Supporting Information

Data acquisition

A commercialized, continuous-wave, functional NIR brain imager (DYNOT, NIRx Medical Technologies, USA) was used to measure hemodynamics of the rat brain. DYNOT can drive up to 31 bifurcated optical optodes and utilize two wavelengths of near infrared light (760 and 830 nm). Signals at two wavelengths were acquired simultaneously and independently. During one data acquisition cycle, each optode was used as light source once, in an order from Optode 1 to the last one. When an optode served as a source, all other optodes (including the source optode itself) detected reflectance of light simultaneously at different locations. The light propagation path is illustrated in Figure 1A, where two detectors are receiving optical signals that are emitted from a source optode. The optical penetration depth is a function of the source-detector separation (Feng et al. 1995). The wider the separation, the deeper the optical signal could reach.

Optode-array arrangement for rat brain measurement

In the rat measurement, 26 optodes (2 mm in diameter) were used and arranged by a plastic frame in a horizontal plane (Figures 1B and 1C). A total number of 676 (26 X 26) data points (signals from all source-detector pairs) were gathered per each data acquisition cycle at one wavelength of light. The sampling rate was about 2 data acquisition cycles per second (2 Hz). This 26-optode array covered a rectangular region with an anterior-to-posterior distance of 16.1 mm and a left-to-right distance of 16.4 mm (Figure 1C). To ensure consistency of array placement, the most posterior row of the array (Optodes 23-26) was aligned by the lambdoid suture; the two middle probes (Optodes 24 and 25) were evenly sided from the midline suture

(shown in Figure 1B). The last check was to make sure Optode 2 on the midline suture (Figure 1B). All of the optodes were in good contact with the rat skull, and a few drops of mineral oil (Equate, US) were applied before the array placement to avoid air gap between optodes and the skull surface.

Image reconstruction: tomography

A NIR data processing program, HOMER (PMI, US), was used to create tomography. This software (Huppert et al. 2009) is based on a widely accepted mathematical model of diffuse light, which is based on two assumptions: (a) the light scattering coefficient (μ_s') of the target (either rat brain or tissue phantom) is constant and much larger than its absorption coefficient (μ_a), and (b) both the spatial and temporal variation of μ_a are small (Durduran et al. 1997; Li et al. 2004). Thus, a change in optical density (ΔOD) is mainly determined by the absorption changes in oxy- (Δ[HbO]) and deoxy- hemoglobin concentration (Δ[Hb]) along the photon pathway inside the tissue, as shown by Eq. 1,

$$\begin{bmatrix} \Delta OD_{\lambda_1} \\ \Delta OD_{\lambda_2} \end{bmatrix} = A \cdot \begin{bmatrix} \Delta[HbO] \\ \Delta[Hb] \end{bmatrix}, \quad (1)$$

where ΔOD is wavelength dependent, and A is a transfer function (a constant matrix) that describes direct relationships between changes in optical densities and hemodynamic chromosphere concentrations (Kim et al. 2005; Li et al. 2004). Since Matrix A is associated with light extinction coefficients of hemoglobin and can be found in literature, [HbO] and [Hb] changes could be solved by multiplying the inversed A on both sides of Eq. 1. To ensure that [HbO] and [Hb] changes are solvable, two or more wavelengths of optical measures are needed.

A diffusion equation has been widely accepted as a photon migration model to quantify the photon density ($P(\boldsymbol{r}, t)$) in diffusive turbid media (Arridge 1999),

47 $$\frac{1}{\boldsymbol{c}}\frac{\partial}{\partial \boldsymbol{t}}\boldsymbol{P}(\mathrm{r},\boldsymbol{t}) - \boldsymbol{D}\nabla^2\boldsymbol{P}(\mathrm{r},\boldsymbol{t}) + \mu_{\boldsymbol{a}} \cdot \boldsymbol{P}(\mathrm{r},\boldsymbol{t}) = \boldsymbol{S}(\mathrm{r},\boldsymbol{t}), \quad (2)$$

48 where c is the light speed in a tissue, $\boldsymbol{r}$ a vector representing a relative distance to the origin (0,0),
49 t the time, D the diffusion coefficient, and S the light source. According to Eq. 2, a photon
50 density at any 3-D location and any time can be determined if all other variables are known. In
51 other words, Eq. 2 describes a quantitative relationship between a regional photon density and
52 the light source.

53 In our study, the measured changes (i.e., ΔOD) by various source-detector pairs can be
54 written as,

55 $$\begin{bmatrix} \Delta OD(\mathrm{Ch}\,1) \\ \dots \\ \Delta OD(\mathrm{Ch}\,n) \end{bmatrix}_{z=0} = B \cdot \begin{bmatrix} \Delta S(x_1 y_1) & \dots & \Delta S(x_1 y_m) \\ \dots & \dots & \dots \\ \Delta S(x_l y_1) & \dots & \Delta S(x_l y_m) \end{bmatrix}_{z=k}, \quad (3)$$

56 where ΔOD(Ch i) is a change in optical density captured by a detector at the tissue surface ($z = 0$)
57 from a source-detector pair, labeled as channel i (i = 1...n); B is a transfer function determined by
58 Eq. 2; ΔS is a perturbation distribution in μ_a at a certain depth along z-axis ($z = k$) inside the
59 tissue. Eq. 3 indicates that if an absorption perturbation distribution is known, respective changes
60 in optical density measured from different source-detector pairs at the tissue surface ($z = 0$) can
61 be determined. Inversely, from a backward perspective, a tissue absorption perturbation map is
62 solvable under two conditions: changes in optical density are known or measured at the air-tissue
63 boundary ($z = 0$); and the inverse of Matrix B exists. However, solving Eq. (3) inversely is ill-
64 posed due to a limited number of optical measurements the surface and the strong attenuation of
65 light in deeper tissue. One of solutions to the inverse problem is to regularize Matrix B. In our
66 study, a regularization method was selected in HOMER (Li et al. 2004). After the tissue

absorption perturbation map at a chosen depth is obtained, changes in hemodynamic parameters, such as Δ[HbO] and Δ[Hb], at each pixel can be further determined by Eq. 1.

