

## ABSTRACT

We introduce a four-wavelength dynamic optical tomography system for small animal studies. A stereotaxic small animal measuring head was developed, which allows imaging of any body site of the animal. We present a setup for non-invasive dynamic optical imaging of the rat brain. The measuring head as well as the fiberoptic are compatible with in-magnet studies.

We give a detailed description of the implementation of the four-wavelength capability and the newly developed imaging head. A variety of system check procedures are presented that were developed to assess instrument functionality and data integrity for the device. Instrument performance parameters that are obtained with these tools—such as noise, shot, and long-term stability—are given. We also show results from a phantom-based calibration procedure to account for inter-channel variations of the system.

We present initial experimental results from dynamic optical measurements of the physiologic response of the rat brain. The hemodynamic response in the brain for different stimulation sites is discussed. The results demonstrate excellent sensitivity of the instrument for measuring hemodynamics in small animal studies.

## INSTRUMENTATION

The instrument developed is based on the NIRx DYNOD imaging platform, which has been described in detail before [1,2]. Key features of the device include:

- Continuous wave (cw) Diffuse Optical Tomographic (DOT) measurements @ 2-75 frames per second depending on number of sources.
- Time division multiplexing of up to 32 sources (S)
- Parallel readout of up to 32 detectors (D)
- Parallel measurement of up to four frequency-encoded wavelengths (WL)
- Dynamic detection range 1:10<sup>6</sup> (90 dB) using source-synchronized detector gain switching
- Current applications: Monitoring, imaging of peripheral vasculature, functional brain imaging, small animal imaging.

## 1) HARDWARE

### 1.1 Four-Wavelength Capability

The following modifications were made to achieve simultaneous four-color laser operation:

- Model 8000 laser diode controller (Newport Corp.) used in DYNOD accommodates modules for independent control of up to four thermoelectrically cooled laser diodes.
- Lasers used: 725 nm (250 mW<sub>max</sub>), 760 nm, 810 nm, 860 nm (all 400 mW<sub>max</sub>)
- Lasers are mounted in compact "sandwich" configuration to save space and to allow efficient heat cooling with fan.
- DYNOD standard power supply can be used without modification.

The optical switch (OS) is identical in design and function to the device in the previously described dual-wavelength imager [1]. Four-color operation of the OS was achieved by developing a new coupling optics with the following features (see Fig. 1):

- Four-wavelength mixer/combiner combines and focuses laser beams into optical switch.
- Custom made dichroic mirrors (Omni-Optical, Inc.) allow efficient (90% typ) wavelength multiplexing.
- Laser delivery fibers and the dichroic mirrors are individually adjustable (translation, tilt) to achieve optimum beam collinearity and centrality with respect to the OS.

The DYNOD programmable multi-channel optical detector (PMOD) is designed to allow simultaneous detection of up to four frequency-encoded optical signals. The following upgrades to the detection side of the system were performed for four-wavelength operation:

- A second lock-in module was added to each of the 32 detector channel boards to upgrade from dual- to quad-wavelength detection.
- A second phase shifter module was installed into the PMOD to condition the two additional reference signals for lock-in detection.
- To acquire data from up to 128 measurement channels (= 4 WL × 32 detectors), a second data acquisition (DAQ) board (National Instruments PC6033E) was installed into the control PC.
- A new interface board was developed for the PMOD that accommodates connections to the two DAQ boards.

### 1.2 MRI compatible Small Animal Imaging Head

The following hardware developments made the instrument compatible with in-magnet studies:

- 12 fiber long fiber optic cables used for light transmission between instrument and target to keep instrument in safe distance from magnet.
- Illumination handle diameter = 0.8 mm, detection handle diameter 1.0 mm, probe diameter diameter = 2.0 mm
- No metal components used in fiber optics (plastic probe ferrules)
- No metal components used in animal holder
- An imaging head was designed to ensure stable position of the animal and good optical probe contact (see Fig. 2)
- Stereotaxic positioning of the animal using ear bars and bite bar
- Fiber holder holds high-density arrangement of probes (3-mm center-to-center spacing)
- Faux cushion in fiber holder allows easy adjustment of probe pressure
- Special mouth piece bends 30° upward to make contact with roof of the mouth
- Fiber probes integrated into ear bars

## 2) SOFTWARE

The DYNOD control software is implemented in the LabVIEW language (National Instruments, Inc.) and has been described in detail before for the case of dual wavelength operation [2]. The software was upgraded to expand all existing hardware capabilities to the case of four-wavelength data sets.

