GCaMP calcium indicators

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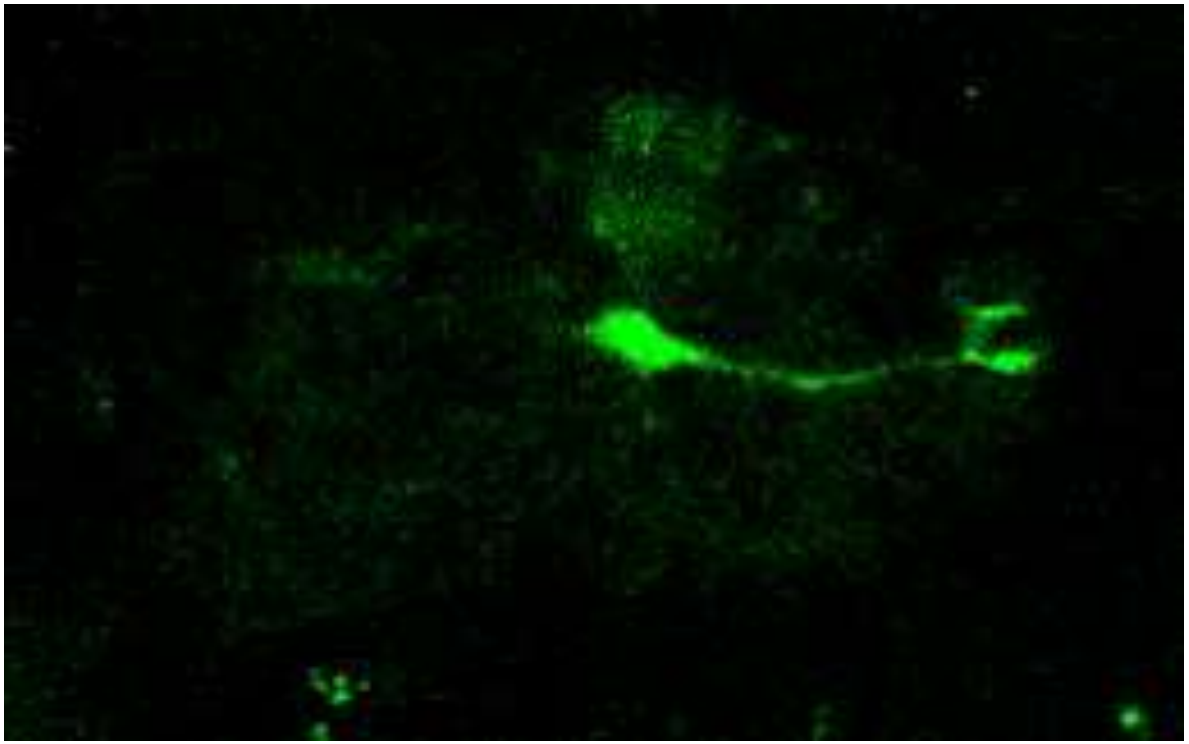
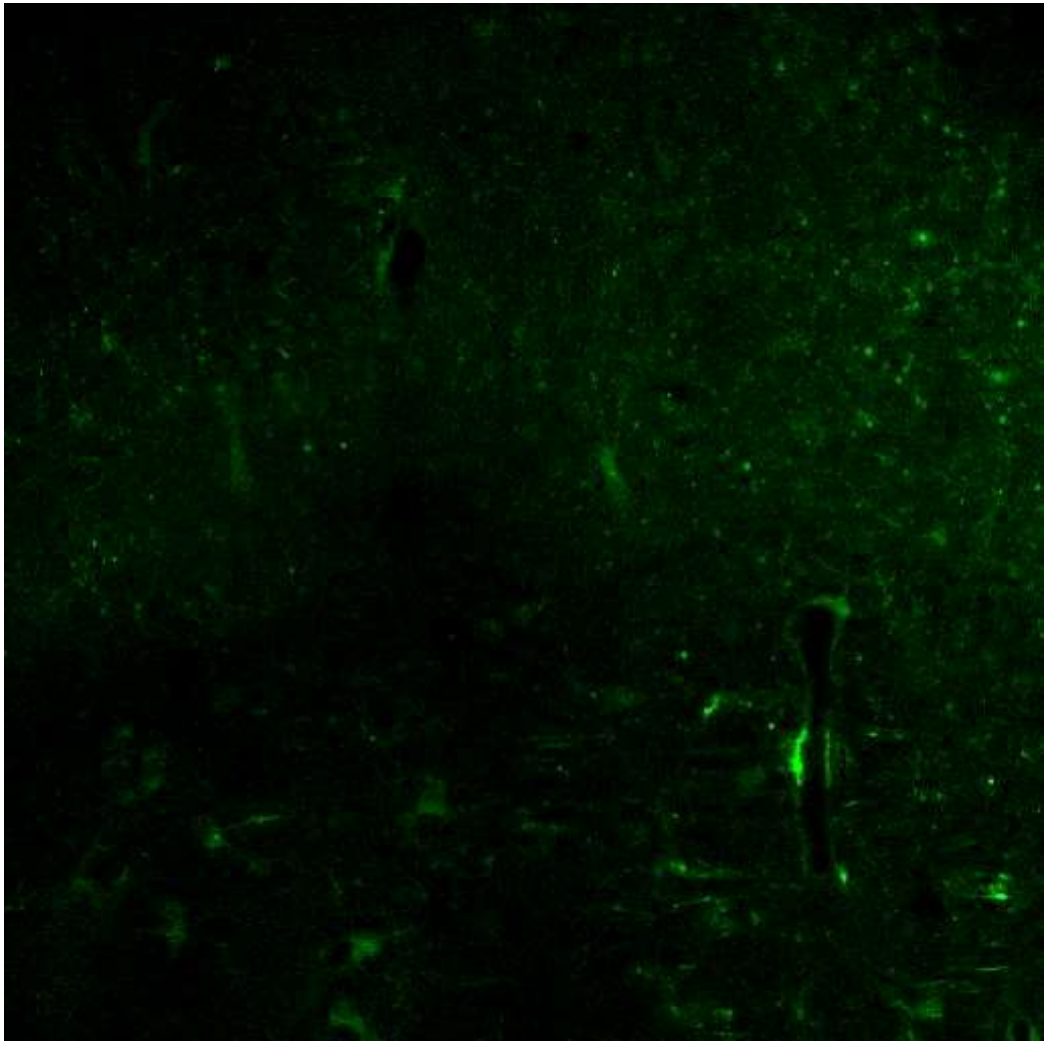
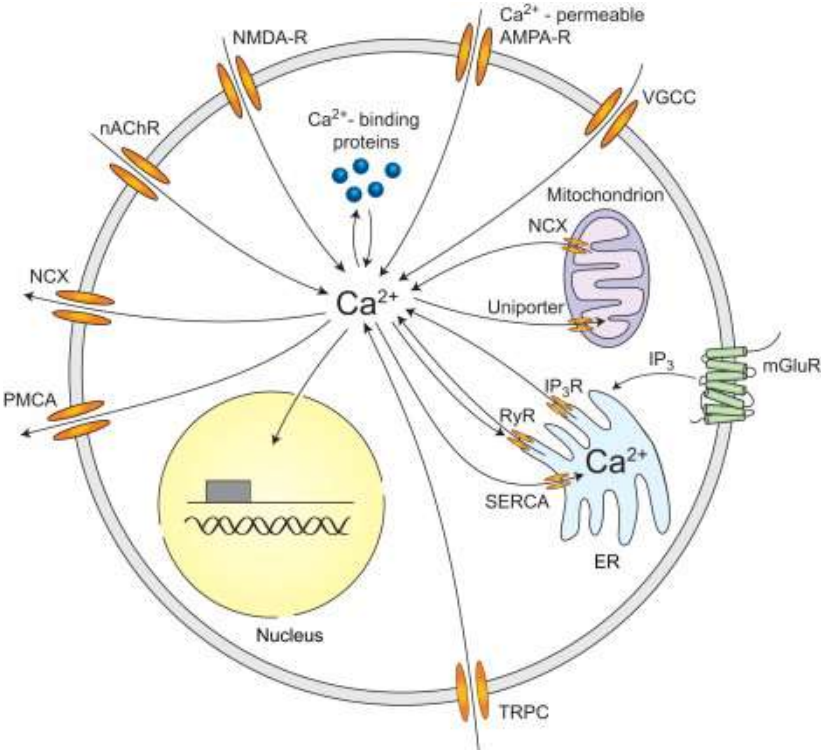
17-Feb-2017



