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Microvascular blood flow monitoring  
with laser speckle contrast imaging  
using the generalized differences algorithm

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**Abstract**

Laser speckle contrast imaging (LSCI) is a full-field optical technique to monitor microvascular blood flow with high spatial and temporal resolutions. It is used in many medical fields such as dermatology, vascular medicine, or neurosciences. However, LSCI leads to a large amount of data: image sampling frequency is often of several Hz and recordings usually last several minutes. Therefore, clinicians often perform regions of interest in which a spatial averaging of blood flow is performed and the result is followed with time. Unfortunately, this leads to a poor spatial resolution for the analyzed data. At the same time, a higher spatial resolution for the perfusion maps is wanted. To get over this dilemma we propose a new post-acquisition visual representation for LSCI perfusion data using the so-called generalized differences (GD) algorithm. From a stack of

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perfusion images, the procedure leads to a new *single image* with the same spatial resolution as the original images and this new image reflects perfusion changes. The algorithm is herein applied on simulated stacks of images and on experimental LSCI perfusion data acquired in three different situations with a commercialized laser speckle contrast imager. The results show that the GD algorithm provides a new way of visualizing LSCI perfusion data.

*Keywords:* Medical and biological imaging, Laser speckle, Image analysis, Blood flow, Microcirculation

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## 1. Introduction

The monitoring of microvascular blood flow can be performed with several optical techniques, among which laser Doppler flowmetry (LDF) and laser speckle contrast imaging (LSCI) are now currently used [1]. In LDF, the tissue under study (skin for example) is illuminated with a low-power laser light. The backscattered light is transmitted to a photodetector. LDF relies on the Doppler frequency shift that appears when light is scattered by moving blood cells (mainly red blood cells). The LDF perfusion is defined from the first moment of the power spectrum of the photocurrent fluctuations (see, e.g., [2, 3]). LDF signals have been the subject of many works (see, e.g., Refs. [4, 5]), but one of the major drawbacks of LDF is that it is a single-point measurement technique. Due to the spatial variations of the microvascular blood flow [6, 7], the reproducibility of the measure is poor [8, 9]. Laser Doppler imaging (LDI) has been designed to prevent this drawback [10]. However, for most of imagers, the image is computed by scanning the area under study which entails long recording times and prevents the monitoring of rapid physiological phenomena. To overcome the latter point, full-field laser Doppler imagers have recently been proposed [11, 12, 13, 14, 15, 16, 17, 18], but require high-speed cameras.

LSCI is another full-field optical technique for the monitoring of microvascular blood flow. It is a real-time method that does not need any scan and uses a normal CCD or CMOS camera [19]. LSCI relies on the following prin-

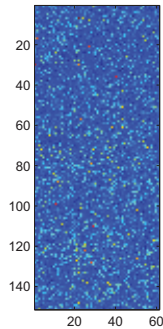


Figure 1: Perfusion image (151 rows and 61 columns) from LSCI technique of a zone on the forearm of a healthy subject.

ciple [20, 21, 22, 23, 24, 25, 26, 27, 28, 29]: when the tissue under study is illuminated by a laser with an expanded beam, the backscattered light forms an interference pattern on the detector (a camera). Due to phase difference  
 25 involved in the backscattered light, there are constructive and destructive interferences. The latter produce a pattern composed of bright and dark areas on the camera. This pattern is known as a speckle pattern. When particles move in the tissue under study, the speckle pattern changes. In LSCI, the changing speckle pattern is recorded with a camera that has an integration time in the  
 30 millisecond range. Due to the long integration time compared to the typical decorrelation time of the speckle pattern, the speckle pattern is blurred in the image. The level of blurring is quantified by the speckle contrast  $K$  that is inversely related to blood flow (see an example in Fig. 1). In the laser speckle contrast imager used in our work, the perfusion is computed from  $1/K - 1$  [30].  
 35 It has been shown that the power spectral density measurements of the light fluctuations derived using LSCI and LDF techniques are equivalent [31].

