LASER SPECKLE CONTRAST IMAGING FOR ANALYSING STATIC SCATTERER CONCENTRATION IN PHANTOM BODY FLUIDS

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Abstract: An adequate amount of blood supply is essential for the proper functioning of all body organs and an impaired supply of blood to the organs can cause various diseases. The concentration of red blood cells (RBC) is an important parameter in analysing the anaemic condition of a person as concentrations of RBC below a certain level leads to severe complications. In this paper, we are presenting Laser Speckle Contrast Analysis (LASCA) as a tool for analysing RBC concentration qualitatively. The preliminary results on this study obtained using body fluid mimicking phantoms are presented here. The technique developed / described here provides a real-time, non-scanning, whole field technique for assessing RBC concentration non invasively.

1. INTRODUCTION

An adequate amount of blood supply is necessary for the proper functioning of all body organs and an impaired supply of blood to the organs can cause various diseases. Blood volume accounts for 7% of human body weight [1] with an average density of approximately 1.054g/cm³ [2] and is a specialized fluid consisting of red blood cells, white blood cells and platelets suspended in a fluid medium known as blood plasma. The blood conveys oxygen from the lungs to the tissues, carbon dioxide from tissues to lungs, supplies nutrients to the tissues, removes metabolic end products and performs immunological functions. Additionally, blood regulates pH and body temperature and maintains the water content of the tissues [1]. The diseases / disorders of the blood that commonly affect human beings can involve any or all of the constituent blood cells. Of these, the concentration of RBC is important determining the tissue oxygenation and hence plays an important role in tissue health. Current method of assessing RBC concentration is invasive as it involves taking out blood from the subject. A non invasive method for RBC assessment will be of great value in offering patient comfort during such tests. In this paper, a non-invasive optical method is given for assessing the RBC concentration qualitatively based on contrast difference in speckle pattern generated in the target by a coherent source.

2. THEORY

When an optically rough target is illuminated with a coherent light source, speckle patterns are formed due to interference of light coming from different scatterers on the target surface. The pattern is random in nature and can only be described by statistics. The speckles obtained from living objects are called as bio-speckles.

In body fluids such as blood, RBC is the major scatterer. When the concentration of the scatterer changes the reflected / scattered intensity also varies. For a lesser concentration of the scatterer, the reflected intensity decreases and vice versa. This information is being utilised in the LASCA method.

The contrast value of a speckle pattern is defined as the ratio of the standard deviation (σ) of the intensity variations to the mean intensity (⟨I⟩). The speckle contrast Ks is given by

\[ K_s = \frac{\text{Standard Deviation of Intensity}}{\text{Mean Intensity}} = \frac{\sigma}{\langle I \rangle} \]  

As reflected intensity is dependent on the amount of scatterers present in the target, a variation in concentration will definitely change the mean intensity and therefore the speckle pattern contrast.

It should be noted that this kind of variation in scatterer concentration can be better analysed in the case of static speckle pattern where no speckle movement is assured. In the case of dynamic speckle, the velocity of scatterers also will contribute to the contrast variation making the resultant contrast dependent on both conc. of scatterers as well as their flow velocity.

Goodman related the variance \( \sigma_s^2 \) (T) of the spatial fluctuations to the time average of the autocovariance \( C_s(\tau) \) of the intensity fluctuations by the relation [3]

\[ \sigma_s^2(T) = \frac{1}{T} \int_0^T C_s(\tau) d\tau \]  

Assuming Lorentzian velocity distribution (because the capillary network is so convoluted) the speckle contrast \( K_s \) is given by

\[ K_s = \frac{\text{Standard Deviation of Intensity}}{\text{Mean Intensity}} = \frac{\sigma}{\langle I \rangle} \]
The measurements were made using a micropipette and the preparation of the solution was made in a standard flask. For set 2, 125ml of intralipid was diluted with 24.875ml of distilled water to make a solution of 0.05% intralipid by volume. The measurements were made using a micropipette and the preparation of the solution was made in a standard flask. For set 2, 125µl of intralipid was diluted with 24.875µl of distilled water to make a solution of 0.5% intralipid by volume. For set 3, 1.25ml of intralipid was diluted with 23.75ml of distilled water to make up a solution of 5% intralipid by volume. For all the cases, 8ml (chosen arbitrary) of the resultant solution mixture is taken in a petri dish which represented the target under zero flow conditions. This target was subjected to illumination from the laser and speckle pattern from the target is imaged through the imaging system explained in Sec. 3.

4. RESULTS AND DISCUSSIONS

The experiments were conducted as described in section 3 on phantom solutions (solutions of intralipid) of three different concentrations. The contrast of the speckle pattern was calculated using the developed algorithm.

Fig. 2(a) and (b) represents the image of the prepared intralipid solution of concentration 0.05% and the corresponding false colour contrast map. It can be seen that the contrast of the speckle pattern from the solution imaged is almost uniform and the average value of the contrast was calculated to be 0.0432. A slight variation seen on some parts of the image is attributed to the lack of exact uniformity in concentration of the solution at these places due to the time delay in imaging after preparation of the sample or clustering of intralipid particles due to slight variations in exact mixing of the intralipid and distilled water. To nullify these effects an average value of the contrast is taken into account. The red colour spots are the reflection from the exposed glass base and rim of the petri dish (shown in the image limits the resolution of the technique. A choice of 5x5 pixels has been used for the study in this paper.