- Setup Screen: Main function is the automated or manual adjustment of detector gain settings for all source-detector pairs to prepare the instrument for a measurement. Contains "Setup Check" feature to confirm probe contact facility, proper hardware function, and appropriate gain settings.
- Measurement Screen: Displays raw data in real time for all wavelengths.
- System Checkout Screen: Contains a variety of automated or semi-automated data and hardware integrity checks, including:
  - Detector dark noise measurement
  - Optical alignment tools for the optical switch
  - Instrument calibration procedures

## DATA INTEGRITY AND PERFORMANCE CHECK PROCEDURES

Because the imaging problem is ill-posed and under-determined, it is of paramount importance that data collection be performed with the highest precision and accuracy possible:

- Requires all hardware components to be intact and to perform according to specified quality standards.
- Requires proper setup of optical measurement e.g. appropriate gain settings, good probe-tissue contact.
- Large number of data channels (i.e., up to 32S × 32D × 4WL = 4096 channels) requires:
  - Automated or semi-automated test procedures to reduce test time and to increase reliability.
  - Easy-to-read visualization of complex results.

## 1) GAIN SETTINGS

Because of each S-D pair need to be established before each measurement. Channels with improper gain setup may experience excessive noise or signal saturation during the measurement of testing the gain setup cycle.

1. Gain settings for each S-D pair are displayed in a color contour plot (see Fig. 3A). Deviations from application-characteristic patterns is indicative of inappropriate gain setting.
2. Because of the reciprocity of light transport, gains for reciprocal S-D channels, (i.e.,  $S_i \rightarrow D_j$  vs.  $D_j \rightarrow S_i$ ) are expected to be similar. For each S-D pair, the difference in gain for its reciprocal pair is calculated and displayed in a color-encoded contour plot. A difference greater than one gain step is indicative of inappropriate setup (see Fig. 3B).

## 2) CORRELATION ANALYSIS

- For a time series of data, the correlation coefficient  $r$  between reciprocal S-D pairs is computed and graphed in a color contour plot.
- Non-vanishing variation of physiologic data leads to high correlation values for reciprocal channels with high signal-to-noise ratios (see Fig. 4).
- Data are low-pass filtered to mitigate the influence of random detection noise (see Fig. 4B).
- Isolated low values are indicative of bad fiber-tissue contact.

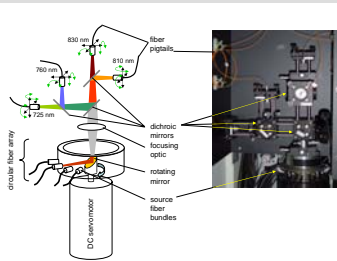


Fig. 1. Optical switch with four-wavelength mixer/combiner.

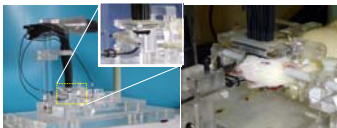


Fig. 2. Stereotaxic rat imaging steps. A: Detailed view of the imaging head and the optical probes. B: Position of animal in the device and placement of optical fibers on the head.

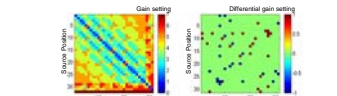


Fig. 3. System setup checks. A: Color encoded display of gain settings; note the symmetric pattern. B: Color encoded display of differences between gain settings of reciprocal channels. Deviation by more than one gain step (shown in red) is indicative of faulty setup.

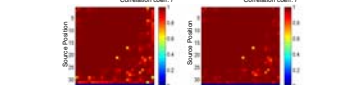


Fig. 4. Correlation values for physiologic time series of reciprocal channels. Strong positive correlation (values greater than 0.9) are expected for reciprocal pairs not showing noise-dominated behavior. A: Unfiltered result. B: Correlation result after low-pass filtering the data. Note the increased values in the lower left compared to A.

