Imaging applications in Neurobiology

Bi177

Wan-Rong (Sandy) Wong

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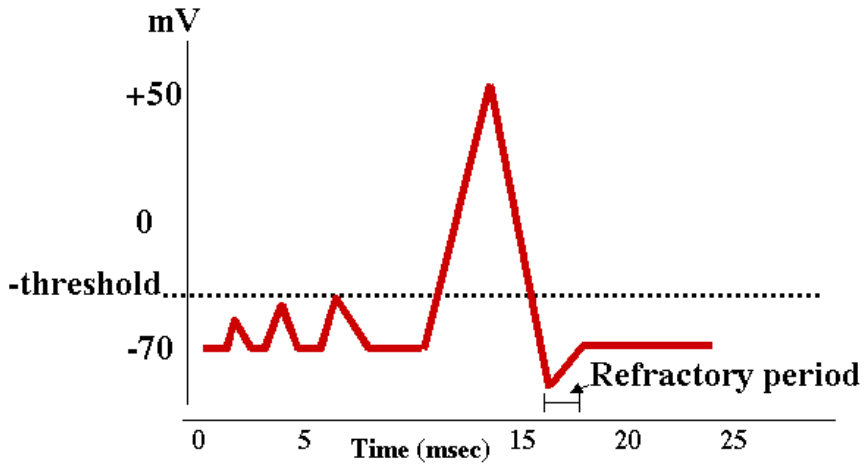
Big Questions in Neurobiology

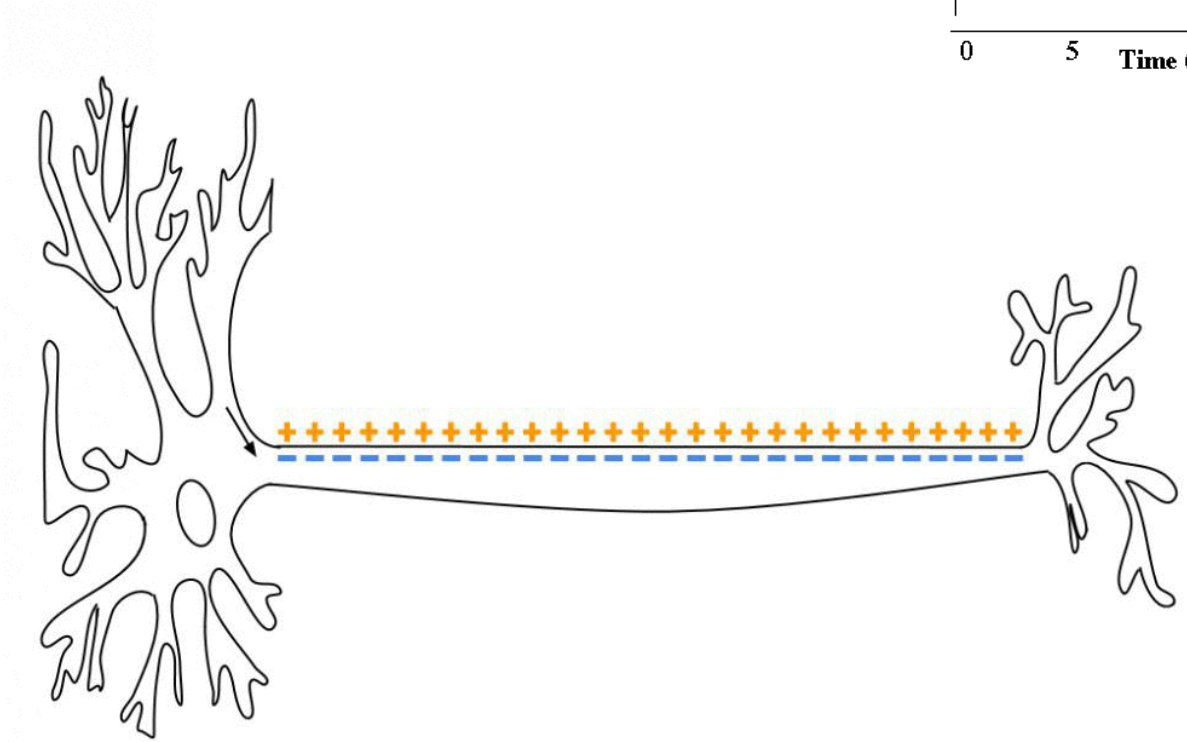
- Connectome: anatomical connection
- Functional connectome
  - differentiate neuron types
  - neural circuits of behavior
  - computational models
- Understand and treat brain disorders

Outline

- Neurobiology recaps
- Types of neuronal activity indicators
- Commonly used devices
- Example: microfluidics in *C. elegans*

Action potential (AP)