Calcium signaling is important and complex





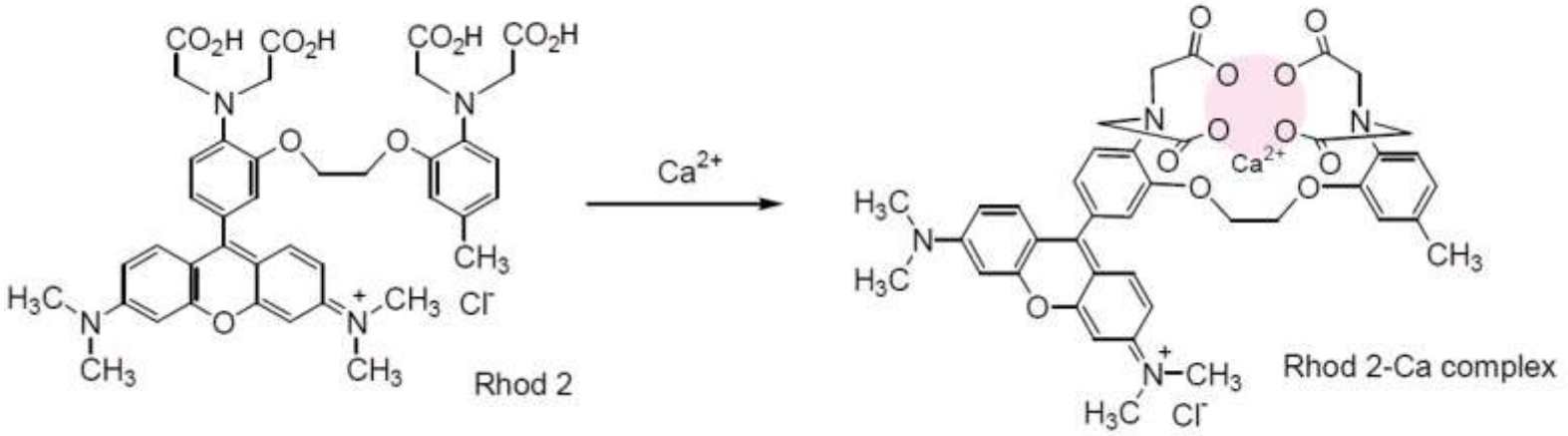
Grienberger and Konnerth *Neuron* 2012

The wish list

- Large dynamic range (F_{max}/F_{min})
- High signal to noise ratio – important if imaging in small cellular compartments or where [indicator] is low
- Affinity (K_d) of the indicator for Ca^{2+} should be appropriate for the Ca^{2+} levels to be measured
- Fast response kinetics to preserve accurate representation of Ca^{2+} transients
- High selectivity for Ca^{2+} over other ions
- Can be targeted to specific cell types or compartments
- Resistant to photobleaching (for long term imaging)

Strategies for imaging calcium

- Synthetic calcium indicators consisting of a calcium chelator + fluorophore
- Load into cells by microinjection, electroporation, or bulk loading

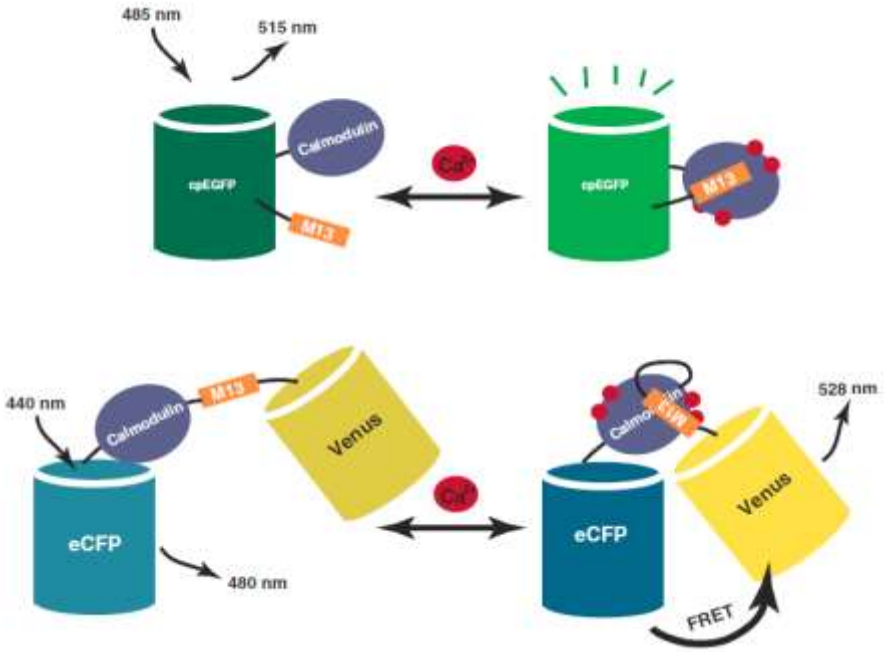


Dojindo Molecular Technologies

- Drawbacks:
  - Cannot be easily targeted to specific cell types or subcellular locations
  - Loading may damage tissue
  - Dyes can leak or be pumped out of cells over time

Use of Genetically Encoded Calcium Indicators (GECIs)