3.1 Preparation of phantom solutions

LASCA is dependent on the reflected / scattered intensity obtained from the scatters present in the target. Taking this into consideration, phantom solutions are made with different scatterer concentration in order to mimic the scatterers present in the body fluid. As this study is basically intended to concentrate on RBC concentration changes and its effects in contrast of the speckle pattern, we have selected intralipid solution as the scattering agent with particle size almost comparable with RBC size [4]. A number of different concentrations were prepared and tested for the study. Of all these, results based on three representative sets of concentrations are presented in this article. These sets were prepared by diluting the intralipid solution with distilled water at appropriate amounts. For set 1, 12.5µl of intralipid solution was mixed with 24.9875ml of distilled water to make a solution of 0.05% intralipid by volume. For set 2, 125µl of intralipid was diluted with 24.875µl of distilled water to make up a solution of 0.5% intralipid by volume. For set 3, 1.25ml of intralipid was diluted with 23.75ml of distilled water to make up a solution of 5% intralipid by volume. For all the cases, 8ml (chosen arbitrary) of the resultant solution mixture is taken in a petri dish which represented the target under zero flow conditions. This target was subjected to illumination from the laser and speckle pattern from the target is imaged through the imaging system explained in Sec. 3.

3. EXPERIMENTAL SETUP

The experimental setup used for the analysis is shown in figure 1. A laser source (671nm red laser, 30mw power) illuminates the target and the resulting speckle pattern is imaged by a zoom lens - CCD camera system (Sony XC 50CE) The captured speckle pattern is digitized by a frame grabber card (DT 3120) and processed on the computer using the developed software.

![Experimental setup for LASCA](image)

**Figure 1. Experimental setup for LASCA**

The processing consists of the following steps

1) Defining a square block of n x n pixels of unit amplitude.
2) Computing the local speckle contrast in the block using equation (1) and assigning the value of this contrast to the pixel representing the centre of this block in a new array.
3) The block is then moved by one pixel and the process is repeated.
4) This is continued until the whole image field has been covered by a set of overlapping blocks.
5) The contrast values are then converted to a false colour contrast map and displayed on the monitor.

The number of pixels used (n) to compute the local speckle contrast can be selected by the user. A choice of a low number of n reduces the validity of the statistics whereas the choice of a higher number

\[ K_s = \frac{\tau_c}{2T} \left(1 - \exp\left(-\frac{2T}{\tau_c}\right)\right) \]

where T is the exposure time of the CCD camera used and \( \tau_c \) is the correlation time of the intensity fluctuations. Having calculated the contrast value \( K_s \) from eqn. (1) the \( \tau_c \) value is calculated using eqn. (3).
using arrows). These spots were omitted while calculating the average value.

Fig. 2 (a) Image of the solution (0.05% intralipid) in the petri dish under laser illumination (b) Corresponding false colour contrast map. X axis and Y axis represent pixel positions of the image in both cases.

Fig. 3(a) and (b) represents the image of the prepared intralipid solution of concentration 0.5% and the corresponding false colour contrast map. It can be seen that the contrast of the speckle pattern from the solution imaged is uniform and the average value of the contrast was calculated to be 0.0327.

Fig. 4(a) and (b) represents the image of the prepared intralipid solution of concentration 0.05% and the corresponding false colour contrast map. Here the reflections from the glass base are weak as due to increased scatterer concentration as evident from the image. The contrast map shows almost uniform nature and the average value of the contrast was calculated to be 0.0212.
Fig. 3 (a) Image of the solution(5% intralipid) in the petri dish under laser illumination (b) Corresponding false colour contrast map. X axis and Y axis represent pixel positions of the image in both cases.

A summary of the results are given in a tabular form (Table 1) for a comparative study. The table includes the contrast value, and correlation time obtained for each solution, along with the scatterer concentration.

<table>
<thead>
<tr>
<th>Scatterer conc. (by volume)</th>
<th>Speckle pattern average contrast</th>
<th>Correlation time (µs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.05 %</td>
<td>0.0432</td>
<td>62.2</td>
</tr>
<tr>
<td>0.5 %</td>
<td>0.0327</td>
<td>35.6</td>
</tr>
<tr>
<td>5%</td>
<td>0.0212</td>
<td>14.9</td>
</tr>
</tbody>
</table>

Table 1: Summary of the experimental and calculated results

From the table, it can be seen that increase in scatterer concentration results in decreased contrast as evident from eq.(1) where the mean intensity increases. The correlation time is decreasing as the contrast is decreasing which is in agreement with the available literature [5].

The study was intended to provide some preliminary results on the use of LASCA technique for the non-invasive assessment of body fluid scatterer concentration. The analysis presented here is helpful for a qualitative analysis on the increase / decrease of concentration of static scatterers, i.e., under zero flow conditions. Further investigations and related analysis is to be carried out for a quantitative analysis of the scatterer concentration.

Apart from static speckle pattern, when a tissue is illuminated with a laser a dynamic speckle pattern is observed which changes with the motion of the RBCs. Such studies have been reported in the literature [6,7]. The variations observed in the contrast value of the bio-speckle pattern when the RBCs move, known as the time-varying speckle, can be utilised for estimating the blood flow and velocity. Any increase in blood velocity is accompanied by a corresponding decrease in speckle contrast and vice versa. Studies on changes in the scatterer concentration in such flow conditions require further research in the future.

### 4. CONCLUSIONS

Some preliminary results on the application of LASCA for analysing the concentration of static scatters are presented here. From the obtained results it is evident that LASCA is indeed a non-invasive technique which can be utilized in assessing the static scatterer concentration in body fluids under zero flow conditions. Possible in-vivo applications of this work include qualitative assessment of RBC concentration to know the progress of a treatment for anemia under conditions of occluded blood flow using a cuff as well as checking the status of RBC concentration after a blood transfer sometimes needed prior to surgery.

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### REFERENCES