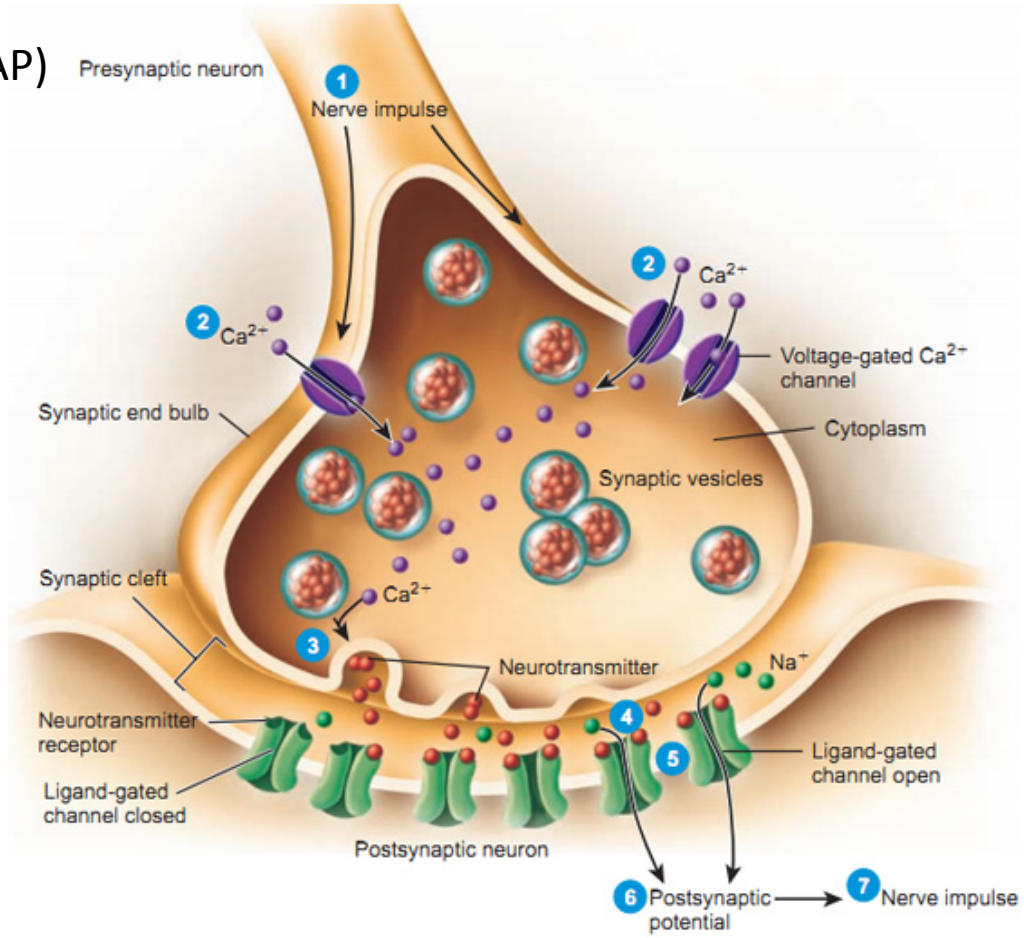


MakeAGIF.com

via Wikimedia Commons

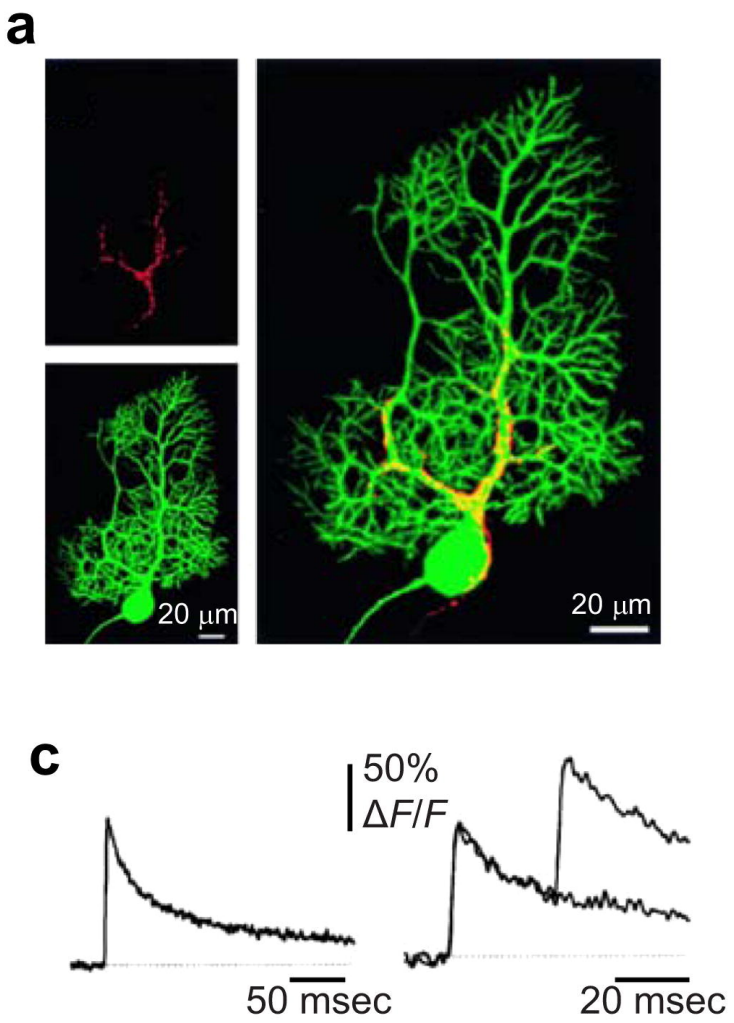
How neurons communicate

action potential (AP)