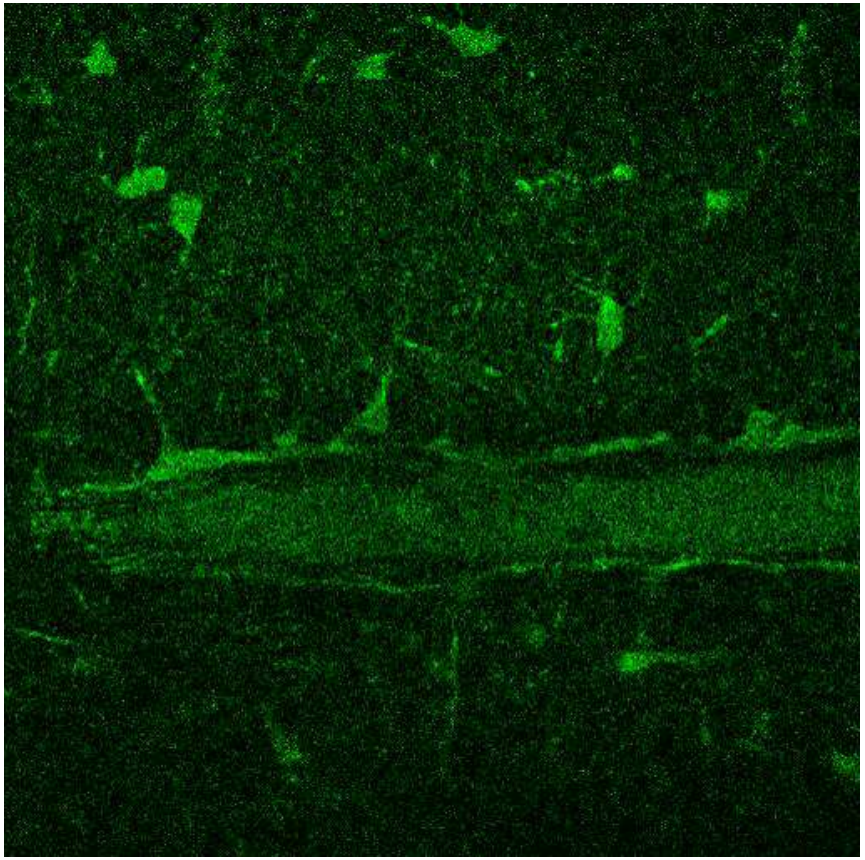
- Ca^{2+}-binding domain + fluorescent protein(s)
- Can be targeted to specific cell populations or subcellular compartments using specific promoters
- Proteins can be imaged repeatedly
- Transgene can be delivered by viruses, in utero electroporation



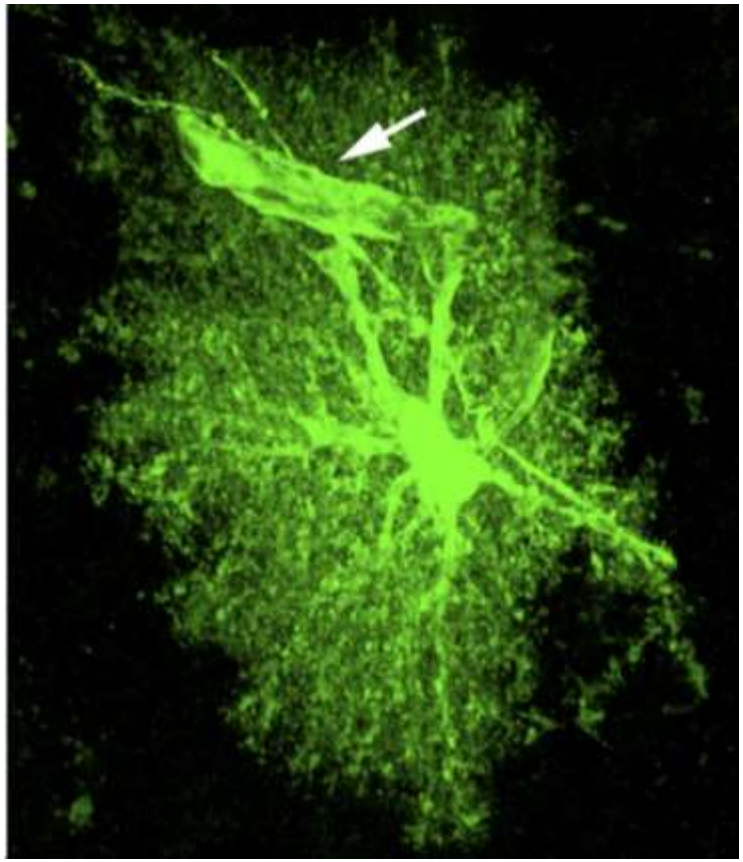
Tian et al. *Cold Spring Harb Protoc* 2012

Bulk loading fails to capture Ca^{2+} signals in fine astrocyte processes

Rhod-2 AM, bulk loading



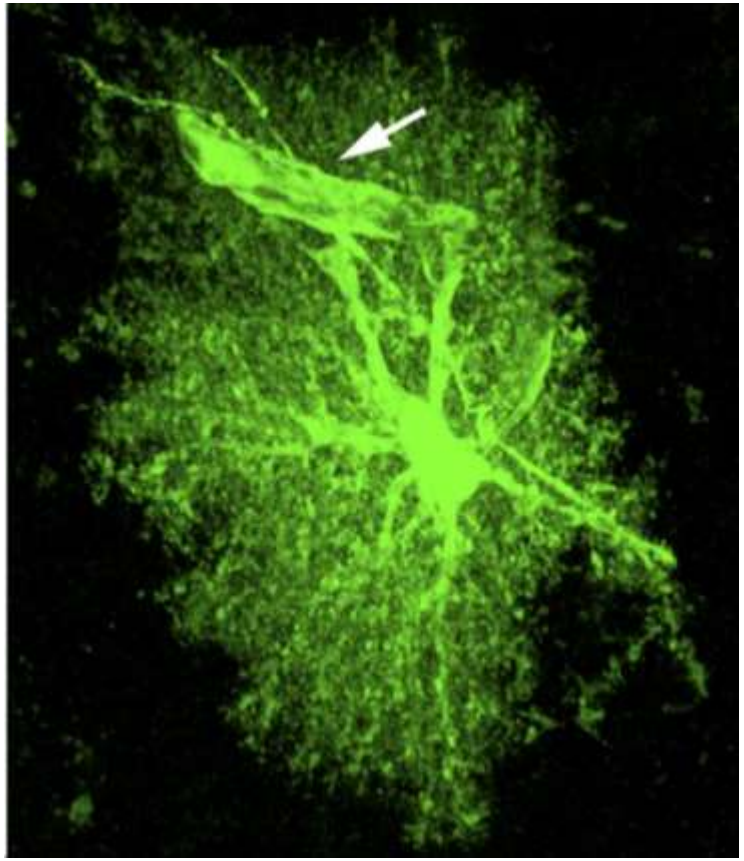
3D reconstruction of dye-filled astrocyte