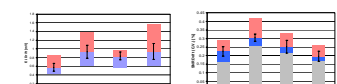


Fig. 5. Long-term offset drift of detectors. Shown for each wavelength are the mean drift, the minimum, the maximum, and the standard deviation of the drift of all channels during three hours of instrument operation. The error bars depict the average drift C.V. of all channels.

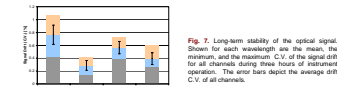


Fig. 6. Long-term drift of noise in the optical signal. Shown for each wavelength are the mean drift, the minimum, the maximum, and the standard deviation of the drift of all channels during three hours of instrument operation. The error bars depict the average drift C.V. of all channels.

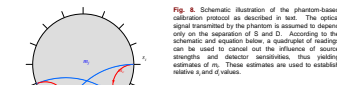


Fig. 7. Long-term stability of the optical signal. Shown for each wavelength are the mean drift, the minimum, the maximum, and the standard deviation of the drift of all channels during three hours of instrument operation. The error bars depict the average drift C.V. of all channels.

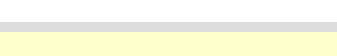


Fig. 8. Schematic illustration of the phantom-based calibration procedure as described in text. The optical signal transmitted by the phantom is assumed to depend only on the separation of S and D. According to the schematic and equation below, a quadrilateral of detectors can be used to cancel out the influence of source strengths and detector sensitivities, thus yielding estimates of  $m_i$ . These estimates are used to calculate relative  $m_i$  and  $d_j$  values.

$$\frac{m_i}{d_j} = \frac{S_i \cdot D_j}{S_j \cdot D_i} \cdot \frac{m_i}{d_j} = \frac{m_i}{d_j} \cdot \frac{S_i \cdot D_j}{S_j \cdot D_i} = \frac{m_i}{d_j} \cdot \frac{S_i \cdot D_j}{S_j \cdot D_i} = \frac{m_i}{d_j} \cdot \frac{S_i \cdot D_j}{S_j \cdot D_i} = \frac{m_i}{d_j} \cdot \frac{S_i \cdot D_j}{S_j \cdot D_i}$$

$$\frac{m_i}{d_j} = \frac{S_i \cdot D_j}{S_j \cdot D_i} \cdot \frac{m_i}{d_j} = \frac{m_i}{d_j} \cdot \frac{S_i \cdot D_j}{S_j \cdot D_i} = \frac{m_i}{d_j} \cdot \frac{S_i \cdot D_j}{S_j \cdot D_i} = \frac{m_i}{d_j} \cdot \frac{S_i \cdot D_j}{S_j \cdot D_i}$$

$$\frac{m_i}{d_j} = \frac{S_i \cdot D_j}{S_j \cdot D_i} \cdot \frac{m_i}{d_j} = \frac{m_i}{d_j} \cdot \frac{S_i \cdot D_j}{S_j \cdot D_i} = \frac{m_i}{d_j} \cdot \frac{S_i \cdot D_j}{S_j \cdot D_i} = \frac{m_i}{d_j} \cdot \frac{S_i \cdot D_j}{S_j \cdot D_i}$$

$$\frac{m_i}{d_j} = \frac{S_i \cdot D_j}{S_j \cdot D_i} \cdot \frac{m_i}{d_j} = \frac{m_i}{d_j} \cdot \frac{S_i \cdot D_j}{S_j \cdot D_i} = \frac{m_i}{d_j} \cdot \frac{S_i \cdot D_j}{S_j \cdot D_i} = \frac{m_i}{d_j} \cdot \frac{S_i \cdot D_j}{S_j \cdot D_i}$$

$$\frac{m_i}{d_j} = \frac{S_i \cdot D_j}{S_j \cdot D_i} \cdot \frac{m_i}{d_j} = \frac{m_i}{d_j} \cdot \frac{S_i \cdot D_j}{S_j \cdot D_i} = \frac{m_i}{d_j} \cdot \frac{S_i \cdot D_j}{S_j \cdot D_i} = \frac{m_i}{d_j} \cdot \frac{S_i \cdot D_j}{S_j \cdot D_i}$$