Imaging neuronal activity

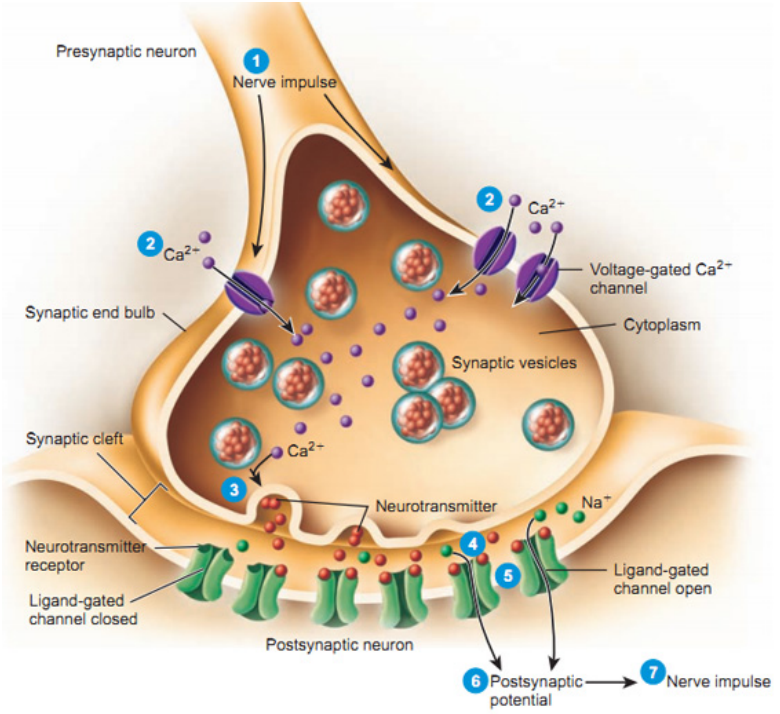
- Electrode recording
- Optical imaging



Grienberger & Konnerth, 2012. Neuron

Indicators of neuronal activity

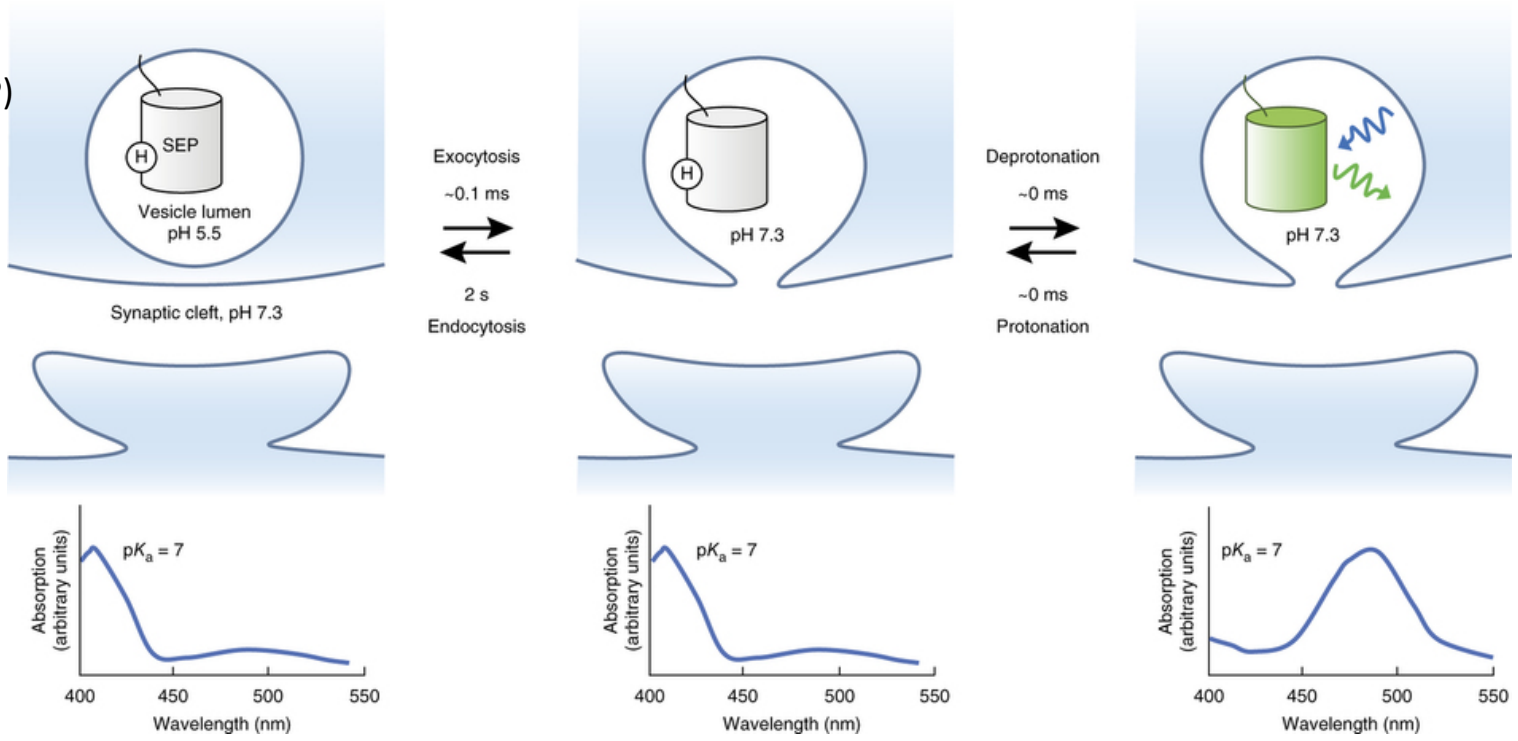
- vesicular release indicators
- neurotransmitter indicators
- voltage indicators
- calcium indicators



Vesicular release indicator

- Vesicularly localized genetically encoded indicators (GEPIs)
- Can visualize responses integrated over large numbers of synapses or APs

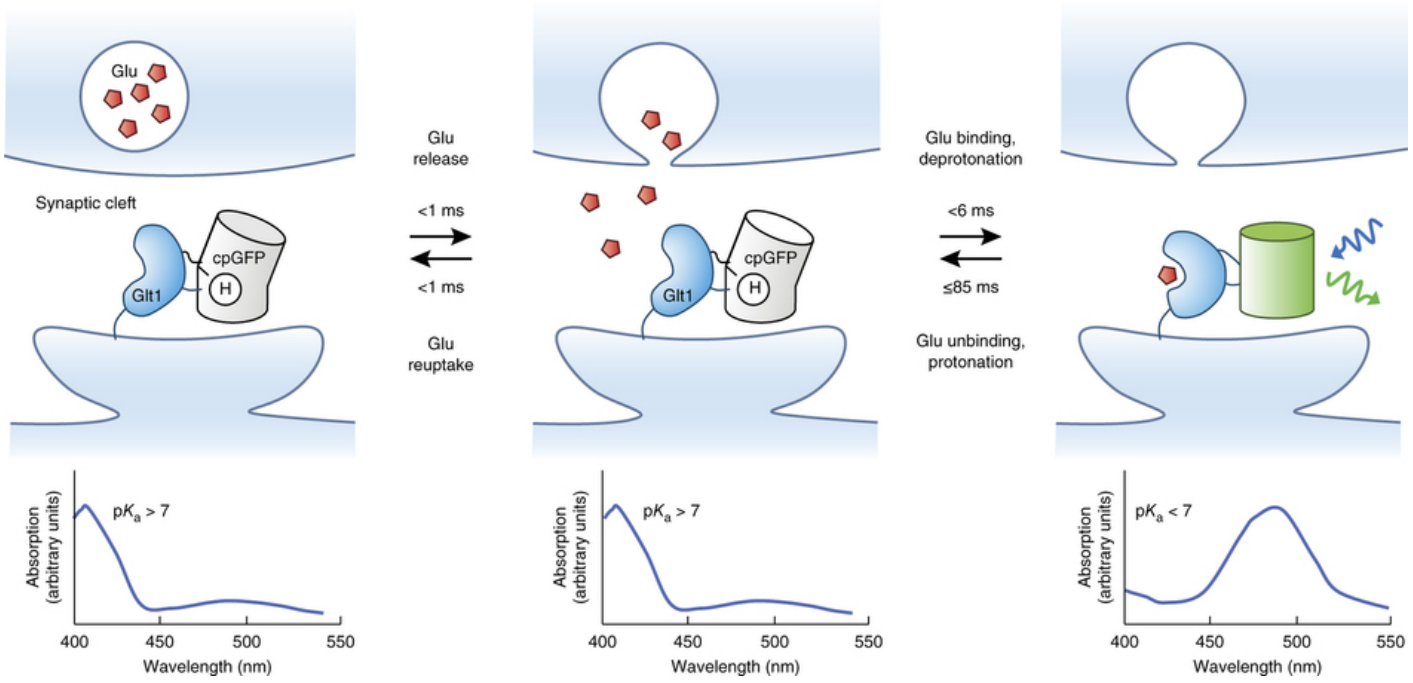
Superecliptic pHluorin (SEP)



Lin & Schnitzer, 2016. Nat Neurosci

Neurotransmitter indicators

- Neurotransmitters are released into synaptic cleft in high concentration and rapid kinetic (removed within 1 ms) manners.
- Glutamate, Acetylcholine, GABA, glycine.