Pekny and Wilhelmsson 2006

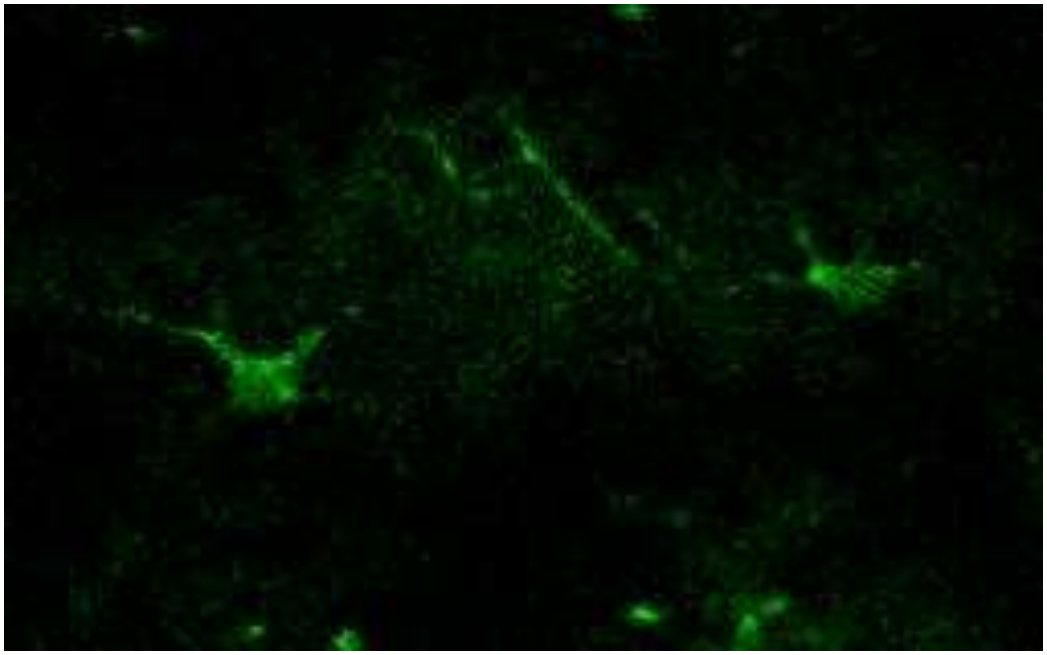
GECIs reveal Ca^{2+} signals in fine astrocyte processes

3D reconstruction of dye-filled astrocyte



Pekny and Wilhelmsson 2006

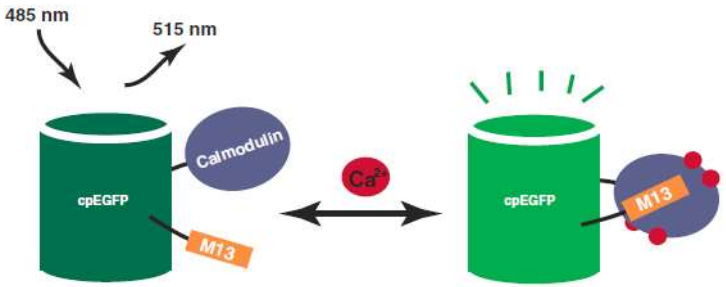
Glast-GCaMP5



GCaMP

- Circularly permuted enhanced GFP (cpEGFP)
- N-terminus linked to M13 fragment of myosin light chain kinase
- C-terminus linked to calmodulin
- Ca^{2+} binding to calmodulin → calmodulin binding to M13 → conformational changes → increased cpEGFP fluorescence

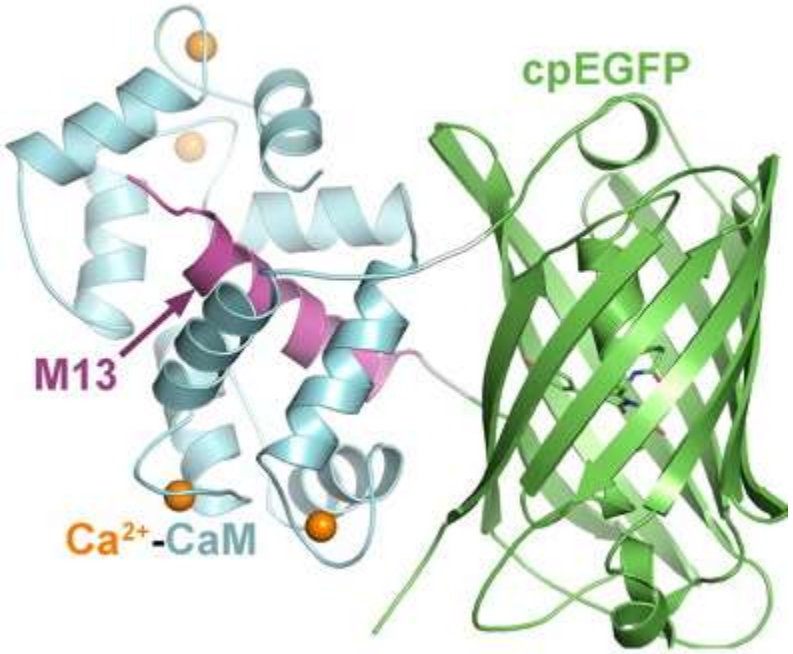
Nakai et al. *Nature Biotechnology* 2001



Tian et al. *Cold Spring Harb Protoc* 2012

Continual optimization of GCaMPs

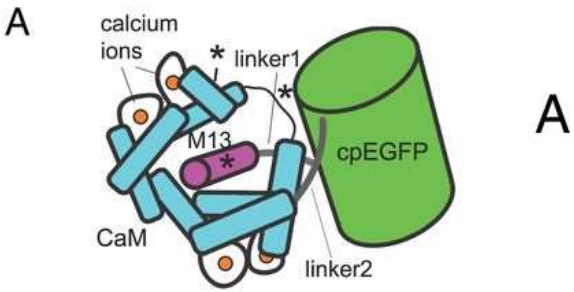
- Initial sensors were dim, folded poorly at 37˚C, pH-sensitive, had poor signal to noise ratio compared to synthetic dyes
- X-ray crystallography analysis of GCaMP in its Ca^{2+}-free and Ca^{2+}-bound states
- Site-directed mutagenesis



