DOI: 10.18721/JPM.12206
УДК 535.36, 535.4, 57.088

MODIFICATION OF LASER CORRELATION SPECTROSCOPY METHOD FOR ANALYZING POLYDISPERSE NANOPARTICLE SUSPENSIONS

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The paper proposes a modification of the laser correlation spectroscopy method to improve the accuracy of determining the size of polydisperse nanoparticles in suspensions. The essence of the modification is to create an original scheme and an experimental data processing algorithm, which makes it possible to determine the size of highly polydisperse as well as nonspherical nanoparticles. A theory is given for calculating the size and shape of nanoparticles, as well as an algorithm for solving the inverse ill-posed problem of laser correlation spectroscopy. The approbation of the developed software and hardware complex is performed using model signals with different noise levels, as well as in the study of monodisperse and polydisperse suspensions of spherical and ellipsoidal particles with known sizes.

Keywords: laser correlation spectroscopy, nanoparticle, dimension, software and hardware complex

Citation: Nepomnyashchaya E.K., Velichko E.N., Modification of laser correlation spectroscopy method for analyzing polydisperse nanoparticle suspensions, St. Petersburg Polytechnical State University Journal. Physics and Mathematics. 12 (2) (2019) 72–85. DOI: 10.18721/JPM.12206

МОДИФИКАЦИЯ МЕТОДА ЛАЗЕРНОЙ КОРРЕЛЯЦИОННОЙ СПЕКТРОСКОПИИ ДЛЯ АНАЛИЗА ПОЛИДИСПЕРСНЫХ СУСПЕНЗИЙ НАНОЧАСТИЦ

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В работе предлагается модификация метода лазерной корреляционной спектроскопии для повышения точности определения размеров полидисперсных наночастиц в составе суспензий. Суть модификации состоит в создании оригинальной схемы и алгоритма обработки экспериментальных данных и позволяет определять размеры сильно полидисперсных, а также несферических наночастиц. Приводится теория для расчета размеров и формы наночастиц, а также алгоритм для решения обратной некорректной задачи лазерной корреляционной спектроскопии. Апробация разработанного программно-аппаратного комплекса производится с использованием модельных сигналов с различным уровнем шума, а также при исследовании монодисперсных и полидисперсных суспензий сферических и эллипсоидальных частиц с известными размерами.

Ключевые слова: лазерная корреляционная спектроскопия, наночастица, размер, программно-аппаратный комплекс

Ссылка при цитировании: Непомнящая Э.К., Величко Е.Н. Модификация метода лазерной корреляционной спектроскопии для анализа полидисперсных суспензий наночастиц // Научно-технические ведомости СПбГПУ. Физико-математические науки. 2019. Т. 12. № 2. С. 73–87. DOI: 10.18721/JPM.12206

Introduction

There is growing interest in synthesis and study of nanoparticles and nanoparticle suspensions. While existing methods such as electron and atomic force microscopy have been recognized as effective, they do not allow to monitor changes in size and dynamics of particle aggregation in real time [1, 2]. Besides, these methods have severe limitations in studies of biological suspensions.

Methods based on dynamic light scattering, including laser correlation spectroscopy (LCS) [1, 3], have proved their efficiency in this situation. LCS is widely used in synthesis of nanoparticles and biomolecules for rapid analysis of particle size distributions. Unfortunately, commercially available laser correlation spectrometers cannot reliably analyze multicomponent polydisperse solutions with sufficient accuracy [4]. Furthermore, all sizes are calculated under the assumption that the scattering particles are spherical, while in fact nanoparticles of other shapes are often found, and biomolecules frequently form non-spherical clusters [5, 6]. In view of these limitations, it is important to improve the existing method of laser correlation spectroscopy and the data processing algorithms for increasing the accuracy with which particle sizes can be measured in polydisperse suspensions and for determining the longitudinal and transverse dimensions of non-spherical particles.

This paper describes the hardware and software system we have developed based on laser correlation spectroscopy. This system makes it possible to solve the problems posed, obtaining the distributions of sizes and their evolution trends for non-spherical particles in polydisperse suspensions.

