Intrinsic Signal Optical Imaging

Introduction

Intrinsic signal optical imaging (ISOI) is a technique used to map dynamics in single cells, brain slices and even – and most importantly – entire mammalian brains. It is label-free, minimally invasive and gives the researcher access to the basic physiology of a functioning brain. All of these benefits come without having to compromise on spatial and temporal resolution which is otherwise only provided by regular imaging techniques that rely on labels and markers. As the use of the latter techniques is undesired in humans, ISOI can be used to confirm observations made with optical imaging techniques in other mammals.

ISOI Background

There is a number of functional brain mapping methods outside of ISOI, including electrical stimulation mapping (ESM), electrophysiological recordings such as electroencephalography (EEG), and functional magnetic resonance imaging (fMRI), each with their strengths and weaknesses.

ESM is an invasive procedure where an electrode is placed on the brain at a specific site to test the function of that site. An electric current is sent through the electrode to stimulate that part of the brain and the resulting physiological response, the movement of a limb, for example, tells researchers for what that part of the brain is responsible. The problem with ESM is that because it requires direct stimulation, it can potentially trigger epileptic activity.

EEG, on the other hand, is non-invasive and involves placing electrodes on the side of the scalp and measuring the voltage fluctuations of neurons in the brain. It can identify certain regions of the brain but the electrodes have poor (~1 cm) spatial resolution.

fMRI is also non-invasive and is performed similarly to a standard MRI and relies on the idea that the area of the brain being used receives more blood. Oxygen-rich and oxygen-starved blood have different magnetic properties which makes it possible to visualize the active areas of the brain based on how much blood is present in that area. It provides identification of targeted areas as well as being able to investigate deep structures. However, an fMRI cannot be taken live during an operation and aligning an fMRI image taken before an operation with the view of the brain during an operation can be complicated by brain shift and swelling. As such, fMRI has an important role in preoperative planning and scientific research but not in the operating room.

ISOI is a brain mapping method that overcomes many of these weaknesses and has the advantage of potential use during an operation, allowing direct visualization of functional brain areas. It takes advantage of the spectral properties of haemoglobin which has different absorption properties when oxygenated or deoxygenated. Using a

scientific camera, neuronal activity is measured by imaging changes in light reflectance of the blood to visualise where the oxygenated blood, and therefore neuronal activity, is located. This is, however, impossible without directly exposing the brain.

So far, ISOI has been used successfully in animal research offering high spatial (~100 μm) and temporal (100 ms) resolution to detect physiological information such as blood flow and oxygen consumption. This has led to important breakthroughs in the organization, physiology, and pathophysiology of the brain. It's hoped that this could lead to the potential clinical use of ISOI in humans.

ISOI Principle

Imaging the intrinsic signal is performed by illuminating the primary cortex with wavelengths between 500 and 650 nm (Figure 1). To get access to the primary cortex, parts of the skull need to be removed, or the bone can theoretically be maximally thinned down. Repeated measurements are required to obtain a reliable signal detection and localization so a window can be permanently implanted. The window could correct the intrinsic curvature of the cortex but applying pressure on the underlying tissue can result in unwanted artefacts making physiologically relevant measurements problematic.

