In Vivo Calcium Imaging of Neural Network Function

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Outline

• Introduction to In Vivo Calcium Imaging
• Calcium Indicator Labeling
• Network Scanning Approaches
• Calcium Signaling in Various Cellular Structures
• Optical Detection of Neuronal Spikes
• Contamination of Somatic Signals by Neuropil
• Outlook
Introduction to In Vivo Calcium Imaging

- Uncover neural networks operating principles
- Spatiotemporal activity patterns
- Limited experimental tools to record from large sets of living cells
- Two-Photon Laser Scanning Microscopy (2PLSM)
- *In Vivo* measurement
- Activity dependent fluorescent markers
In Vivo Labeling of Cellular Networks with Calcium Indicator
Laser Scanning Modes for In Vivo Two-Photon Microscopy

A

B

C

D

E

F
Signal Components in Bulk-Loaded Neocortical Network

A

B

C

D

\[ \Delta [\text{Ca}] \]

Neurons

Astrocytes

Neuropil

Sensory stimulus

\[ \Delta F/F \]

20%

Sensory stimulus

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Example

A

B

C

Figure 1.11: In vivo calcium imaging of neural network function

A. Schematic of raster scanning at a particular focal depth.

B. Left: Two-photon image of a single focal plane with selected regions of interest corresponding to individual cells or cell clusters. Cells were unspecifically loaded with the green calcium-sensitive marker OGB-1 AMs. Note the high level of background fluorescence. Astrocytes were counterstained with the astrocyte-specific red fluorescent dye sulforhodamine. Scale bar = 5 µm.

Right: Single ∆F/F image from a time series of spontaneous activity. See also Supplementary Movie.

C. Spontaneous calcium signals for the same cells highlighted in B. The time point of the ∆F/F image in B is indicated by a dashed vertical line.

D. Scheme of fast straight line scan through dendritic cross-sections.

E. Left: Two-photon image of single dendrites. The cell was stained with the calcium-sensitive dye OGB-1 by electroporation. The position of the straight-line scan through two dendrites is indicated by the red horizontal lines. Scale bar = 5 µm.

Right: Entire set of linescan data (top) and time course of the dendritic calcium signal in ∆F/F for the two sampled dendrites (bottom). Same color code as indicated above on the left. Vertical scale bar = 5 µm.
Example

Movie
Action Potential-Evoked Somatic Calcium Signaling

A

Spike Trains

1
2
3

Single-AP evoked calcium transients

Δ[Ca]

Reconstructed Spike Train

1
2
3

ΔF/F

Instantaneous Spike Rates

1
2
3

Convolution

Reconstruction Algorithm

Added Noise

10%
AP Description

\[ \Delta [\text{Ca}] \propto \frac{\Delta F}{F} \propto \frac{1}{1 + \kappa_S + \kappa_{\text{dye}}} \]

\[ \tau \propto (1 + \kappa_S + \kappa_{\text{dye}}) \]
Fluorescence Signal Dependence on Calcium Indicator Concentration

\[
\Delta F \propto \frac{F}{1 + \kappa_S + \kappa_{\text{dye}}} \approx \frac{\kappa_{\text{dye}}}{1 + \kappa_S + \kappa_{\text{dye}}}
\]

\[
\text{SNR} = \frac{\Delta F}{\sigma_F} \propto \frac{\kappa_{\text{dye}}}{(1 + \kappa_S + \kappa_{\text{dye}}) \sqrt{\kappa_{\text{dye}}}} = \frac{\sqrt{\kappa_{\text{dye}}}}{(1 + \kappa_S + \kappa_{\text{dye}})}
\]
Finding Optimal Dye Concentration

\[ \kappa_{max} = (1 + \kappa_S) \approx \kappa_S \]
Simulation of Neurupil Contribution to Somatic Fluorescence Signals

A: Soma \rightarrow \text{Neurupil} \rightarrow \text{Convolution} \rightarrow \text{Image}

B: Relative intensity (%)

C: Beam profiles

D: Contamination (% of neurupil)

NA 0.3, NA 0.5, NA 0.8

Effective numerical aperture

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Outlook

• Fast 3D measurement
• Improved GECIs
• Reliable single spike detection from large numbers of neurons
• Awake behaving animals
• Light activated channel proteins
References
