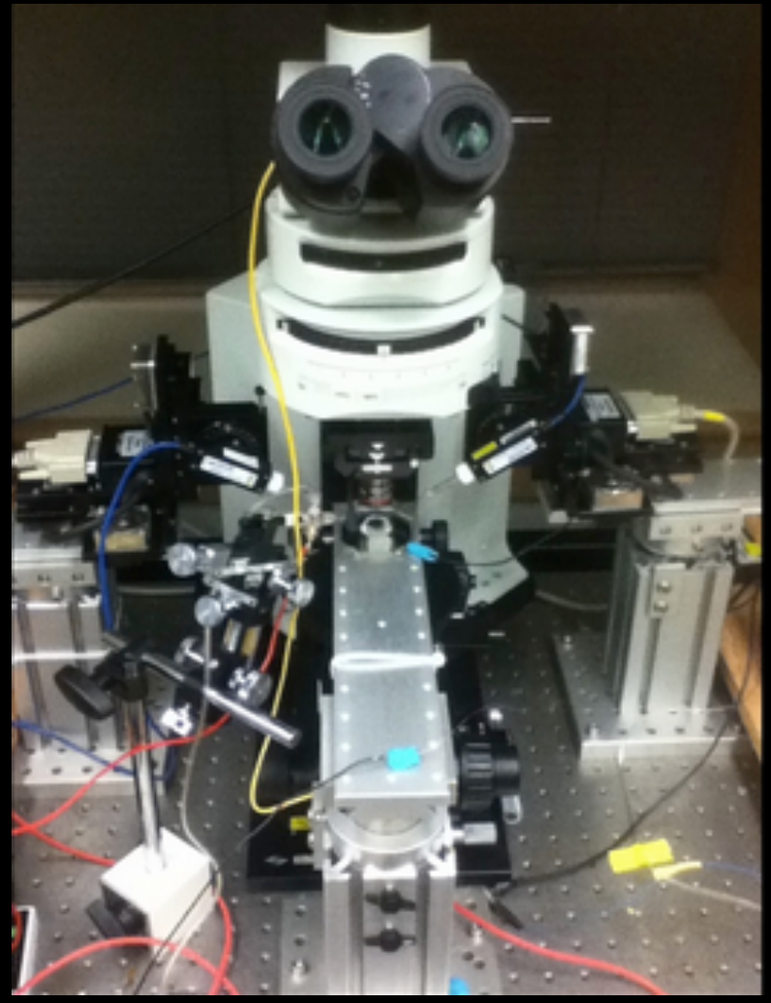
-Type
-Controller
-Speed

ii. Objective

-Magnification
-Medium

iii. Detector

-Type
-Speed



Objective required for imaging in saline

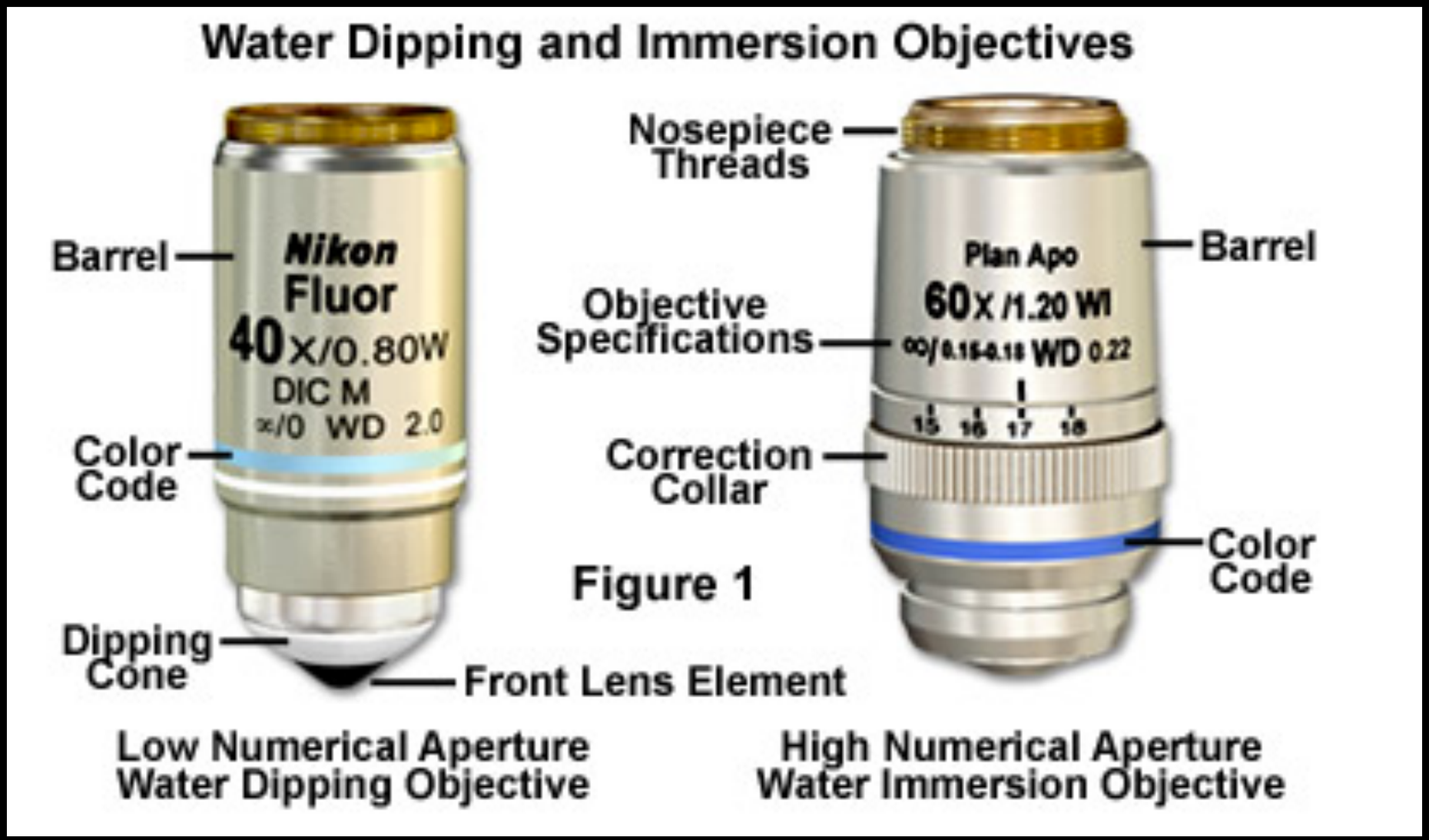


Figure 1

Nikon microscopy website

Detectors: CCD (wide-field) or PMT (confocal)

