Structure and refractive index of fibrin protofibril aggregates according to laser phase microscopy accompanied by DLS and AFM

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Abstract: The structures, sizes, and refractive indices (RI) of protein aggregates formed in a fibrinogen-thrombin system are examined using laser phase microscopy (LPM) accompanied by dynamic light scattering (DLS) and atomic force microscopy (AFM) measurements. Fibrin aggregates found in pure fibrinogen and fibrinogen with thrombin solutions by the DLS method, after drying the sample, form complex structures of different shapes and sizes on a glass surface. The LPM reveals submicron-sized dimeric structures in the pure fibrinogen solution, elongated micron-length structures, and rectangular structures in the fibrinogen-thrombin sample. AFM measurements show that the elongated structures form branched fibers, which in turn assembly into rectangular structures. All sizes obtained by LPM and AFM are consistent with DLS measurements. The refractive indices of all the structures, estimated by optical thickness, vary from 1.53 to 1.62, which indicates that they are fibrinogen derivatives. Effective visualization of the structure and determination of the optical properties for fibrin gel indicate that laser phase microscopy is capable of tissue imaging and characterization.

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1. Introduction

The study of the properties and structure of fibrin gels is of great interest. Fibrin gel is the final component of a complex coagulation cascade, which is involved in the formation of blood thrombi and clots and determines the viability of human beings. The application of optical methods for the study of fibrin gel structure and optical properties can provide information on the state of the coagulation system as a whole and enzyme activity in particular and the effect of various drugs (including nanoparticles, which are actively used for the hemostasis correction). In addition, visualization of fibrin hydrogel by new methods of microscopy (for example, laser phase microscopy) can be useful for the aims of tissue engineering. Fibrin gel scaffolds are known to be biocompatible, soft, flexible, and, moreover, fibrin contains cell-binding sites, which enhance cell adhesion. The work [1] shows the application of fibrin hydrogel matrices produced based on fibrinogen with thrombin conjugated to iron oxide nanoparticles as a scaffold for the cultivation of nasal olfactory mucosa (NOM) cells and their further implantation into spinal cord injuries.

Fibrin hydrogel is a natural biopolymer arising from the conversion of fibrinogen into its insoluble form – fibrin in the reaction of its proteolytic cleavage by thrombin. Generally, gel formation occurs in several stages, and these stages are the subject of various studies [2,3]. Some fundamental works in this area were conducted using light scattering methods (dynamic and static). For example, R. Kita et al. [4,5] showed that the autocorrelation function (ACF) and the intensity of the scattered light (Is) are changing during the process of the fibrin gel formation. In the total scattered light intensity time course, R. Kita distinguished the first plateau corresponding
to the stage of thrombin interaction with fibrinogen followed by the cleavage of fibrinopeptides from its molecule and the appearance of insoluble fibrin monomers. The next stage is a sharp increase of the scattered light intensity, which manifests the interaction of fibrin molecules with each other with the formation of two-stranded half-staggered protofibrils. Then protofibrils themselves laterally aggregate to produce more thick fibrin fibers [3]. In the third stage, fibers form a fibrin network, and in the scattered light intensity, one can see a plateau with intensity oscillations. As for the autocorrelation function, its form changes from exponential to a power law at the point of transition of the sample from a liquid state to a gel [6].

In our previous work, using dynamic and static light scattering techniques, we showed [7] that the formation of fibrin gel under preliminary incubation of thrombin with iron oxide nanoparticles obtained in acoustoplasma discharge is significantly faster (20-30 times) than without nanoparticles. We also assessed the fractal dimensions of fibrin gel samples from light scattering using Martin’s theory [6] and Muthukumar’s equation [8]. We found out that an increase of thrombin concentration or its activation by iron oxide nanoparticles leads to the increased fractal dimension of formed gels. E. Ryan et al. [9] showed by atomic force microscopy that increased thrombin concentration leads to the formation of the fibrin gel consisting of thinner fiber with more cross-links. The thinner the protofibril of which the gel is composed, the higher its fractal dimension. For our samples of fibrinogen with thrombin of higher concentration or activation with nanoparticles, we obtained higher values of the fractal dimension Df, which is consistent with the results obtained by E. Ryan.

