MULTI-EXPOSURE, CONTINUOUS LASER SPECKLE CONTRAST IMAGING OF MOUSE BRAIN ENABLED BY A NOVEL SINGLE PHOTON AVALANCHE DIODE (SPAD) ARRAY

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OBJECTIVES: Multi-exposure laser speckle contrast imaging (MESI) measures absolute microvascular blood flow by using the speckle contrast data acquired sequentially, at a wide range of exposure times[1]. To minimize the sensitivity to physiological changes, there is a need for fast data acquisition. Here we present a new single shot multi-exposure laser speckle contrast imaging approach on the mouse brain enabled by a novel, high frame-rate single-photon avalanche diode (SPAD) array. The method provides sensitive, robust data acquisition compared to conventional laser speckle imaging and hence is suitable for longitudinal studies.

METHODS: SPAD array is a high frame rate (up to 100000 fps) imager with single-photon counting sensitivity. There is no readout noise and, therefore, it is possible to acquire a continuous stream of very short frames and to estimate all exposure times for single-shot MESI by summation of the acquired frames, instead of performing different set of acquisitions for each exposure time. In these experiments, full-field illumination was achieved with a continuous-wave laser diode (785 nm). Three month old male mice (C57/BL6, 30g) were placed on a stereotaxic frame, anesthetized with isoflurane, and scalp was removed. Hypercapnia ($20\%O_2+75\%N_2O+5\%CO_2$) and hyperoxia ($100\%O_2$) were utilized to cause bulk changes in the brain for validation followed by sacrifice. First single-shot MESI data was acquired, followed by standard approach.

RESULTS: The speckle contrast was calculated for both single-shot and standard methods. The decorrelation time (τ_C) was obtained by fitting the data against a theoretical model [1]. Blood flow was assumed to be inversely proportional to τ_C and relative blood flow was used to compare two approaches as shown in Table 1. The standard and continuous methods are in agreement in absolute values as well as in changes.

Table 1: Fitted values of τ_C and changes of cerebral blood flow for each challenge

Challenge	τ_c (sec)		Relative blood flow (%)	
	Single-shot	Standard	Single-shot	Standard
Baseline	$(3.8\pm0.4)10^{-4}$	$(3.41\pm0.32)10^{-4}$	100	100
CO_2	$(3.10\pm0.29)10^{-4}$	$(3.04\pm0.26)10^{-4}$	121.6±2.0	112.1±4.6
Recovery	$(3.17\pm0.33)10^{-4}$	$(3.10\pm0.23)10^{-4}$	118.9±1.6	109.7±3.6
O_2	$(3.23\pm0.33)10^{-4}$	$(3.24\pm0.24)10^{-4}$	98.3±4.0	95.8±4.7
Recovery	$(2.83\pm0.26)10^{-4}$	$(2.89\pm0.24)10^{-4}$	112.0±3.3	107.5±4.3
Biological Zero (post mortem)	$(259\pm4)10^{-4}$	$(519\pm89)10^{-4}$	0	0

CONCLUSION: We have introduced a new laser speckle contrast imaging approach enabled by a novel SPAD array that is able to provide multi-exposure data in a fast single-shot acquisition. To validate the approach, measurement of absolute and relative blood flow during baseline, hypercapnia

and hyperoxia in the mouse brain were utilized showing close agreement between the two methods. This high performance method will be further demonstrated during functional activation of the whisker barrel cortex. Future potential for this technology will be discussed.

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