Implementation scheme of modified LCS method

The LCS method is based on detecting and analyzing the light scattered by particles in Brownian motion in liquid. The scattered light forms a dynamic speckle pattern in the observation plane [7]. The intensity of this pattern at a point varies with time due to motion of scattering particles in liquid. Monitoring the intensity variation of the speckle pattern in a small region, we can assess the motion of particles and their sizes [8]. In this case, the motion of particles is characterized by the diffusion coefficient.

The scheme of the hardware and software system developed is shown in Fig. 1. A single-mode laser module *1* with 5 mW continuous-wave output power and a wavelength of 650 nm was chosen as the radiation source. Power was supplied to the module through a rechargeable battery, providing highly stable lasing output. An aspheric short-focus lens *3* is used, allowing to focus the beam to a diameter of 500 μm, with a caustic length in solution equal to 5 mm. Scattered radiation can be detected in a range of angles from 5 to 175°. The aperture and multimode fiber serve to limit the region from which radiation is

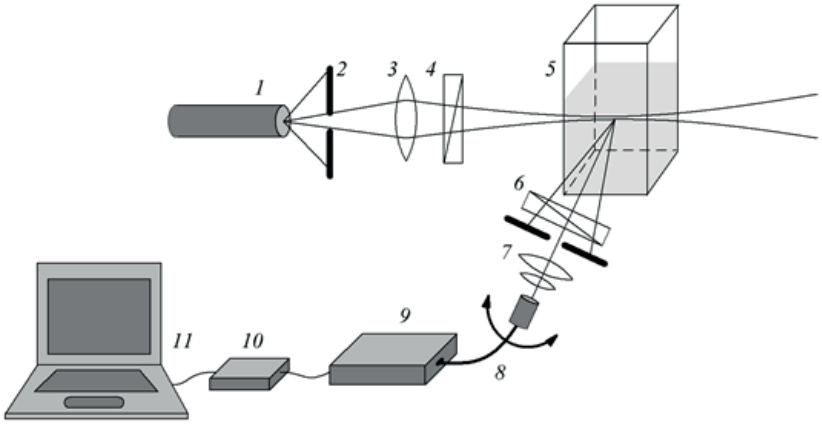

Fig. 1. Scheme of laser correlation spectrometer:
laser *1*, screen *2* with aperture, focusing lens *3*, polarizer *4*,
cell *5* with test solution, output rotary analyzing polarizer *6*,
collimating system *7*, input connector *8* of single-mode optical fiber,
photomultiplier *9*, ADC module *10*,
computer *11* with processing software

scattered (setting the effective scattering volume). The effective scattering length, calculated for a scattering angle of 90°, is 4.8 mm. The signal from photomultiplier *9* is digitized by ADC module *10* at frequencies of 50 kHz–50 MHz and processed with the software.

The hardware components could be fit in a small package with the given modification of the LCS method (the dimensions were 25 × 15 × 5 cm), allowing to construct a portable laser correlation spectrometer (weighing up to 2 kg) [9].

Polarizing plates *4* were added to the scheme for analyzing non-spherical nanoparticles [10].

The scattered radiation was detected in two orthogonal positions of the analyzing polarizer, so that the translational and rotational diffusion coefficients could be calculated separately. The longitudinal and transverse dimensions of non-spherical particles were estimated by both of these components.

Analysis of nanoparticle sizes using autocorrelation functions

The signal recorded using the given scheme is a pseudo-random dependence of the intensity of scattered radiation on time. The frequency of this signal depends on intensity fluctuations in the light scattered in the observation plane, which depend, in turn, on the coefficient of Brownian diffusion of particles in the solution. Fourier transforms can be used to find the characteristic frequencies but this approach does not yield accurate results because signals from several particles are recorded simultaneously and noise is generated. As a rule, time-domain rather than frequency-domain representation of the signal spectrum is used in such cases, i.e., the autocorrelation function is calculated.

Let us consider the diffusion of particles in solution in more detail. Brownian motion is a random process, so when a cell with the solution is illuminated with a laser beam, the number of scattering particles in the measuring volume is random, as is the intensity of the scattered light.

The scattering signal is processed in LCS by calculating the autocorrelation function of the signal:

$$G^{(1)}(\tau) = \langle E_S^*(t) E_S(t+\tau) \rangle, \qquad (1)$$

where $E_s(t)$ is the light field in the observed region; $G^{(1)}(\tau)$ is called the autocorrelation function of the first kind; τ is the correlation time.