For the study of fibrin gel structures formed at different stages of its formation, one can use microscopy (confocal light microscopy and scanning electron microscopy), as well as small-angle X-ray scattering. Despite the number of different works on fibrin gel formation, the latter stages of the thick fiber and fibrin network formation are not fully understood. The paper [10] presents the results of the structural study of fibrin fibers using small-angle X-ray scattering (SAXS) with computational molecular modeling of protofibrils. The authors show that the fibrin fibers are partially ordered with a fractal structure formation.

In the present work, for the first time, we have studied the structures formed in the samples of human fibrinogen with thrombin (in low concentration) using laser phase microscopy (LPM), dynamic light scattering (DLS), and atomic force microscopy (AFM). Our work is focused on the new application of modern optical methods in biomedicine, particularly, laser phase microscopy to determine the structure of fibrin gel and its optical properties. These methods can also be used to examine other tissues or cells.

2. Materials and methods

2.1. Sample preparation

Human fibrinogen lyophilized powder (Sigma Aldrich) has been dissolved in the Tris-HCl buffer to the final concentration of 3 mg/ml. Human thrombin lyophilized powder (Sigma Aldrich) was prepared according to the instruction and added to the fibrinogen solution. The final concentration of thrombin in the sample was approximately 0.00125 NIH/ml. We have chosen such a low concentration to stop fibrin gel formation on the stage of protofibrils and fibrin fibers formation. Fibrinogen was mixed with thrombin, and after several minutes, the sample (still liquid) was inflicted on a glass slide (for Laser phase microscopy and Atomic force microscopy). For LPM measurements, we abandoned the standard cover glass technology because we were unable to obtain a high-quality image. The initial drop (the initial thickness of which was 1 mm) was slightly dried to obtain a thin layer several microns thick. Despite drying, the structure under study remained completely surrounded by water. For DLS measurements, a sample of the solution was filtered and placed in a dust-free cylindrical cell. We used filters PVDF with a pore diameter of 0.22µ.
2.2. Laser phase microscopy (LPM)

LPM experiments were performed using a phase modulation interference microscope MIM-310 (manufactured by Amphora Labs, Russia) operating at a wavelength of 405 nm. The microscope is a two-channel device in which one channel is a conventional white light microscope and the other is a high-resolution laser interferometer; the optical layout of the interferometer is given in Fig. 1.

**Fig. 1.** Optical layout of MIM 310 phase-modulation laser interference microscope: laser with a wavelength of 405 nm (L), half wave plate (HWP), polarizing beam splitter (PBS), reference phase-modulation piezoelectric mirror (PM); telescopic system (T); beam splitters (BS1, BS2), mirrors (M1, M2); polarization modulators (PM1, PM2); objectives (O1, O2); matrix photodetector (D), sample (S).

The collimated beam from the laser L is passed through the half-wave retardation plate HWP and is split by the polarizing beam splitter PBS. The elements PM1 and PM2 control the beam polarization in both arms of the interferometer. Each of them is a combination of λ/4 and λ/2 phase plates. The redistribution of the laser intensity between the object and the reference beams is controlled by rotating HWP to ensure the optimum interference contrast regardless of the optical density of the object. After the object beam is reflected by the mirror M1 and split by the beam splitter BS1, the beam portion illuminating the sample placed on the stage S is directed into the objective O1. The light reflected by the sample prepared on a reflective substrate is collected by O1 and directed through BS1 and the telescopic system T into the photodetector D. The reference beam produced by PBS is passed through the beam splitter BS2 and directed into the objective O2, which is similar to O1. The reference beam is reflected back by the piezoelectric mirror PM located near the focal plane of the objective O2, and then directed by BS2 and the mirror M2 to BS1, where it is added to the object beam to obtain an interference pattern on the CMOS sensor of the photodetector D. The dynamic interference pattern received by D is processed by a personal computer.

The measurements of the 2D spatial distribution of the phase shift between the reference and object waves in a plane transverse to the beam axis are performed as follows. The light intensity
measured by the matrix D in each pixel is described by the formula

\[ I = I_1 + I_2 + 2\sqrt{I_1 I_2} \cos \delta, \]

where \( I_1 \) and \( I_2 \) are the intensities of the reference and object waves, respectively, \( \delta \) is the intensity of the background (incoherent) radiation, \( \delta \) is the phase shift between the reference and the object waves. To determine the value of \( \delta \) for each pixel of the matrix D, sequential intensity measurements are carried out for four fixed values \( \Delta \delta_i \) \((i = 1, 2, 3, 4)\) of the phase shifts associated with a change in the length of the reference arm of the interferometer using the piezoelectric mirror PM. Specifically, the system of four equations is solved for each pixel:

