Chapter 57
Simultaneous Imaging of Cortical Blood Flow and Haemoglobin Concentration with LASCA and RGB Reflectometry

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Abstract We demonstrate a system for the simultaneous imaging of cortical blood flow and haemoglobin oxygenation by laser speckle contrast analysis (LASCA) and RGB reflectometry. The sensitivity of the system was tested by observing changes of haemoglobin oxygenation and blood flow in rats in response to ischaemic stroke, hypercapnia, hyperoxia, hypoxia, cortical spreading depression and cortical activation following forepaw stimulation.

57.1 Introduction

Optical imaging is widely used as a tool for the assessment of brain function and pathological tissue. Two dominant aims in neurological research are the quantification of cortical haemoglobin oxygenation and blood flow changes as the key parameters for an understanding of neurometabolic-vascular coupling [1]. Illuminating the exposed cortex with a continuous wave light source and observing the backscattered light with a CCD camera is a suitable approach for mapping of haemoglobin changes [2]. Another more reliable spectroscopic solution is accomplished by combining a RGB (red, green, blue)-LED and a RGB-(colour) camera. It has already been shown that RGB reflectometry is able to record small changes in haemoglobin oxygenation associated with neurometabolic and neurovascular coupling [3].

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We now extend this by integrating laser speckle contrast analysis (LASCA) to enable a simultaneous measurement of both parameters, haemoglobin and blood flow changes. For this aim we exploit the fact that these two methods use different spectral ranges. A commercial 2-CCD camera separates the reflected NIR (near infrared) laser light (for LASCA) and the reflected visible light (for haemoglobin) by means of a dichroic prism and images; these different wavelength ranges on two separate CCDs sensors. By the use of GPU (graphical processor unit), computing a temporal resolution of up to 15 Hz is achieved.

The sensitivity of the combined system was tested by imaging haemoglobin oxygenation and blood flow changes in cortical tissue of rats.

57.2 Methods and Setup

57.2.1 RGB Reflectometry

The standard approach for the analysis of reflectance spectra is based on the Lambert-Beer equation. When both the (RGB-) light source and the (CCD-) detector have broad, overlapping spectra, the extinction coefficients matrix of haemoglobin can be expressed by integrating the product of the known parameters of the tissue and spectroscopy system over the wavelength range used:

$$E_{ji} = \int \varepsilon_j(\lambda) \cdot D_i(\lambda) \cdot S(\lambda) \cdot L(\lambda) \cdot d\lambda \tag{57.1}$$

with the index i for the colour sensors of the camera, j for the single chromophores and ε the extinction coefficients of oxygenated and deoxygenated haemoglobin (oxyHb and deoxyHb). $D_i(\lambda)$ is the sensitivity spectra of the camera, $S(\lambda)$ the normalised intensity spectrum of the light source and $L(\lambda)$ the mean optical path length. The unknown concentration changes Δc can then be obtained by matrix inversion from the experimental attenuation changes ΔA: $\Delta c_j = E_{ji}^{-1} \cdot \Delta A_i$.

57.2.2 Laser Speckle Contrast Analysis

When illuminating a surface with coherent laser light, it will appear granular to the observer. This effect is commonly known as speckle effect. A scattering of the laser light by moving particles like blood cells will cause fluctuations of the speckle pattern. If these fluctuations are observed by an integrating system like a camera with fixed exposure time, the pattern will appear blurred. This blurring is generally quantified as speckle contrast SC and defined as the quotient of the standard deviation of the averaged intensity of a small pixel window inside the pattern. Goodman gave a statistical description of speckle patterns and defined the correlation between the

speed of the moving particle and the speckle contrast [4]. More information about the methods used can be found in [5].

57.2.3 Setup of the Imaging System

A 2-CCD camera (AD-080GE, Jai A/S, Denmark; 1,024×768 pixels of 4.65^2 μm^2 size, 12 bit dynamic, 30 frames/s) in conjunction with a standard zoom-camera lens with f = 5.6–32 mm and f/# = 1:5.6 (Computar Corp., Japan) allowed simultaneous imaging of the RGB and NIR wavelength bands.

A ring of six RGB-LEDs (LZ4-00MC10, LedEngin Inc., USA) served as illumination for RGB reflectometry and an AlGaAs laser diode (ADL78901TL, Arima Lasers Corp., Taiwan) with a wavelength of 785 nm and 90 mW for LASCA. The speckle contrast was calculated as the ratio of standard deviation divided by the averaged intensity within sub-windows of 7×7 pixels. Image acquisition and control of all camera functions was programmed in LabVIEW 2010 (National Instruments Inc., USA). The software running on a workstation (i7 950, Intel; GTX 480, NVIDIA) allowed an on-line calculation and display of the haemoglobin and flow images at up to 15 Hz.

57.3 Evaluation Measurements

For evaluation of the combined imaging system, changes in cortical blood flow and haemoglobin concentration were mapped with the combined imaging system. Information about the animal preparations can be found in [5, 6].