LSCI data have shown to have excellent reproducibility [9, 32, 33, 34]. The rapid adoption of LSCI in clinical research is also probably due to the relative  
 40 ease and low cost of building an instrument, compared to other techniques such

as MRI or CT [19, 35]. The developments of the technique are again the subject of many studies (see, e.g., [36],[37],[38, 39, 40],[41],[42, 43, 44, 45, 46, 47],[48]). LSCI is now used in many medical fields such as dermatology, cardiology, vascular medicine, diabetology, neuroscience, ophthalmology [26], among others. In cardiovascular studies, LSCI can be used to analyze the impairment of tissue blood supply provoked by pathologies such as diabetes, Raynaud's phenomenon, or peripheral vascular diseases. Monitoring blood flow with LSCI can therefore allow early diagnoses or an evaluation of the evolution for such diseases. However, LSCI (as LDF and LDI) gives blood flow values in arbitrary units: no absolute values as  $\text{ml.g}^{-1}.\text{min}^{-1}$  tissue are possible [22, 49], as again pointed out recently (see, e.g., [29]). Another drawback of the LSCI technique is that it leads to a large amount of data: the frequency sampling of the images can be of several Hz (depending on the application) and the recordings usually last several minutes. In order to monitor the blood flow, the clinicians have therefore a stack containing many images (perfusion maps) reflecting the temporal evolution of the blood flow on the area under study. To analyze the data easily and rapidly, one or several regions of interest (ROIs) are often chosen on the first image of the image stack, and an average of the blood flow is performed in this ROI. The same ROI is chosen on the other images of the stack and thus an average of the blood flow can be studied in this ROI in time, at the rate of the image frequency acquisition [26, 32]. This leads to a 1D-signal reflecting the time evolution of the average value for the blood flow in the ROI (see an example in Fig. 2). In the same time, algorithms or methods to obtain a higher spatial resolution for the images are still proposed (see, e.g., [50, 51, 52, 53]). However, this spatial resolution is "lost" by the averaging procedure performed in the ROIs, in clinical routine, to evaluate the evolution of the blood flow in a simple and rapid way. To get over this dilemma we propose herein a new way of visualizing LSCI perfusion data from commercialized imagers. Our procedure uses the so-called generalized differences (GD) algorithm. This leads to the computation of a new *single perfusion image* reflecting by itself the *variations* of the blood flow on the whole images of the stack. Moreover, this new im-

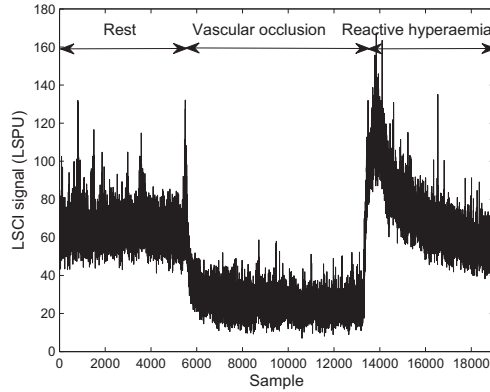


Figure 2: Laser speckle contrast signal (perfusion signal) of a zone on the forearm of a healthy subject, at rest, during vascular occlusion and post-occlusive reactive hyperaemia, computed from spatial averaging on a region of interest of  $7 \times 7$  pixels<sup>2</sup> in laser speckle contrast images (perfusion images).

age has the advantage of possessing *the same spatial resolution as the original images*. Our work therefore corresponds to a post-acquisition perfusion image processing procedure. Our goal is not to propose an imaging pre-processing method on raw speckle images. The problem raised by large volumes of data when imaging blood flow is of importance. Moreover, the visualization of blood flow data remains a challenging task (see, e.g., [54, 55]).

Among the algorithms that are often used in the evaluation of the activity for laser speckle images we find the Fujii's method [56, 57] and the temporal difference method [58]. However, unlike the GD method (see below), the Fujii's algorithm does not take into account the difference between nonconsecutive images. This presents a drawback in the case of laser speckle perfusion evaluation as variations of perfusion are slow compared to the frequency of the image acquisition; the variations are therefore low between two consecutive images. Comparisons between perfusion images differing by short times are not reliable because this prevents a salient analysis of slow phenomena when a relatively

short duration is studied (a few seconds). Moreover, as for the Fujii's method, the temporal difference method is not appropriated for microvascular blood flow characterization with laser speckle perfusion data because the difference between nonconsecutive perfusion images are not taken into account. Moreover, the temporal difference method leads to a stack of  $N - 1$  difference images which does not correspond to our goal to obtain a new single image. These two methods have therefore been discarded from our work.

In what follows, we first present the GD algorithm. Afterwards, the proof of concept of our work and the measurement procedure used to acquire experimental laser speckle contrast images in different situations are described. The results obtained on simulated and experimental data with the GD algorithm are detailed and discussed. Moreover, the results are compared with those given by other algorithms.