CCD

~10 frames per second (fps)

~100 fps

~1,000 fps

PMT

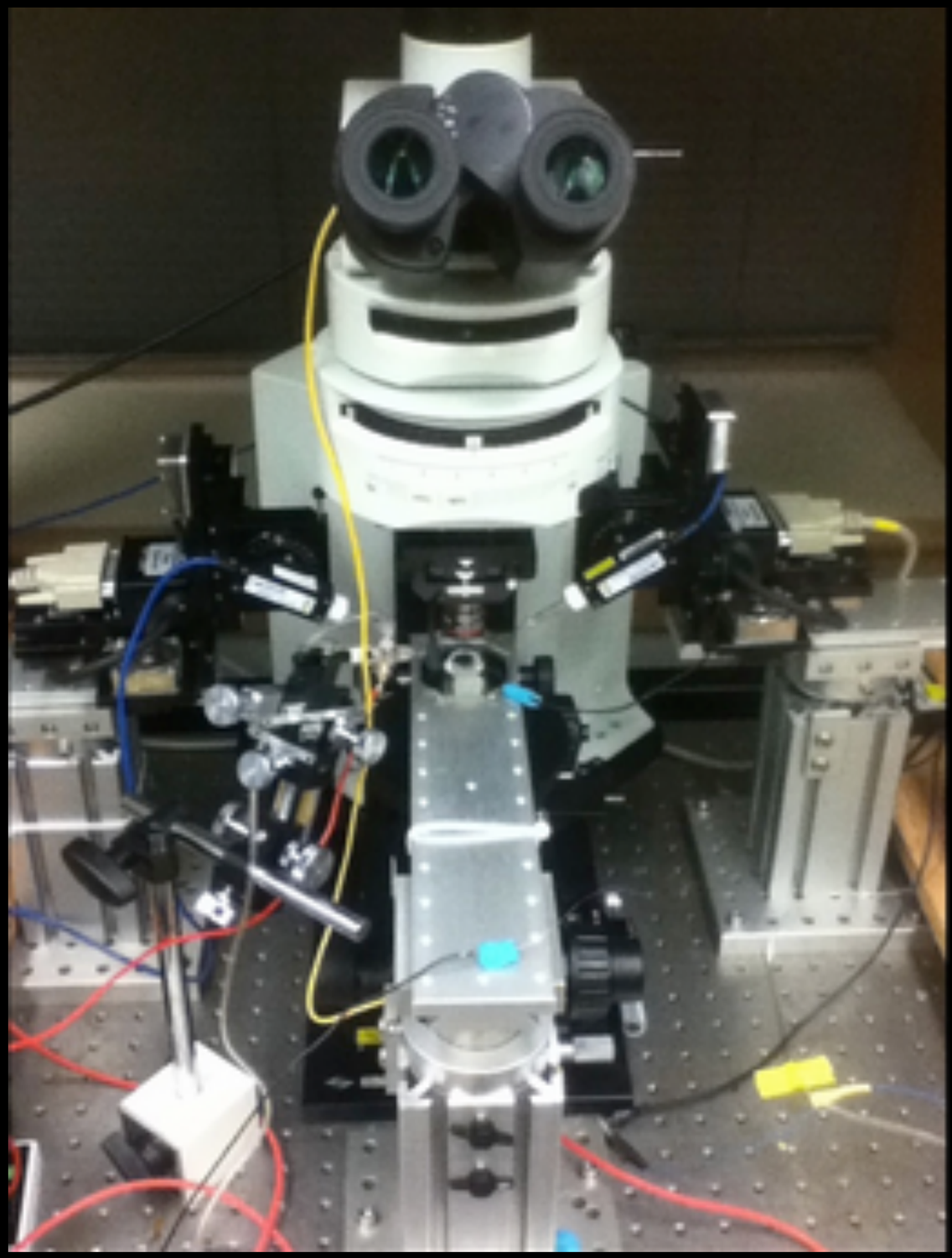
12,000 Hz
(line scan frequency)

