

# Evaluation of blood plasma coagulation dynamics by speckle analysis

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**Abstract.** Analysis of speckle dynamics is frequently used to study the motion of scattered objects or liquids. By assessing the increase in contrast on the speckle field produced by a blood plasma sample, illuminated by a laser during a coagulation test, as well as the slowing down of speckle fluctuations, we measured the time required for blood plasma coagulation *in vitro* and evidence the process dynamics. Then, we compared this noninvasive method with a mechanical viscosity-based detection system classically used in hematology laboratories; our results show good correlation and could provide more information about the blood clotting process. © 2004 Society of Photo-Optical Instrumentation Engineers. [DOI: 10.1117/1.1645799]

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## 1 Introduction

Laser light-induced granularity can be observed either in free space (an objective speckle) or on the image plane of a diffuse object illuminated by the coherent source (a subjective speckle).<sup>1</sup> This random distribution intensity of light contains information about the dynamics of scattering media such as translation movement of a scattering object<sup>2</sup> and Brownian motion of scattering particles or scattering flow.<sup>3</sup> In recent years there has been growing interest in using laser speckle techniques in medical applications in order to, for example, evaluate biological liquid dynamics as blood flow.<sup>4,5</sup> Measurements used correlometric methods or laser speckle contrast analysis.<sup>6–8</sup>

This paper reports on the use of speckle phenomenon in the real-time investigation of blood plasma coagulation dynamics. Clinical evaluation of the status of a coagulation system is of paramount importance in the diagnosis of imbalance, bleeding disorders, e.g., hemophilia, and in monitoring the administration of drugs. To determine coagulation parameters, e.g. clotting time or clotting dynamics, an activation agent must be added to anticoagulated, i.e., calcium-free, plasma samples. In hospitals, clotting times are mainly measured by mechanical methods (based on an increase in plasma viscosity, e.g., Diagnostica Stago Industry's STA-R coagulometer) or by optical transmission measurement (Biomerieux Industry's MDA coagulation analyzer). Other studies have also investigated blood plasma coagulation by surface plasmon resonance<sup>9</sup> or by using a piezoelectric quartz crystal sensor.<sup>10</sup>

### 1.1 Plasma Coagulation

To measure clotting time, we mixed plasma with coagulation factors to form a blood clot, with the final product a result of a complex series of reactions. Called a "clotting cascade" it consists of two multistep pathways in which elements, mainly

proteins, termed coagulation factors, are involved. In one of them clotting is initiated further to cell injury and the clot forms after factor VII activation.<sup>11</sup> Then, an activation-generated enzyme converts prothrombin (factor II) into thrombin (factor IIa); the latter, in turn, converts fibrinogen into fibrin and activates a factor that solidifies the clot by crosslinking the fibrin polymers (Fig. 1).

*In vitro*, factor VII is activated by addition of thromboplastin and the clotting time measured is the "quick" time.

### 1.2 Speckle Field

The objective speckle effect is observed (Fig. 2) when a mixture of plasma and thromboplastin is illuminated by a coherent light source. The light propagation in this medium is characterized by the single and multiple scattering from blood platelets ( $20\,000/\text{mm}^3$ ) and proteins. So photons travel along random optical paths. The associated wavelets are randomly dephased, and then produce random interference that induces statistical distribution of the light intensity, that is, the speckle. During the liquid phase of mixing, the Brownian motion of particles causes random agitation of the speckle ("boiling of the speckle"<sup>12</sup>). So analysis of clotting process dynamics is made from a dynamic objective speckle field.

