Overall goals and objectives

The ability to observe neuronal activity at the macroscopic scale (on the order of millimeters to centimeters) has been advancing rapidly through the development of several non-invasive techniques including PET, fMRI, EEG, and MEG. Although macroscopic neuroimaging can address numerous important questions about human brain function at the systems level, it is often difficult to relate these results to the underlying biophysics and cellular organization of the brain at the microscopic scale. The overall goal of this project is to develop an integrated suite of technologies to bridge this critical gap.

Our main objectives are to improve the spatial and temporal resolution of non-invasive technologies, so that they can resolve more discrete (e.g., column and laminar level) neural units which bridge the systems and cellular levels; and to clarify the mechanisms which relate the biophysics of neuronal activity to “observables” in our imaging measurements. These observables include direct and indirect measures of neuronal activity such as electrophysiological and hemodynamics (blood flow, blood volume, and blood oxygenation). Developing realistic models of brain function requires instruments capable of extreme sensitivity to these observable physiologica and biophysical parameters, with high spatial and temporal resolution to measure direct and indirect consequences of brain activity at near-microscopic scales.

Aim 1: Improve fMRI Spatial Resolution in the macaque and rat brain through development of pulse sequence methods, RF array coil technology and gradient coils for ultra-high field imaging (9.4T for rodents and 7T for primates).

Figure 1. Laminar resolution of 7T Structural MRI in primates.

The line of Gennari (layer 4b in V1) is clearly visible in the primary visual cortex of the anesthetized macaque. The left panel shows a sagittal slice, from a 250 µm x 250 µm x 750 µm 3D MPAGE T1 weighted scan at 7T using a 6.5 mm two-dimensional surface coil over primary visual cortex (area V1) in macaque. The right panel shows an intensity profile taken orthogonal to the cortical surface along the black line overlaid on the image in the top panel. In both the MR image (top) and the corresponding line profile (bottom), the highly-myelinated layer 4b (the area of Gennari) is prominently visible within the center of the gray matter, across three folds of primary cortical cortex.

Figure 2. Functional imaging of awake behavior using monkey fMRI at 7T.

Time course of visual activation produced by a large flickering checkerboard stimulus, recorded without averaging from a small (1.25 mm diameter) telemetrically connected cortex depth electrode in an awake behaving macaque scanned at 7T. The checkerboard contrast stimulus was presented 4 second blocks separated by blocks of a uniform gray stimulus, while the animal was fixating the center of the stimulus screen.

Figure 3. 3 T channel receive-only primate array with dedicated transmit coil.

The array consists of 3-mm-diameter actively detuned coil elements cut out on a CAD controlled circuit board milling machine. The array is shown over the monkey head model made from MR data and 3D printing methods to act as a support in construction of custom fitted helmets for the individual monkeys used in the fMRI experiments.

Figure 4. Rat fMRI at 9.4T.

Electrical stimulation of right (A) and left (B) whisker pad 30 sec at 3 Hz produces extensive activation of the somatosensory cortex region observable using ultra-high field imaging with a 3.5 mm two-dimensional surface coil and 0.4mm isometric resolution spin-echo sequences. The timecourse of activation from active pixels in A is shown in C.

Aim 2: Improve the spatial resolution of Laminar Optical Tomography (LOT) for brain imaging.

Figure 5. The instrument for simultaneous measurement of blood flow, volume and oxygenation.

We have developed an instrument that provides simultaneous spectroscopic imaging of intrinsic signals and laser speckle imaging of blood flow. A schematic representation is shown on the left, a close-up image of the experimental setup is on the right.

Figure 6. Multispectral imaging of intrinsic signals.

A. Ratio images of activation following electrical forepaw stimulation for each of the 6 filters (wavelength indicated above) were calculated by dividing the response at the peak by the baseline image. B. Corresponding maps of oxy-hemoglobin (HBO), deoxy-hemoglobin (Hb) and total hemoglobin (HBT). Below are time courses of the row signal (left) and calculated Hb(380)/HbT(right).

