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## Introduction

Visualization of blood vessels is a fundamental task to evaluate the health and biological integrity of the tissue. Laser Speckle Contrasts Imaging (LSCI) is a noninvasive technique to determine the blood flow in superficial or exposed vasculature. However, the high scattering of biological tissue, hinder the visualization of those structures. In this paper, we propose the use of Principal Component Analysis (PCA) in combination with LSCI to improve the visualization of deep blood vessel. Using PCA, it be separated and filtrated by selecting the most significant principal components. This analysis was applied to in vitro samples, and our results demonstrate that this approach allows the visualization and localization of blood vessels as deep as $1000 \mu \mathrm{~m}$.

## Laser speckle imaging

The local contrast $K$ [1]. is computed typically in a sliding window of $5 \times 5$ pixels through the equation

$$
K=\frac{\sigma}{\langle l\rangle}
$$

where $\sigma$ is the standard deviation and $\langle I\rangle$ is the mean intensity of the pixels in the sliding window, this local contrast value is assigned to the central pixel.
The contrast equation (1) can be expressed [2] as a function of the correlation time $\tau_{c}$ of the backscattered light from the sample and the exposure time $T$ of the CCD camera[3]:
$K^{2}(x)$
$=\beta \rho^{2} \frac{\exp (-2 x)-1+2 x}{2 x^{2}}+4 \beta \rho(1-\rho) \frac{\exp (-x)-1+x}{x^{2}}+\beta(1-\rho)^{2}$
where $x \equiv T / \tau_{c}, \rho$ is the fraction of the dynamically scattered light and $\beta$ is a correction factor that depends on the ratio of speckle and pixel size. When $x \gg 1$, the contrast reaches an asymptotic value ( $K_{S}$ ) given by:

$$
\begin{equation*}
\left.K^{2}(x)\right|_{x \gg 1} \equiv K_{s}^{2}=\beta(1-\rho)^{2} \tag{3}
\end{equation*}
$$

Separate out $K_{S}^{2}$ from $K_{D}^{2}$ and therefore improve the visualization of deep blood vessels.

## PCA

PCA is a statistical technique that uses an orthogonal transformation to describe a set of correlated observations in terms of new uncorrelated variables, called principal components (PC's), which are linear combinations of the original variables [4]. The procedure to obtain the PC's begins with the organization of the data in a matrix $\Gamma$ of dimension Mx N . Here M represents the number of observations and N the number of variables


Fig. 1 a) organization of images in a new data matrix a) a set of 30 raw speckle images are arrangement into matrix $\Gamma$, given by Eq. 4 . N corresponds to number of image ( $\mathrm{N}=1$ to 30 ) and $M$ corresponds to image pixel ( $M=1$ to $640 \times 480$ ), b) original space, and PC's space.

## Materials and method



Fig. 2 a) LSI system , b) top-layer thicknesses[5] (TLTs) of $\delta=0,190,510,311$ and $1000 \mu \mathrm{~m}$. We used a syringe pump to infuse intralipid at $3 \%$ in water as a blood substitute into the channel at speed of $5 \mathrm{~mm} / \mathrm{s}$, glass capillary tube, with an inner diameter of $550 \mu \mathrm{~m}$ and Region of interest (ROI) centered


Fig. 3 A) Eigenvalue vs the number of PC , the thick red horizontal line establishes the separation by means of the Guttan-Kaiser B). Contrast images for groups C;A and C-A. C) Experimental $K^{2}$ (symbols) as function of $T$ for different $\delta$ and its theoretical adjustmen.


Comparison between the traditional LSCI (C) and our proposal C-A with their corresponding segmentation by K-means (2nd and 4th columns).


Fig. 5. a) Location of the capillary [6] (vertical lines) of the profiles for $C, b$ ) similarly for $C-A, c$ ) Actual and estimated capillary (vessel) width for different $\delta$ values

## Conclusions

In this work, we demonstrate that PCA applied to LSCI allow us to separate out the static component from the dynamic component in the raw speckle images achieving visualization of blood vessels as deep as $1000 \mu \mathrm{~m}$. In addition, employing kurtosis on the dynamic region (such as flow in the capillary) we demonstrated a more accurate estimation of the actual vessel width. It is important to mention that our proposal works well when the variance between the raw speckle images is enough to be distributed among all the PCA, for example when the dynamic of the sample is relatively low or for short exposure times. Otherwise the first component attracts most of the information compared with the rest of the component and the filtering process fails.

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