Quadratic detectors (photomultipliers) are typically used in real experiments; they register fluctuations of scattered radiation intensity rather than the field. In this case, an autocorrelation function of the second kind is calculated, taking the following form:

$$G^{(2)}(\tau) = \langle E_S^*(t) E_S(t) E_S^*(t+\tau) E_S(t+\tau) \rangle. \qquad (2)$$

If the scattered light is a stationary Gaussian random process, the autocorrelation function of the second kind is connected to the autocorrelation function of the first kind by the Siegert relation [11]:

$$G^{(2)}(\tau) = \left| G^{(1)}(\tau) \right|^2 + 1. \qquad (3)$$

This relation allows to make a transition from the measured function $G^{(2)}(\tau)$ to the function $G^{(1)}(\tau)$. The autocorrelation function for identical spherical diffusers whose positions are not correlated can be rewritten in the following form [11]:

$$G^{(1)}(\tau) = S(\mathbf{q}, d) \langle e^{i\mathbf{q}(\mathbf{r}(\tau) - \mathbf{r}(0))} \rangle e^{-i\omega_0 \tau}. \qquad (4)$$

In this equation, $S(\mathbf{q},d)$ is the amplitude of the scattered radiation; $\mathbf{q}$ is the scattering vector whose modulus is calculated as follows:

$$|\mathbf{q}| = \frac{4\pi n_0}{\lambda_0} \sin\left(\frac{\theta}{2}\right). \qquad (5)$$

This expression is further simplified for free and isotropic diffusion:

$$G^{(1)}(\tau) = S(\mathbf{q}, d) \langle e^{-\mathbf{q}^2 D_T \tau} \rangle e^{-i\omega_0 \tau}. \qquad (6)$$

Here D_T is the translational diffusion coefficient, which, according to the Stokes–Einstein formula, is given as follows [11]:

$$D_T = 2k_B T / 6\pi\eta d, \qquad (7)$$

where η, Pa·s, is the viscosity of the liquid; k_B, J/K, is the Boltzmann constant; T, K, is the temperature; d, m, is the hydrodynamic diameter of the diffusers.

The above formulas are sufficient for calculating the diffusion coefficients and the size of molecules in equilibrium. Additionally, agglomerations of biological molecules or metals can be monitored, allowing to qualitatively characterize the activity of various molecules, assessing the composition of the solution.

This theory is only valid for spherical scatterers and in the absence of polarizability anisotropy of radiation. Rotational diffusion also has to be taken into account for more detailed study. If we abandon the spherical approximation and assume that the amplitudes of the scattered light field depend on particle orientation in space, the expression for the

autocorrelation function for objects with rotational symmetry (cylinders, ellipsoids) is rewritten as follows [10, 12]:

$$G^{(1)}(\tau) = S_1(\mathbf{q}, d_1, d_2)e^{-q^2 D_T \tau} + \\ + S_2(\mathbf{q}, d_1, d_2)e^{-(q^2 D_T + 6D_R)\tau}, \quad (8)$$

where $S_0(\mathbf{q},d)$, $S_1(\mathbf{q},d)$ are the constant and the variable amplitude of scattered radiation, respectively, the latter depending on particle rotation; d_1, d_2 are arbitrary values of diameter and length of particles, D_R is the translational diffusion coefficient.

The first term in this expression is responsible for ordinary translational diffusion, the second usually depends on rotational diffusion.

Angular dependences of $S_0(q,d)$ and $S_1(q,d)$ suggest that only translational diffusion makes a significant contribution to the scattering signal at small angles (in our case, $\theta < 60°$) [12]. As the viewing angle increases, the contribution of rotational motion increases as well, but angular dependences of scattering should be measured to separate translational from rotational motion.

In case of polarizability anisotropy of the scatterers (or in case of non-spherical particles), the autocorrelation function of the depolarized scattering component can also be measured:

$$G^{(1)}_{Dep}(\tau) = S_{Dep}(\mathbf{q}, d)e^{-(q^2 D_T + 6D_R)\tau}. \quad (9)$$

Evidently, the component responsible solely for translational motion is absent, which makes it possible to avoid measuring the angular dependences and limit measurement of polarized and depolarized components at small (in the range of 20–50°) angles.

