From two-photon microscopy to fMRI: BOLD transients as a function of single-vessel dilation and constriction

Richard B. Buxton*, Peifang Tian†, Ivan C. Teng‡, Kun Lu§, Miriam Scadeng¶, Larry D. May**, Ron Kurz*, David A. Boas†, Anders M. Dale‡ and Anna Devor*†

Departments of Radiology and Neurosciences, University of California, San Diego, CA; 2Martinos Center for Biomedical Imaging, MGH, Harvard Medical School, Charlestown, MA

Summary and conclusions
We used in vivo optical imaging and BOLD fMRI to investigate the transformation of single-vessel diameter and blood flow velocity changes across vascular compartments into depth-resolved BOLD signal change. Two-photon microvascular measurements in cortical layers I-III (down to ~550 μm), CCD-based optical imaging of blood oxygenation/flow and cortical layer-resolved BOLD fMRI were performed under identical conditions in a chloralose-anesthetized rats responding to forepaw stimulation (~4mA, 300 usec, 3 Hz, 2 or 20 sec).

Our BOLD results indicate that:
1) The earliest rise of the positive BOLD response is observed in middle cortical layers (consistent with Silva&Koretzky PNAS 2002)
2) The initial dip is confined to top layers
3) BOLD amplitude decreases with increasing cortical depth
4) In the cortex with our previous study; there is a negative transient response in the contralateral primary somatosensory cortex (SI) accompanied by a negative response in ipsilateral SI
5) There is a post-stimulus undershoot in response to both 2- and 20-sec stimulus

These results are in register with 2-photon measurements of the layers I-III showing that:
1) The deepest measured arterioles and capillaries have the fastest dilation onset and time-to-peak
2) Dilation response of the capillary bed in top layers is delayed relative to the deeper layers
3) The largest dilation is observed at the surface arterioles
4) There is more constriction in the surround region of contralateral SI and virtually only constriction in the ipsilateral SI
5) There is a post-stimulus constriction of arterioles with the temporal profile of the diameter change closely resembling that of the BOLD post-stimulus undershoot.

In addition, our results show no evidence for swelling of the venous vessels down to 550 μm.

Methods
Animals: Cannulas were inserted in the femoral artery and vein. Anesthesia was maintained with 50 mg/kg intravenous bolus of α-chloralose followed by continuous intravenous infusion at 40 mg/kg·h−1. HR, BP and body temperature were continuously monitored. Respiration was maintained with 35% and 45 mHg. In CCD optical imaging experiments an area of skull overlying SI was exposed and the skull was thinned. In 2-photon experiments the skull and dura mater were removed, and the space between the exposed brain surface and the coverglass was filled with ~1% (v/v) agarose/medium in ACSF.

Image acquisition:
2-photon microscopy: 1) 77×0.5 cm horizontal bore scanner (BioSpec 70/30 USR, Bruker)
2) EPI sequence
3) FOV = from 1.64 mm to 2.64 mm
4) Flip angle = 30°
5) In-plane resolution = from 200x500 μm to 300x500 μm
6) Matrix = 80x80
7) Slice thickness = 1 mm
8) TR = 1 sec
9) 5 adjacent slices
10) 10-mm diameter transmit/receive surface RF coil positioned over the SI
11) coronal slices

2-photon imaging is described in [1,2]. ~0.3 ml of 5% (w/v) solution of 2M Dexasfluorescein-conjugated dextran (FD-2000S, Sigma) in saline was injected i.v. Images were obtained using 4-chip Ultima 2-photon microscopy system from Prairie Technologies using 900 nm illumination.

CCT imaging is described in [1,2]. Spectral white light from a tungsten-halogen source was directed through a 6-position rotating filter wheel (560, 570, 580, 590, 600 and 610 nm). Images were acquired by cooled 12 bit CCD camera at ~15Hz. The spectral data were converted to percent change maps for oxy-, deoxy- and total hemoglobin (HbO, Hb and HbT). Spectral: diode laser at 640 nm, 40 mW was used for illumination. Images were acquired by 8 bit CCD camera (~250Hz).

Two-photon imaging is described in [1,2]. Spectral white light from a tungsten-halogen source was directed through a 6-position rotating filter wheel (560, 570, 580, 590, 600 and 610 nm). Images were acquired by cooled 12 bit CCD camera at ~15Hz. The spectral data were converted to percent change maps for oxy-, deoxy- and total hemoglobin (HbO, Hb and HbT). Spectral: diode laser at 640 nm, 40 mW was used for illumination. Images were acquired by 8 bit CCD camera (~250Hz).

Acknowledgements
We gratefully acknowledge support from the NINDS (NS-051188 and NS-057198) and NIBIB (EB-00918) to Anna Devor