## 2. Materials and Methods

### 2.1. Generalized differences

In the method called generalized differences, a new image  $PU_{GD}$  is constructed from a sequence of  $N$  (perfusion) images  $PU_k$  ( $1 \leq k \leq N$ ) as [59]

$$PU_{GD}(i, j) = \sum_{k=1}^{N-1} \sum_{l=k+1}^N |PU_k(i, j) - PU_l(i, j)|, \quad (1)$$

where  $PU_k(i, j)$  is the perfusion value for the pixel situated at the coordinates  $(i, j)$  in the  $k$ -th image of the perfusion sequence (a similar notation is used for  $PU_l(i, j)$ ). In the new image  $PU_{GD}$ , regions differing in their activity are displayed as different gray levels. From Eq. 1, we observe that the GD algorithm does not depend on the order of the images. Moreover, the pixel  $(i, j)$  in image  $PU_{GD}$  is zero (minimum value) when all the  $PU_k(i, j)$  values are equal (no movement at this pixel).

Another variant (denoted as GD\*) considers not absolute values but square  
 115 values of the difference [60]. The new image  $PU_{GD^*}$  is therefore computed as

$$PU_{GD^*}(i, j) = \sum_{k=1}^{N-1} \sum_{l=k+1}^N (PU_k(i, j) - PU_l(i, j))^2. \quad (2)$$

It has been reported that one drawback of the methods based on GD is  
 that they require many images and that the investigated phenomenon must be  
 slow as compared with the sampling frequency [60]. However, for LSCI perfu-  
 sion data, several seconds and even minutes are commonly recorded to monitor  
 120 microvascular blood perfusion. Moreover, the frequency acquisition is usually  
 more than 10 Hz and the phenomena of interest are often slow compared to this  
 frequency acquisition. The above-mentioned drawback for the GD algorithm is  
 therefore not a problem for studies like ours. Moreover, one of the advantages of  
 the GD and GD\* representations is that a range of times is encoded in a single  
 125 frame.

## 2.2. Proof of concept

In order to show the proof of the concept used in our work (from a stack of  
 perfusion images, we want to generate a new *single perfusion image* with the  
 130 same spatial resolution as the original images (perfusion maps) and this new  
 image reflects perfusion changes) we apply the GD algorithm on deterministic  
 examples: we first generated a stack of 15 images,  $30 \times 30$  pixels<sup>2</sup> each. On  
 these 15 images, all the pixel values were identical and equal to 10 (simulating  
 blood flow with no variations at all, or no blood flow (biological zero)), except  
 135 on images 5 to 9 where a square of  $6 \times 6$  pixels<sup>2</sup> had higher values, simulating  
 a transient increase of blood flow in this region of interest (see Fig. 3): values  
 of 20, 40, 60, 45, 25 on the square of images 5, 6, 7, 8 and 9 respectively. We  
 then applied the GD algorithm on the stack of 15 images with the aim to see if  
 it is able to detect the transient variation of pixel values.

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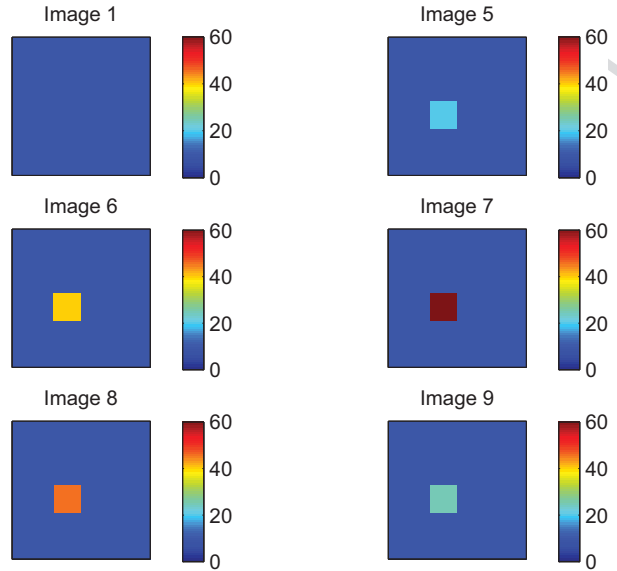


Figure 3: Representation of images 1, 5, 6, 7, 8, and 9 of the simulated images (see text for details).