The diffusion coefficients depend on the shape of the diffuser. The diffusion coefficients for ellipsoids of revolution, which are the main form of nanoparticles observed in experiments, can be written as follows [12]:

$$D_T = \frac{k_B T}{3\pi\eta d_a} F(d_a, d_b),$$

$$D_R = \frac{k_B T}{\pi\eta d_a^3} \frac{\left(2 - \left(\frac{d_b}{d_a}\right)^2\right) F(d_a, d_b) - 1}{1 - \left(\frac{d_b}{d_a}\right)^4},$$

$$F(d_a, d_b) = \frac{1}{\sqrt{1 - \left(\frac{d_b}{d_a}\right)^2}} \times \\ \times \ln\left(\frac{1 + \sqrt{1 - \left(\frac{d_b}{d_a}\right)^2}}{\frac{d_b}{d_a}}\right), \quad (10)$$

where d_a, d_b are the semi-axes of the ellipsoids.

Based on measuring the translational and rotational diffusion coefficients, we can separately calculate the sizes of non-spherical particles.

Thus, the task of determining the sizes of nanoparticles consists in constructing the autocorrelation function of the scattering signal, solving the inverse ill-posed problem to find the diffusion coefficients and calculating particle sizes by Eq. (7) or (10).

Methods for solving the inverse LCS problem

As already noted, the problem of approximating experimental data is simple for the given case of light scattering by monodisperse spherical particles. However, it is more difficult to interpret the experimental data if the samples are polydisperse. Only two or three parameters of the polydisperse distribution can be obtained for achievable measurement accuracy: the average particle size, the width of the distribution, and its asymmetry.

The form of the correlation curve of the field function, which is an exponential function in case of monodisperse spheres, i.e.,

$$|g^{(1)}|\ (\tau) = e^{-\Gamma}$$

(where $\Gamma = D_T q^2$), changes for polydisperse particles and is generally written as a superposition of exponential functions:

$$\left|g^{(1)}(\tau)\right| = \int_0^\infty F(\Gamma) e^{-\Gamma\tau} d\Gamma, \quad (11)$$

where $F(\Gamma)$ is the contribution to total intensis ty from the radiation component scattered by particles of the same size.

Eq. (11) is solved by finding a set of diffusion coefficients for each particle size. The expression for the function contains experimental errors, which leads to a systematic error in the desired distribution $F(\Gamma)$.

There are many methods for finding solutions to equations of type (11) [13]. These methods can be divided into several main categories: statistical, variational, iterative and projective. The specific method selected for a particular task of LCS depends on its advantages and disadvantages for each case.

Projective methods. These methods are used for increasing the stability of the problem and are based on projection of an unstable functional on a compact. However, it is too difficult to define a compact for solving the right-hand side of the equation in real problems. So projective methods are commonly used to apply a priori limitations to the desired solution. A method that can be used for integral equations is the Fourier transform. If we know the right-hand side of the equation only approximately, then the Fourier transform using the filter function can suppress the effects of high frequency.

Statistical methods. These methods are based on a priori statistical information about the properties of the matrix represented as an approximate integral operator. The discrete equivalent of integral equations is taken in these methods:

$$Ax + \xi = y.$$

A common method in industrial operation is the method of cumulants [14]. It is quick and easy, so this method is described in the international standard ISO 13321:1996. However, only the average diffusion coefficient and its moments can be found by this method without a priori information. What is more, the method of cumulants is verifiable with a unimodal distribution, yielding distorted results in case of polydisperse solutions [9].

The distribution of decay rates $F(\Gamma)$ is found from the condition that $F(\Gamma)d\Gamma$ is the fraction of the total scattering intensity due to molecules whose $D_T q^2$ values lie in the interval between Γ, $\Gamma + d\Gamma$

$$\int_0^\infty F(\Gamma)\,d\Gamma = 1.$$

Cumulants $K_m(\Gamma)$ of the distribution $F(\Gamma)$ were calculated by the experimental data in [15] based on Koppel's approach. The first cumulant ($m = 1$) gives the "Z-average" value of the diffusion coefficient, the second ($m = 2$) characterizes the width of the distribution, the third ($m = 3$) characterizes the asymmetry, etc. Cumulants are also used as a sensitive method for finding deviations from monodispersity.

This method is still the most popular in commercial production, but it does not yield accurate measurement results when the expected particle sizes are unknown. Besides, commercial spectrometers often produce incorrect results for polydisperse multicomponent mixtures.