The general scheme to produce a tomographic image of the rat's brain was to: (a) convert the DYNOT outputs to a specific format to be compatible with HOMER; (b) normalize each channel with respect to the co-located source-detector channel (i.e., source-detector separation = 0 for the latter channel); (c) adjust contra-and-ipsilateral presentations (i.e., always put the left side of image as the contralateral); (d) set a low pass filter with a cutoff frequency at 0.1 Hz; (e) set the 5-s measure before stimulation as baseline; (f) for mechanical stimuli, average 5 consecutive blocks for each animal; (g) determine a reconstruction depth for a specific region of interest; (h) generate reconstructed images (21-by-21-pixels) at two wavelengths, respectively; and (i) use Eq. 1 to get hemodynamic responses in terms of Δ[HbO], Δ[Hb], and Δ[HbT] (i.e., Δ[HbO] + Δ[Hb]) in an arbitrary unit. The pixel size was 0.77 X 0.78 mm^2 (divide the *x*- or *y*-axis length by 21). All processing procedures were completed in either HOMER or Matlab (MathWorks, US).

Image reconstruction: topography

Topography is a simple way to interpret hemodynamic responses. As shown in Figure 1A, the signal from one source-detector pair reflects general hemodynamic responses within the associated banana-shaped photon path. By using properly selected pairs, a 2-D hemodynamic response image can be obtained. One assumption is made that the light intensity change is primarily due to the absorption perturbation, but not due to the light scattering change.

Twelve source-detector pairs were used (Figure 1D). Of those pairs, Optodes 10, 15, and 20 (on the midline) served as sources, and the rest of the chosen optodes (Optodes 1, 4, 8, 13, 18,

23 on the left and Optodes 3, 7, 12, 17, 22, and 26 on the right) acted as detectors (Figure 1B). The distance between the sources and detectors ranged from 6.5 to 8.2 mm. The brain image was, therefore, divided into 12 subdivisions (Figure 1D). Hemodynamic responses in each subdivision were only determined by one geometrically affiliated source-detector pair. The signal change in a subdivision represents a comprehensive effect of all local optical property changes (i.e., absorption) along the full length of the photon pathway depicted in Figure 1A.

The general scheme to produce a topography image was to: (a) convert the DYNOT outputs into logarithmic values for each of different source-detector pairs; (b) normalize each channel by subtracting the baseline measure (i.e., the median value of 5-s measure prior to injection); (c) run a median filter (every 20 data points, or 10 s); (d) adjust contra-and-ipsilateral presentations (i.e., always align the left side of an image as the contralateral); and (e) use Eq. 1 to obtain the hemodynamics. Temporal resolution of the topographic image was then reset at 10 s (0.1 Hz) due to the temporal averaging (i.e., the median filter). Topography was generated in Matlab.

Depth location of an activated area in NIR tomography

It is important to realize that the current fNIRI results in only 2-D imaging and is capable to form only lateral images accurately, without the ability to localize the depth of activation. In general, the depth localization of brain activation is pre-determined or chosen approximately based on actual human or animal neural anatomy (Boas and Dale 2005) with the assumption that the hemodynamic changes come only from the cortical (for both human and animal) and sub-cortical (for animal only) structures. In addition, recent efforts have been made in developing regularization-modified algorithms in order to quantify depth localization and thus to form 3D

fNIRI images (Culver et al. 2003; Niu et al. 2010b; Pogue et al. 1999). However, this study does not plan to involve such mathematical advancement and fuses on 2D-based fNIRI. Specifically, based on rat's brain anatomy, we selected depth=~5 mm during image reconstructions to form respective fNIR images at statistically significant levels (Figs. 2 and 3 in the paper).

Although our method (using a 26-optode array) can detect a cerebrovascular perturbation up to 1 cm below the rat's dorsal skull, which is supported by both theoretical calculations (Feng et al. 1995) and tissue phantom experiments (data not shown), we are not able to provide the exact physical depth where a hemodynamic response occurred in 2D tomography. Thus, our current work focused on the performance of fNIR and the interpretation of fNIR-based hemodynamics on the lateral plane. Facing the limitation of the depth resolution, we dampened discussions on individual regions of interest, but emphasized a synergistic picture of several regions of interest in response to different peripheral stimulations. In fact, the depth specificity of fNIR-based hemodynamics is under active research. A recent development (Niu et al. 2010a; Niu et al. 2010b) has introduced a depth compensation algorithm (DCA) to counterbalance the decay nature of light propagation in tissue. Our future studies will validate some advanced reconstruction methods to process the *in vivo* data, and promote this technology as an alternative neuroimaging tool with a capability to produce 3D tomography.

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Figure Caption

Figure 1. General information on optical theory and instrumentations. **A**. Light propagation paths from a source optical probe to two detectors. Photons initially enter into the tissue, traveling through a banana-like pathway, and finally emit out which is sensed by detectors. **B**. The placement of the optode array (containing 26 optodes) above the rat's skull (Paxinos 1998). **C**. Physical dimensions of the 26-optode array and the image orientation: rostral, caudal, contra-, and ipsilateral. In particular, the ipsilateral side is the right.