We then generated another stack of images in order to analyze the influence of possible experimental noise on the resulting GD image. For LSCI, the experimental noise usually arise from movement. For this purpose, we generated 15 simulated images,  $30 \times 30$  pixels<sup>2</sup> each. On these 15 images, all the pixel values are identical and equal to 10 (simulating blood flow with no variations at all, or no blood flow (biological zero)), except on images 5 to 9 where a square of size  $6 \times 6$  pixels<sup>2</sup> had the same values as previously (see Fig. 3), simulating a transient increase of blood flow in this region of interest. Moreover, on images 4 to 8, three smaller squares ( $4 \times 4$  pixels<sup>2</sup>) of different amplitudes have been inserted: the first square contains pixels with random amplitudes varying from 25 to 27. The second square contains pixels with random amplitudes varying from 45 to 55, whereas the third square contains pixels with random amplitudes varying from 200 to 220. Different random values were generated for each of the images 4 to 8. These three “noisy” squares have therefore pixel amplitudes

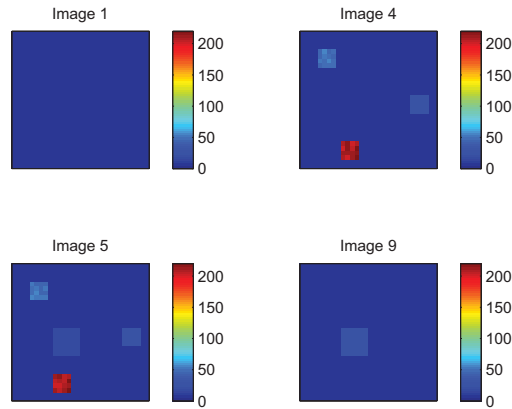


Figure 4: Representation of images 1, 4, 5, and 9 of the simulated images used to analyze the influence of movement artefacts (see text for details).

155 varying from half to more than four times larger than the simulated transient increase of blood flow, simulating transient increases of pixel amplitude due to movement artefacts (see Fig. 4).

### 2.3. Experimental image acquisition

160 Three kinds of experimental LSCI recordings have been performed to evaluate the performance of the GD algorithm in the visualization of perfusion changes from real LSCI data. All the image acquisitions have been done in laser speckle perfusion units (*LSPU*) with a PeriCam PSI System (Perimed, Sweden) having a laser wavelength of 785 nm and an exposure time of 6 ms.

165 The first recording has been performed on the two hemispheres of a mouse, recorded through the skull. The experiment has been performed in compliance with institutional guidelines and international standards on animal welfare and approved according to local and national regulation for animal care and use for research purposes [63]. The mouse was anesthetized with isoflurane (2.5%

170 induction, 1.5-2% during surgery) in 70% N<sub>2</sub>O and 30% O<sub>2</sub>. After end of  
preparation isoflurane anesthesia was reduced to 1.1-1.2%. Temperature was  
continuously measured and maintained at 37.8°C by use of an automatic con-  
trolled homeothermic blanket system. The mouse was placed in a stereotaxic  
frame and the skin was removed above both hemispheres to enable cerebral  
175 blood flow measurements by LSCI. At the parietal bone a small craniotomy  
(~3 mm lateral from midline/lambda and rostral to the lambdoid suture; diam-  
eter: ~1.2 mm) was made by use of a dental drill (SI-923 implantMED, W&H  
Deutschland GmbH, Laufen Germany) for spreading depression induction. The  
LSCI recordings have been performed with a sampling frequency of 0.1 Hz and  
180 the distance between the laser head to skull was set at 10.4 cm [64] which gave  
images with a resolution around 0.02 mm. The provocation used to obtain  
perfusion variations was a pinprick in one of the hemispheres that induced a  
cortical spreading depression/depolarization (CSD). CSD is a self-propagating  
wave of depolarization which corresponds to a slow-moving ionic and metabolic  
185 disturbance that propagates in cortical brain tissue [65]. The perfusion map  
stack processed was composed of 10 images.

The second experimental LSCI recordings have been performed on the fore-  
arm skin of a healthy subject. The variations of the perfusion have been induced  
190 by a local heating. The latter leads to a vasodilation and therefore to an increase  
of the perfusion values [66, 67, 68]. The recordings have been performed with  
a sampling frequency of 5 Hz and the distance between the laser head to skin  
was set at 14.7 cm [64] which gave images with a resolution around 0.14 mm.  
The perfusion map stack processed was composed of 100 images.