Bayesian methods use a posteriori probability density as a function of uncertainty $P(x|y)$ for the solution vector x and the experimental data y. This method is very effective and can provide the required solution with any background noise in the matrix and on the right-hand side of the equation. The only problem is that the most complete a priori information about the desired solution has to be obtained to apply the method, which is currently impossible in experiments with biological fluids.

Iterative methods. The central idea behind these methods is to formulate an iterative scheme converging to an exact solution if $\delta = 0$ on the right-hand side of the equation or there are no errors in the operator, or if $\delta \neq 0$ when a divergent iterative process is interrupted for a number of iterations. Nonlinear optimization (the Levenberg–Marquart method) can be used for solving the inverse problem in polydisperse solutions. Unfortunately, this method requires accurate a priori information about the form and number of components in the distribution, so it is not particularly useful in actual processing.

The non-negative least squares method is also iterative. It is useful as part of other algorithms but the data it yields may be too fragmented [15]. The simplest and most effective of the iterative methods is the Friedman method [16] allowing to take into account almost any a priori information about the required solution. On the other hand, this method's robustness against noise in the original data is poor and, besides, it is not quite suitable for solving equations with an exponential kernel.

Variational methods. Substantial progress in solving ill-posed problems was made through Tikhonov's general theory of regularization [17]. The method consists in finding a solution not in the class of all integrable functions but in a narrower class that satisfies some additional conditions. Until recently, CONTIN was one of the most popular methods. However, this method is too specific for the regularization parameter and does not allow for narrow peaks to be resolved. We compared Tikhonov's regularization and CONTIN in our previous paper [4].

Tikhonov's regularization stabilizes the deviations of the theoretical curve from the ex-

Table 1

Comparison of benefits and drawbacks of different methods for solving inverse ill-posed problems

Methods	Benefits	Drawbacks
Projection (e.g., Fourier filtering)	1. No need for a priori information. 2. No need for least-squares fitting procedures	1. Oscillations present 2. Filtering function has to be selected 3. Adjacent lines with different intensities are difficult to reconstruct 4. Negative values present in solution
Statistical (e.g., Bayesian)	1. Yield necessary solution with any background noise 2. Default level can be set individually for each matrix element	Greatest amount of a priori information needed
Iterative (e.g., Friedman method)	Virtually any a priori information can be taken into account.	1. Large number of iterations 2. Values of expected results have to be set 3. Possible errors accumulated
Variational (e.g., Tikhonov's method)	1. Cross-functionality 2. Minimum a priori information required 3. Smooth solution	1. Regularization parameter has to be chosen 2. Narrow lines are difficult to reconstruct

perimental one by means of an additional composite stabilizing functional $\Omega(x)$. The main advantage is cross-functionality of the method, as it uses a minimum of a priori information. Another variational method is truncated singular-value decomposition [18]. Its algorithm is close to Tikhonov's method, but it can be used to reduce rounding errors.

Comparison of the methods. The above methods are summarized and compared in Table 1. As evident from the data, variational methods are optimal for use in LCS problems with no a priori information about the scattering particles. The existing drawbacks of these methods can be avoided by introducing some modifications in the common algorithms and conducting model experiments to refine the regularization parameter.

Algorithm developed for solving inverse problem of LCS

As noted above, if suspended particles of different sizes are found in the liquid, their sizes can be determined by solving the inverse LCS problem (11), that is, reconstructing the function $F(\Gamma)$ from the known function $g^{(1)}(\tau)$. This inverse problem is ill-posed in the sense that the small error of the experimental data produces a large error in the calculated dependence $F(\Gamma)$.

Because the dependence $F(\Gamma)$ is measured in a discrete and finite set of points, and because a numerical solution has to be obtained for (11), the problem is reduced to a system of equations that is written in matrix form as

$$A\mathbf{f} = \mathbf{g} \qquad (12)$$

Certain a priori conditions which differ depending on the problem solved are imposed on the solution of this system if regularization is used. The conditions that the solution be non-negative (bounded in a compact set $M \geq 0$) and smooth (i.e., without outliers) are commonly accepted.