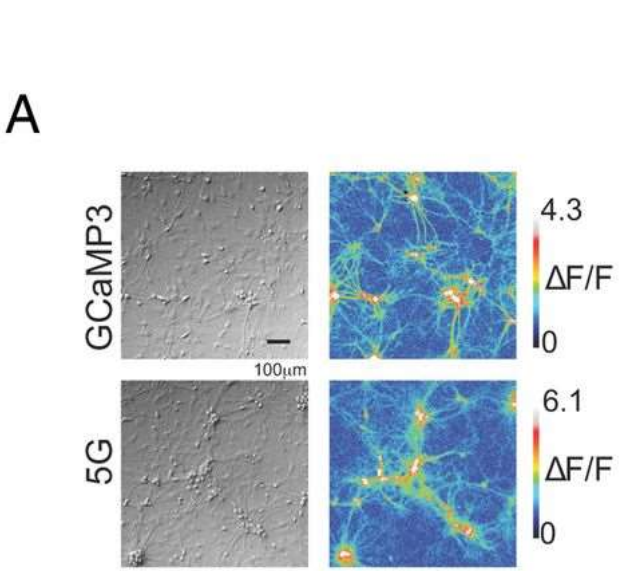
Akerboom et al. *J Biol Chem* 2009

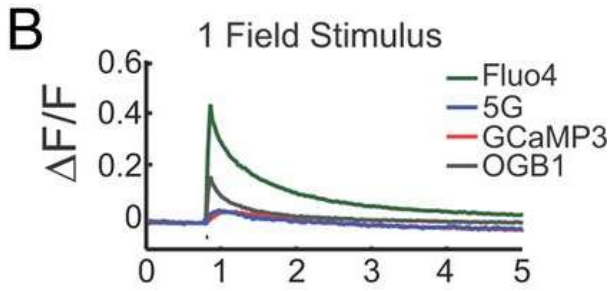
Development of GCaMP5

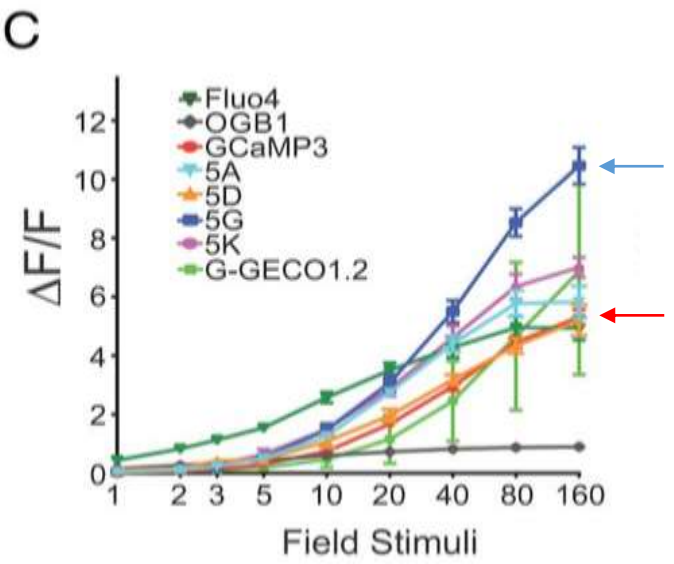
- Generated a series of GCaMPs by mutating GCaMP3 in different locations
- Transfect GCaMPs into neuronal culture and measured fluorescence in response to electrical stimulation



	M13pep	linker1		cpEGFP	linker2		CaM	
GCaMP3	A	L	E		T	R	D	R
	52	59	60		302	303	380	392
GCaMP5A	-	-	-		-	-	Y	-
GCaMP5B	-	H	P		-	-	-	-
GCaMP5C	-	Q	P		-	-	-	-
GCaMP5D	-	-	-		L	P	-	-
GCaMP5E	-	H	P		L	P	-	-
GCaMP5F	-	Q	P		L	P	-	-
GCaMP5G	-	-	-		L	P	Y	-
GCaMP5H	-	Q	P		L	P	Y	-
GCaMP5I	-	H	P		L	P	Y	-
GCaMP5J	-	H	P		-	-	Y	-
GCaMP5K	-	-	-		-	-	Y	G
GCaMP5L	V	-	-		L	P	-	-



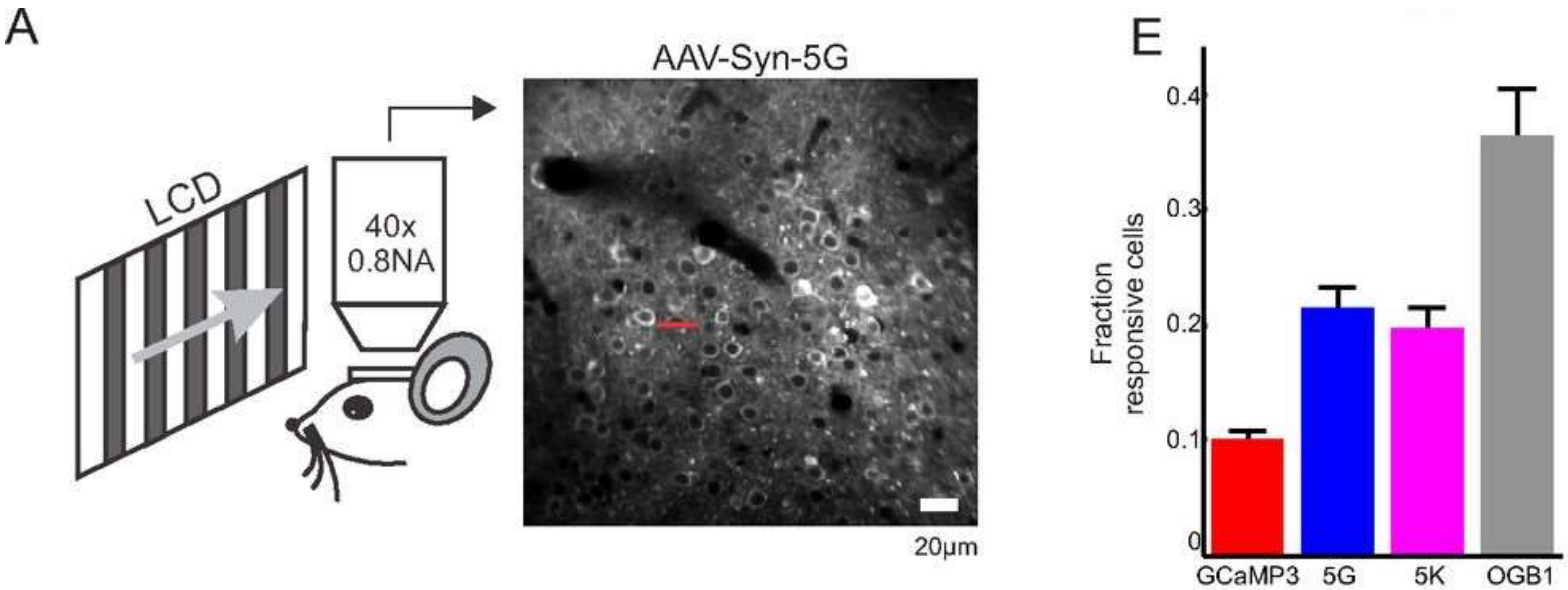




Akerboom et al. *J Neurosci* 2012

Development of GCaMP5

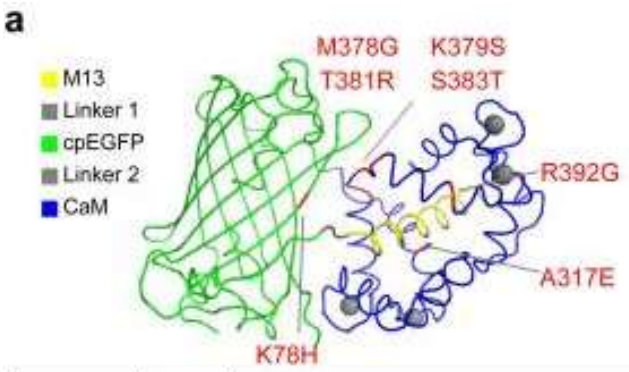
- Characterized in a number of model organisms *in vivo*
  - C. elegans chemosensory neurons
  - Drosophila larval neuromuscular junction
  - Zebrafish retina and tectum
  - Mouse visual cortex