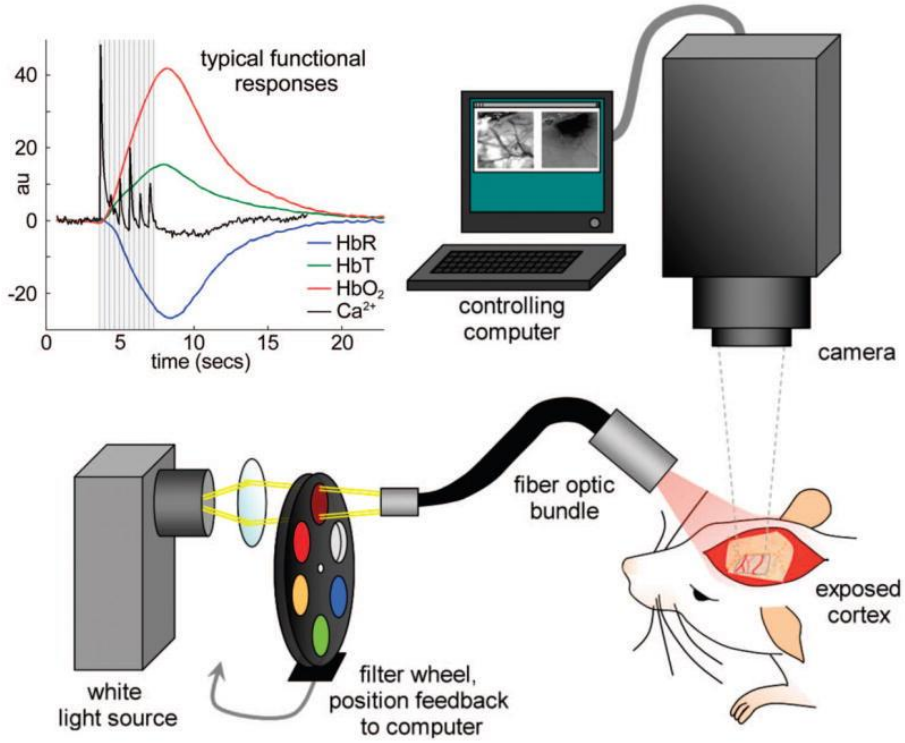

Figure 1: Typical setup for camera-based imaging of the exposed cortex. Inset shows typical hemodynamic and calcium-sensitive responses in rat somatosensory cortex to ~1-mA forepaw stimulus delivered in 3 ms pulses at 3 Hz for 4 s. (modified from Hillman EMC, 2007)

The most important reporter used is haemoglobin which has different absorption properties when it is oxygenated or de-oxygenated. The general signal for blood flow is imaged by exciting at 540 nm whereas the de-oxygenated excitation is imaged at 600 nm. As the extinction coefficient of de-oxygenated blood is higher at this wavelength compared to oxygenated, less light will be reflected and reach the camera. In this way, areas of the brain with higher blood flow can be localized.

The results are based on calculating the difference between an unstimulated baseline and various time points recorded post stimulation (ΔR/R). As signals can be very marginal, the real signal will become obvious only after multiple repetitions of the experiment and averaging across the same time points. Several trials with a repetition of the stimulus paradigm have to be averaged.

Structural changes of the tissue can have a large influence on the reflective properties and hence the outcome of the measurement. During stimulation of the animal, brain areas of interest will enlarge their volume also caused by fusion of secretory vesicles with the pre-synaptic membrane (exocytosis). The excess membrane will be retrieved sooner or later by endocytosis and the tissue volume will normalize again. But for this reason, it is necessary to collect multiple images and use averaging to determine whether the reflective properties are truly indicative of changes in blood characteristics.

