Low frame rate laser Doppler holography

Léo Puyo,1,2,3 Michel Paques,2,3 and Michael Atlan4

1Corresponding author: gl.puyo@gmail.com
2Centre Hospitalier National d’Ophtalmologie des Quinze-Vingts, INSERM-DHOS CIC 1423. 28 rue de Charenton, 75012 Paris France
3Institut de la Vision-Sorbonne Universités. 17 rue Moreau, 75012 Paris France
4Institut Langevin. Centre National de la Recherche Scientifique (CNRS). Paris Sciences & Lettres (PSL University). École Supérieure de Physique et de Chimie Industrielles (ESPCI Paris) - 1 rue Jussieu. 75005 Paris France

(Dated: April 2, 2020)

Laser Doppler holography (LDH) is an interferometric blood flow imaging technique based on full-field measurements of the Doppler broadening. So far, LDH has been demonstrated in the retina with ultrafast cameras, typically at 75 kHz. We show here that it can be implemented with camera frame rates 10 times slower than before. Low and high frequency local power Doppler signals have opposite variations due to energy conservation. A simple contrast inversion of the low frequency power Doppler reveals fast blood flow largely beyond the camera detection bandwidth for conventional laser Doppler measurements. Relevant blood flow variations and color composite power Doppler images can be obtained with camera frame rates down to a few kHz.

Laser Doppler holography (LDH) is a digital holographic method where blood flow is measured from the interference of coherent light backscattered by the eye with a reference beam [1–3]. The Doppler broadening is measured over the full-field array of an ultrafast camera thanks to the coherent gain brought by the reference beam. The power Doppler is calculated pixel-wise as the integral of the high-pass filtered Doppler power spectrum density (DPSD) to reveal blood flow from the larger Doppler broadening of light scattered in blood vessels. By using a sliding short-time window, variations of retinal blood flow over cardiac cycles can be measured in power Doppler units with a few milliseconds of temporal resolution [4]. The method was previously introduced in ophthalmology to image retinal and choroidal blood flow in the human eye [4–6]. LDH measurements demonstrated so far have been performed with ultrahigh camera frame rates, as required to sample Doppler shifts up to a few tens of kHz. Undersampling the Doppler broadening otherwise leads to artifactual blood flow measurements, for example in large vessels lumen or during systole [4]. This frame rate requirement is a serious limitation for the technique as ultrafast camera are expensive, and generate large amounts of data that are challenging to deal with. In this Letter, we demonstrate that blood flow measurements of similar quality can be performed with frame rates 10 times slower than before by taking advantage of the energy conservation of the power spectrum density. We have found that low and high frequency local power Doppler signals have opposite variations, and that simply reversing the contrast of the very low frequency power Doppler allows to obtain relevant blood flow measurements with camera frame rates down to a few kHz. This scheme allows to reveal fast blood flow that is largely beyond the camera detection bandwidth for conventional laser Doppler measurements [7, 8]. The method is first validated by using the low frequency content of ultrahigh speed measurements and comparing it to the usual blood flow measurements obtained from high frequency power Doppler, which serves as ground truth. The technique is then demonstrated with real low frame rate measurements.

We use the LDH setup presented in [4] to image retinal and choroidal blood flow in human eyes. Informed consent was obtained from the subjects, experimental procedures adhered to the tenets of the Declaration of Helsinki, the study authorization was obtained from the appropriate local ethics review boards CPP and ANSM, and the clinical trial was registered under the references IDRCB 2019-A00942-5, and NCT01419021. Interferograms are recorded using a CMOS camera (Ametek - Phantom V2511, quantum efficiency 40%, 12-bit pixel depth, pixel size 28 μm), which is used in a 512 × 512 format with ultrafast (i.e. between 60 and 75 kHz), and slow (i.e. between 4 and 8 kHz) frame rates. The ocular exposure to the 785 nm single frequency laser diode is constant, and the camera exposure time is set to the maximum possible. The data processing is essentially the same for both the fast and slow frame rates. The digital holograms are numerically propagated by angular spectrum propagation, and analyzed by short-time Fourier transform. In order to remove the Doppler contribution of eye motion, each short-time window is first filtered by singular value decomposition (SVD), as detailed elsewhere [9]. The eigenvectors of the holograms space-time matrix associated to the eigenvalues of highest energy are rejected, which allows the access to low frequency blood flow signals, otherwise dominated by spurious contributions. Then, for each time point \( t_n \) the power Doppler \( M_0 \) is calculated as the integral of the power spectrum density \( S \) over a given frequency range \( [f_1, f_2] \):