195  
The third experimental LSCI recordings have been performed on the forearm  
skin of another healthy subject. The variations of the perfusion have been  
induced by a local pinching. The latter was manually done and performed on  
the ventral face of the forearm. The pinching induced a local and transient  
200 increase of the perfusion. The recordings have been performed with a sampling

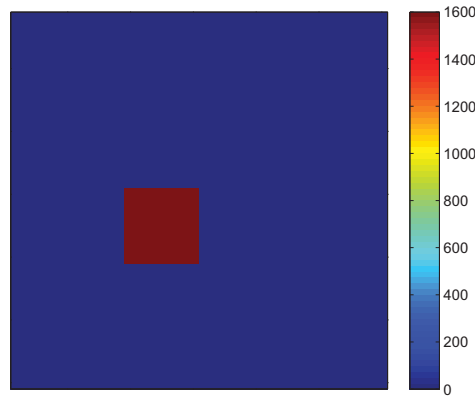


Figure 5: Image computed with the generalized differences algorithm from 15 simulated images (see Fig. 3).

frequency of 5 Hz and the distance between the laser head to skin was set at 15.9 cm [64] which gave images with a resolution around 0.15 mm. The perfusion map stack processed was composed of 100 images.

### 3. Results and Discussion

#### 3.1. Simulated images

The result of the GD algorithm for the simulated data shown in Fig. 3 is represented in Fig. 5. From the latter, we observe that the GD image is zero everywhere except in the square that was present in images 5 to 9 of the stack. In the new image  $PU_{GD}$ , the pixels are zero (minimum value) when all the pixel values are equal in the stack (no movement at this pixel) and regions differing in their activity are displayed as different gray levels. This result is therefore a proof of our concept for the use of the GD algorithm to detect perfusion changes in LSCI data.

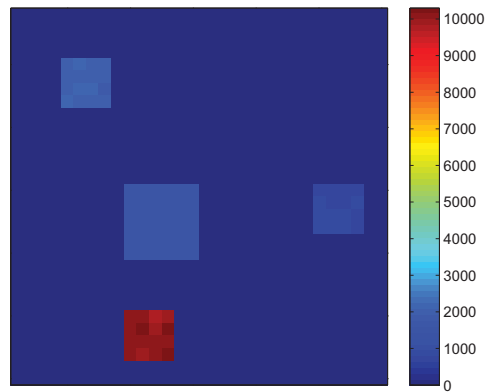


Figure 6: Image computed with the generalized differences algorithm from 15 simulated images (see Fig. 4).

215 The results of the GD algorithm for the stack of 15 images containing movement artefacts (Fig. 4) is shown in Fig. 6. The results show that the image generated with the GD algorithm reflects all the variations: all the four squares are visible in the new image, the one due to the transient increase of blood flow and the three squares reflecting the movement artefacts. Therefore, the  
 220 GD algorithm keeps the transient increase of blood flow visible, but also reflects pixel amplitude variations due to movement artefacts. However, LSCI is by definition very sensitive to movements. That is why LSCI experiments have to be performed with still subjects to avoid experimental noise.

### 225 3.2. Experimental images

The first perfusion image of the recordings for the three experimental cases is presented in Figs. 7 to 9. The results of the GD algorithm for the three cases are shown in Figs. 10 to 12. For each of these latter figures, we can observe in a single frame where the perfusion has changed (due to the stimulus). For  
 230 case 1 (see Fig. 10), we note that the perfusion has mainly changed in the right

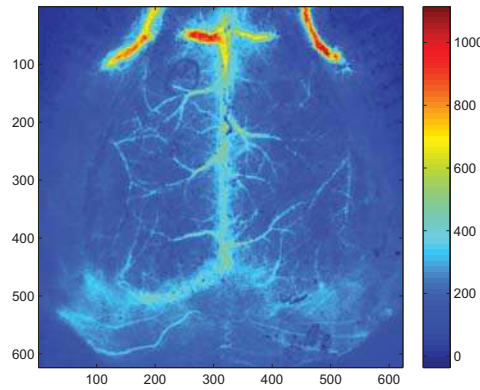


Figure 7: Perfusion map (623 rows and 623 columns) representing the two hemispheres of a mouse, recorded through the skull [63].

part of the image. For case 2 (see Fig. 11), we observe that the variations of the perfusion due to the local heating mostly appear in the bottom left part of the image. Finally, for case 3 (see Fig. 12), the perfusion variations are globally localized on all the image. As mentioned above, Fig. 10 has been computed from 10 images and Figs. 11 and 12 have been computed from 100 images.