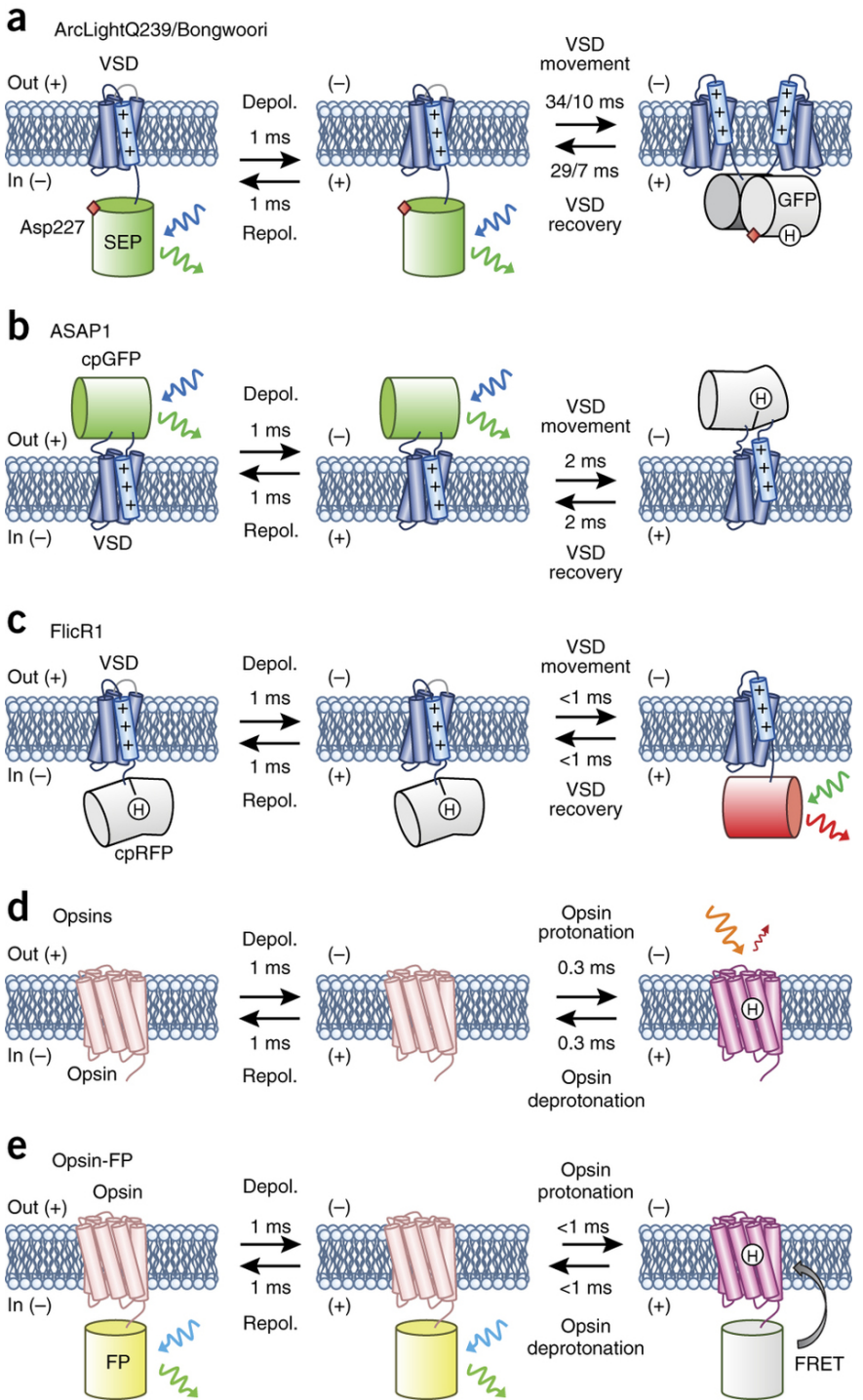


Lin & Schnitzer, 2016. Nat Neurosci

Voltage indicators

- Detect transmembrane voltage changes
  - transient depolarization
  - spike generation

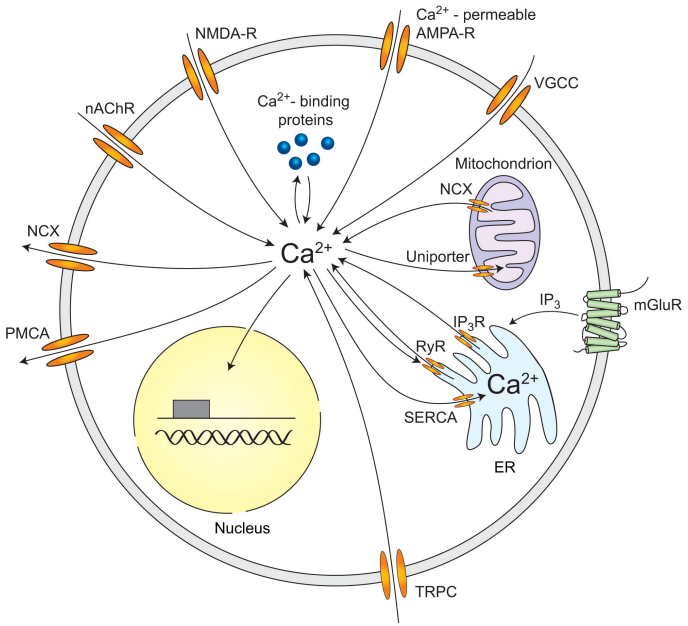
- Often highly phototoxic
  - incapable of long-term imaging of single cell
  - no genetically targeted delivery