## 2 Coagulation Characterization by Speckle Fluctuation Measurement

### 2.1 Experiment

To induce clotting, we added twice the volume of thromboplastin to the volume of anticoagulated, calcium-free, plasma ( $200\ \mu\text{L}$  for  $100\ \mu\text{L}$  plasma) previously heated at  $37\ ^\circ\text{C}$ . The experimental setup is depicted in Fig. 3: the sample is illuminated by a HeNe laser that emits at  $632.8\ \text{nm}$  with a coherence length of about  $20\ \text{cm}$ . Temporal variation of light intensity,  $I(x_0, y_0, z_0, t)$ , is measured at the point  $(x_0, y_0, z_0)$

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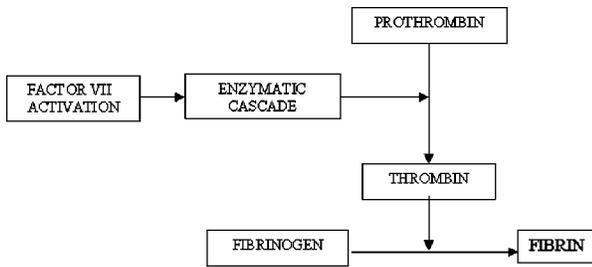


Fig. 1 Clotting cascade.

during  $\Delta t$  by a photomultiplier placed behind a pinhole whose diameter is smaller than the size of the speckle.<sup>3</sup>

The signal is numerized by a numerical oscilloscope and processed by an algorithm to give the clotting time, which corresponds to the exact time at which the speckle field becomes static. In addition, the software algorithm calculates the cumulative sum of signal fluctuation extrema after filtering (Fig. 4). A low pass filter was adjusted to eliminate the high frequencies of the raw signal due to the measurement noise and turbulence produced by the addition of thromboplastin. This algorithm permits us to follow the dynamics of coagulation.

Experimental data for this noninvasive optical technique, i.e., speckle fluctuation measurement (SFM), were compared to those obtained by a viscosity-based detection system (VBDS) (Diagnostica Stago Industry's STA-R coagulometer) often used in hematology laboratories. In this system, the magnetic field-induced oscillations of a little steel ball placed in the plasma are recorded. Detection begins at the time of thromboplastin addition, and the chronometer is started when the ball starts to oscillate. As a clot is forming, the viscosity increases and the oscillations amplitude decreases. The chronometer is stopped at a time determined by different algorithms, and gives the "quick" time even if the clot is weak, and even if the ball is still in motion.

Five samples were analyzed in triplicate; for each of them the results were averaged to obtain the SFM results. The times given by the optical technique we used and the mechanical method are not the same: the former (SFM result) corresponds to the time needed for completion of the clotting process whereas the latter (VBDS result) is measured slightly after

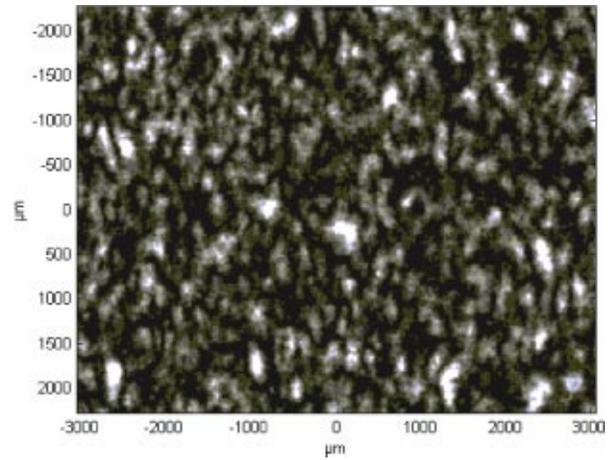


Fig. 2 Example of a speckle.

start of this process. The total dynamics of coagulation are not taken into account by VBDS and the exact start of the clotting process is not known.

## 2.2 Results

The temporal evolution of a typical signal during a coagulation experiment is displayed in Fig. 5(a) [Fig. 5(b) is an enlarged view of the signal displayed in Fig. 5(a)]. It shows the following three parts:

- first, a section where fluctuations are slow (plasma only is present);
- a second section of fast fluctuations that slow down with as the clotting process develops; and
- a final section with no fluctuations (only noise), once the clotting process is over.