$$\frac{m_i}{d_j} = \frac{S_i \cdot D_j}{S_j \cdot D_i} \cdot \frac{m_i}{d_j} = \frac{m_i}{d_j} \cdot \frac{S_i \cdot D_j}{S_j \cdot D_i} = \frac{m_i}{d_j} \cdot \frac{S_i \cdot D_j}{S_j \cdot D_i} = \frac{m_i}{d_j} \cdot \frac{S_i \cdot D_j}{S_j \cdot D_i}$$

$$\frac{m_i}{d_j} = \frac{S_i \cdot D_j}{S_j \cdot D_i} \cdot \frac{m_i}{d_j} = \frac{m_i}{d_j} \cdot \frac{S_i \cdot D_j}{S_j \cdot D_i} = \frac{m_i}{d_j} \cdot \frac{S_i \cdot D_j}{S_j \cdot D_i} = \frac{m_i}{d_j} \cdot \frac{S_i \cdot D_j}{S_j \cdot D_i}$$

$$\frac{m_i}{d_j} = \frac{S_i \cdot D_j}{S_j \cdot D_i} \cdot \frac{m_i}{d_j} = \frac{m_i}{d_j} \cdot \frac{S_i \cdot D_j}{S_j \cdot D_i} = \frac{m_i}{d_j} \cdot \frac{S_i \cdot D_j}{S_j \cdot D_i} = \frac{m_i}{d_j} \cdot \frac{S_i \cdot D_j}{S_j \cdot D_i}$$

$$\frac{m_i}{d_j} = \frac{S_i \cdot D_j}{S_j \cdot D_i} \cdot \frac{m_i}{d_j} = \frac{m_i}{d_j} \cdot \frac{S_i \cdot D_j}{S_j \cdot D_i} = \frac{m_i}{d_j} \cdot \frac{S_i \cdot D_j}{S_j \cdot D_i} = \frac{m_i}{d_j} \cdot \frac{S_i \cdot D_j}{S_j \cdot D_i}$$

$$\frac{m_i}{d_j} = \frac{S_i \cdot D_j}{S_j \cdot D_i} \cdot \frac{m_i}{d_j} = \frac{m_i}{d_j} \cdot \frac{S_i \cdot D_j}{S_j \cdot D_i} = \frac{m_i}{d_j} \cdot \frac{S_i \cdot D_j}{S_j \cdot D_i} = \frac{m_i}{d_j} \cdot \frac{S_i \cdot D_j}{S_j \cdot D_i}$$

$$\frac{m_i}{d_j} = \frac{S_i \cdot D_j}{S_j \cdot D_i} \cdot \frac{m_i}{d_j} = \frac{m_i}{d_j} \cdot \frac{S_i \cdot D_j}{S_j \cdot D_i} = \frac{m_i}{d_j} \cdot \frac{S_i \cdot D_j}{S_j \cdot D_i} = \frac{m_i}{d_j} \cdot \frac{S_i \cdot D_j}{S_j \cdot D_i}$$

$$\frac{m_i}{d_j} = \frac{S_i \cdot D_j}{S_j \cdot D_i} \cdot \frac{m_i}{d_j} = \frac{m_i}{d_j} \cdot \frac{S_i \cdot D_j}{S_j \cdot D_i} = \frac{m_i}{d_j} \cdot \frac{S_i \cdot D_j}{S_j \cdot D_i} = \frac{m_i}{d_j} \cdot \frac{S_i \cdot D_j}{S_j \cdot D_i}$$

$$\frac{m_i}{d_j} = \frac{S_i \cdot D_j}{S_j \cdot D_i} \cdot \frac{m_i}{d_j} = \frac{m_i}{d_j} \cdot \frac{S_i \cdot D_j}{S_j \cdot D_i} = \frac{m_i}{d_j} \cdot \frac{S_i \cdot D_j}{S_j \cdot D_i} = \frac{m_i}{d_j} \cdot \frac{S_i \cdot D_j}{S_j \cdot D_i}$$

