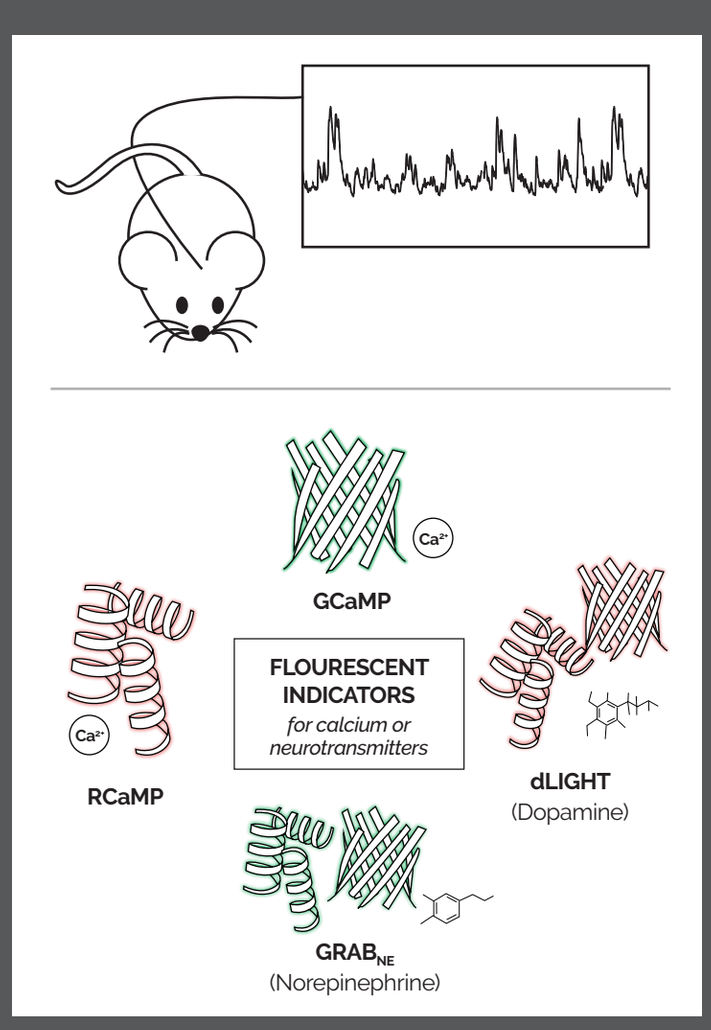
Intro to Fiber Photometry

FROM NEUROPHOTOMETRICS

What is fiber photometry?

Fiber photometry is an optical technique for measuring chemical signaling in the brain of a freely behaving animal.

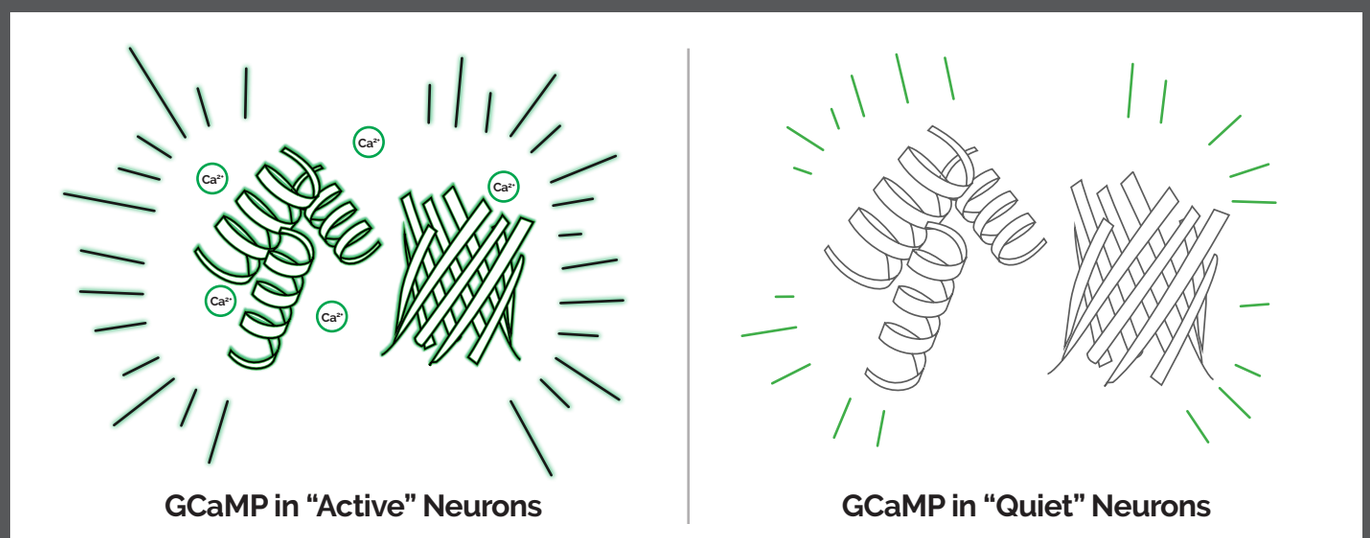
Traditionally, photometry has been used to measure calcium signaling using genetically encoded fluorescent indicators such as GCaMP. More recently, a growing array of indicators has become available, allowing for measurements of many different kinds of chemical signals.