One should note a decrease in fluctuation amplitude concomitant with the decrease in optical transmission by the sample during the coagulation process. So the light intensity needs to be relatively strong. Figure 5(a) shows that, in the range of 32–35 s, the signal during turbulence fluctuations looks truncated because of oscilloscope overloading. But such trunca-

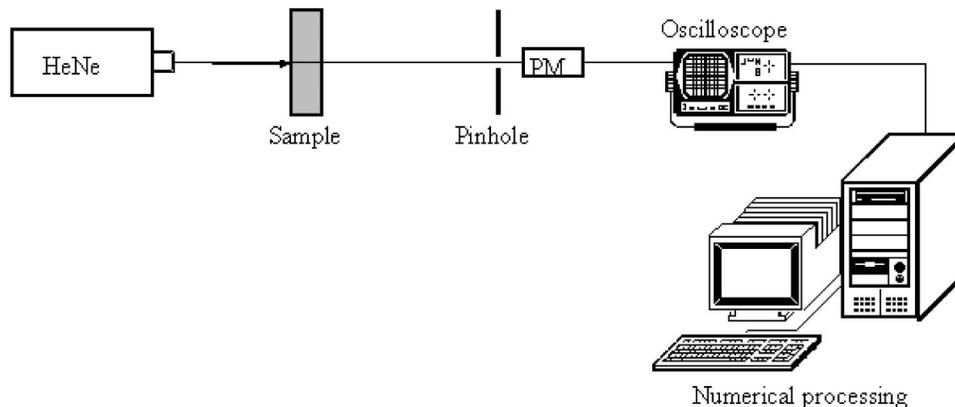


Fig. 3 Experimental setup. PM: Photomultiplier.

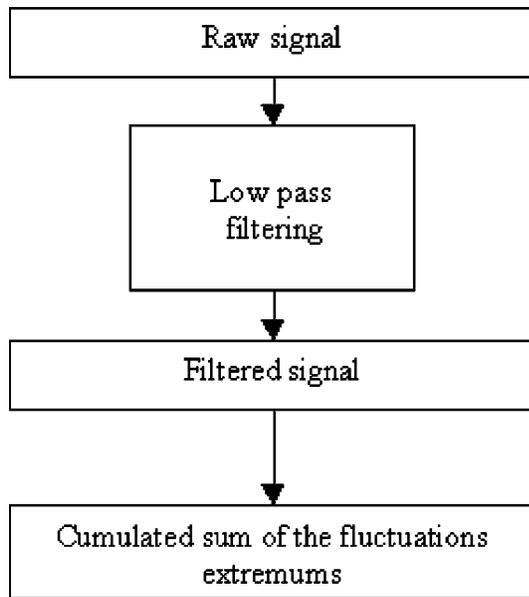


Fig. 4 Numerical processing. The raw signal comes from the oscilloscope.

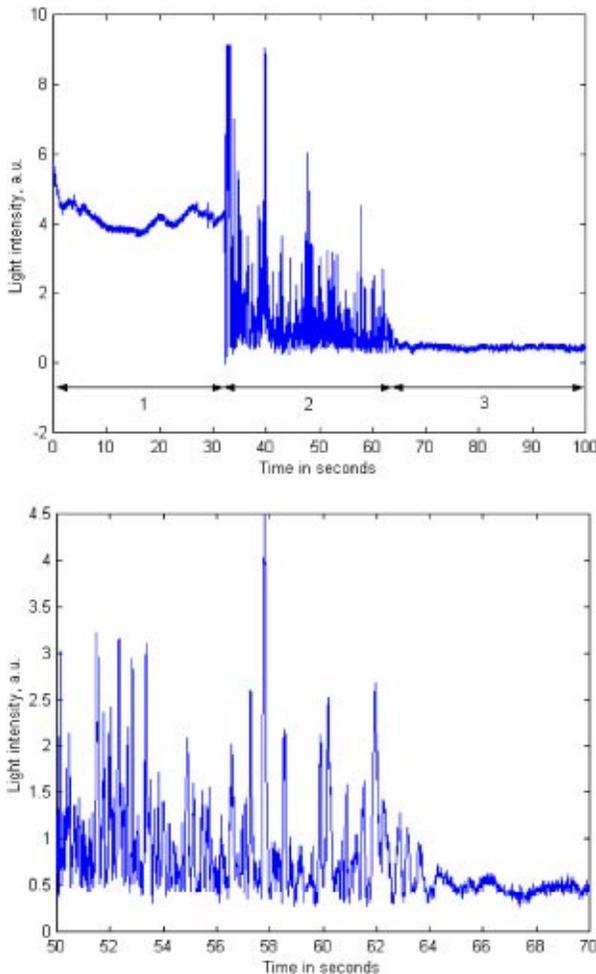


Fig. 5 (a) Light signal versus time (raw signal). (b) Zoom in.