57.3.1 Ischaemic Stroke Caused by Injection of Macro-spheres

An ischaemic stroke was induced by injecting two macro-spheres of 300–360 μm in diameter into the carotid artery [6]. Figure 57.1 displays false colour images of changes in deoxyHb, oxyHb and flow for four times (frames) during the first minute after occlusion onset. Haemoglobin parameters and flow are displayed as changes with respect to the baseline before occlusion onset. There is a clear increase in deoxyHb concentration and a decrease both in oxyHb and blood flow for two different areas. A dominating PCAO (posterior cerebral artery occlusion) and a smaller ACAO (anterior cerebral artery occlusion) located nearby could be identified due to the spatially resolved images. The observed pattern of cerebral haemoglobin and blood flow changes is consistent with the physiological concept of an ischaemic reaction following an artery occlusion by the injection of the macro-spheres.

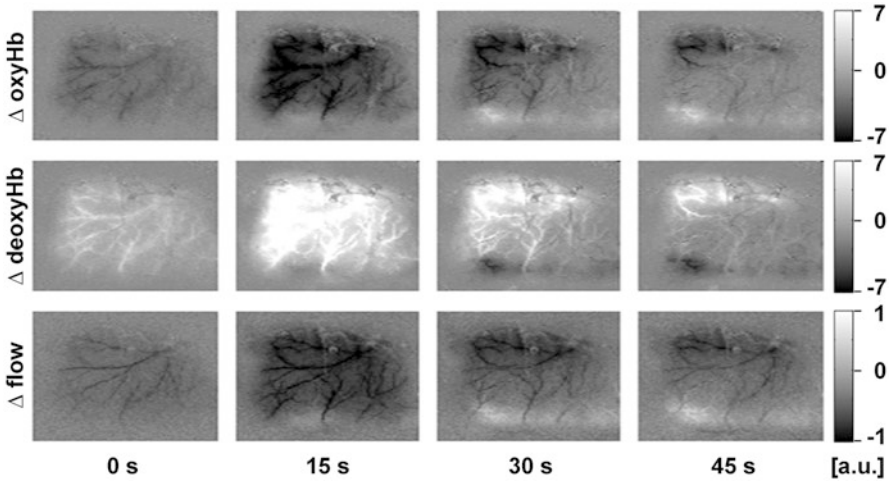

Fig. 57.1 False colour images of deoxyHb, oxyHb and flow changes following anterior cerebral artery occlusion and posterior cerebral artery occlusion caused by two macro-spheres

57.3.2 Hypercapnia, Hyperoxia and Cortical Spreading Depression

The imaging system was further evaluated under different oxygen supply conditions. The experiment was separated into four parts for hypercapnia, hyperoxia, cortical spreading depression (CSD) and hypoxia (Fig. 57.2). The haemoglobin parameters were averaged for three regions of interest (ROIs), with the first two on the left hemisphere close to the excitation point of the CSD and the third on the right hemisphere. During hypercapnia, CO_2 concentration in the supplied air mix was increased to 5 % with a similar systemic effect on the blood parameters in all ROIs. Due to its triggering effect on cortical blood flow, CO_2 and flow are correlated and concurrent with an increase in oxyHb and a decrease in deoxyHb. During the ventilation conditions of hyperoxia and hypoxia (100 % and 0 % O_2 in the supplied air, respectively), there are large changes in the haemoglobin concentrations while the effect on blood flow is small. This experiment demonstrates that the three measurement parameters are independent with no simple correlation between them. While hypercapnia, hyperoxia and hypoxia induce systemic changes, a CSD was triggered by a needle prick to generate a self-propagating wave of depolarization of neurons and its glial cells. This wave spreads over the left hemisphere, which gives rise to a time shift for the signal of the first two ROIs. For the third ROI on the right hemisphere, only very small changes were observed. During CSD the increased blood flow is correlated with an increase in oxyHb and a decrease in deoxyHb.