Due to the spatial heterogeneity of the microcirculation [6, 7] and because clinical cases require perfusion analyses with a high spatial resolution (see, e.g., Ref. [69]), LSCI is an interesting optical technology. However, the visualization of LSCI perfusion images should be easy and rapid. Due to the high amount of images generated by several minutes of recordings, the GD image is an interesting way to visualize the variations of the perfusion (post-acquisition procedure) with a spatial resolution as high as the original perfusion maps.

As shown in Fig. 6, the GD image reflects perfusion changes but also movement artefacts. This is also shown in Fig. 13 where the GD image has been computed from perfusion data recorded on the forearm of a healthy subject during a local heating while movements were performed by the subject. We

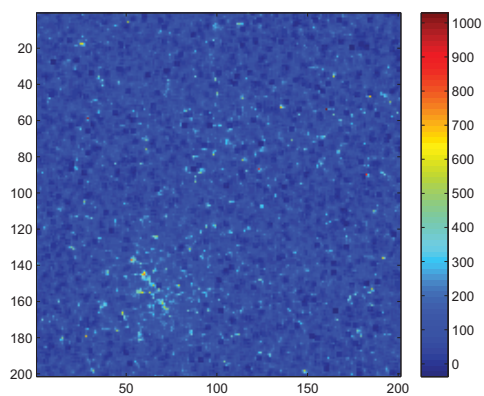


Figure 8: Perfusion map (201 rows and 201 columns) representing the forearm skin at the very beginning of a local heating.

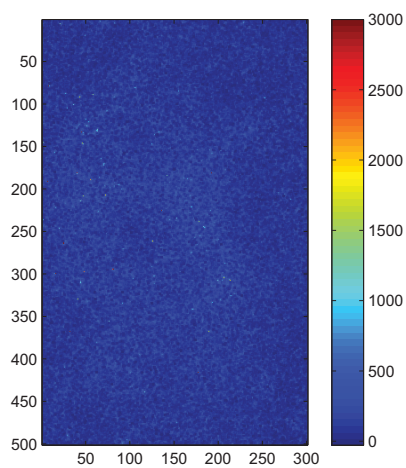


Figure 9: Perfusion map (501 rows and 301 columns) representing the forearm skin just after a local pinching.

note that both perfusion changes and movement artefacts lead to high pixel  
250 values in the GD image. In order to avoid misinterpretation between perfusion  
changes and movement artefacts, the acquisitions have to be performed when

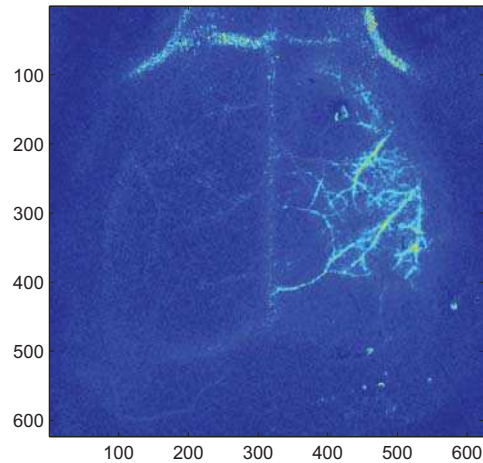


Figure 10: Image (623 rows and 623 columns) computed with the generalized differences algorithm from 10 perfusion images recorded during a pinprick in one of the hemispheres that induces a wave of ischemia (see text for details).

the subject is totally still.

We also compared our results given by the GD algorithm on the experimental  
 255 LSCI data with the ones given by the GD\* algorithm and by the Fujii's method.  
 The results given by the GD\* algorithm are shown in Figs. 14 to 16. The ones  
 given by the Fujii's method are shown in Figs. 17 to 19. From these figures, we  
 observe that the perfusion changes (due to the stimulus) are much less visible  
 than the ones given by the GD algorithm. The GD algorithm therefore appears  
 260 as the most interesting. No comparison with the temporal difference method  
 is possible because, with the latter method, a sequence of  $N$  images leads to a  
 new sequence of  $N - 1$  difference images, and this does not corresponds to our  
 goal.