Table 1 Comparative results of SFM and VBDS.

Sample	1 (s)	2 (s)	3 (s)	4 (s)	5 (s)
SFM results	24.4	32.3	33.5	34	37.9
VBDS results	17.7	22.2	23.3	18.7	25.2

tion is not paramount because the quick turbulence fluctuations are not taken into account in the signal processing thanks to the use of a low-pass filter.

Samples were ranked in ascending order according to their clotting time obtained by SFM.

Table 1 clearly shows no full agreement in the ranking order of the sample clotting times between the two methods: according to VBDS measurement: the classification is 1, 4, 2, 3, and 5. As mentioned above, the parameter “time” measured by the two methods is not the same, which explains the difference for sample 4; the start of coagulation and the duration of the clotting process are not interrelated although both depend on the characteristics of the plasma under test. This is highlighted by plotting versus time the cumulative sum of filtered signal fluctuation extrema from a single coagulation experiment for samples 1, 3, 4, and 5 (samples 2 and 3 have like behavior). Figure 6 clearly shows three zones.

1. The first and linear sections correspond to the filtered quick fluctuations of the raw signal; they result from the turbulence phenomena induced in the sample by thromboplastin addition and Brownian motion at low viscosity. Before coagulation, Brownian movement is constant. After low-pass filtering and smoothing of Brownian fluctuations, the frequency distribution of the filtered signal is constant over the course of this first section. So the cumulative sum of the extrema versus time forms a linear section.

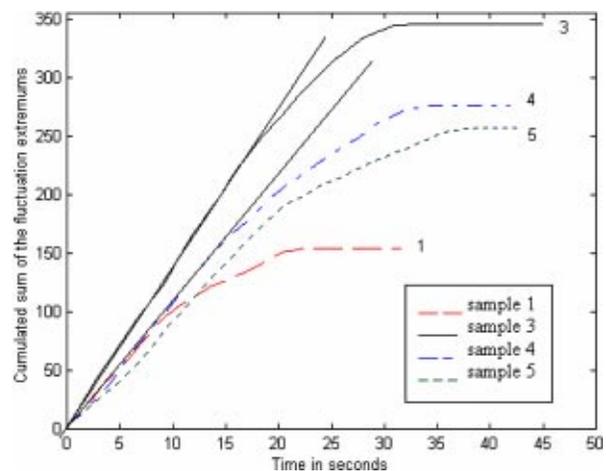
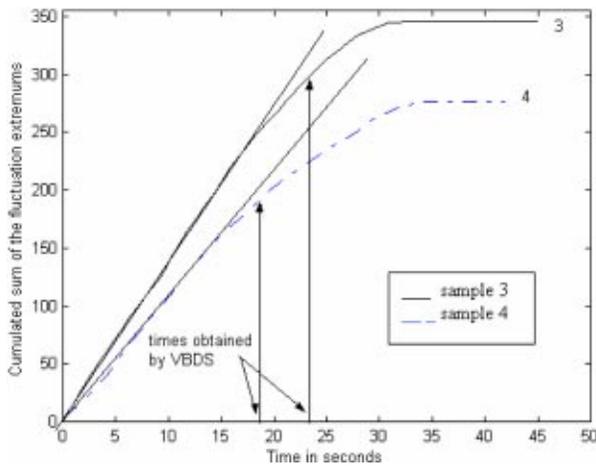


Fig. 6 Evolution of the cumulative sum of the fluctuation extremums versus time during a coagulation test. Time 0: Thromboplastin addition.