Microscopy setup combining optogenetics and electrophysiology

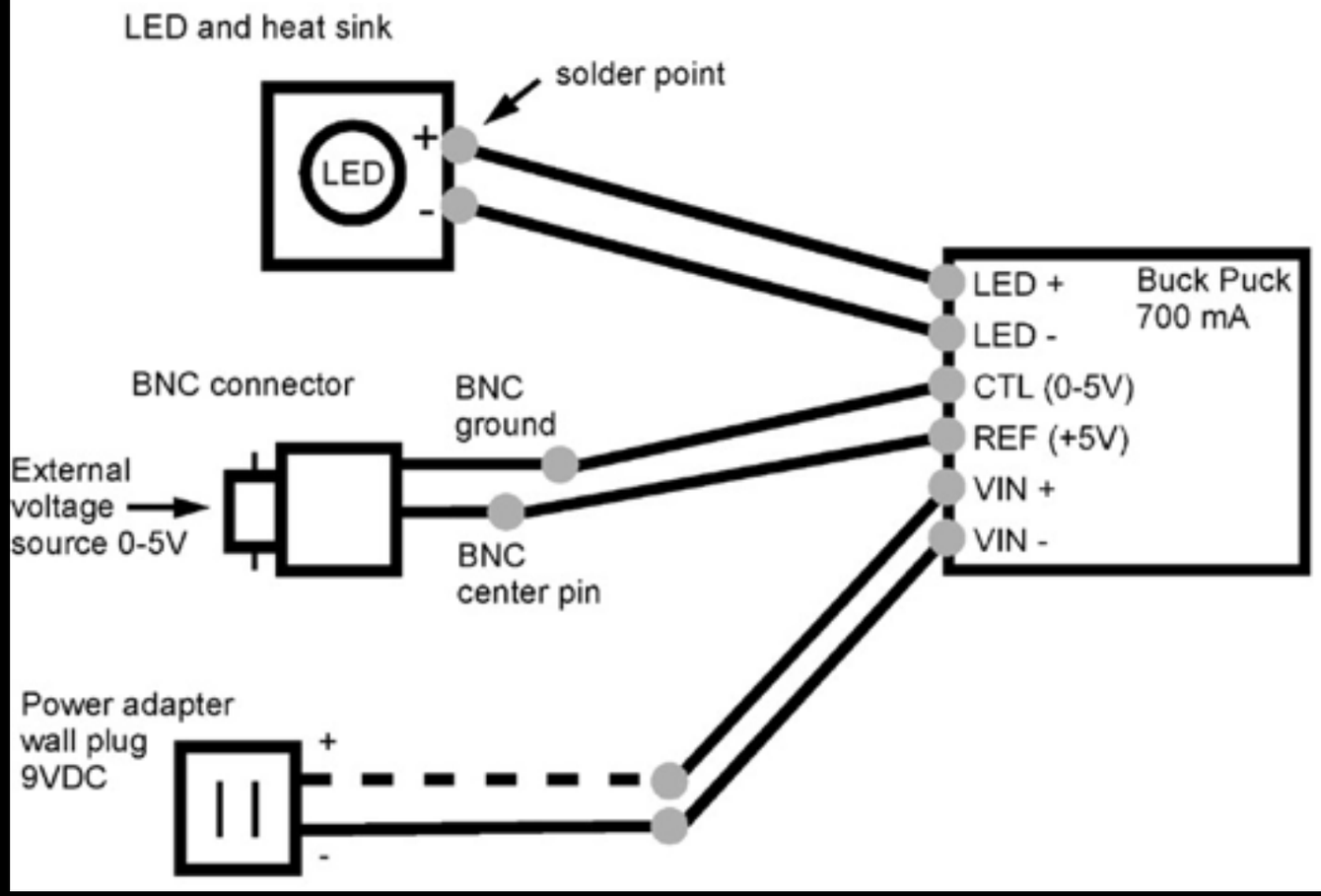
<u>Stimulus</u>
<u>Light source</u>

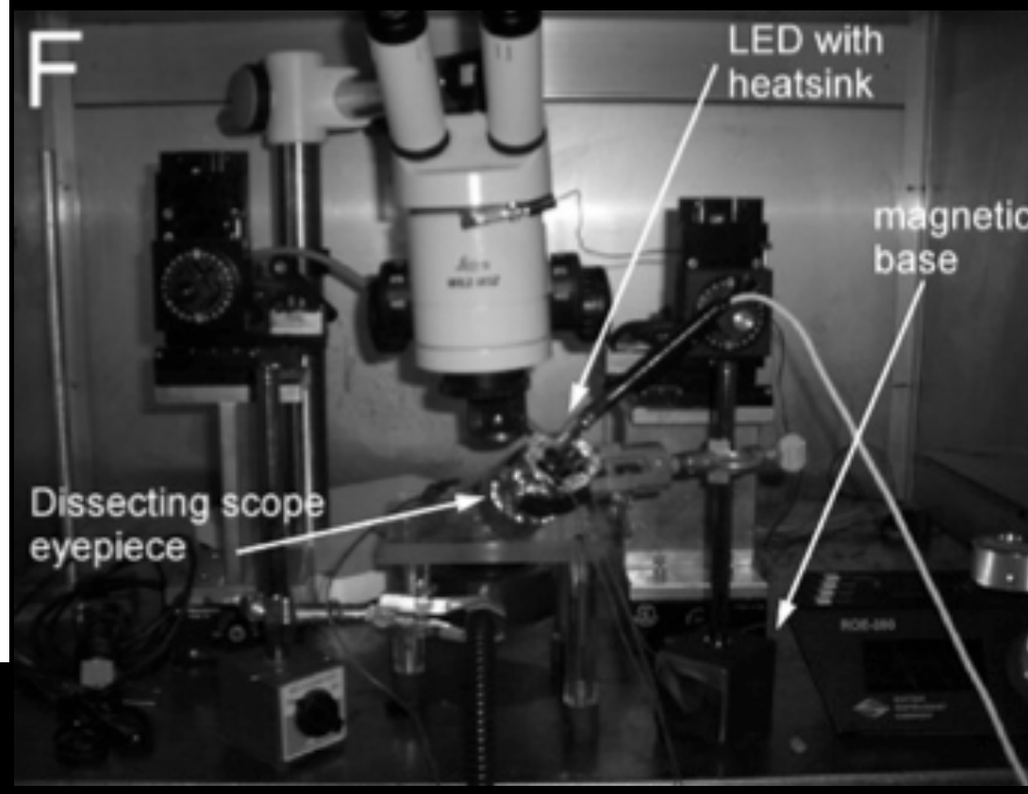
-LED
~1-10mW/mm^2
~470nm

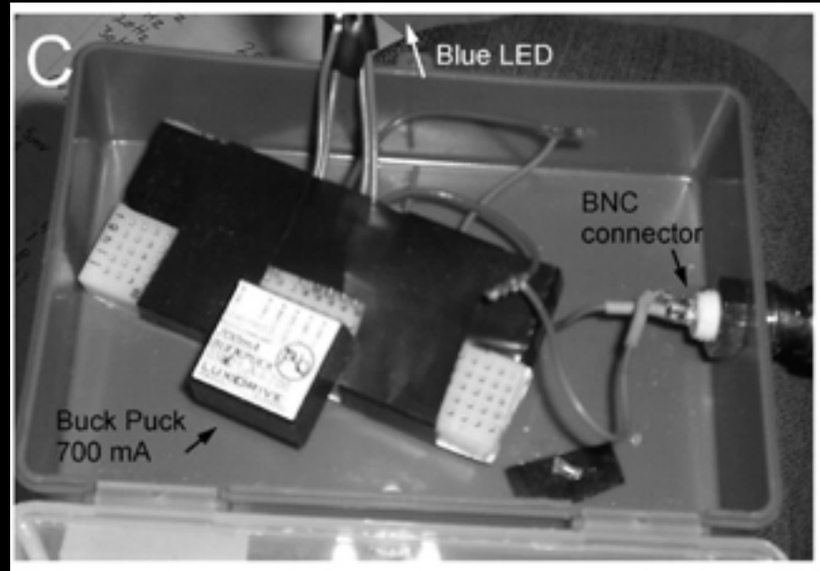
-Wide-field and laser illumination also work