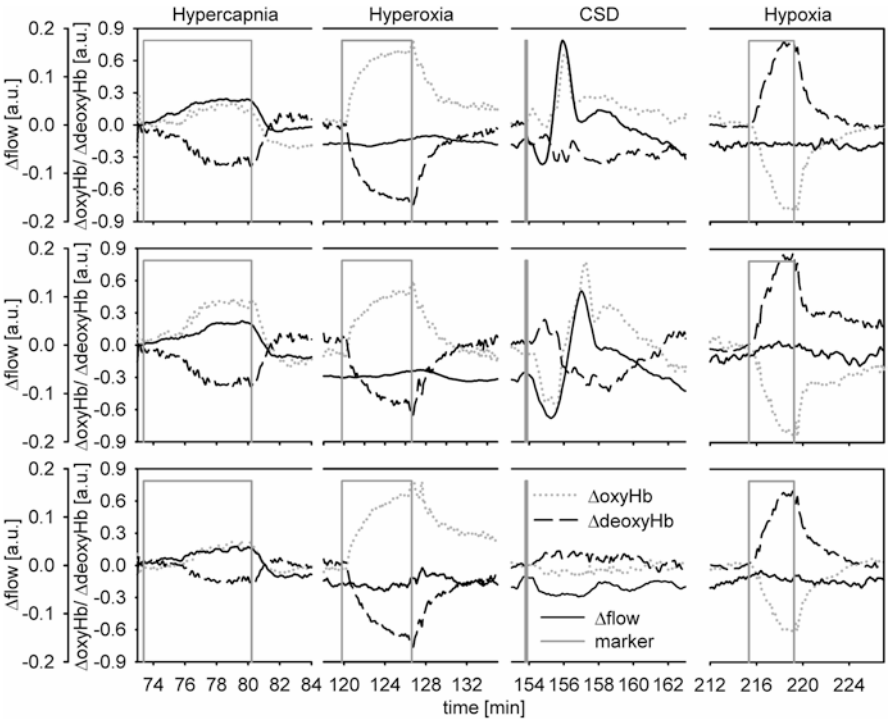

Fig. 57.2 Time course of changes in blood flow as well as oxyHb and deoxyHb averaged for ROIs on the left (*upper* and *middle traces*) and right (*lower part*) hemisphere of a rat during hypercapnia, hyperoxia, cortical spreading depression (*CSD*) and hypoxia

57.3.3 Cortical Activation

To activate a somatosensory stimulation, two needle electrodes were inserted into the skin of the left forepaw between digits 2 and 4 and rectangle pulses (0.3 ms duration at amplitude of 1.6–1.8 mA at 3 Hz) delivered in stimulation trains (length 16–20 s). The interstimulus interval was 45 s to 2 min.

In Fig. 57.3 the haemoglobin and blood flow response following cortical activation are shown for ten stimuli. The p-values (superimposed on the anatomical images) indicate a very high sensitivity combined with a high spatial resolution in the images. When averaging over the ROI, each stimulus is linked to prominent and strong changes in oxyHb, deoxyHb and flow. The observed pattern of a decreased deoxyHb concentration and an increase in both oxyHb and flow is consistent with the concept of neurometabolic-vascular coupling which links blood flow as well as blood volume and oxygenation to the higher oxygen extraction after cortical activation.

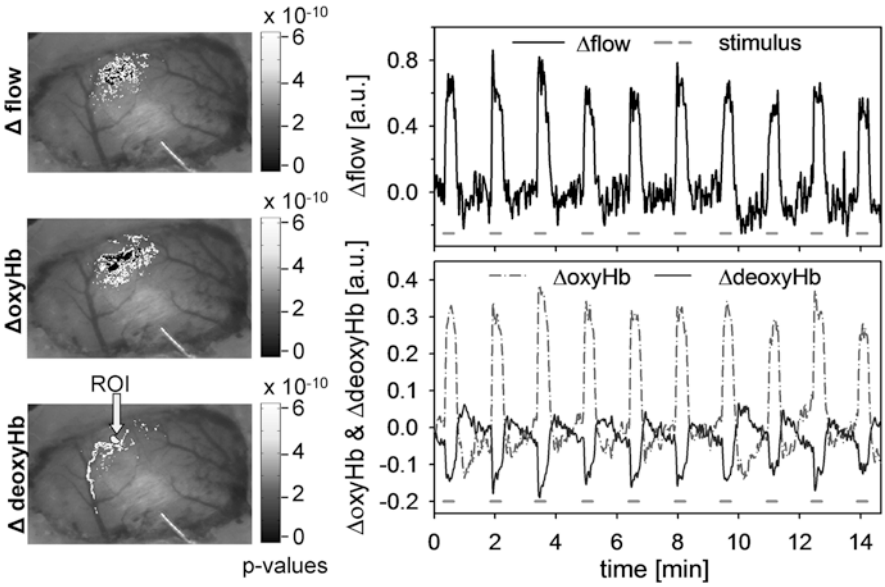

Fig. 57.3 Haemoglobin and blood flow response following cortical activation as observed with the system. *Left*: anatomical images with a selected region of interest (*ROI*) and *p*-values. *Right*: time courses of changes in oxyHb, deoxyHb and flow as averaged values from the ROI for ten single stimuli

57.4 Conclusion

In conclusion, a system for simultaneous spatially and temporally resolved imaging of haemoglobin oxygenation and blood flow with RGB reflectometry and LASCA was presented. It consists of a RGB-LED and a low power diode laser as light sources. The system is based on a two-CCD camera to separate the VIS and NIR spectral bands, allowing a continuous illumination and observation of signals from both light sources. We demonstrate that the system allows haemoglobin and blood flow changes to be imaged in cortical tissue for different conditions like ischaemic stroke, hypercapnia, hyperoxia, hypoxia, cortical spreading depression and cortical activation. The new system is demonstrated to have a high sensitivity combined with good temporal and spatial resolution. Its design offers significant advantages in terms of technical simplicity, reliability and robustness compared to other approaches.

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