**Fig. 7** Comparison of SFM measurements to times obtained by VBDS for samples 3 and 4.

2. Increase of the sample viscosity during the coagulation process slows down fluctuations of the raw signal followed by the filtered signal; it is depicted by the second section in Fig. 6. This part corresponds to a reduction in speckle grain agitation and then to monitoring of the clotting process.
3. The plateau starts as soon as the clot forms and the speckle is fixed.

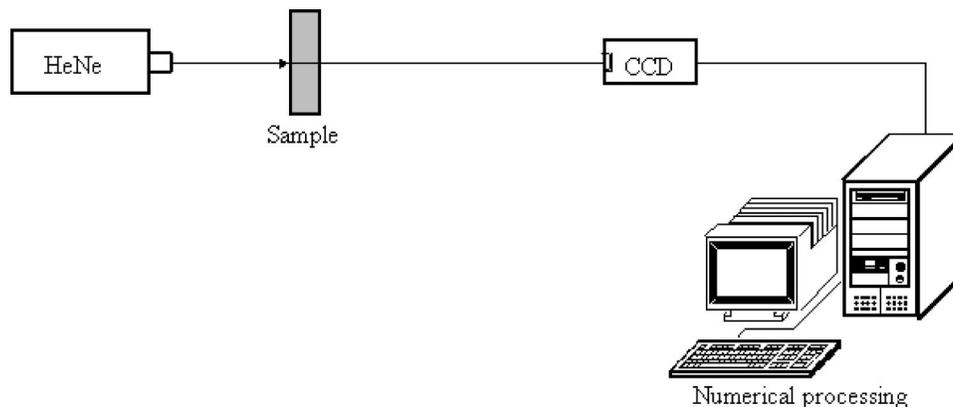
In Fig. 7 superimposed are the times obtained from VBDS measurement (VBDS data) by the kinetics produced by samples 3 and 4: sample 4 begins to coagulate before sample 3 (VBDS data); in addition, the second section (slowdown of speckle motion) begins earlier (VBDS data) and ends later (SFM data) than sample 3.

So the differences noticed between the two series of data come from the coagulation dynamic properties of samples.

### 3 Coagulation Characterization by Speckle Contrast Measurement

#### 3.1 Experiment

Speckle contrast can be measured by a charge coupled device (CCD) camera which detects the light distribution in a plane



**Fig. 8** Experimental setup for contrast measurement.

over a certain time. The CCD allows one to follow the clotting process through real-time contrast measurement, since contrast is affected by speckle motion during the time needed for image acquisition (a 60 ms acquisition time, in our experiment). For example, it is increased as a result of a slowdown in speckle movement. At the end of coagulation, the sample contains the clot, the speckle is fixed, and the contrast is optimal. But, because contrast may become constant before the end of speckle agitation, it induces some error in the measurement when the speckle moves slowly. In this section we set that the end of the clotting process corresponds to constant contrast.

In Fig. 8 is the experimental setup we used. The light intensity,  $I(x,y)$ , is measured on the CCD sensor surface, and contrast  $C$  is calculated from the following relation:

$$C = \frac{\sqrt{\langle I^2 \rangle - \langle I \rangle^2}}{\langle I \rangle},$$

where the angled brackets denote a spatial average.

An image contains  $268 \times 748$  pixels that are  $8 \mu\text{m} \times 8 \mu\text{m}$  in size. A large number of speckles in each image sample is necessary for meaningful statistical evaluation, but the speckles must be large compared with the pixel size so that speckle intensity variations can be resolved. In our study, the diameter of a speckle was approximately 5–7 pixels (25–50 pixels/speckle).

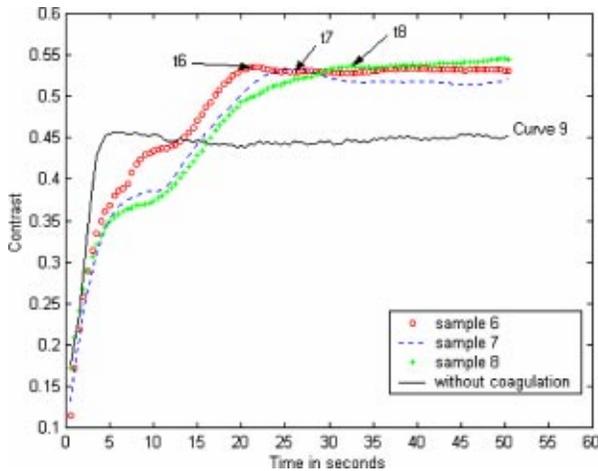
Three new samples were analyzed and the coagulation reaction described in Sec. 2.1 was carried out. The clotting time was determined by speckle contrast measurement (SCM) on contrast curves at the end of the clotting process when the contrast was constant.