\[
\begin{align*}
I(1) &= I_1 + I_2 + 2\sqrt{I_1 I_2} \cos(\delta + \Delta \delta_1), \\
I(2) &= I_1 + I_2 + 2\sqrt{I_1 I_2} \cos(\delta + \Delta \delta_2), \\
I(3) &= I_1 + I_2 + 2\sqrt{I_1 I_2} \cos(\delta + \Delta \delta_3), \\
I(4) &= I_1 + I_2 + 2\sqrt{I_1 I_2} \cos(\delta + \Delta \delta_4).
\end{align*}
\]

By measuring the value of \( \delta \) for each pixel, the distribution of the phase shift over the entire matrix is actually obtained. The area of the sample, which can be processed in this way, is determined by the magnification factor of the objective O1; Moving the stage S step by step in the plane transverse to the optical axis and recording the interference pattern at each step, it is possible to obtain the distribution of the phase shift over the entire investigated sample.

The phase shift between the reference and object waves for a transparent (weakly absorbing) object can be expressed as:

\[ \delta = \frac{4\pi}{\lambda} (n - n_0)L, \]  

where \( n_0 \) is the refractive index of the medium, \( \lambda \) is the wavelength \((\lambda = 405 \text{ nm})\), \( L = L(x, y) \) is the vertical size (along the beam axis) of the investigated object (particle, structure), depending on the coordinates of the transverse plane. If \( n > n_0 \), then the form of \( \delta \) dependence on the transverse coordinates will be convex or, otherwise, concave. The distribution of the phase difference in the plane transverse to the laser beam allows us to determine the size and the refractive index of particles simultaneously.

In phase microscopy, the phase shift is actually measured in units of the optical path difference (OPD) between the reference and object waves. According to [11], the OPD \( \Delta h \) for a spherical particle is related to its size (diameter \( d \)) and refractive index \( n \):

\[ \Delta h = \frac{\delta \lambda}{2\pi \alpha} = \frac{d \Delta n}{\alpha}, \]

where \( \alpha \) is an apparatus coefficient depending on the particle size, determined experimentally for latex spherical particle in paper [11]. We will use [Exp. (2)] for refractive index estimation of elongated particles, under assumption that the average contour of their section is close to a circle, that is, the vertical and horizontal sizes of the particle in the section are approximately the same.

In this study, we used an objective with a numerical aperture (NA) of 0.9. Lateral resolution of MIM 310 (in XY plane transverse to the beam axis) varies from \(~10\) to 100 nm depending on the phase contrast of the object. The vertical resolution (along the beam axis \( Z \)) is determined by the accuracy of measuring the phase of the object wave by the interference with the reference wave and can reach 1 nm.

2.3. Dynamic light scattering (DLS)

In our experiment, we use a conventional setup for dynamic and static light scattering consisting of He-Ne laser (632.8 nm, 5-10 mW), a cylindrical cuvette (with a diameter of 15 mm) placed in an
immersion cuvette (with a diameter of 35 mm) on the subject table of the goniometer, which allows us to set various scattering angles from 0 to 180°, photodetector (avalanche photodiode), correlator (Photocor-Fcm, manufactured by LLC “Photocor”, Russia), an optical system ensuring spatial coherence of recorded light. The cuvette was thermostated at the temperature of 37.0 ± 0.2°C. The setup is equipped with PC software for recording the autocorrelation function (ACF) of the scattering intensity (Photocor-Fcm program) and processing the ACF to determine the scattering intensity distribution over the scatterer sizes (DynaLS program).

2.4. Atomic force microscope (AFM)

For measuring the height distribution of fibrinogen structures on a glass surface, we used AFM, which consists of an NPX200 scanner unit, having a vertical resolution (Z) of 0.3 nm and movement range: lateral (XY) 500 nm – 800 µm, vertical (Z) ± 800 nm, and a Nanoptics 2100 controller.