\[
M_0(x, y, t_n) = \int_{f_1}^{f_2} S(x, y, t_n, f) \, df \quad (1)
\]
Finally, power Doppler images are corrected for non-uniform illumination, and the baseline signal is subtracted to the power Doppler movie \[ S = 67 \text{ kHz} \]. To study blood flow variations with low frame rate acquisitions, the negative power Doppler is considered, which amounts to reversing the contrast of images (inversion of the image grayscale). This process is illustrated in Fig. 1 with a LDH measurement performed at \( f_S = 67 \text{ kHz} \). As noted in previous work \[ S^1 \text{ or } S^2 \], blood vessels are visible with a dark contrast on low frequency power Doppler images. This is shown in Fig. 1(a) with a 0-1 kHz power Doppler image: the power Doppler is lower in vascularized structures than in the surrounding tissue. In Fig. 1(b), the contrast of the power Doppler image has been inverted, and this reverse contrast (RC) power Doppler image is very similar to the usual high frequency (6-33 kHz) power Doppler image shown in Fig. 1(c). Retinal and choroidal blood vessels with fast blood flow can be observed on the two images with a similar contrast. This phenomenon can be explained by energy conservation. All pixels correspond to retinal areas that have received the same amount of energy from the laser beam illumination, and the Parseval-Plancherel identity states that the total power Doppler (area under the curve of the power spectrum density) is a quantity that is conserved in the temporal and spectral domains. Thus, aside from the difference of reflectivity and absorption discussed further, the total area under the curve of the DPSD should be the same in vascular and tissue structures because it corresponds to the laser beam energy. In blood vessels, the power spectrum energy is more broadened towards higher frequencies due to the larger Doppler shifts induced by faster scattering parti-
when blood flow is increased, the energy is shifted towards higher frequencies, which means the low frequency power Doppler is depleted. Thus, probing the negative power Doppler variations at low frequency allows to retrieve information about the positive variations of high frequency power Doppler.

The results shown in Fig. 1 and 2 suggest that using a low frame rate and reversing the power Doppler contrast could be used to perform blood flow measurements similar to those obtained from ultrafast frame rates LDH acquisitions. This technique is verified in Fig. 3 with a LDH measurement recorded at low frame rate. In order to access the frequency band 0-4 kHz, the sampling frequency was set to $f_S = 8$ kHz. It is possible to see on the RC power Doppler image shown in Fig. 3(a) that fast blood flow can indeed be revealed as expected. The corresponding variations of negative power Doppler in an artery (red) and a vein (blue) are plotted in Fig. 3(c). It is possible to observe the systolic increase, diastolic notch, and diastolic decrease in the artery, and a cycloidal flow profile in the vein, which are the waveforms typically measured with high frequency LDH. The coefficient of variation map that was previously introduced to differentiate retinal arteries and veins is shown in Fig. 3(b), and shows a quality similar to what was obtained at high frequency in our previous work [6]. The results presented in this Fig. demonstrate that perfusion maps and blood flow variations of fast blood flow largely beyond the detection bandwidth of the camera can be revealed by inverting the contrast of low frame rate LDH measurements. The results shown in Fig. 1 and 2 are obtained from high frequency LDH measurements, where it can be assumed that the Doppler broadening is properly sampled. This means the measured power spectrum density reflects the real Doppler broadening of light. In the case where the measurement is done at low frequency, the undersampled part of the spectrum is aliased, and it could be expected the ability of the RC low frequency power Doppler to reflect positive high frequency power Doppler would be jeopardized. However the results obtained with the low frame rate LDH measurement shown in Fig. 3 show that it is not the case.