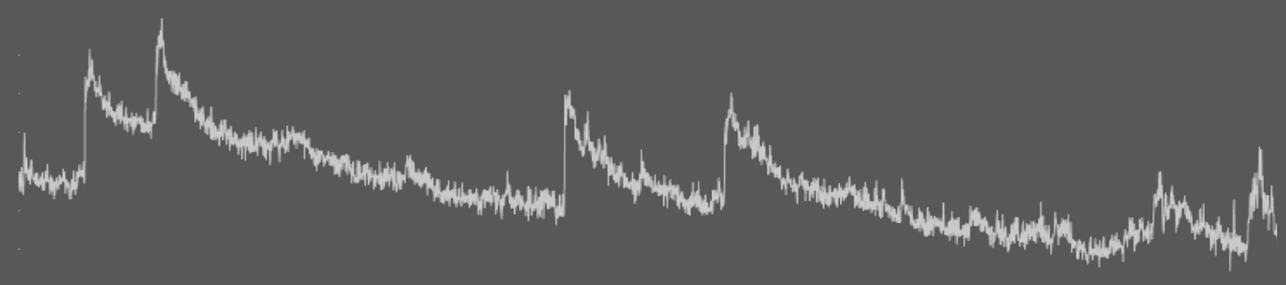
What are we *really* measuring?

The fundamental measurement in fiber photometry is fluorescence.

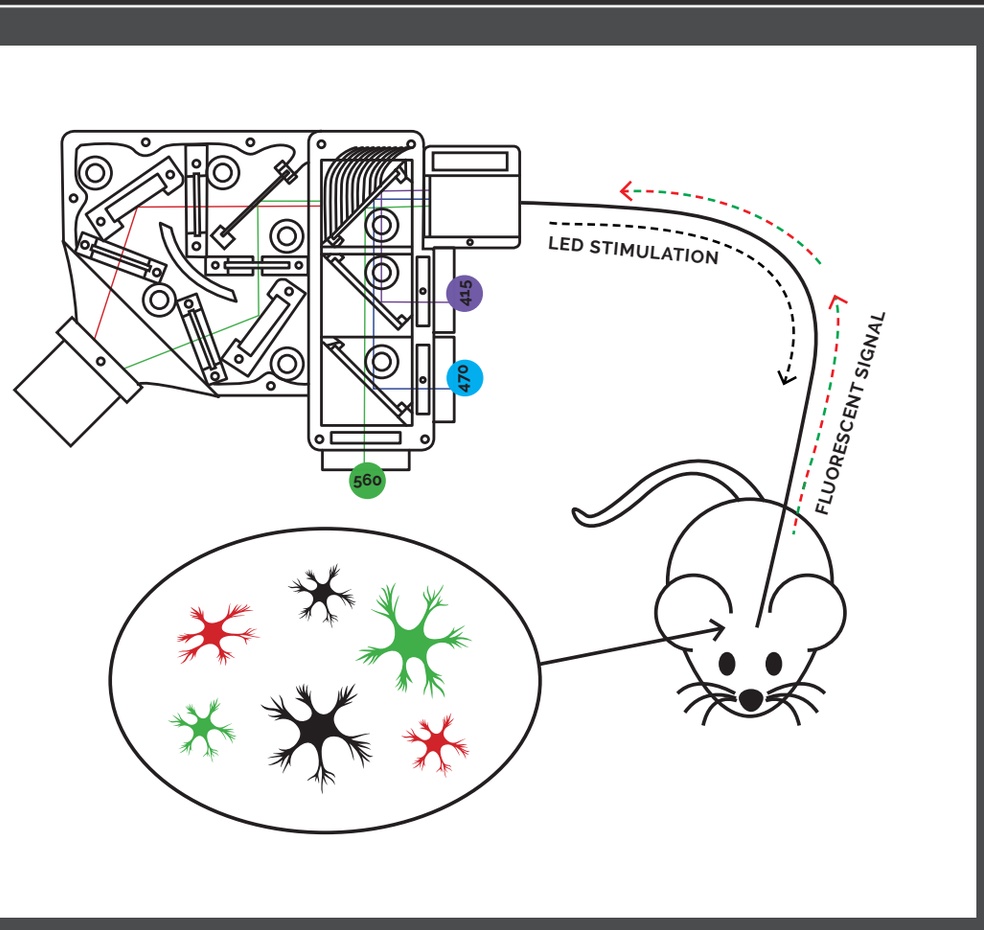
In fiber photometry, fluorescence acts as a representation of neural activity. A genetically-encoded fluorescent indicator – like GCaMP – is excited by a light source in the fiber photometry system and in turn emits its own light.