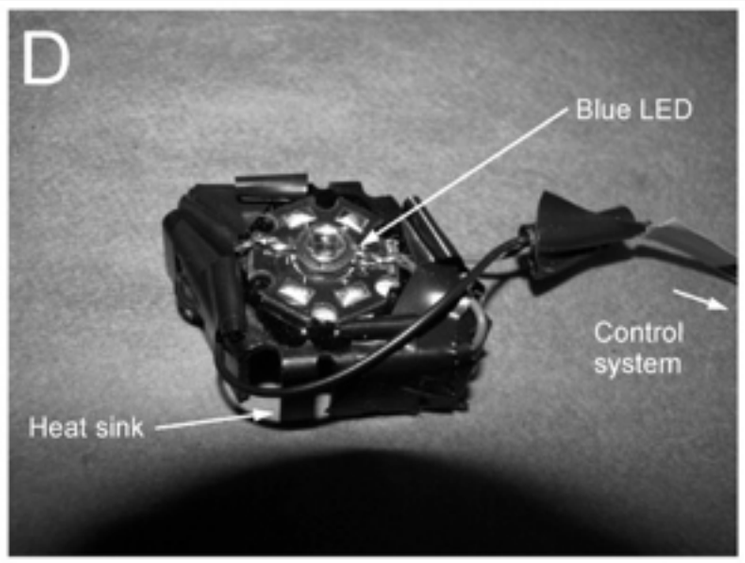


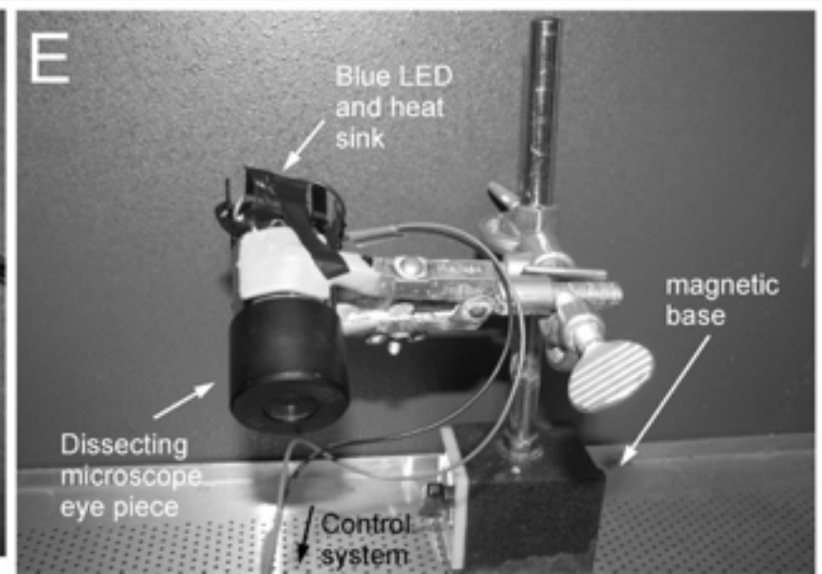
Pulver et al., 2011- Adv Phys Edu











