

# Assessing ocular blood flow during water drinking test by using laser speckle flowgraphy

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#### **Abstract**

How to measure ocular blood flow during water drinking test

**Citation:** Tong Boon Tang Assessing ocular blood flow during water drinking test by using laser speckle flowgraphy.

protocols.io

dx.doi.org/10.17504/protocols.io.impcc5n

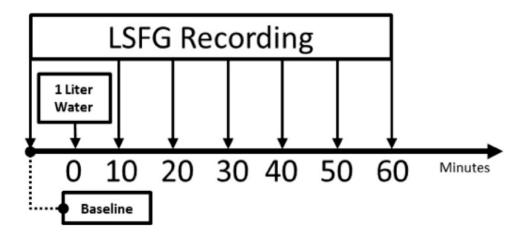
Published: 26 Jun 2017

#### **Protocol**

## **Experimental Flow**

## Step 1.

All subjects were familiarized with the LSFG-NAVI system (Softcare Co., Ltd., Japan) and the various procedural details. The LSFG-NAVI system (Softcare Co., Ltd., Fukuoka, Japan) was adjusted according to the eye position of each participant, and each was given sufficient time to relax before the experiment commenced. All the recordings were conducted in a darkroom. The first LSFG recording was performed before the participant drank 1 liter (1000 mL) of water. The participant was instructed to consume the entire liter within a few minutes. The next LSFG recording was performed 10 minutes after commencement of water intake. Five subsequent recordings were obtained at 10-minute intervals until 60 minutes after water intake. The time intervals for all recordings are shown in figure below. Three consecutive recordings were acquired at each time point, and the average of these three values was used for further analysis.



**Fig. 3.** Timeline for LSFG recordings according to experiment design. A baseline LSFG recording before drinking 1 liter of water, followed by six further recordings at 10-minute intervals.

#### Data Analysis

## Step 2.

The LSFG-NAVI system provided MBR pulse waveforms as measurements of ocular blood flow. Pulse waveform parameters were obtained using the proprietary analysis software provided with the LSFG-NAVI system. MBR was computed for two regions of interest in each participant: the entire ONH and the avascular region. The ONH region was segmented into its constituent vascular and tissue areas. The pulse waveform parameters of the MBR waveform for the whole ONH, the vascular part of the ONH, the tissue part of the ONH and the avascular region were exported from the LSFG analysis software for further analysis. Moreover, heart rate was also computed from the MBR waveform using the LSFG analysis software, and is reported in this study.

The values were normalized according to baseline readings in each participant, and statistical analysis was performed to assess any significant difference. Statistical analyses were conducted using SPSS statistical software. Based on visual inspection, utilizing histograms, QQ plots and box plots, the data was found not to follow normal distribution. Therefore, a nonparametric test called Wilcoxon Signed Rank test is carried out on the dataset, and asymptotic significance (2-tailed) was computed at P < 0.05. Wilcoxon signed-rank test is as powerful for non-parametric data as is the t-test for parametric data.