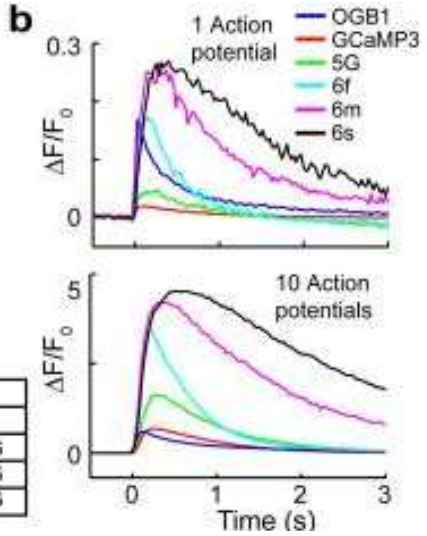
Akerboom et al. *J Neurosci* 2012

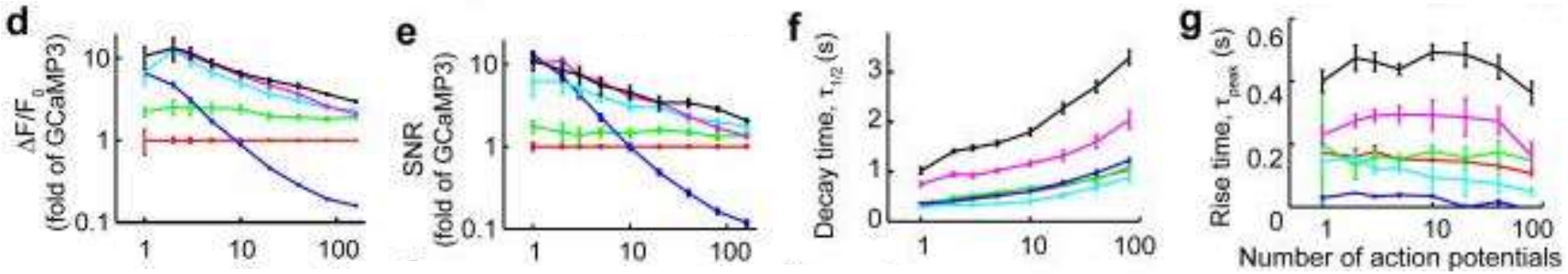
Development of GCaMP6

- Further optimization of GCaMP to improve its sensitivity and speed



	cpEGFP	CaM					
GCaMP5G	K78	A317	M378	K379	T381	S383	R392
GCaMP6s	K78H				T381R	S383T	R392G
GCaMP6m			M378G	K379S	T381R	S383T	R392G
GCaMP6f		A317E			T381R	S383T	R392G





Chen et al. *Nature* 2013

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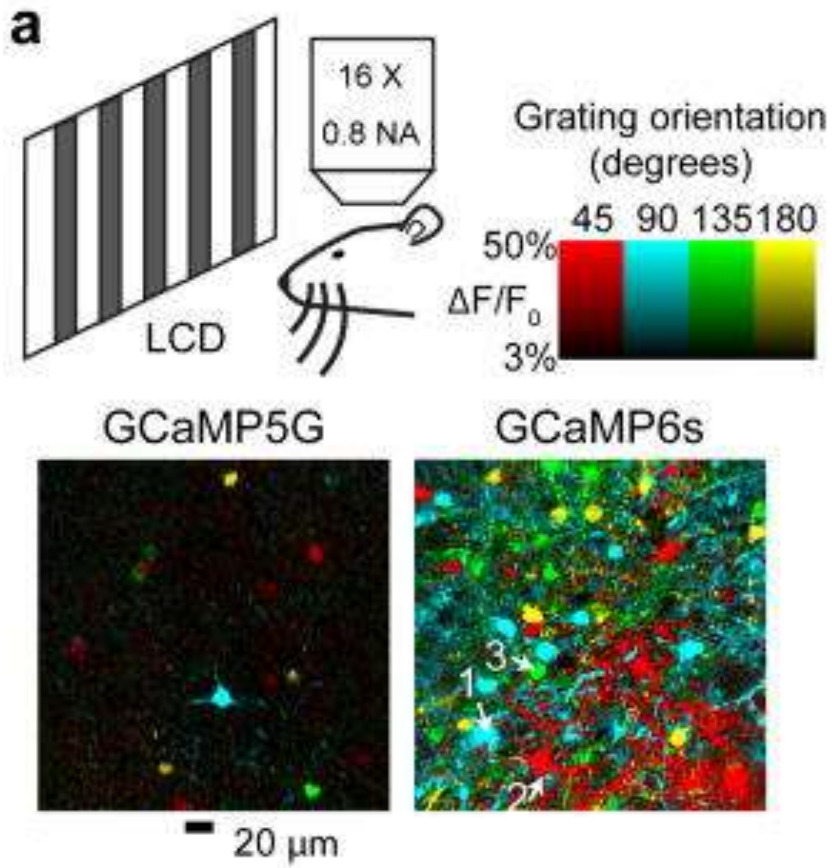