Lin & Schnitzer, 2016. Nat Neurosci

Calcium indicators

- Capture the [Ca^{2+}] dynamic

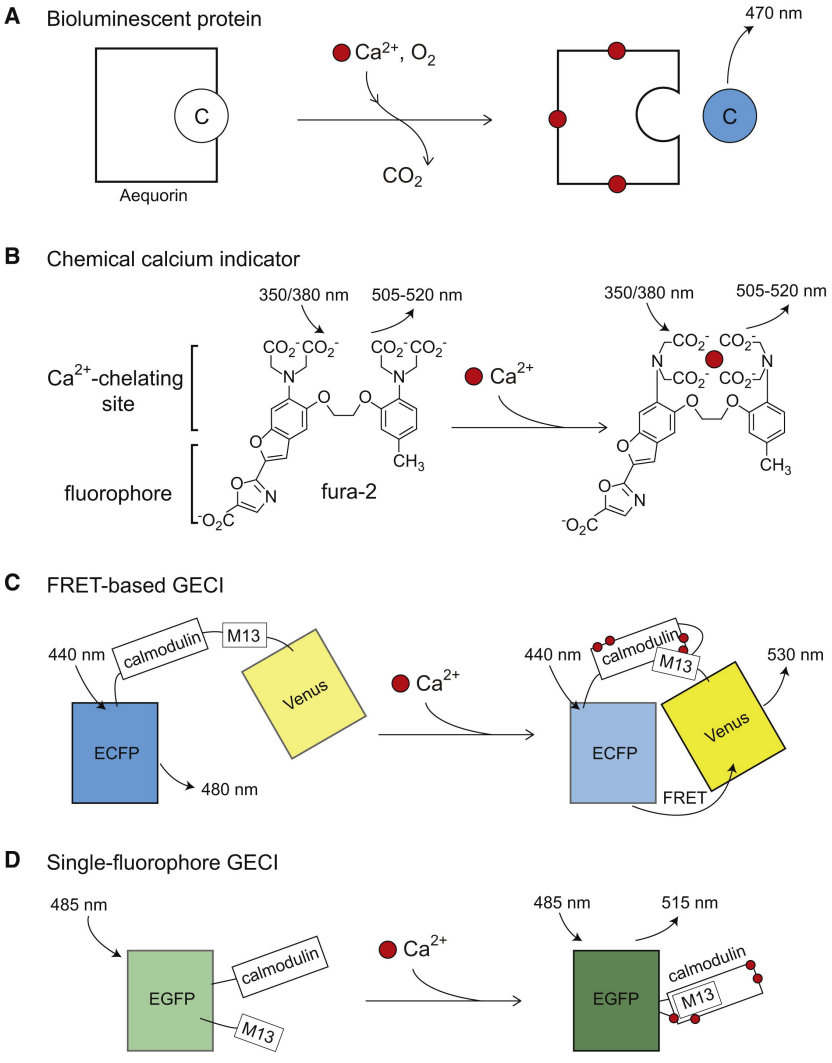


- extracellular Ca^{2+} influx
- release of internal storage
- removal of Ca^{2+}

Grienberger & Konnerth, 2012. Neuron

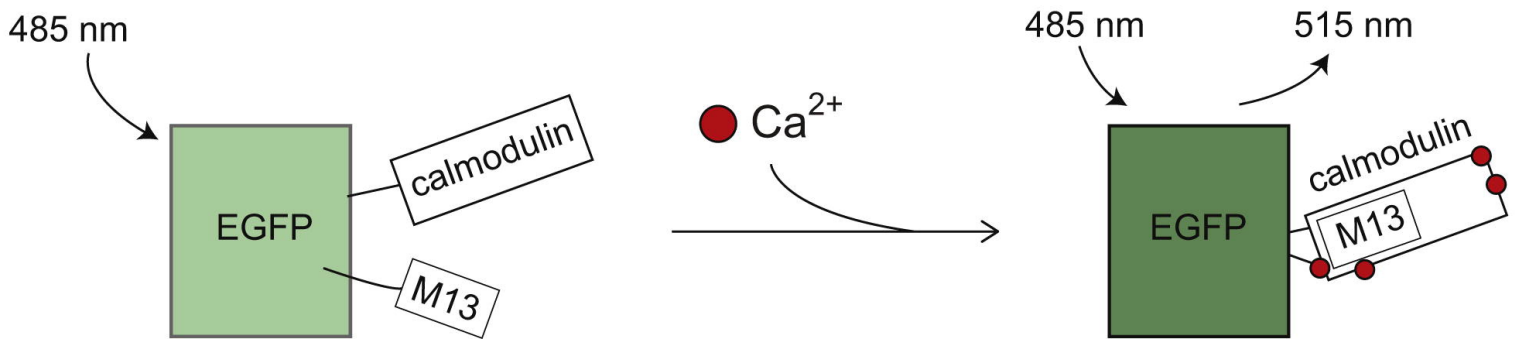
- Allow long-term time-lapse imaging
  - Sampling intervals 30-60 ms
  - single AP: 3-5 ms

Ca^{2+} indicators



Grienberger & Konnerth, 2012. Neuron

GCaMPs



Grienberger & Konnerth, 2012. Neuron

- Genetically Encoded Calcium Indicator (GECIs)
- Calmodulin (CaM) is an important post-synaptic density protein that binds Ca^{2+}
- Ca^{2+}binds to CaM and causes a conformational change that causes GFP fluoresence

GECIs

GECI	Maximum $\Delta F/F$ *in vitro*[a]	Ca^{2+}-free brightness (mM^{-1} cm^{-1})[b]	Ca^{2+}-saturated brightness (mM^{-1} cm^{-1})[b]	K_d *in vitro* (nM)[c]	$\Delta F/F$ per AP in tissue[d]	Half-decay rate in tissue (ms)[e]	Refs.
YC3.60	–0.66 (ECFP)	8.8[f]	3.1	780	–0.01	410	137,138
	+0.77 (cpVenus)	2.4[f]	11		+0.02		
YC3.60 3GS	–0.66 (ECFP)	8.8[g]	3.1	140	–0.01	470	139,140
	+0.77 (cpVenus)	2.4[g]	11		+0.01		
D3cpV	–0.46 (ECFP)	7.3[h]	3.6	530	–0.03	9,500	141,142
	+1.1 (cpVenus)	4.8[h]	10		+0.02		
TN-XXL	–0.5 (ECFP)	9.6[i]	5.4	800	–0.01	1,600	142,143
	+1.0 (cpCitrine)	1.5[i]	10		+0.02		
Twitch-2B	–0.77 (mCerulean3)	22[j]	5.8	200	–0.12	2,100	82,142
	+0.87 (cpVenus)	0.83[j]	12		+0.12		
GCaMP3	+12	1.8	23	540	+0.14	650	114,142
GCaMP5k	+9.4	ND	ND	190	+0.04	270	72,144
GCaMP6f	+52	0.70	37	380	+0.22	140	72,91
GCaMP6s	+63	0.66	42	140	+0.25	550	72
R-CaMP2	+4.8	2.3 (1.6)[k]	11	69	+0.60	150	106
jRGECO1a	+11	1.0 (0.74)[k]	12	150	+0.19	200	7
jRCaMP1b	+6.2	4.0 (4.0)[k]	29	712	ND[l]	ND	7

Lin & Schnitzer, 2016. Nat Neurosci

Advantages of optical imaging

- Less biased than electrodes
  - unfavorable cell morphology, weak electrical dipoles, extracellular tissues
- Reveal spatiotemporal activity pattern
  - dendritic integration, voltage propagation, dendritic spiking
- Minimizing neuronal damages
- Genetically encoded