265 For other dynamic vascular phenomena, it could be possible to compute the  
 new GD image on more, or less, images in order to visualize slow or rapid phe-



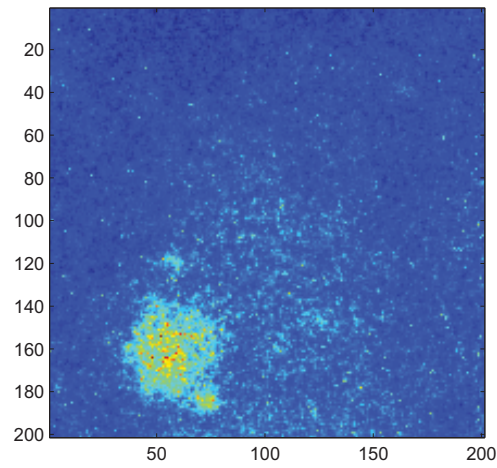


Figure 11: Image (201 rows and 201 columns) computed with the generalized differences algorithm from 100 perfusion images recorded during and after a local heating (see text for details).

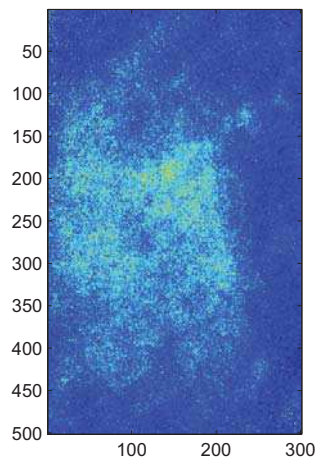


Figure 12: Image (501 rows and 301 columns) computed with the generalized differences algorithm from 100 perfusion images recorded just after a local pinching (see text for details).

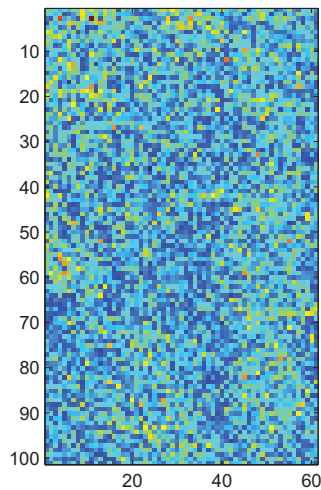


Figure 13: Image (101 rows and 61 columns) computed with the generalized differences algorithm from 100 perfusion images recorded during and after a local heating, while movements were performed by the subject (see text for details).

nomena. A normalization could be useful to compare results coming from GD calculated from different numbers of images.

270 Moreover, by definition, the GD algorithm loses the time dimension. Therefore, it is not directly possible to obtain a GD measure in time. If one wants to obtain information on the time evolution of the perfusion changes, it could be possible to split the stack of images under study in several sub-stacks of images and to compute the GD image for each sub-stack. However, this splitting would  
275 lead to several GD images. Therefore, the number of sub-stacks should not be too large as this would lead to many GD images and would prevent an easy and rapid examination of perfusion changes.

The visualization of perfusion data remains a challenging task and our work  
280 is the first one that proposes a post-acquisition procedure for visualizing LSCI

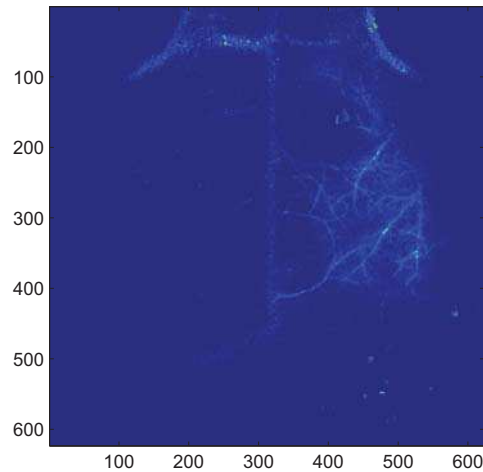


Figure 14: Image (623 rows and 623 columns) computed with the modified generalized differences algorithm ( $GD^*$ ) from 10 perfusion images recorded during a pinprick in one of the hemispheres that induces a wave of ischemia (see text for details).

perfusion maps coming from commercialized imagers. The GD algorithm appears as being an interesting tool in the evaluation of blood flow with high spatial resolution, which is of the utmost interest for clinical cases.

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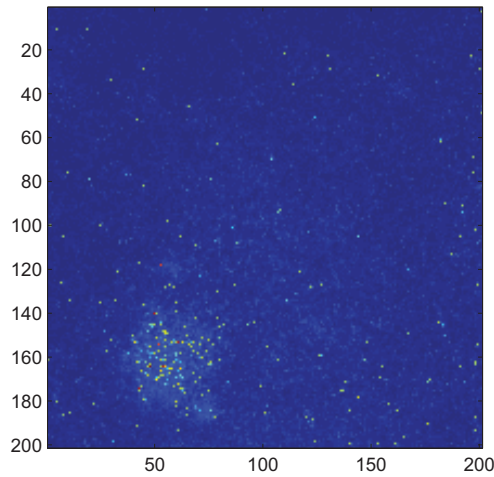


Figure 15: Image (201 rows and 201 columns) computed with the modified generalized differences algorithm (GD<sup>\*</sup>) from 100 perfusion images recorded during and after a local heating (see text for details).