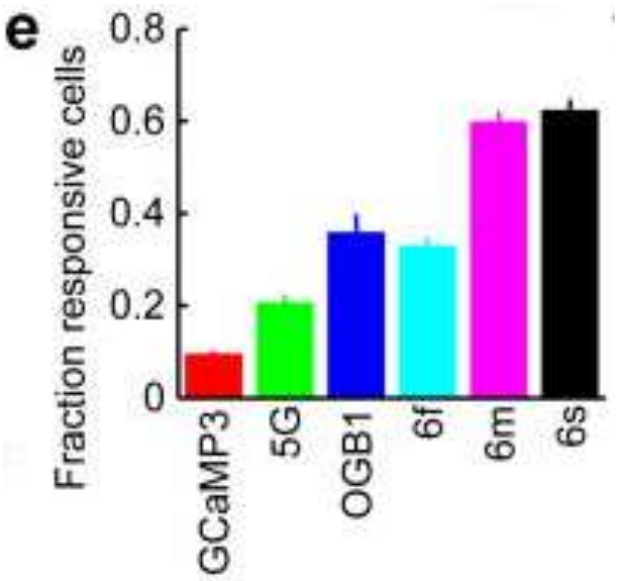

Chen et al. *Nature* 2013

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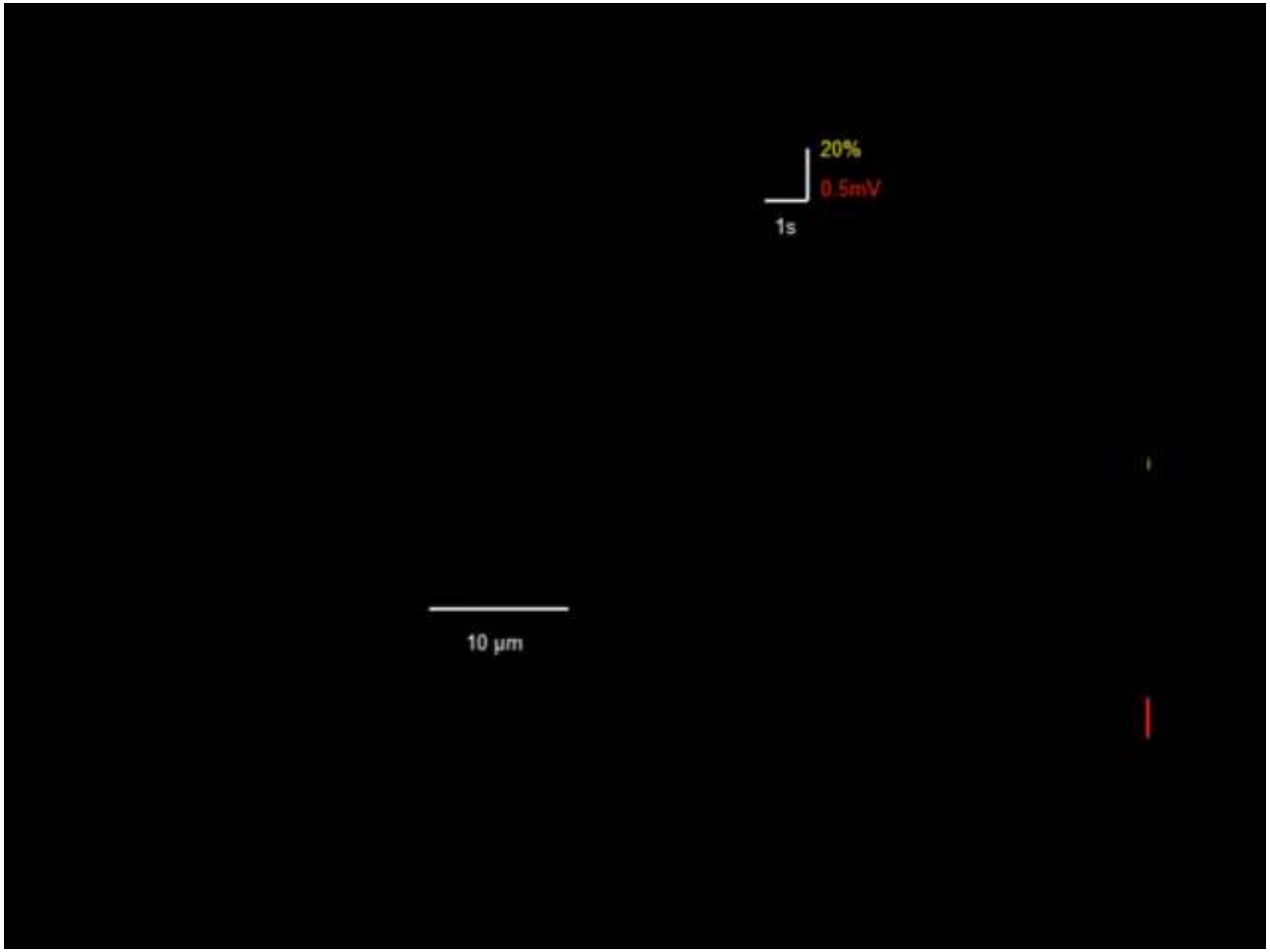

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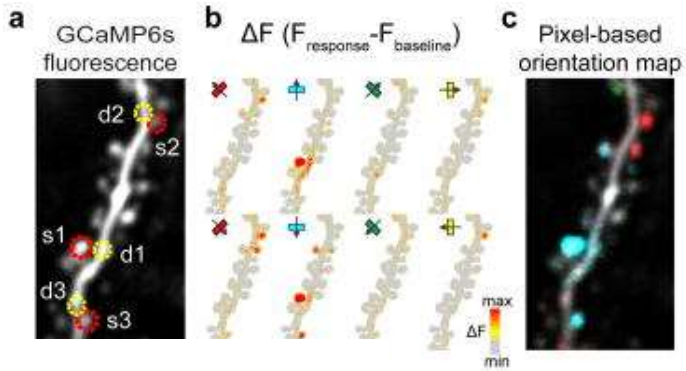
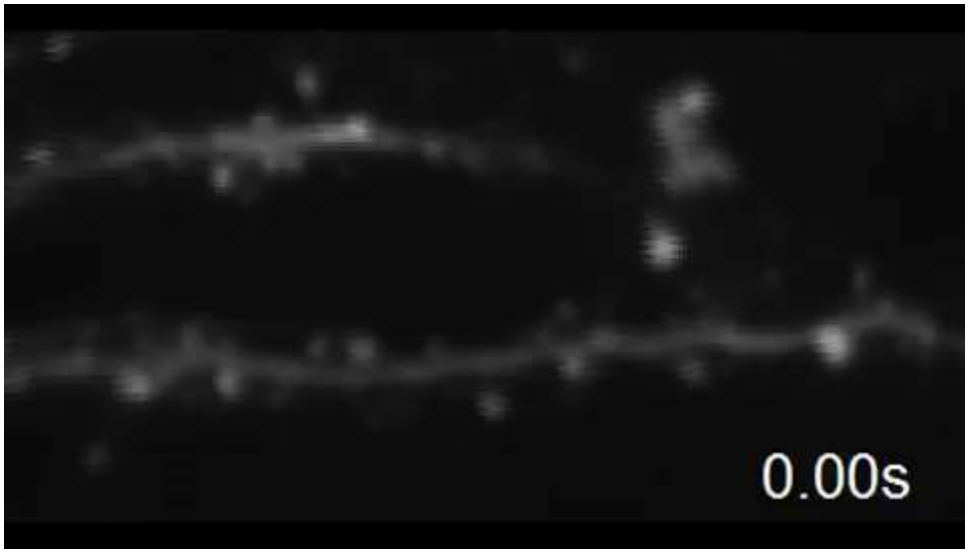




Chen et al. *Nature* 2013

Factors to consider when choosing a GCaMP

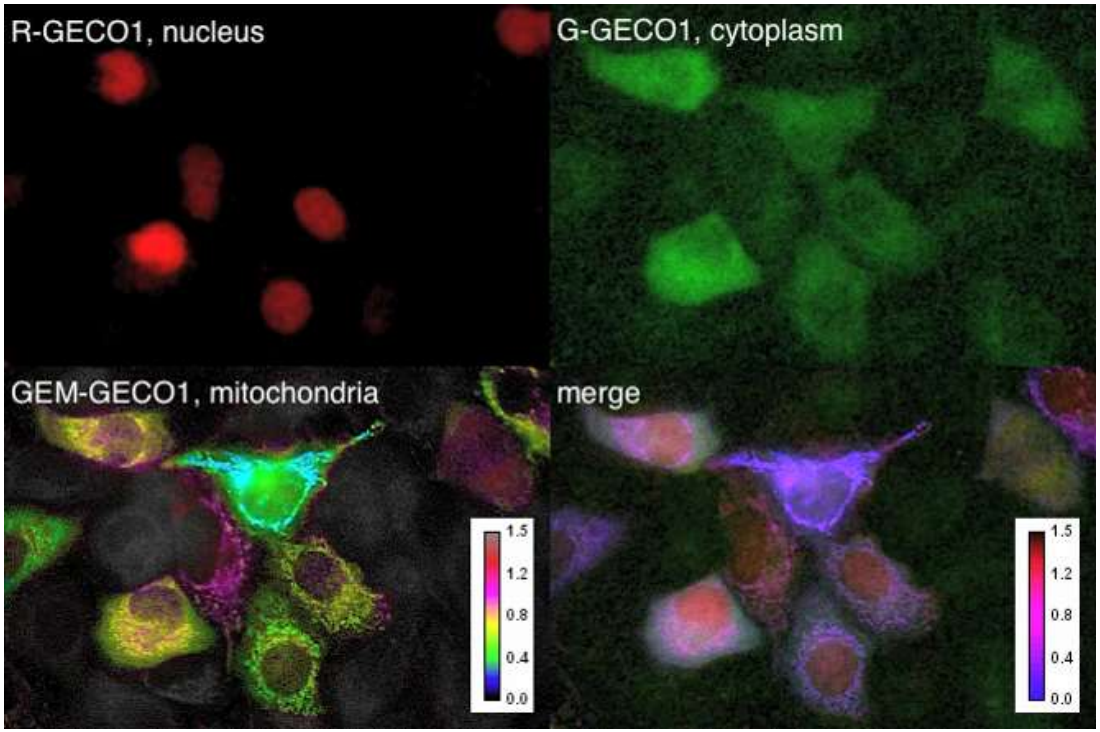
- Expression in your favourite species / cell type / organelle
- What kind of signal are you interested in?
  - Sparse APs- high affinity indicator to generate larger fluorescence changes to small transients, but will saturate more easily if spikes are long-lasting, or closely spaced
- What else do you want to be imaging at the same time?