When that indicator is bound to a ligand, as GCaMP binds to calcium during neural activity, it emits more light than when it is unbound.



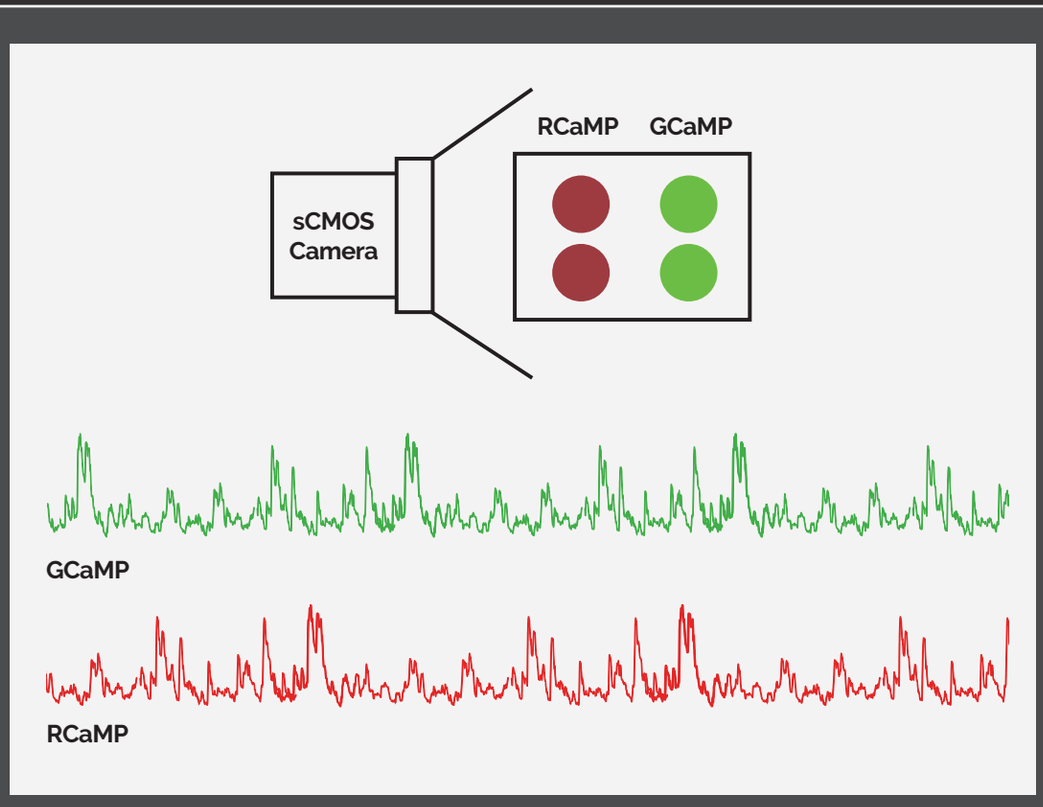
The recorded result is a trace of relative fluorescence that is sensitive to the amount of ligand present for your indicator. In this case, the trace is sensitive to the amount of calcium, which will increase in a way that correlates with action potentials in your target neurons.



What is the fiber photometry system doing?

A fiber photometry system has two jobs: provide a light source to excite the fluorophore and quantify the light emitted by the fluorophore.

The photometry light source is an LED or laser which passes through a series of filters to ensure a tight excitation band at a wavelength specific to the fluorophore you are targeting. The light is routed into a fiber optic cable and then into the brain. The fluorophore – which should be specifically expressed in a cell type or region of interest – is excited by the light and in turn emits its own light signal that travels back up the same patch cord in the opposite direction. This emitted light is then filtered and focused onto a camera or photodiode for measurement.



How do we "read" fluorescence as activity?

Fiber photometry systems can read the emitted fluorescence with one of two tools: a photodiode or a camera.

Our Neurophotometrics systems use an sCMOS camera to read changes in fluorescence as differences in the brightness values of pixels. Systems that use a photodiode convert fluorescence changes to measurable differences in current. Whether you're looking at pixel values or current, it's all used as a proxy for changes in fluorescence.

It is important to note that the units we use to describe changes in fluorescence – F or ΔF – are arbitrary. This means that there is no one value that represents an objective "zero" amount of emitted fluorescence (i.e. activity) because the fluorophore is always emitting some amount of light – what's important is how much that amount changes when there is activity. Therefore, you'll always need a baseline fluorescence measurement to calculate these relative changes in fluorescence.

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