In our previous work [4], we showed that composite power Doppler images from low and high frequency ranges that reveal slow and fast blood on a single image can be very useful. The typical frequency ranges used for these images are 1-6 kHz and 6 kHz - $f_S/2$, where $f_S$ is usually between 60 and 75 kHz. Arterial and venous choroidal vessels are differently contrasted on these composite images, thanks to the natural discrepancy of flow between these vessels. We demonstrate in Fig. 4 that composite images with very similar contrast can be generated from RC low frequency LDH measurement. LDH measurements were performed in the optic nerve head
FIG. 4: Making low/high flow composite images with high and low frame rates. (a), (b), and (c) show the usual composite images previously demonstrated [5]. (d), (e), and (f) show the result with a 8 kHz frame rate: the high flow image is obtained from the RC low frequency power Doppler. The composite movies are shown in Visualization 3 and Visualization 4.

FIG. 5: $f_s = 4$ kHz LDH measurement. (a) RC power Doppler image on the 0-2 kHz range, the corresponding movie is shown in Visualization 5. (b) The high dynamic range composite color image reveals both slow and fast blood flow in the ONH microvasculature and in large vessels.

(ONH) of a same eye with sampling frequencies of 67 kHz and 8 kHz. In Fig. 4(a) and (b) are shown the usual power Doppler images for the low and high flow for $f_s = 67$ kHz, and in Fig. 4(c) the resulting composite. Then for the measurement at $f_s = 8$ kHz, we show in Fig. 4(d) that a similar low flow power Doppler image is obtained from the 1-4 kHz frequency range. Then, as shown in Fig. 4(e), the RC power Doppler image on the range 0-1 kHz produces an image equivalent to the very high frequency power Doppler, which reveals the fast flow. Consequently, combining these two images into the color composite Doppler image displayed in Fig. 4(f) leads to an image contrasted very similarly to the composite image obtained with the 67 kHz measurement. Retinal vessels can be observed on both images with the same contrast. More importantly, as shown by the arrows ‘CA’ and ‘CV’, the choroidal artery and vein in the vicinity of the ONH are similarly differentiated on both composite images. This shows that colored composite images of equivalent quality to those obtained from ultrafast LDH measurements can be obtained from low frame rate LDH measurements.

In Fig. 5, we demonstrate blood flow measurement with RC-LDH by using a camera frame rate set to $f_s = 4$ kHz. The average RC power Doppler image for the 0-2 kHz range shown in Fig. 5(a) is able to reveal the perfusion map of fast blood flow. To obtain a color composite Doppler image from this measurement, the 0-1 kHz RC power Doppler and the 1-2 kHz power Doppler have been used to reveal fast (red) and low (cyan) flow. The resulting image shown in Fig. 5(b) reveals the ONH microvasculature as well as fast blood flow in large vessels. The 0-1 kHz range in RC-LDH produces perfusion maps of satisfying quality. However, the pulsatile variations of blood flow in large vessels are revealed by higher frequencies than those revealing the steady component of blood flow [6]. This is why we used the 0-4 kHz frequency range to measure pulsatile variations in Fig. 3 and 4. When the local velocity is greater, the Doppler shifts are greater, so the spectrum energy is broader and the spectral depletion revealing blood flow by RC power Doppler is increased. This means that higher velocities are also revealed by greater frequencies in RC-LDH. Roughly speaking, we found that blood flow revealed with normal power Doppler in the range of a few kHz and tens of kHz, seems to be revealed with RC power Doppler in the range of a few hundreds of Hz, and a few kHz, respectively. Slow blood is very challenging to reveal with the RC power Doppler because ultralow frequencies are very corrupted by spurious contributions. Thus, it is advantageous to use frequencies of a few kHz for the dual purpose of measuring the pulsatile variations of blood flow with RC power Doppler, and revealing slow blood flow with normal power Doppler. Interestingly, the low frequency range, around 1 to 4 kHz, is able to reveal...
both slow and fast blood flow, by using RC or normal power Doppler. This frequency range is very affected by spurious interferometric contributions, so the use of spatio-temporal filtering is critical to reveal the signal. We have found the SVD to be efficient to reject the specular reflections from instrument diopters and the anterior segment. It is particularly important to filter the pulsatile axial motion of the eye because they are synchronous with blood flow and occur in the direction that maximizes the Doppler effect.