$$\frac{m_i}{d_j} = \frac{S_i \cdot D_j}{S_j \cdot D_i} \cdot \frac{m_i}{d_j} = \frac{m_i}{d_j} \cdot \frac{S_i \cdot D_j}{S_j \cdot D_i} = \frac{m_i}{d_j} \cdot \frac{S_i \cdot D_j}{S_j \cdot D_i} = \frac{m_i}{d_j} \cdot \frac{S_i \cdot D_j}{S_j \cdot D_i}$$

$$\frac{m_i}{d_j} = \frac{S_i \cdot D_j}{S_j \cdot D_i} \cdot \frac{m_i}{d_j} = \frac{m_i}{d_j} \cdot \frac{S_i \cdot D_j}{S_j \cdot D_i} = \frac{m_i}{d_j} \cdot \frac{S_i \cdot D_j}{S_j \cdot D_i} = \frac{m_i}{d_j} \cdot \frac{S_i \cdot D_j}{S_j \cdot D_i}$$

Fig. 4.7 illustrate the measured noise and drift performance of the instrument.

## INSTRUMENT PERFORMANCE

### 1) Detector electronics noise/stability

Dark noise test of detector electronics:

- Detection of:
  - Component failure/laging in PMOD.
  - Problems with DAQ electronics
  - Detrioration of grounding connections, shielding
  - External sources of EM interference
  - Dark current is measured for each channel dependent on gain settings.
  - Display of noise level (STD) and noise power spectrum.

### 2) Illumination noise/stability

- Illumination noise and drift is caused by:
  - Laser instabilities:
    - Typically long term (sec - min)
    - Minimized by TEC control of laser operating temperature
    - Corrected by monitoring laser output power
  - Variation of the incohering efficiency of the OS
  - Long term: thermal effects (heating of OS motor)
  - Short term: Frame-to-frame variations in illumination strength due to repeatability limits of the mechanical beam-steering mechanism.

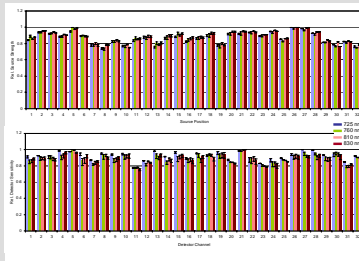


Fig. 9. Calibration results for 4-wavelength imager. A, B: coefficients for four calibration measurements performed over the course of two days. All calibrations were performed on a 2.0mm dia (OD 65 mm, ID 27 mm) filled with diluted Intralipid (LI). LI concentration was 0.5% for measurements 1,3 and 10% for the last measurement. Error bars indicate minimum and maximum values of the four experiments. B: grayscale for each wavelength.

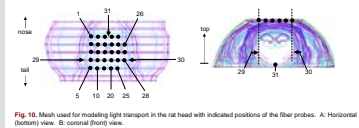


Fig. 10. Mesh used for modeling light transport in the rat head with indicated positions of the fiber probes. A: Horizontal cross-section view. B: coronal frontal view.

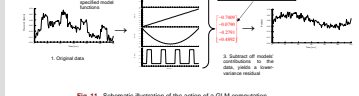


Fig. 11. Schematic illustration of the action of a GLM computation.

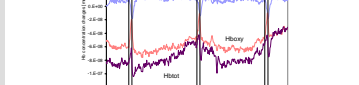


Fig. 12. Time series of the spatially averaged imaging results for the three Hb states. Shown are three consecutive epochs of left front paw stimulation (by averaging over multiple spots). Indicated are start and end of stimulation epochs. An early increase in HbO2 can be seen, followed by a delayed decrease in Hb. The rise in HbO2 ends at the cessation of the stimulus, while HbO2 shows delayed peaking. A biphasic HbR response is observed.

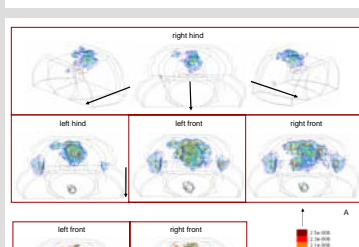


Fig. 13. GLM results for the reconstructed image time series for HbO2 (A) and HbR (B). Shown are spatial maps of the GLM coefficients for the four fiber channels representing periods of brain activation as estimated from the spatial averages of the reconstructed images. The top row in A shows three different views to illustrate the spatial position of the activated area, and the bottom row shows it in a top-down view of the head. Arrows point toward the nose.



