Combining imaging and patch clamp recording in slices

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Fluorescence microscopy
**Figure 1** - Jablonski diagram. Horizontal lines indicate the various electronic configurations of a molecule and the energy of the molecule is represented on the vertical axis, with a higher vertical position indicating a larger amount of energy in the molecule.
Figure 2 – Fluorescence excitation spectrum.
Figure 3 – Electronic transitions from the excited state during fluorescence
Figure 4 – Fluorescence emission spectrum. Note that the emission spectrum is always shifted to longer wavelengths relative to the excitation spectrum. This shift is termed the Stokes Shift.
Figure 5 – Arc lamps. A, components of an arc lamp. B, emission spectrum of mercury arc lamp. C, emission spectrum of xenon arc lamp. Note different vertical scales for B and C.
Figure 6 – Basic optical filters used in fluorescence microscopy.
Figure 7 – Epifluorescence microscopes use the objective lens both to deliver excitation light and to collect emitted fluorescence.
Figure 8 – Dichroic mirrors are the key to epifluorescence microscopy. A, optical properties of a dichroic mirror. B, Diagram of the position of a dichroic mirror in the light path of a epifluorescence microscope.
Figure 9 – Two examples of filter sets useful for imaging fluorescein on an epifluorescence microscope.
Figure 10 - Fluo-3, a fluorescence Ca$^{2+}$ indicator that does not shift its spectral properties upon binding calcium.
Figure 11 - Other fluorescence indicators that do not shift their spectral properties upon binding calcium. Note differences in affinity and excitation/emission spectra between indicators.
\[
[Ca^{2+}] = K_d \left( \frac{F - F_{\text{min}}}{F_{\text{max}} - F} \right)
\]

**Figure 12** - Relationship between fluorescence emission of fluo-3 and calcium concentration.
**Figure 13** - Fura-2 and Indo-1, fluorescence indicators that shift their spectral properties upon binding calcium.
\[ [\text{Ca}^{2+}] = K_d \left( \frac{R-R_{\text{min}}}{R_{\text{max}}-R} \right) \left( \frac{S_{f2}}{S_{b2}} \right) \]

**Figure 14** - Relationship between fluorescence excitation ratio of fura-2 and calcium concentration.
Figure 15 - AM ester loading of organic indicator dyes.
**Figure 16** - Fluorescence resonance energy transfer (FRET) depends on close proximity of two fluorophores.
**Figure 17** - Cameleon, a genetically encoded Ca²⁺ indicator.
Fluorescence imaging in brain slices
**Figure 18** – Water immersion objectives provide the long working distance required for combining patch clamp recording with imaging in brain slices.
Table 1
Specifications of the microscope objectives used in our lab

<table>
<thead>
<tr>
<th>Objective</th>
<th>Mag.</th>
<th>Immersion Medium</th>
<th>NA</th>
<th>WD</th>
<th>UV Transmission</th>
<th>Price</th>
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<tr>
<td>Zeiss Neofluar</td>
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Abbreviations: Mag. = magnifications; NA = numerical aperture; WD = working distance; UV = ultraviolet light (364 nm); ICS = infinity-corrected optics

* = only used objectives are sold now; infinity corrected replacements are available.
Figure 19 – An example of a set-up used for combining patch clamp recording with imaging in brain slices.
Figure 20 – Proper application of positive pressure is critical when using dye-filled patch pipettes in brain slices.
Figure 21 – Time-dependent diffusion of dye solution from a patch pipette to the dendrites of a cerebellar Purkinje neuron.
Figure 22 – Ratiometric measurement of [Ca] in a brain slice neuron filled with fura-2.
Figure 23 – Experimental arrangement for stimulating parallel fiber synapses in cerebellar slices.
Figure 25 – Changes in Purkinje cell [Ca] produced by stimulation of parallel fiber (PF) synapses. A – control response; B – responses after AMPA-type glutamate receptors are blocked by CNQX; C - responses after AMPA-type glutamate receptors are blocked by CNQX and metabotropic glutamate receptors are blocked by MCPG.
Figure 26 – Localized [Ca] responses produced in Purkinje cell synaptic spines following PF activity. A – fluorescence image; B,C – zoomed image of spiny dendrites, with changes in [Ca] produced by parallel fiber activity indicated by pseudocolor scale. AMPA-type glutamate receptors were blocked by CNQX treatment.
Figure 27 – Photolysis of a “caged” IP3 compound produces a rapid jump in IP3 concentration.
**Figure 28** – Set-up for combining fluorescence imaging with uncaging in brain slices.
Figure 29 – Uncaging IP3 at sites of active PF synapses. Left - Local rise in [Ca] indicates location of active PF synapses. Right – Focusing a spot of UV light at the same location uncages IP3 and yields a local release of intracellular Ca.
Figure 30 – Uncaging IP3 causes long-term depression of PF synaptic transmission. C – PF synaptic currents recorded from a Purkinje cell before and after uncaging IP3. D – Time course of changes in PF synaptic currents following uncaging of IP3 at 0 timepoint. E – Long-term depression is only observe when IP3 is uncaged at site of active PF synapses.
References