Sensor	Baseline fluorescence F_0, relative to GCaMP3	Dynamic range ($\Delta F/F_0$ at 160 AP)	Kd (nM)	Decay $\tau_{1/2}$ 10 AP (ms)	Rise τ_{peak} 10 AP (ms)	SNR 1 AP (fold GCaMP3)	SNR 10 AP (fold GCaMP3)
GCaMP3	100±3%	565±6%	345±17	597±8	137±4	1.0±0.1	1.0±0.03
GCaMP5G	72±3%	1085±66%	447±10	667±43	166±20	1.7±0.3	1.4±0.2
GCaMP6s	74±5%	1680±48%	144±4	1796±73	480±24	11.2±2	4.6±0.8
GCaMP6m	54±4%	1177±30%	167±3	1162±55	280±48	10.3±1.7	3.1±0.3
GCaMP6f	67±5%	1314±56%	375±14	400±41	80±35	6.2±2	4.1±0.3

Chen et al. *Nature* 2013

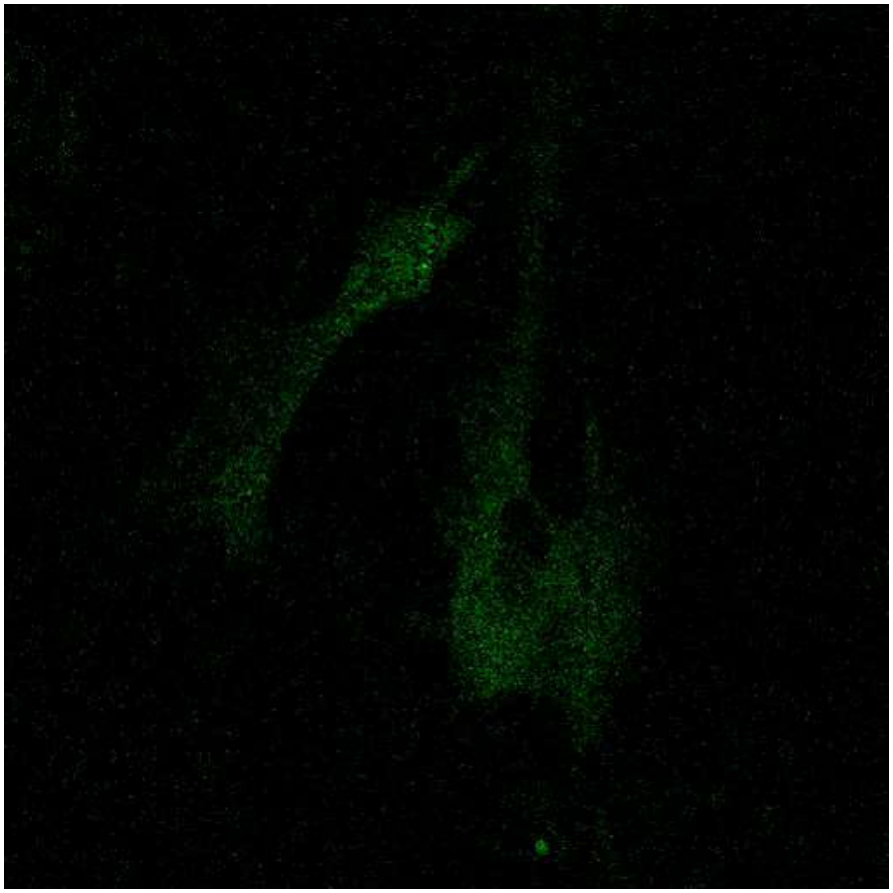
Red-shifted GECIs

- Two-colour imaging to monitor signals simultaneously
  - Within different compartments of the same cell
  - Between two populations of cells
  - Calcium – calcium, calcium – glutamate etc.
- Combine with optogenetics experiments
- Reduce phototoxicity, blood-related artifacts
- RCaMP (Akerboom et al. *Front Mol Neurosci* 2013), R-GECO1 (Zhao et al. *Science* 2011)

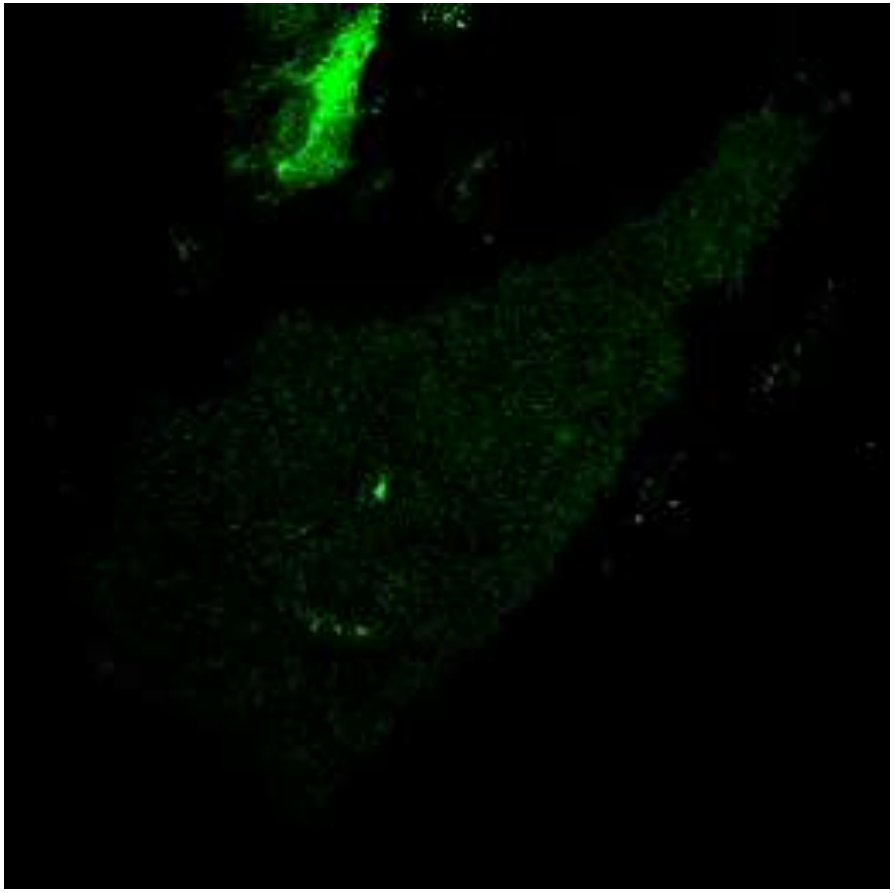


Cytosolic versus membrane-tethered GECIs in cultured astrocytes

GCaMP6, cytosolic



Lck-GCaMP5, membrane-tethered



Limitations of using GCaMPs

- GECIs may form aggregates or become calcium-insensitive
- High levels or chronic expression of GECIs can change physiology of the cells
- Use inducible expression systems