Fig. 14. Correlating sum of the number of image nodes versus change in Hb state. Results are shown for the four fiber channels (Right, Left, Front, Back) for HbO2 and HbR (top), and for HbO2 and HbR (bottom). The graphs indicate clear differences in the hemodynamic response to different stimulation sites. For example, a HbO2 change of 0.25 μmol/l or stronger is seen in ~5% of the pixels for HbR stimulation and in ~10% of the pixels for HbR stimulation. Likewise, a HbO2 increase of 0.25 μmol/l more is found in 6% of the image values for HbR stimulation and in 18% of the pixels for the HbR measurement.

## 3) Calibration protocol

A phantom measurement based calibration procedure has been introduced before, which allows determination of the relative inter-channel variations of the imager [3]:

- For a symmetric phantom, the light intensity transmitted from a source to a detector depends only on S-D separation (see Fig. 5).
- Deviations in the measured readings for different S-D pairs having the same probe separation can be attributed to instrumentation influences. These are numerically separated into differences in source strengths (expressed in source strength factors,  $0 \leq S_i \leq 1$ ) and differences in detector sensitivity (expressed in detector sensitivity factors,  $0 \leq D_j \leq 1$ ).

For equalization placement of the probes along the perimeter of a cylindrical phantom (see Fig. 5), the measured signals can be modeled by matrix equation  $\mathbf{R} = \mathbf{M} \cdot \mathbf{D}$  (R: matrix of the measured readings; M: matrix containing the fraction of intensity transmitted from S to D through the medium; S, D: Matrices of source strength coefficients and detector sensitivity factors, respectively.)

- For a symmetric medium, M is always a constant-diagonal (TOPLITE) matrix.
- For a circular geometry, the symmetry of M can be used to obtain estimates of the entries  $m_{ij}$  of a matrix  $\mathbf{M}^T = \mathbf{M}$ .
- By creating the ratio  $r_{ij} = m_{ij} / m_{ji} = a_i / d_j$  and dividing each resulting element by its row sum, a number proportional to  $d_j$  is obtained.  $d_j = m_{ij} / r_{ij}$ . Likewise, dividing elements  $a_{ij}$  by their column sum yields estimates of the source strength coefficients  $r_{ij} = a_i / s_j$ .

An electronic calibration was implemented to account for relative inter-channel variation of the detector gain settings.

Fig. 5 shows calibration results for the 4-WL imager.

## FOUR-COLOR OPTICAL IMAGING OF THE RAT BRAIN

### 1) METHODS

1. Experimental protocol
  - A male rat (approx. 400 g) was anesthetized, intubated, and positioned in the stereotaxic imaging head as seen in Fig. 2.
  - 31 fiber optic probes (placed on the head as schematically indicated in Fig. 10):
    - 28 probes (channels numbers 1-28) were placed on the top of the head in a five-by-eight rectangular grid with two corner positions left unoccupied.
    - Two probes (nos. 29, 30) were placed in the ears.
    - One probe (nos. 31) was placed inside the mouth.
  - After instrument setup, a baseline measurement was performed for the duration of 1300 frames (~ 12 min).
  - The baseline measurement was followed by electric stimulation of each of the four paws in the order right hand, left hand, right front, left front. Each paw was stimulated for six epochs which were applied every 150 imaging frames (79 s), with each epoch lasting six images (frames ~ 3 s). Stimulation was performed with 5 mA ac current (10 Hz).