3. Experiments and discussion

3.1. Laser phase microscopy measurements

We measured the OPD for a sample of pure fibrinogen solution. After drying the sample on a glass surface, we registered a complex structure consisting of two subunits. Figure 2 shows an image of the 2D distribution of OPD for the structure (Fig. 2(a)) and the corresponding transversal OPD cross-section along the Y-axis for the first subunit (Fig. 2(b)) and the second subunit (Fig. 2(c)). The subunits diameter \( d \) is determined as the width at a half-height of the OPD. For the first subunit, the estimated diameter is approximately equal to 400 nm (in the section perpendicular to the axis of binding), and the OPD value \( \Delta h \) is equal to 45 nm. For the second subunit, the diameter \( d \) is 500 nm, and OPD \( \Delta h \) is 45 nm. Since the shape of the profile is close to the OPD profile of the calibration spherical particles, we assume that the geometric section of our particles can be approximated by a circle, and therefore we can use [Eq. (2)]. Estimation of \( \Delta n \) following [Eq. (2)] gives the values 0.276 and 0.207 for the first and second subunits at a wavelength of 405 nm. From the characteristic behavior of \( n(\lambda) \) for protein and water, it follows that \( \Delta n \) at a wavelength of 589 nm will be 0.009 less. Therefore, the refractive indices \( n \) are 1.597 and 1.529 for the first and second subunits at a wavelength of 589 nm. From the literature data, the refractive index of anhydrate fibrinogen calculated by the Lorentz-Lorentz equation is 1.602 [12]. Since the refractive index of the first subunit practically coincides with the literature, and the second is slightly lower, we can conclude that the registered structure is a derivative of fibrinogen, apparently, an assembly of two protofibrils. The difference in the refractive indices of these two subunits (protofibrils) can be explained by the fact that they themselves are aggregates of fibrin monomers with a fractal dimension [13,14]. According to our previous work [15,16], aggregates of nanoparticles of metal oxides or diamonds are not continuous, but an assembly of individual NPs with interlayers of a dispersion medium. Aggregates with a larger diameter have a lower density (fractal dimension) and a smaller difference in refractive indices between an aggregate and water. An analogous picture is observed for the structure shown in Fig. 2, that is, the larger subunit has a lower RI value, and the smaller subunit has a higher RI, which can be conditioned by the different amounts of water in the aggregate subunit in the under-dried sample.

In the dried sample of fibrinogen with thrombin, we observed structures of elongated and rectangular shapes. Figure 3 presents the two-dimensional distribution of OPD of an elongated structure (a) and the corresponding section along the X-axis (b) and the Y-axis (c). The OPD profiles along the X-axis give the \( \Delta h \) value of 50 nm and the diameter of 400 nm. The Y-axis profile shows several sequentially related subunits with the OPD values \( \Delta h \) from 40 to 65 nm and diameters of 500–700 nm. The estimation of \( \Delta n \) following [Eq. (2)] gives the value 0.247. The obtained refractive index of the subunits in the structures is 1.58, which is close to the RI values
of subunits presented in Fig. 2. As we mentioned above, according to the literature data, the refractive index of fibrinogen is 1.602. Therefore, we can conclude that the elongated structure is an associate of protofibrils, namely, a fiber. The total length of the fiber is more than 3 \( \mu m \).

Figure 4 presents a rectangular structure found in the dried sample of fibrinogen with thrombin. Figure 5 shows the OPD distribution for a similar structure. The structure consists of several long associates of individual subunits. From the OPD profiles along the X-axis (Fig. 5(b)) and the Y-axis (Fig. 5(c)), we can obtain the value of the diameters (400–700 nm) and OPD (40–70 nm) for subunits of the structure. These values are very close to those observed for separate elongated structures (Fig. 3). We suppose that the structure is an associate of individual elongated structures analogous to presented in Fig. 3, namely, fibrin fibers. The sizes of this fiber associate are \( 1.6 \times 1.7 \mu m \).

In another sample of fibrinogen with thrombin, we observed similar rectangular structures consisting of significant number of elongated structures. Figure 6 demonstrates the 2D distribution of OPD (a) and profiles of this structure along the X-axis (c) and Y-axis (d). Along the X-axis, the profile of the structure has more pronounced differences between adjacent areas. This section shows that the OPD values are 35–45 nm, and the mean diameters are 300–400 nm. Estimation of \( \Delta n \) according to [Eq. (2)] gives the value 0.29. Therefore, the refractive index of the structure is 1.62, which is also close to the RI of fibrinogen and indicates that this structure consists of connected protofibrils. The sizes of this rectangular structure are \( 2.6 \times 3.0 \mu m \).
Fig. 3. Elongated structure detected by LPM in the dried sample of fibrinogen with thrombin. (a) two-dimensional distribution of optical path difference; (b), (c) corresponding one-dimensional profile along the X-axis, the Y-axis.