#### 3.2 Results

The contrast curves plotted for the three new samples (samples 6, 7, and 8) (Fig. 9) enable one to also visualize the coagulation dynamics. A fourth curve (curve 9) corresponds to a blank (thromboplastin, but no plasma): the low contrast is caused by turbulence, and contrast becomes constant when the agitation is stationary (Brownian movement).

The sample curves exhibit four zones that correspond to

- slowdown of turbulence after thromboplastin addition,



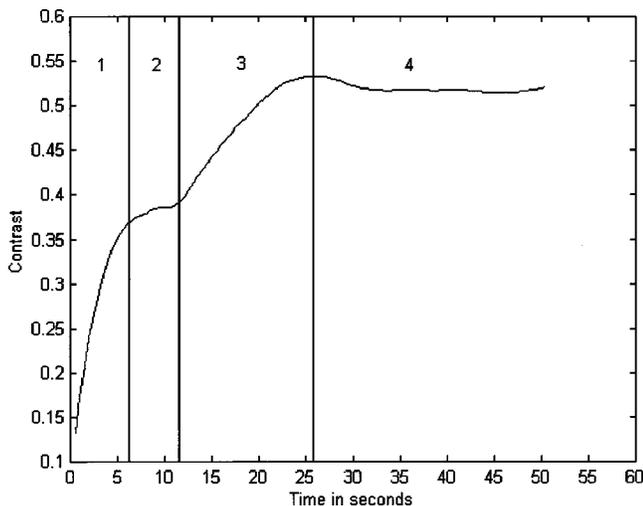
**Fig. 9** Evolution of contrast versus time during a coagulation test.  $t_6$ ,  $t_7$ ,  $t_8$ : Coagulation times. Curve 9 corresponds to a blank (thromboplastin, but no plasma).

- stabilization of the agitation,
- the process of coagulation (increase of the viscosity), and
- the end of this process, that is, the final clot.

Figure 10 illustrates this kinetics for sample 8.

Measurement of the coagulation time at the beginning of the fourth zone for the different samples gave

- 21 s ( $t_6$  in Fig. 9) for sample 6 (13.2 s by VBDS measurement),
- 26 s ( $t_7$  in Fig. 9) for sample 7 (16.1 s by VBDS measurement), and
- 33 s ( $t_8$  in Fig. 9) for sample 8 (48 s by VBDS measurement).



**Fig. 10** Evolution of contrast in real time during clot formation for sample 8. Time 0: Thromboplastin addition.

The differences in the times we measured and those produced by the VBDS technique come from the criteria used to determine the coagulation time. On the other hand, both methods produced similar ranking orders for the samples from their clotting times.

### 4 Conclusion

Analysis of the motion of the speckle field produced by the scattering of a laser beam by blood provides helpful data on the motion of blood platelets and proteins in this biological fluid; in particular, it allows observation and analysis of plasma coagulation dynamics by a noninvasive technique. Two methods (speckle fluctuation measurement and speckle contrast measurement) based on speckle properties were used to visualize these dynamics and assess the duration of the clotting process. These techniques were validated through comparison with measurements made by another classical methodology often used in hematology laboratories.

The first method (SFM) brings information on the duration of the coagulation process and plasma properties: for example, coagulation in a given sample starts earlier but finishes later than another one. These specifics can be helpful when used for clinical treatment.

Detection of the end of coagulation by the second method (SCM) could be optimized by a longer image acquisition time. Contrast should be highest only when the speckle is fixed.

Finally, this analysis of the dynamic speckle field produced by human blood can be used as a real-time cheap technique by which to get data on blood coagulation dynamics.

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