Disadvantages of optical imaging

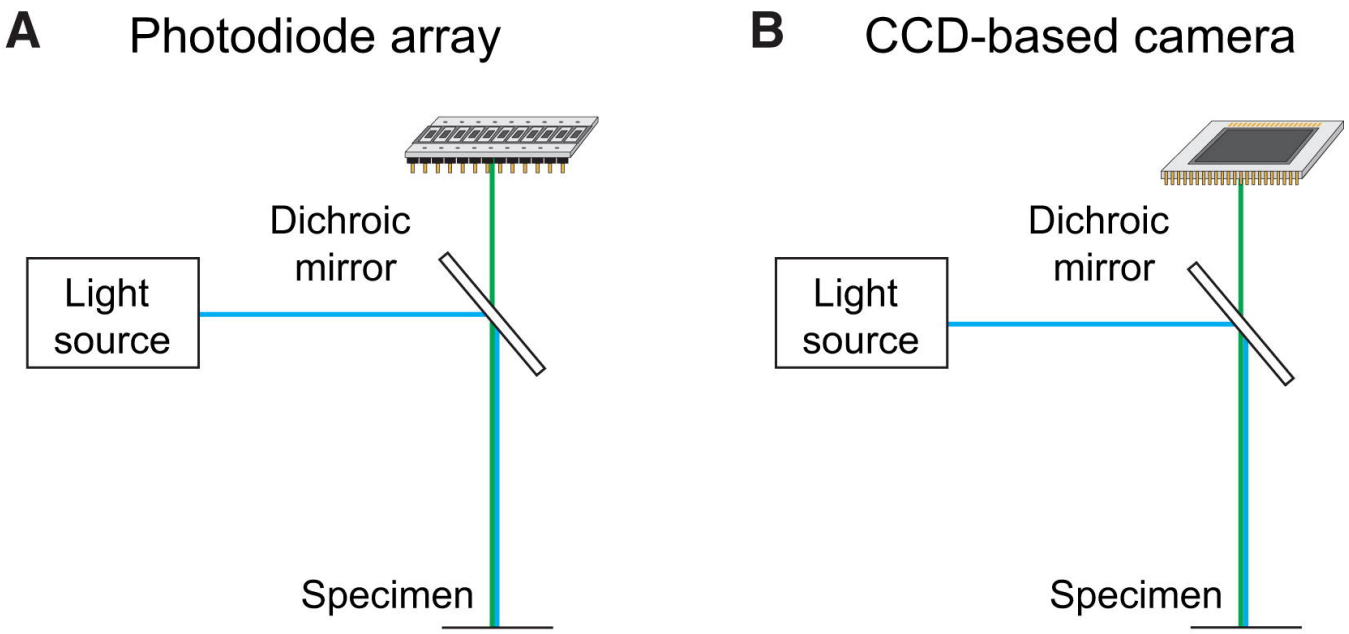
- Transduction efficiency and toxicity
- Limited by the inherent quantum mechanical randomness of photon emission and detection
- Influenced by indicator's fluorescence response curve and response kinetics
- Good representation of neuronal activity?

Outline

- Neurobiology recaps
- Types of neuronal activity indicators
- Commonly used devices
- Example: microfluidics in *C. elegans*