According to Tikhonov's method, an approximate solution of system of linear algebraic equations (12), resistant to small changes in the right-hand side, is found by replacing system (12) with the minimization problem with the added regularizing term:

$$\left\| A\mathbf{f} - \tilde{\mathbf{g}} \right\|^2 + \alpha\Omega(\mathbf{f}) \to \min, \qquad (13)$$

where α is the smoothing parameter ($\alpha > 0$); $\Omega(\mathbf{f})$ is the stabilizing functional that is selected separately for each problem; $\tilde{\mathbf{g}} \to \mathbf{g}$.

The stabilizing functional is chosen in laser correlation spectroscopy to reduce the jumps of the zero derivative for obtaining smooth solutions:

$$\Omega(\mathbf{f}) = \|\mathbf{f}\|^2 .$$

Minimization of this kind stabilizes the solution of the system, improving its conditioning; the agreement between the real and desired solutions is also increased. However, this choice often leads to excessive smoothing of solutions; if the solution is a combination of several narrow peaks, it can be difficult to separate them.

The regularizing parameter α is selected based on the input data, which is to say that a too high α produces "smoothed" solutions, and a too low value makes the problem unstable.

The starting value of the parameter α in our algorithm was chosen to be 1% of the maximum diagonal element of the matrix *A*. After the first iteration of solving system (13), the residual $\|A\mathbf{f} - \tilde{\mathbf{g}}\|^2$ was calculated, the parameter α was reduced by 90% of the initial value and the system was solved again. After the second iteration, the residual was again calculated and compared with the residual obtained at previous iteration. If they differed by more than 10%, the parameter α was again reduced by 90%, and the next iteration started. Since the proposed algorithm uses comparison of residuals, there is no need to set the noise level in the experiment, which is often not known exactly [19].

System of equations (13) is solved by the modernized Gauss method with the eigenvalues of the matrix shifted towards higher values because a regularizing term is introduced; this makes the solution more robust to noise.

After the solution cycle ends and the final distribution **f** is obtained, the solution is checked for negative components. Two different methods for eliminating negative solutions are included in the software.

In the first case, with $f_j < 0$, we take $f_j = 0$ for all extreme values of j in this Gaussian and exclude these points from further calculations. After that, we return to setting the initial value of α.

Values of f_j exceeding 60% of the minimum, rather than extreme points, are excluded from the calculations in the second method. This calculation is faster, but yields less accurate results, therefore it is suitable for preliminary analysis of particle size distributions. System (13) is recalculated until all negative components are completely eliminated.

Setting a fairly high initial value of α could produce unnecessarily smoothed solutions, however, the regularization algorithm eliminates positive values in addition to discarding negative values. Extreme points in the Gaussian are excluded from the solution one by one until a given width is reached, which makes it possible to calculate the sizes in highly polydisperse mixtures with resolutions up to 0.5 nm.

There is constant background illumination in addition to the variable component in real experiments. As a result, the autocorrelation function does not drop to zero at infinity, but rests on a pedestal whose height is proportional to the intensity of the background noise. A cycle removing the constant component was added to the program's algorithm to eliminate background noise.

The algorithm can be described as follows:

Step 1. Set initial (sufficiently high) value of α.

Step 2. Solve system of equations (13) and find the solution **f**.

Step 3. Calculate the residual

$$\|A\mathbf{f} - \tilde{\mathbf{g}}\|^2$$

(decrease α by 90% after the first iteration and return to Step 1).

Step 4. Compare the residuals by the inequality

$$\|A\mathbf{f} - \tilde{\mathbf{g}}\|_i^2 < 0{,}1 \cdot \|A\mathbf{f} - \tilde{\mathbf{g}}\|_{i-1}^2 ;$$

if it is satisfied, reduce α by 90% and return to Step 1, if not, go to Step 5.

Step 5. Check for negative components of the solution **f**. If there are $f_i < 0$, assume that $f_j = 0$ and return to step 1. The corresponding component is excluded from further calculations.

Step 6. If there are no $f_i < 0$, check the number of distribution points $n > N$ (set prior to solution); if yes, then set $f_{\min} = 0$ and return to Step 1; if no, end the calculation.

The domain of expected solutions and the desired accuracy should be set before start to speed up the calculations. Solution in the entire range of permissible values is also possible but it takes much longer because there is a cubic relationship between the time to solve the problem by the Gauss method and the number of points. The method for eliminating negative values (as described above) and the number of points left at the peak (more are taken if there are weak components) should be also chosen. The correlations obtained from experimental data can be averaged for greater accuracy.