### ii. Data analysis and image reconstruction

1. Data pre-processing:
  - Coefficients of variation (CV) were computed for all channels, and channels having CV values larger than 15% were excluded from further processing.
  - Raw data in the remaining channels were corrected for variations in laser power, which is monitored during the experiment.
  - For each channel, readings were normalized to the temporal mean value during the initial baseline period.
  - Data are subjected to an adaptive median filter to remove instantaneous negative spikes caused by measurement noise.
2. Image reconstruction
  - The weight matrix was computed using a finite element method (FEM) to numerically solve the diffusion equation. The FEM mesh (shown in Fig. 10) representing the anatomy of the rat brain was adapted from [4]. For the current calculations, an optically homogeneous model was used.
  - Image reconstruction was achieved using the Normalized-Difference Method [5].
  - Truncated singular value decomposition (SVD) is used to solve the linear perturbation equation [6].
  - The reconstructed absorption coefficient images were used to compute image time series for oxygenated, deoxygenated, and total hemoglobin (HbO2, Hb, HbR, respectively) concentration changes.
3. Post-processing
  - A general linear model (GLM algorithm [7]) was used to find the best fit of each image time series to a linear combination of four model functions, as schematically illustrated in Fig. 11.
  - The four model functions used were a constant (offset) term, linear and quadratic baseline drift functions, and a two-state boxcar function representing the idealized hemodynamic response of the brain to the stimulation.
  - The boxcar function was generated to reflect the periods of brain activation as determined from the raw spatial-averaged detector time series.
  - The GLM coefficients for the boxcar function yield a representation of the spatially varying hemodynamic response to the stimulation.
  - No averaging over multiple epochs was performed for the analysis.

### ii. Hemoglobin state time series

Fig. 12 shows the spatial average of the reconstructed image time series for the three Hb states during three consecutive stimulation epochs of the left front paw. Onset and cessation of each epoch are indicated.

- HbO2 concentration shows increase during stimulation:
  - Immediate rise (within one time frame = 0.5 s) at the beginning of stimulus.
  - Steady increase during stimulation, peaking at the end of epoch.
  - Return to baseline starts immediately at end of epoch; return time ~ rise time.
- Hb concentration shows decrease during stimulation:
  - Similar (but inverted) onset and duration of the HbO2 curve.
  - Notable consistent time delay (~ one frame = 0.5 s) between stimulation and response start.
  - Notable consistent time delay (~ one frame = 0.5 s) between stimulation end and response peak.
- HbR concentration shows biphasic behavior:
  - Immediate rise (within one time frame = 0.5 s) at epoch start.
  - Peaking after 4-5 frames (i.e. 2-3 s).
  - Underneath of baseline following stimulation.

### iii. Reconstructed images

- The HbRD changes during stimulation occur primarily in a central region of the head that coincides with the location of the cortex. Some HbR response can be seen in the outer regions of the head.
- Classic somatosensory activation is expected to be centralized for hind paw stimulation and lateralized for front paw stimulation. Right side stimulation is expected to activate the left portion of the cortex, and vice versa. Some spatial variation of the response to different stimulation sites—mostly in the extent of the response—is seen, but a clear somatosensory localization cannot be conclusively demonstrated.
- To gain a more quantitative understanding of the spatial extent of activation, we calculated the cumulative sum of image nodes showing a given change in Hb state (Fig. 14). The results show significant variation in the activated brain region for different stimulation sites.

## CONCLUSION

- We presented data demonstrating the short and long term precision of the newly developed rat imager. Physiologic time series results show excellent measurement sensitivity to event related changes in Hb states in the rat brain.
- A phantom based calibration protocol and calibration results were presented.
- We demonstrated the capability of measuring individual activation epochs with high repeatability and signal quality allowing analysis of individual events without the requirement of averaging over multiple epochs.
- We proposed the following next steps to observe somatosensory localization of brain activity in the reconstruction results:
  - The stimulation parameters such as amplitude and duration need to be explored further.
  - We plan the refinement of the analysis. Possible strategies include the use of more sophisticated model functions, the reconstruction of temporal features (such as delay times), the application of rate equations, and the introduction of a more accurate optical model of the rat head.

## REFERENCES

[1] C. H. Schmitz, M. Leeker, J. M. Lankford, A. H. Heflinger, R. L. Barbour, "Tomographic for fast functional optical tomography," *Proc. of SPIE*, Vol. 4939, pp. 4939-4939 (2002).

[2] C. H. Schmitz, H. L. Graber, R. L. Barbour, J. M. Lankford, A. H. Heflinger, Y. Pei, "A multi-line system for dynamic optical tomography," *Proc. of SPIE*, Vol. 4939, pp. 4939-4939 (2002).

[3] C. H. Schmitz, H. L. Graber, H.