Common imaging devices

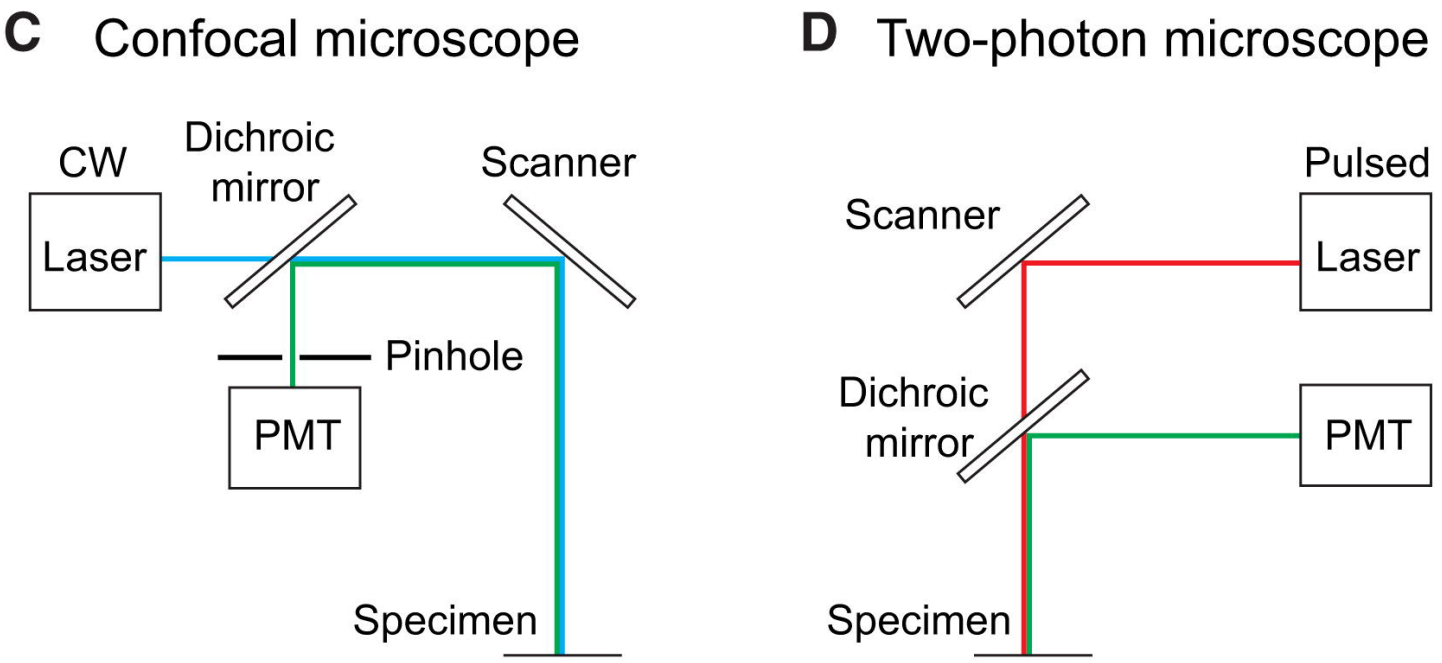
- Wide-field microscopy



Grienberger & Konnerth, 2012. Neuron

Common imaging devices

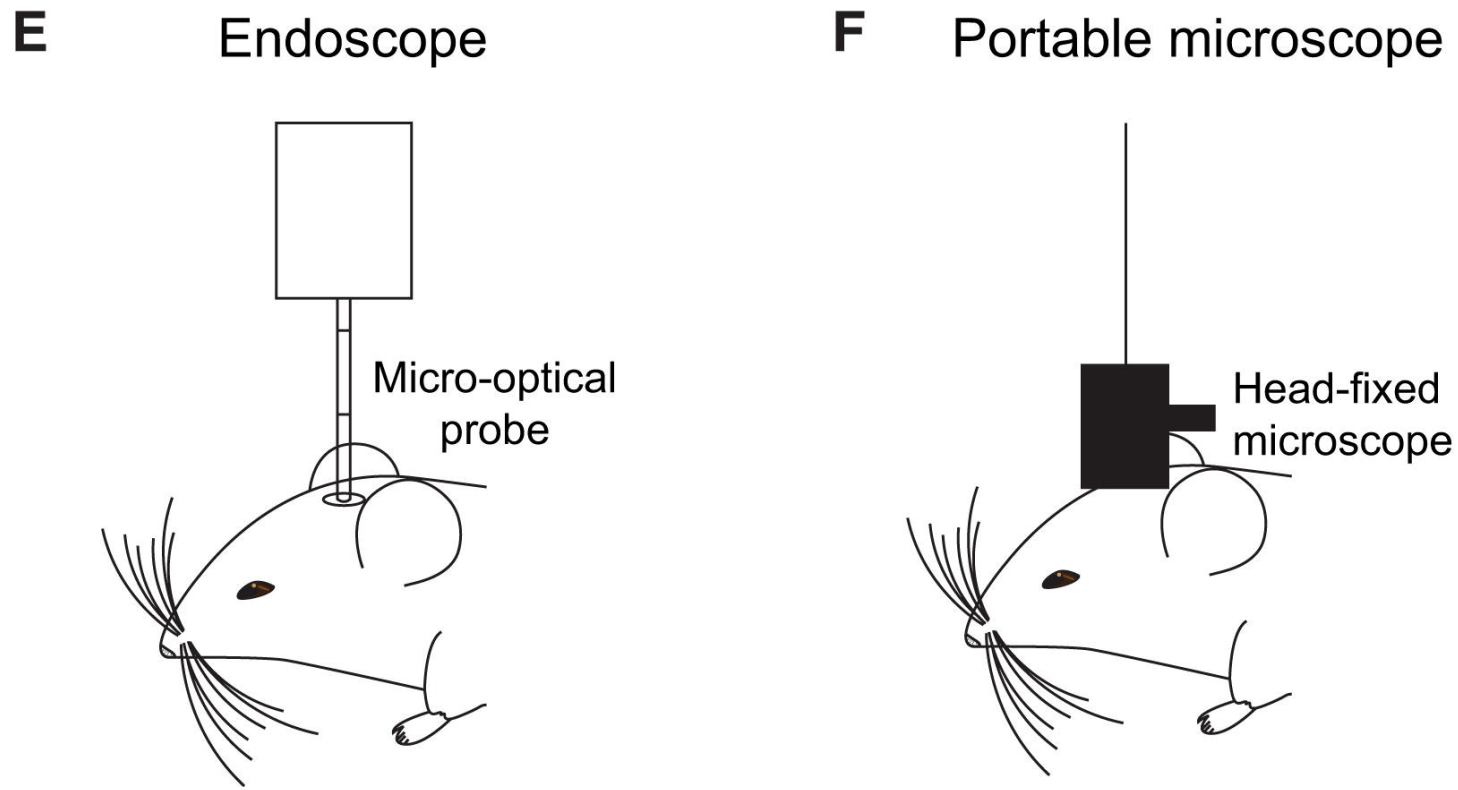
- Laser scanning microscopy



Grienberger & Konnerth, 2012. Neuron

Common imaging devices

- Recording in freely moving animals



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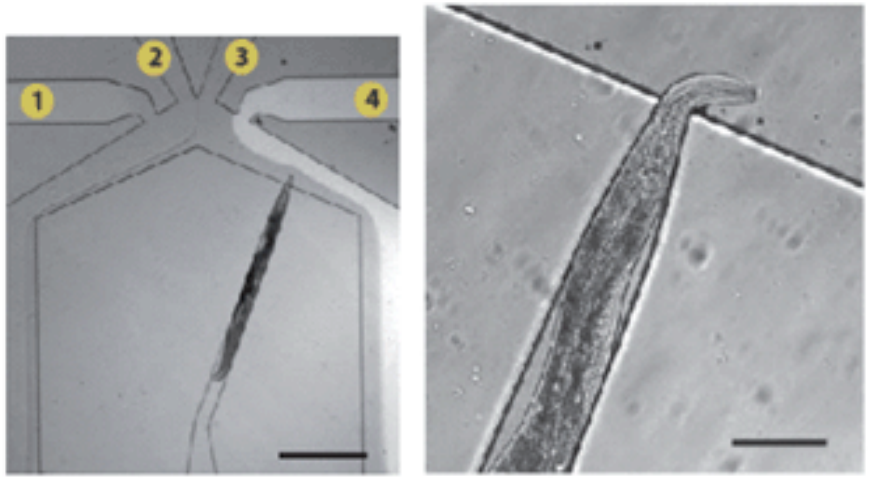
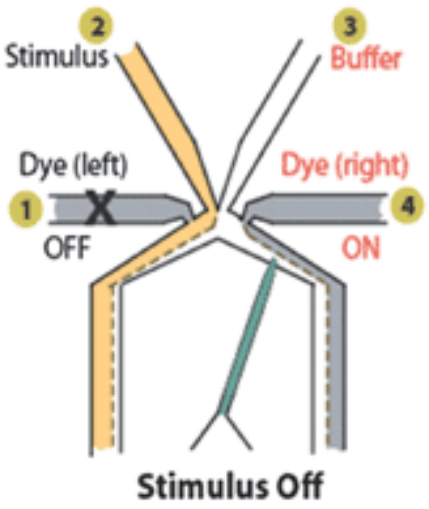
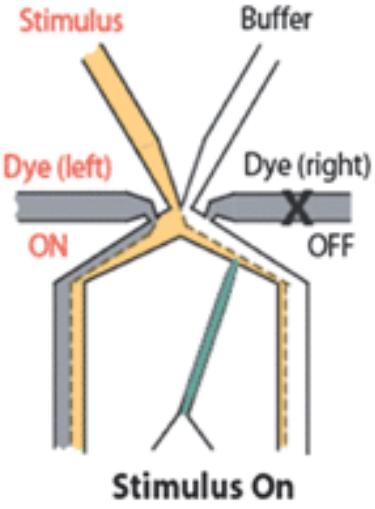
Microfluidics in *C. elegans*