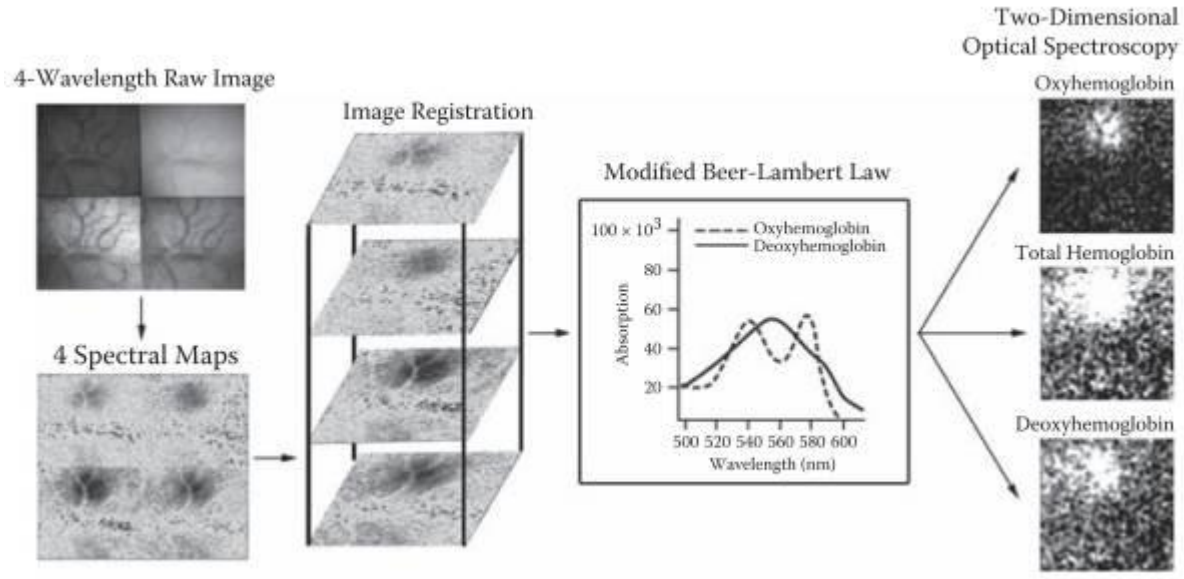

Figure 2: 2D optical spectroscopy (2DOS). Images are collected simultaneously at four wavelengths (560 nm, 570 nm, 577 nm, 610 nm) using a four-way beam splitter. Images are spatially registered, and grayscale changes are normalized. Each registered pixel thus has four grayscale values (one for each wavelength) which change over time. For each pixel at each time point, least squares analysis is used to fit the measured absorbance changes to changes in oxy- and deoxyhemoglobin concentration using a modified form of the Beer–Lambert law. Oxy- and deoxyhemoglobin absorption coefficients for each wavelength are derived from an in vitro phantom, thus accounting for wavelength-dependent path differences in the brain. (from Brennan KC and Toga AW, 2009)

To perform ISOI, a mechanically stable setup is required as well as a light source with stable output characteristics. Previously, tungsten-based light sources with nearly no fluctuations in intensity were used but they could result in a reduced signal-to-noise ratio as the variability of the signal could be partially attributed to those fluctuations. Today, the better options are state-of-the-art LED illumination devices which offer high flexibility in terms of wavelength and higher stability over the time course of an experiment.

The biggest problem is to distinguish stimulus-dependent changes in activity from basic activity. Furthermore, although this technique gives unprecedented access in time and space to a brains activity, it'll only enable the researcher to visualize primary cortices. The imaging depth is dependent on the penetration depth of the used light and typically not more than 500 μm. Areas of interest which are below the cortical surface are out of reach for this technique.

Conclusion

ISOI is capable of fast, contract-free data acquisition using a standard scientific camera with high spatial resolution. It is used in research as a near to none invasive technique and also for diagnostic purposes. Most functional imaging techniques, such as fMRI, have very coarse spatial resolution whereas ISOI is very fine. Furthermore, electrophysiological methods such as single cell recordings may provide the researcher with excellent knowledge about the individual signal but don't show the functional context which ISOI does.

Results from ISOI generally correlate well with results obtained from ESM, EEG and fMRI, highlighting its effectiveness as a technique.

ISOI Camera Choice

ISOI relies on the detection of signal changes which can occur on the scale of <1% of the signal intensity. Therefore, a high signal to noise ratio to ensure signal detection and a large full-well capacity to detect small signal changes are some of the most desired characteristics of the camera.

Most commonly, CCD cameras are used for ISOI. The high quantum efficiency and broadband sensitivity provide a strong imaging performance. However, CCD cameras tend to have high read noise characteristics which necessitate longer exposure times to have a high enough signal to noise ratio to reliably detect the small changes in signal. Long exposure times are a problem when imaging the live brain because brain movement results in blurred images. The small full-well capacity of CCD cameras can also be an issue for some ISOI applications. For these reasons, some Scientific CMOS cameras are also used.

Scientific CMOS cameras typically have equivalent or better quantum efficiency (up to 95%) than modern CCD cameras with far lower read noise characteristics, giving a much-improved signal to noise ratio. The full-well capacity is also equivalent or better and because of the lower noise characteristics the dynamic range of scientific CMOS cameras is therefore higher.

Most modern scientific CMOS cameras also boast larger fields of view (up to 25 mm diagonal) and higher speeds (up to 100 fps, can be made faster by choosing smaller regions). These are typically less important features for ISOI but may be useful for some implementations.

For most ISOI applications, CCD cameras are ideal but for those applications where higher sensitivity and dynamic range are required, Scientific CMOS cameras may be the better choice.

References

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