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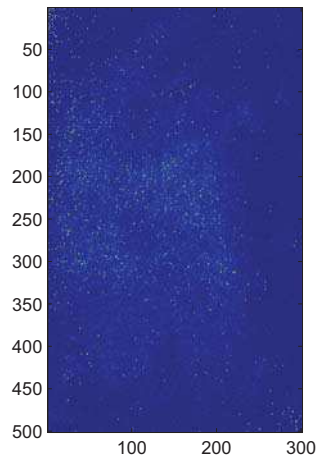


Figure 16: Image (501 rows and 301 columns) computed with the modified generalized differences algorithm (GD\*) from 100 perfusion images recorded just after a local pinching (see text for details).

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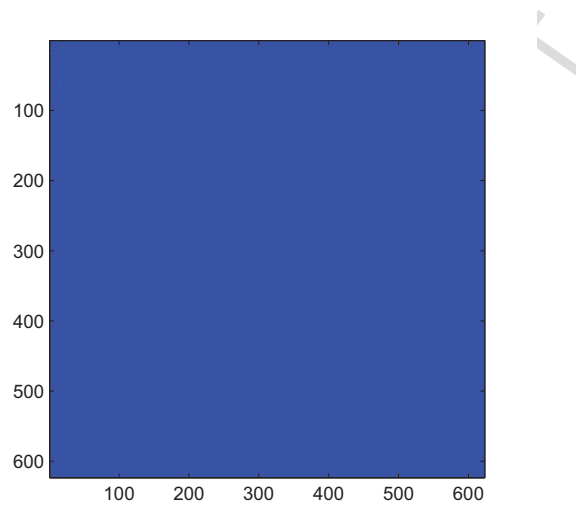


Figure 17: Image (623 rows and 623 columns) computed with the Fujii's method from 10 perfusion images recorded during a pinprick in one of the hemispheres that induces a wave of ischemia (see text for details).

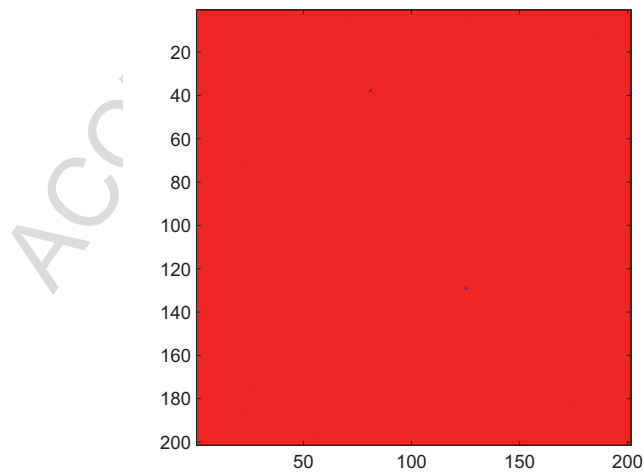


Figure 18: Image (201 rows and 201 columns) computed with the Fujii's method from 100 perfusion images recorded during and after a local heating (see text for details).

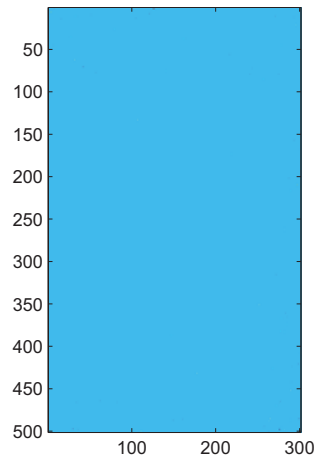


Figure 19: Image (501 rows and 301 columns) computed with the Fujii's method from 100 perfusion images recorded just after a local pinching (see text for details).

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**Highlights**

- Laser speckle contrast imaging (LSCI) enables to monitor microvascular perfusion
- Large amount of data are often recorded leading to a long process of analysis
- Spatial averaging is therefore often performed in regions of interest (ROI)
- The spatial averaging reduces the spatial resolution of the images
- We propose a new visual representation of LSCI data using generalized differences