Fig. 4. Laser light image of rectangular structures in the sample of fibrinogen with thrombin. The size of the white square is 8.8 µm x 8.8 µm.
Research Article

Vol. 12, No. 5 / 1 May 2021 /

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Fig. 5. Protofibrils assembly detected by LPM in the dried sample of fibrinogen with thrombin: (a) two-dimensional distribution of optical path difference; (b), (c) corresponding one-dimensional profile along the X-axis and the Y-axis.

Figure 7 presents another OPD distribution obtained in the sample of fibrinogen with thrombin. One can see that the form and internal structure of this object are very similar to those presented in Fig. 3–5. However, the structure has a more significant size equal to 3.0 × 4.0 µm and greater OPD value than those presented in Fig. 3–5. The OPD values for these structures, especially on the edges, reaches 120-150 nm. We believe that the formed structure consists of several layers of associates similar to those shown in Fig. 5 and 6, that is, it has a multi-layer architecture, which can be part of the formed gel.

3.2. Dynamic light scattering measurements

To assess the extent to which the results obtained with the LPM are in agreement with other methods of investigation, we conducted a study of liquid samples of fibrinogen and fibrinogen with thrombin using the DLS method. Figure 8 demonstrates the distribution of the scattered light intensity over particle sizes in the solution of pure fibrinogen obtained at the scattering angle of 45°. One can see that the distribution has three peaks with mean hydrodynamics radii equal to 13, 157, and 3024 nm. The first peak corresponds to fibrinogen monomers [17], which scatter the more significant part of the scattered intensity. We believe that the other two peaks represent natural self-assembled fibrinogen aggregates. It is known [18, 19] that fibrinogen tends to spontaneously modification of its carboxyl ends in such a way that flexible polymer filaments are formed. These filaments then twist into coils, which in turn can form branched aggregates.

DLS data are in good agreement with LPM data. We were unable to observe the fibrinogen monomers with the LPM because they are too small. However, the peak with a hydrodynamic
radius of about 160 nm is in good agreement with the sizes of individual subunits (protofibrils) of the structure shown in Fig. 2 (size about 400 nm). The third peak with a size of about 3 µm can correspond to one of the structures shown in Fig. 3–5, which are associates of individual protofibrils.

To monitor the gelation process, thrombin was added to pure fibrinogen solution. Further, the autocorrelation functions of the scattered light intensity and the corresponding size distributions of the intensity were measured over a period of time (about an hour). Figure 9 presents the distribution of the scattered light intensity over particle radii in the solution of fibrinogen in 45 minutes after thrombin adding. One can see that the distribution of pure fibrinogen (Fig. 8) is changed dramatically, and there are three peaks with the opposite intensity-radii relation. We have described such redistributions of the scattered light intensity over the particle sizes in a fibrinogen-thrombin system in our previous work [7]. During the interaction of thrombin with fibrinogen, the number of fibrinogen monomers (first peak) decreases, and the number of large fibrin aggregates increases in the solution. One can see that the third peak with a radius of 5500 nm corresponds in order of magnitude to the rectangular structures registered by LPM (Fig. 7). We explain the appearance of such structures in the sample (either in liquid or under-dried) by the formation of parts of the gel, consisting of a large number of fibrin fibers. However, the position of this peak corresponds to twice the size of the subunits observed by other methods. Therefore,
Fig. 7. Protofibrils multilayer assembly detected by LPM in the dried sample of fibrinogen with thrombin: (a) two-dimensional distribution of optical path difference; (b), (c) corresponding one-dimensional profile along the X-axis and the Y-axis.

Fig. 8. Scattered light intensity distribution over particle hydrodynamic radii obtained by DynaLS software from the ACF in the solution of pure fibrinogen. The scattering angle is 45°.
another possible explanation for the appearance of this peak in the distribution will be given in the next section.

![Fig. 9. Scattered light intensity distribution over particle hydrodynamic radii obtained by DynaLS software from the ACF in the solution of fibrinogen with thrombin in 45 minutes after its adding. The scattering angle is 45°.](image)

3.3. Atomic force microscopy (AFM)

Using atomic force microscopy, we also examined under-dried samples of fibrinogen solution with thrombin and observed subunits with a characteristic size of about 300-400 nm, which assemble into aggregates of branched and rectangular form, see Fig. 10 and 11. The subunit sizes correspond to the hydrodynamic radii of the second peak in the scattered light intensity distribution over particle sizes obtained by DLS for native fibrinogen aggregates (Fig. 9). It is also in good agreement with the diameter of the subunits of the elongated structure presented in Fig. 2 and Fig. 3.