The comparisons we have conducted between RC power Doppler and normal power, from low and high frequency LDH measurement, empirically validate the use of the contrast inversion. Considering there is a 10 fold frame rate reduction, the similarity between the obtained blood flow traces and color composite images is very satisfying. For measurements of a same duration, it seems that the signal quality of blood flow measurements is slightly lower with RC power Doppler, possibly because there are less pixels involved in the signal detection. However this is counterbalanced by the possibility to perform longer acquisitions. The quality of the perfusion maps, blood flow variations, color composite Doppler images obtained by RC-LDH seems of sufficient quality for clinical use. As current streaming cameras have an available throughout allowing measurement at frame rates of a few kHz, this work leaves the way open towards a real-time LDH system.

In conclusion, the detection of low frequency light fluctuations by digital holography enables efficient characterization of high frequency Doppler broadening beyond the Shannon-Nyquist sampling limit. Perfusion maps and pulsatile blood flow variations of flow velocities largely out of the reach of conventional wideband laser Doppler measurements can be obtained. Color composite Doppler images of low/high flow can also be produced with surprisingly low camera frame rates.

**Funding Information**

This work was supported by the European Research Council (ERC Synergy HELMHOLTZ #610110), the Institut Hospitalo-Universitaire ForeSight (ANR 18-001), and the Sesame program of the Region Ile-de-France (4DEye project). The Titan Xp used for this research was donated by the NVIDIA Corporation.

**Disclosures**

The authors declare no conflicts of interest.

[1] M. Simonutti, M. Paques, J. A. Sahel, M. Gross, B. Samson, C. Magnain, and M. Atlan. Holographic laser doppler ophthalmoscopy. *Opt. Lett.*, 35(12):1941–1943, 2010.
[2] Caroline Magnain, Amandine Castel, Tanguy Boucneau, Manuel Simonutti, Isabelle Ferezou, Armelle Rancillac, Tania Vitalis, José-Alain Sahel, Michel Paques, and Michael Atlan. Holographic laser doppler imaging of microvascular blood flow. *JOSA A*, 31(12):2723–2735, 2014.
[3] Mathilde Pellizzari, Manuel Simonutti, Julie Degardin, J.-A Sahel, Mathias Fink, Michel Paques, and Michael Atlan. High speed optical holography of retinal blood flow. *Optics Letters*, 41(15):3503–3506, 2016.
[4] L. Puyo, M. Paques, M. Fink, J.-A. Sahel, and M. Atlan. In vivo laser doppler holography of the human retina. *Biomedical Optics Express*, 9(9):4113–4129, Sep 2018.
[5] Léo Puyo, Michel Paques, Mathias Fink, José-Alain Sahel, and Michael Atlan. Choroidal vasculature imaging with laser doppler holography. *Biomedical Optics Express*, 10(2):995–1012, 2019.
[6] Léo Puyo, Michel Paques, Mathias Fink, José-Alain Sahel, and Michael Atlan. Waveform analysis of human retinal and choroidal blood flow with laser doppler holography. *Biomedical Optics Express*, 10(10):4942–4963, 2019.
[7] T. Tanaka, C. Riva, and I. Ben-Sira. Blood velocity measurements in human retinal vessels. *Science*, 186:830–831, November 1974.
[8] M. D. Stern. In vivo evaluation of microcirculation by coherent light scattering. *Nature*, 254(5495):56–58, March 1975.
[9] Leo Puyo, Michel Paques, and Michael Atlan. Spatio-temporal filtering in laser doppler holography. arXiv preprint, 2020.