0.000 sec



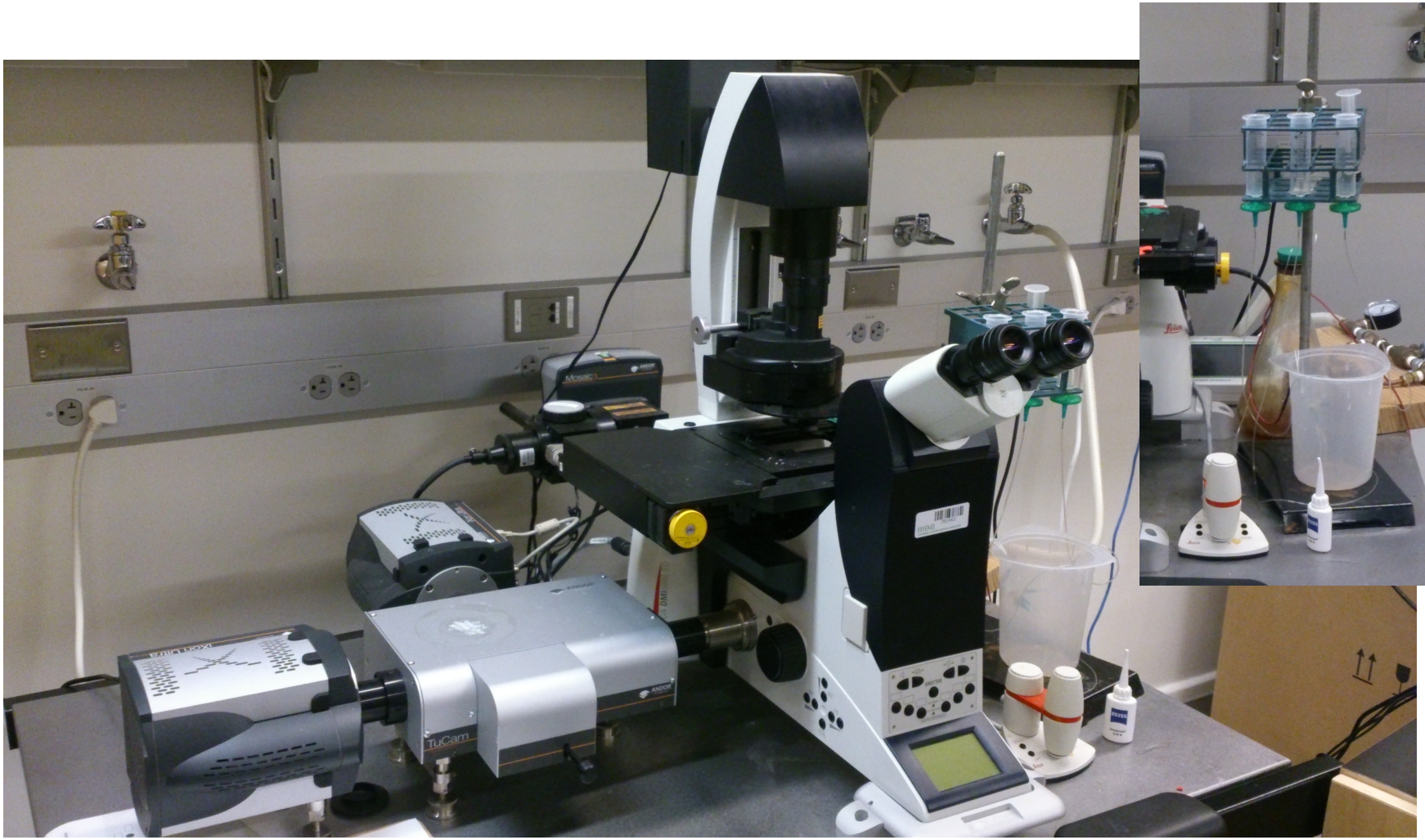




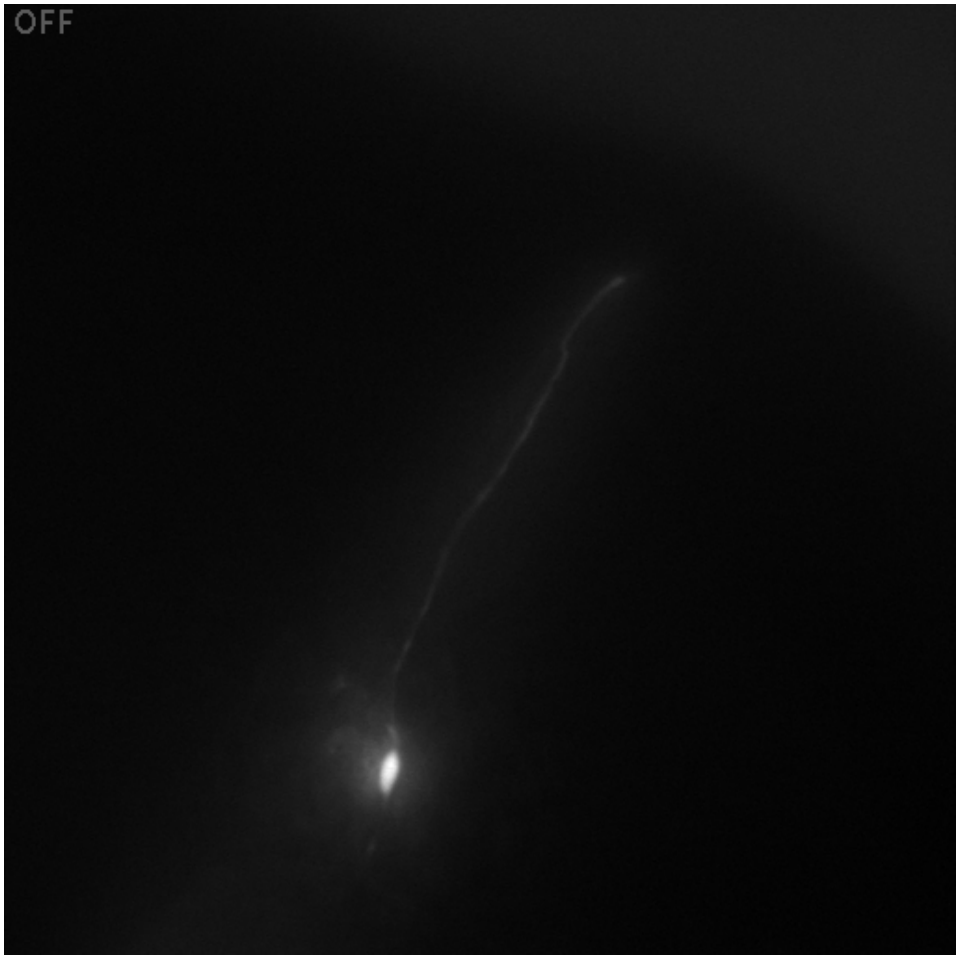
aversive avoidance
response to copper

Chronis, 2009

Ca^{2+} imaging device



ASH Video



Future approaches to technical challenges

- Protein engineering:

Voltage-sensors, calcium indicators, and other indicators of neuron activity with fast kinetics and large changes in fluorescence

- Optics:

- LEDs with specific wavelength and constant illumination
- Filters/dichorics for unwanted wavelengths
- Multimirror arrays for targeted illumination
- Cameras that can capture images quickly
- Big data processing

Questions?


The accommodations here are awful, I'd like to check out early.
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