![Fig. 10. Branched structures detected by AFM in the dried sample of fibrinogen with thrombin: (a) two-dimensional image, (b) profile along the section, (c) table with parameters.](image)

As already shown using the LPM method, protofibrils form elongated structures (Fig. 3). However, the size of phase images in the phase microscope is only 8.8 x 8.8 microns, and with
Fig. 11. Elongated structures of protofibrils detected by AFM in the dried sample of fibrinogen with thrombin.

this method, we can only see a small part of the structure. AFM makes it possible to see a more complete picture (Fig. 10). A branched structure with a total length of more than 30 µm is visible. Figure 11 includes the table with characteristic parameters, which shows that the transverse dimension of these branched elongated structures (yellow line, distance 723 nm, which is two mean diameters of subunits) corresponds to those in Fig. 3. These values are close to the size of fibrin aggregates obtained using DLS (the second peak in Fig. 9, as was mentioned above). Simultaneously, the third peak of the distribution in Fig. 9, p = 5.5 µm (or tau = 0.235 s), is more than two times larger than the sizes of the largest individual subunits recorded using LPM and AFM. And this is despite the fact that when using LPM and AFM, the concentration increases during the drying process. A possible explanation is that this third peak in the distribution (Fig. 9) is not due to the diffusional motion of particles but to vibrations of the branched structure shown in Fig. 10.

In samples with a long period of drying, it was also possible to register by the AFM method rectangular structures similar to those visualized using LPM (Fig. 5–7). Figure 11 shows an example of such structures. Unfortunately, it was impossible to determine their real height by the AFM method because the needles just broke. Such fact testifies that the thickness of such rectangular structures is more than 500 nm.

Indeed, if, to estimate the geometric thickness of a rectangular structure, we assume Δn = 0.24, α = 2.5 and maximum OPD Δh = 150 nm (Fig. 7), then we can use [Eq. (2)], rewriting it in the form:

\[ d = \frac{\Delta h \alpha}{\Delta n}, \]  

Then the geometric thickness of this structure will be of the order of d = 1300 nm.

Note that a similar estimation for the geometric thickness of the elongated structure in Fig. 2 (but with α = 2.2) gives d = 220 nm, which coincides with the AFM.
4. Conclusions

Summarizing, we, for the first time, have performed measurements of the optical phase distribution for the structures formed in the samples of fibrinogen with thrombin on a glass surface using laser phase microscopy, accompanied by dynamic light scattering and atomic force microscopy. We have shown that fibrin aggregates found by the DLS method in solutions of pure fibrin and fibrin with thrombin form complex structures when the solutions are dried on the glass. Using LPM, we observed the rectangular and elongated structures forming branched fiber, which are also visible by AFM. The refractive indices of the registered structures vary from 1.53 to 1.62, which is in good agreement with the literature data for the fibrinogen refractive index (1.602).

Based on the refractive indices obtained, we conclude that all the structures are derivatives of fibrinogen, namely, protofibrils, their dimers, and elongated associates, forming branched fibers and multilayer aggregates of fiber parts. Such branched structures can be formed already in solution, and the relaxation time of their bend fluctuations is about 0.24 s. From the data obtained, we can conclude that the forming gel consists of branched fibrin fibers and multilayer rectangular aggregates. They have an ordering of subunits on a submicron scale with a characteristic distance of the size of protofibril aggregates, similar to the ordering of fibrin monomers in protofibrils on a nanometer scale [10]. LPM showed the variability of intermediate forms of fibrin aggregates in the process of gelation, and the ability to control the appearance and development of these forms using optical methods. Modern optical methods such as laser phase microscopy can be used to assess the state of the blood coagulation system, the effect on it of various drugs (including nanoparticles), to visualize fibrin hydrogel scaffolds, and, consequently, other tissues and cells as well.

Funding. Ministry of Science and Higher Education of the Russian Federation (075-15-2020-912).

Acknowledgments. This work was supported by a grant of the Ministry of Science and Higher Education of the Russian Federation (075-15-2020-912) for the organization and development of a World-class research center “Photonics”.

Disclosures. The authors declare no conflicts of interest.

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