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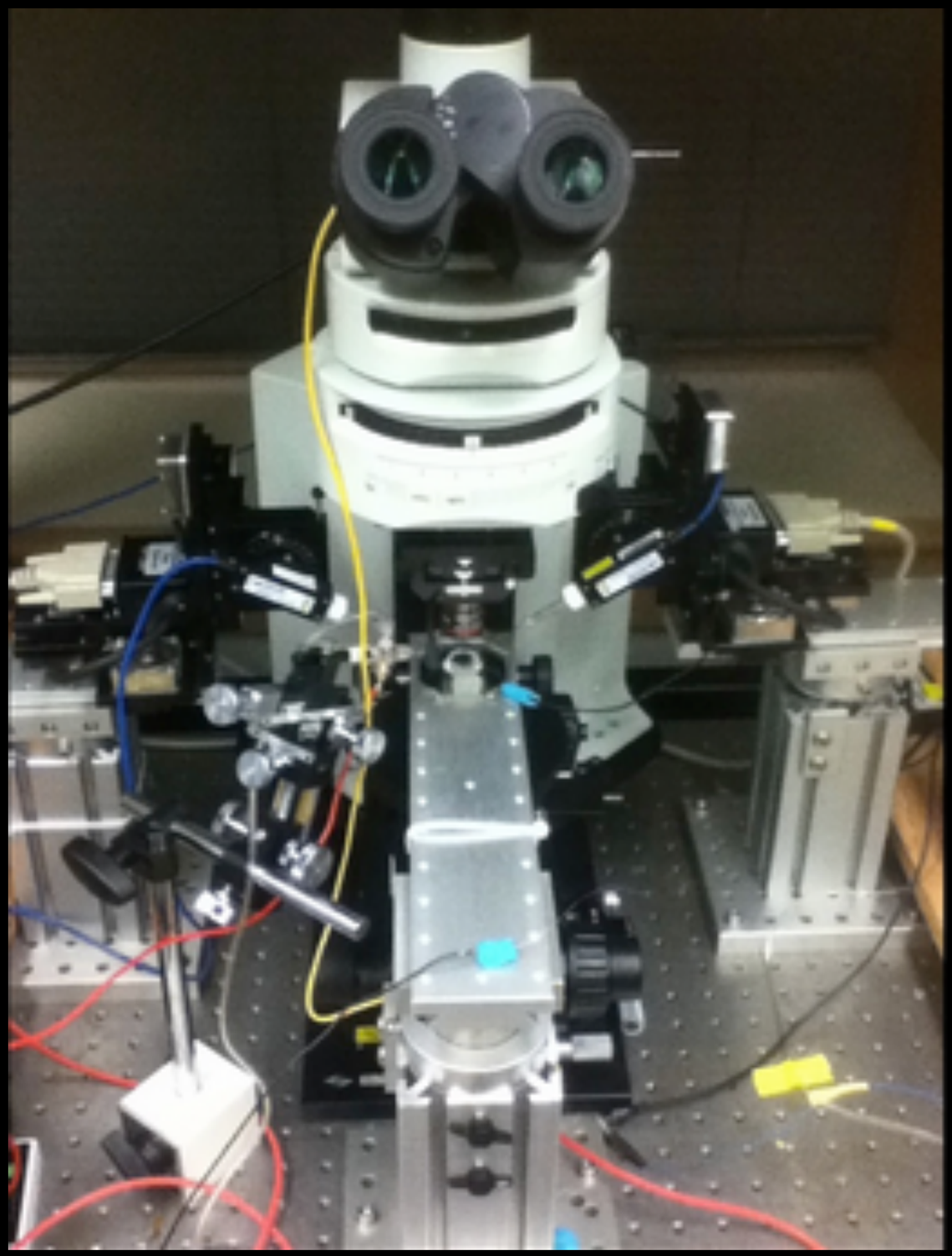
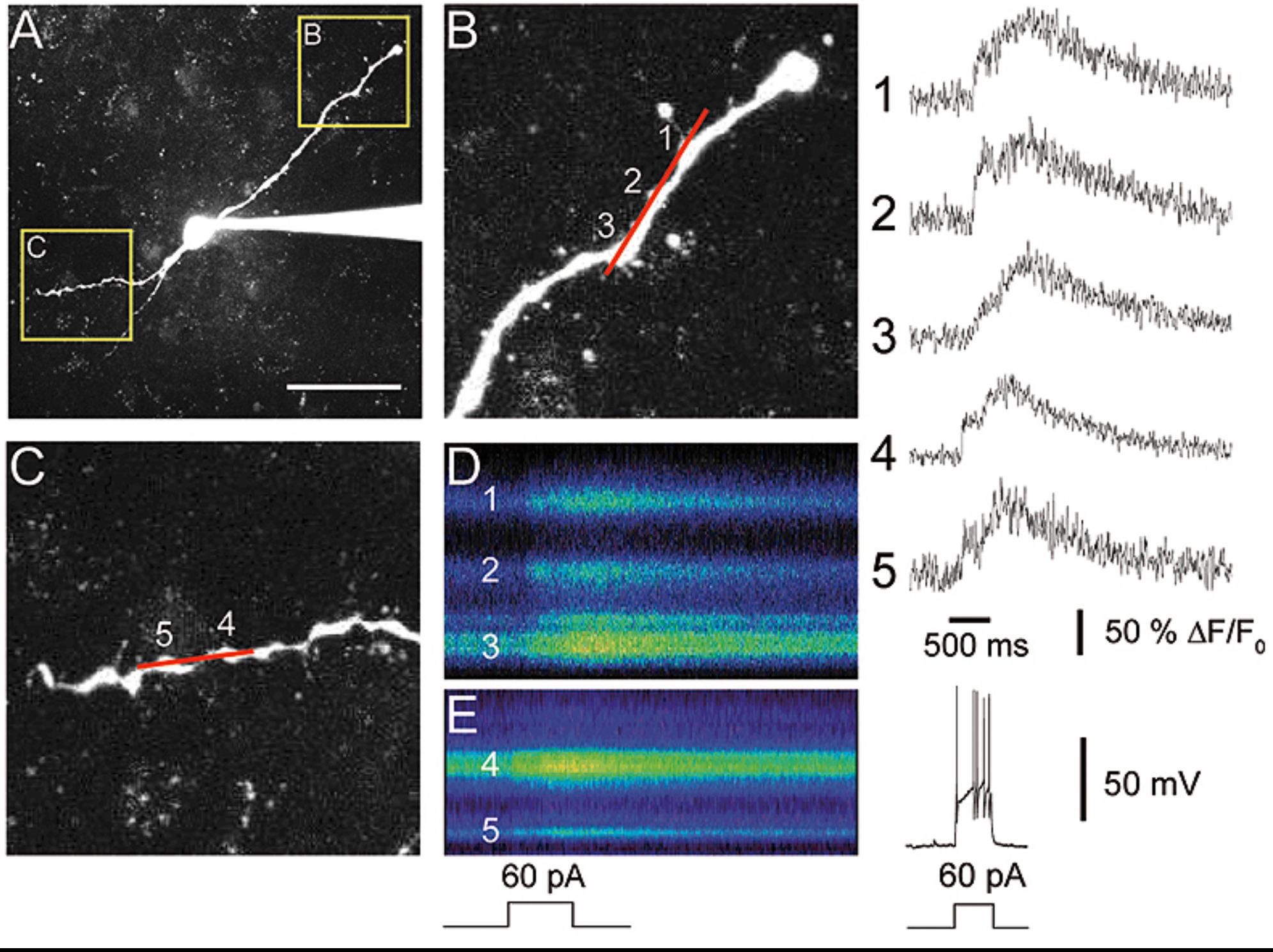