Figure 7. Laser speckle contrast imaging of cerebral blood flow.

Ratio images of blood flow response following electrical forepaw stimulation were calculated by dividing the response at the peak by the baseline image. The changes are displayed for all pixels with an increase in flow greater than 5%. Signal timecourse is shown below.

Figure 8. Calculation of CMRO2 using simultaneous spectroscopic and speckle contrast imaging.

CMRO2 = CR (f - S0) = CR (f - S0) / \int h(t) dt = \int h(t) dt

A tomographically reconstructed image at the peak activation time for different brain regions in the visual cortex. D. Data extracted from the whole time-series of reconstructed images, demonstrating that the centroid of the resolved response is around 650-700nm, which corresponds well to cortical layer IV. The LOT results also indicate that the temporal shape of the activation is in fact different at different depths, something that is not revealed using the raw data or the CCD data alone.

Aim 3: Apply the new technologies to image functional activation.

Correlation between hemodynamic imaging signals and the biophysics of neuronal activity.

Figure 9. Laminar Optical Tomography (LOT).

LOT uses a system similar to a confocal microscope. The LOT system design allows detection not only of light emerging from the point being illuminated, but also of the scattered light emerging from different relative distances from the illumination point. This light has traveled more deeply in the tissue, and therefore provides images of structures that are much deeper than can be visualized with Confocal microscopy. In addition to the raw images, the data can be used to perform a reconstruction that actually resolves the depth of the features seen.

Figure 10. Signal timecourses of Hb, HbO, and HbT calculated from the data in figure 10.

Hemodynamic signals monotonically increase (close to linear) throughout the range of stimulus intensities.

Figure 12. Laminar recordings of neuronal activity using 23 channel depth array.

In order to address the laminar pattern of electrophysiological activity, multiple unit activity (MUA) and local field potential (LFP) were measured using a linear array multi-electrode. The figure shows single trial laminar recordings of LFP in response to tactile whisker stimulation. Each trace represents a recording from a single contact as a function of time. The inter-contact distance 100 µm. The upper most contact is on the cortical surface. Stimulus waveform underlines the traces represents a control signal for the piezoelectric stimulating device. Stimulus onset is indicated by a dashed line.

Figure 13. Simultaneous recordings from cortex and thalamus.

We performed laminar recordings from the active cortical column (principal barrel in Barrel cortex) and the corresponding input location (the corresponding barrel in the thalamus). A. Thalamic (top) and cortical layers (bottom) response deflections of a single whisker. Responses using different spatial (amplitude) are superimposed. B. Peak response amplitudes as a function of stimulus intensity. Superimposed are the cortical layer IV response (red), the hemoglobin (blue). Bottom: spatiotemporal layers (green and blue) saturate at a slower rate than layer IV(red).

Figure 14. Hemodynamic response is influenced by neuronal activity in neighboring cortical columns.

Simultaneous recordings from two neighboring cortical columns (barrels) show that the response in surrounding barrel (red) is proportional to the amount of stimulus intensity saturates at a slower rate. A. An image of vasculature with two electrodes recorded from the surface (xw) and surrounding (sw) barrel. B. A ratiogram image of response at 380 nm - CD LFP and MUA. E. Peak amplitude of LFP (left) and MUA (right) in the (blue) and surrounding (red) barrel. Note that the red curve do not reach an asymptote.

Figure 15. Neurovascular function relationship.

An increase in the hemodynamic response beyond saturation of local post synaptic activity indicates that the neuronal responses do not have a single correlation to hemodynamic response. C. Point measurement of neuronal response and the hemodynamic response activity. However, the relationship between local hemodynamic activity and neuronal activity in the same area is superimpose. D. The neuronal activity cannot be inferred from the point hemodynamic measurement. E. Surrounded hemodynamic “inhibition” does not correspond to a decrease in neuronal activity.