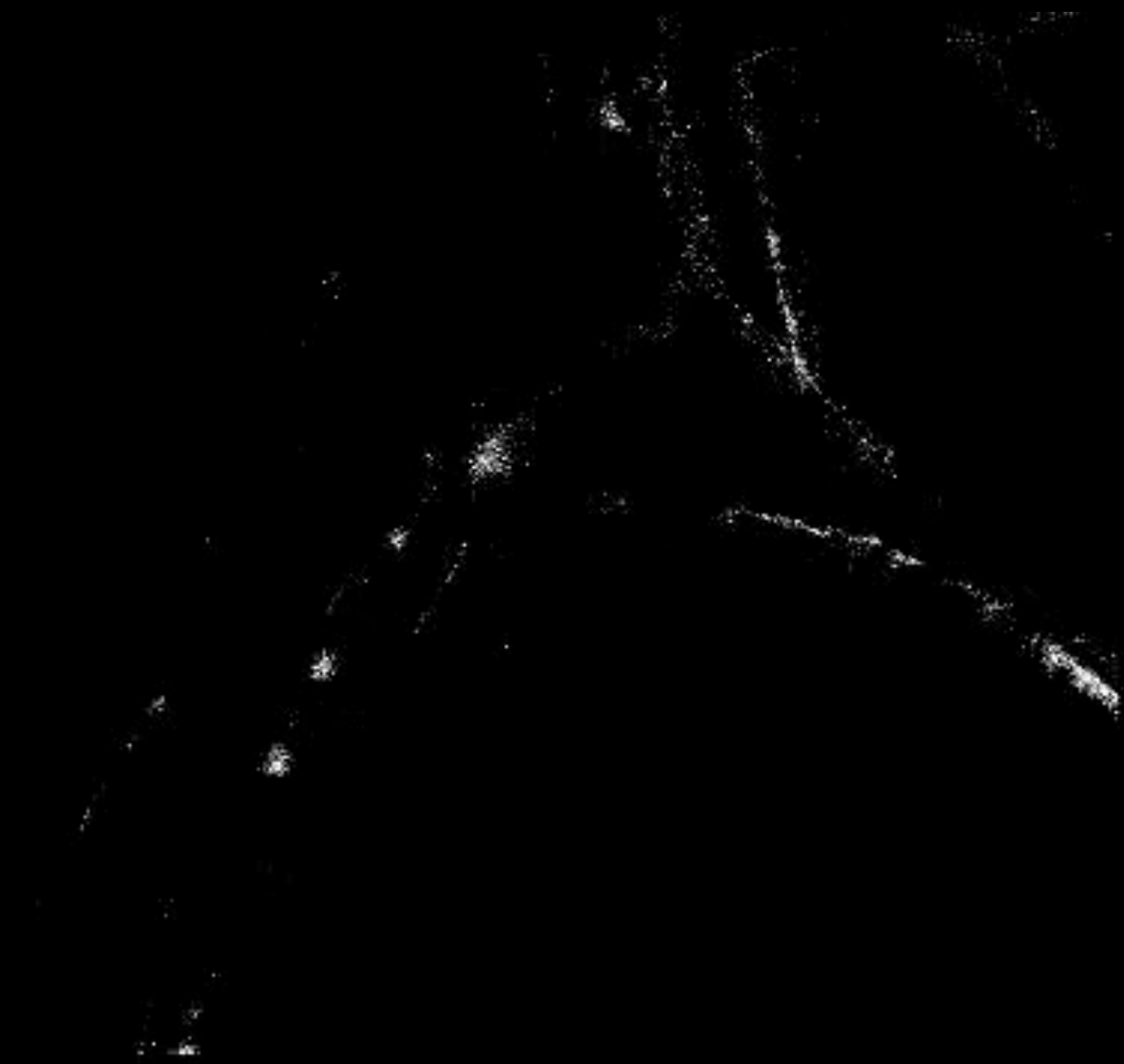
<u>Acquisition</u>
<u>Light source</u>

Confocal or wide field

Frame vs line scanning

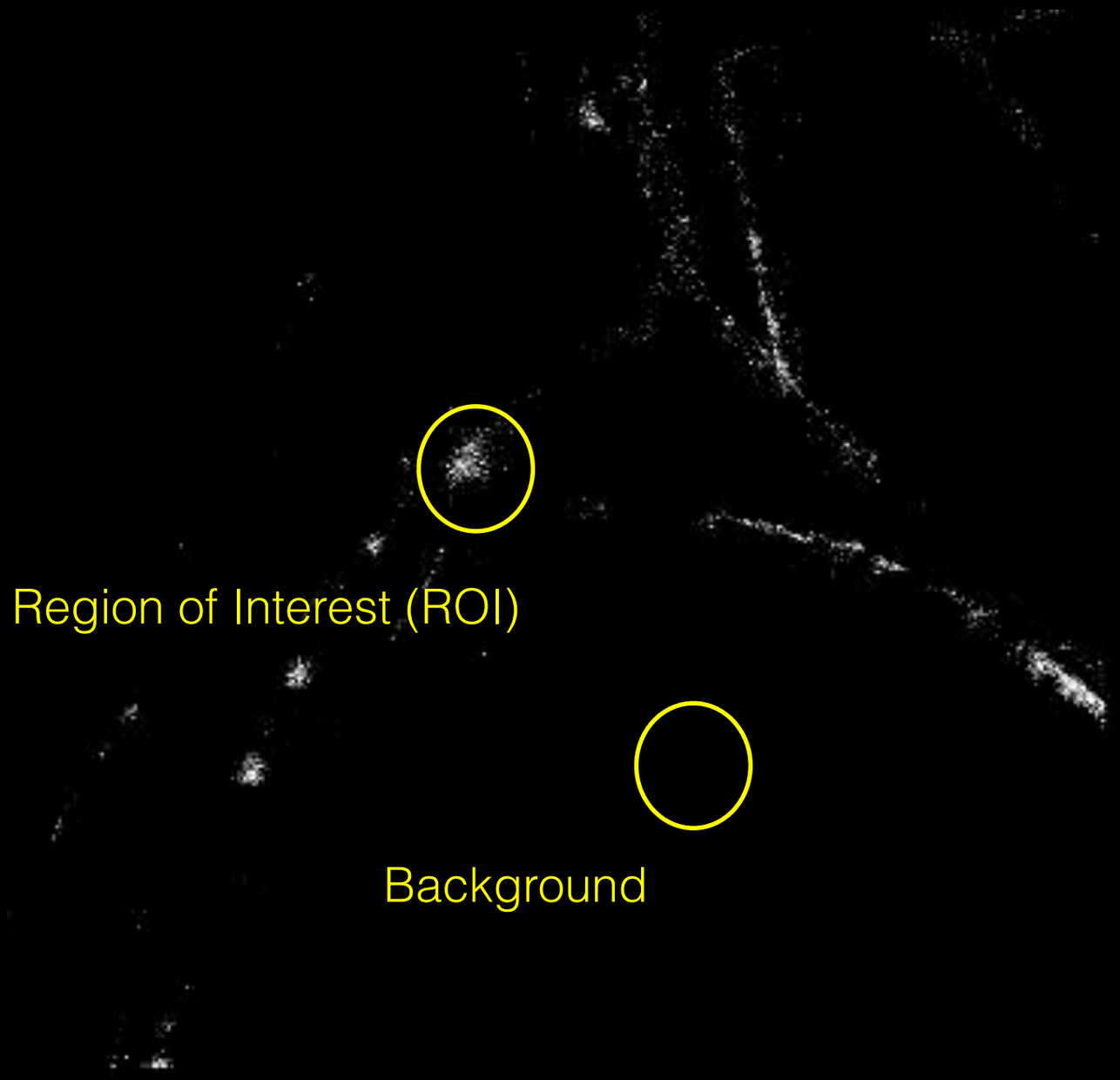


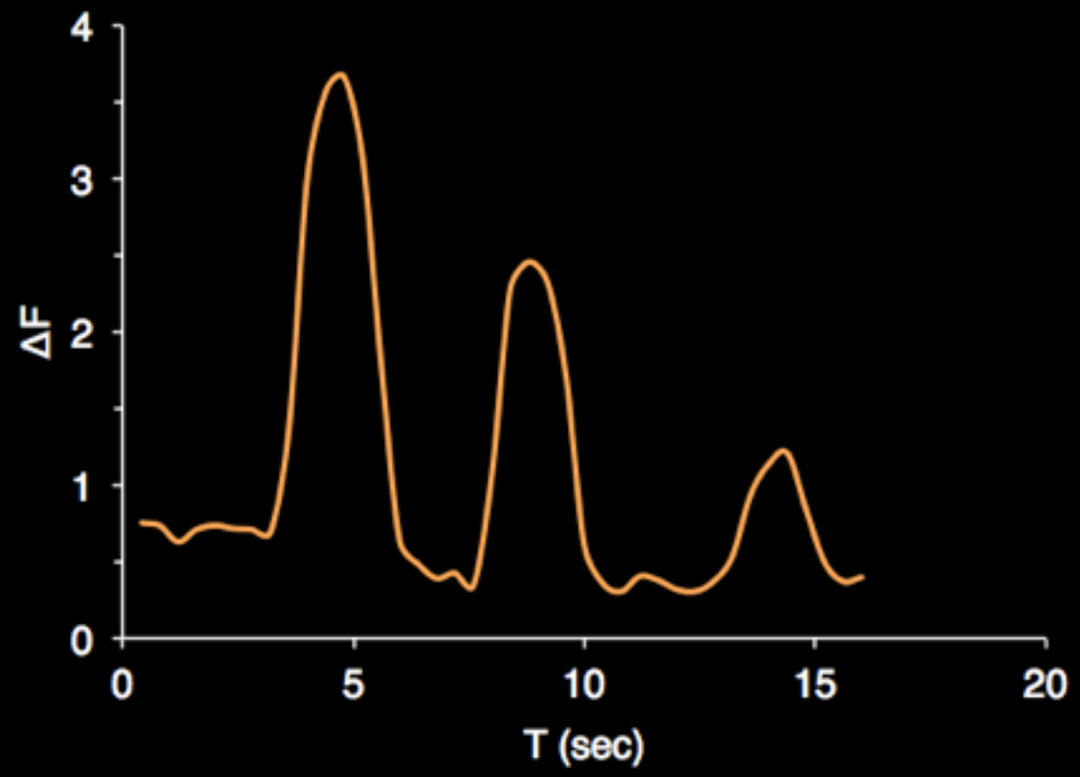
Frame vs line scanning



Confocal
40x
3 frames/sec
15 sec

Extracting the data

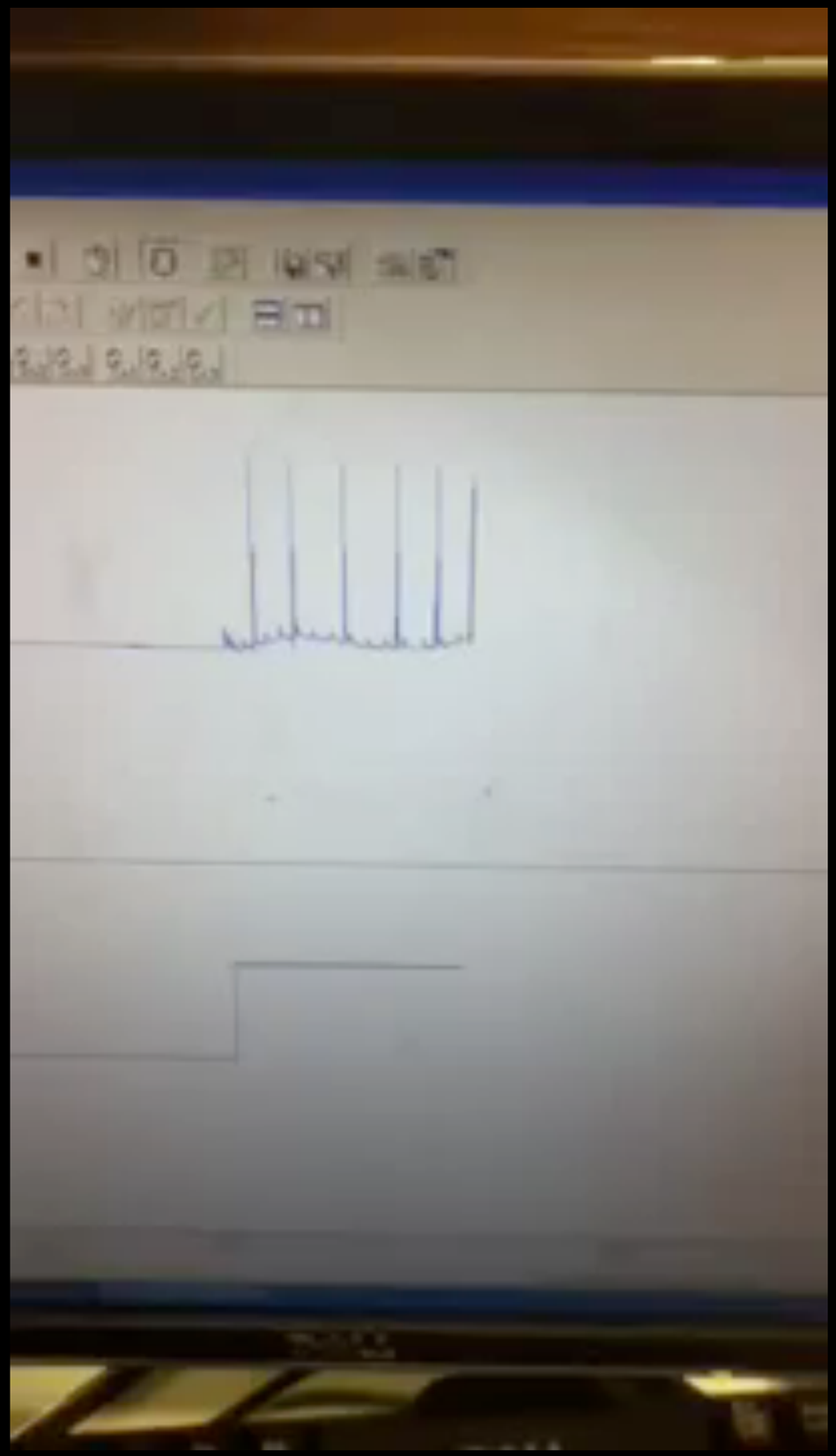


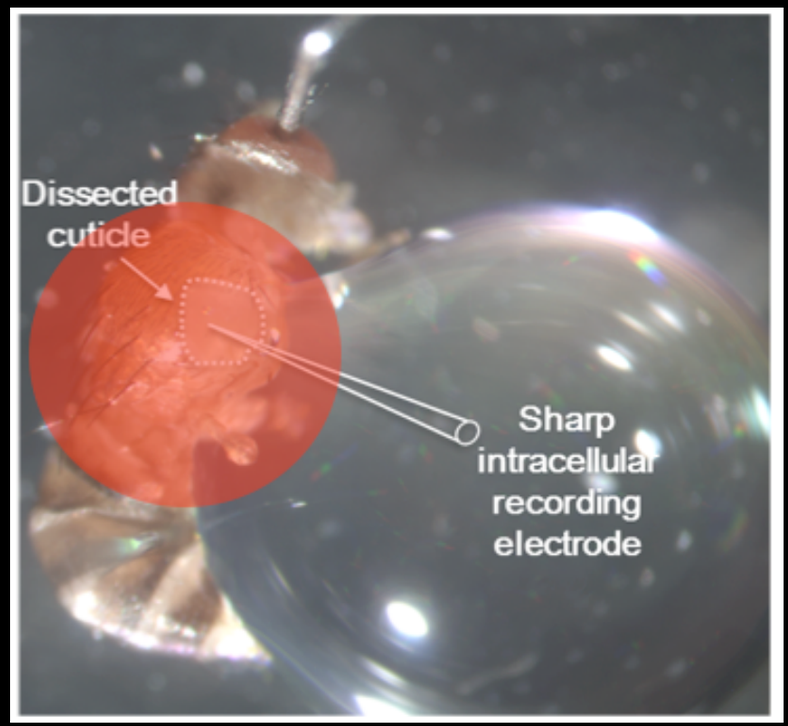


Confocal
40x
3 frames/sec
15 sec

Controls for optogenetic experiments

Control for viability





Comparison of genetically-encoded and synthetic reagents

Genetically-encoded sensors/effectors

Pros
-Increased spatial resolution

Cons
-Suboptimal optimal kinetics (Ca2+ indicators)
-Developmental defects (proteins are mis-expressed)

Synthetic sensors/effectors

Pros
-No genetics required
-No developmental effects
-Usually better kinetics/less photobleaching

Cons
-Low spatial resolution
-Toxicity

Take home messages

i. Genetics and microbiology can be exploited to provide elegant tools for manipulating/measuring biological systems

ii. Accessory specs $\geq$ microscope specs

Last slide