Verification of the method

We tested the developed hardware and software system using computer simulation and experiments with objects of known diameters, polydisperse biological suspensions and suspensions containing non-spherical clusters of particles.

The first stage of testing involved analyzing model signals with different noise levels (from 0 to 10% of the useful signal) and with a different number of components. The results indicate that the accuracy with which the center of the Gaussian is reconstructed for unimodal distributions is close to 100% for any values of the noise level. The noise value of 10% for model signals corresponds to a signal-to-noise ratio of 21.6 dB in actual experiments (for a single measurement). To increase the signal-to-noise ratio, we recorded from 50 to 100 signals whose autocorrelation functions were averaged. This considerably reduced the contribution of the noise component to the useful signal.

Since the main purpose of the hardware and software system was to study the dynamics of cluster formation in polydisperse biological fluids, we found it interesting to calculate the accuracy with which model signals containing information about polydisperse particles with sizes from 1 to 100 nm are reconstructed. {Fig. 2 shows the reconstructed size distributions (calculation) for a signal with the given particle sizes, nm: 4, 10 and 21 (model).

Evidently, relative concentrations are not always reconstructed correctly but the position of the central peak and its width is calculated with an error not exceeding 5.7%.

The actual experiments were carried out using a quasi-monodisperse suspension of

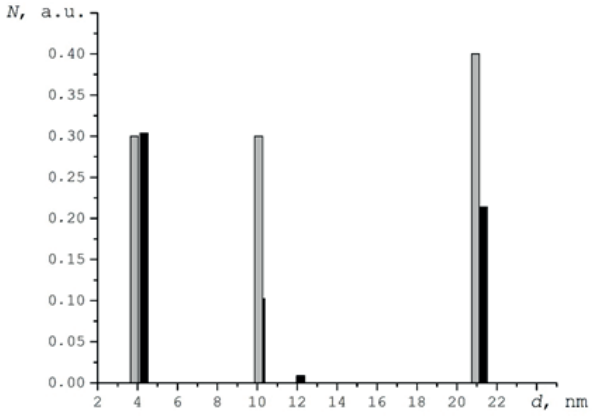

Fig. 2. Reconstructed distributions of model concentrations for particles with sizes of 4, 10 and 21 nm
Black columns correspond to calculated values, gray columns to model values

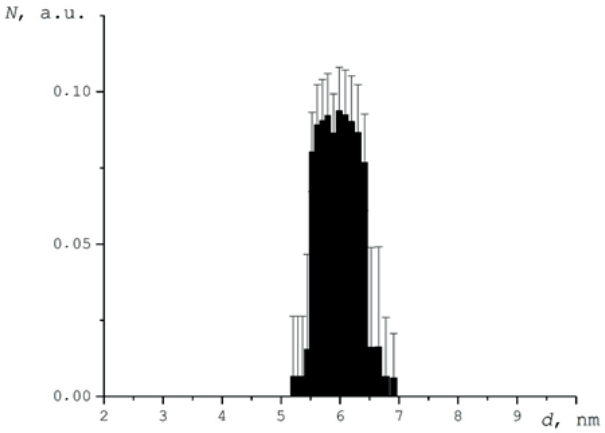

Fig. 3. Calculated particle size distribution in aqueous suspension of albumin protein at neutral pH

egg albumin protein in water. According to theoretical data, the albumin molecule has a diameter $d = 6$ nm in equilibrium (at neutral pH) [20]. Fig. 3 shows the size distribution of albumin in an aqueous suspension. It can be seen that the central position of the peak corresponds to the theoretical value, and the shape is described by the Gaussian curve.

To confirm that it was possible to describe the dynamics of particle aggregation in solutions, we measured the sizes of albumin protein aggregates in solutions with a varying pH. Albumin tends to aggregate forming large clusters near the isoelectric point (pH = 4.8) [21]. As the pH of the solution decreases further, protein aggregates disintegrate and the protein denatures [22]. The pH value in our experiments varied from 8.0 to 1.6 [23]. The calculated average sizes of aggregates are given in Table 2.

Table 2

Average particle sizes in albumin solutions with different pH values

pH	*R*, nm
8.0	6.00.4±
7.0	6.00.4±
6.0	9.00.5±
5.0	29.02.3±
4.2	30.02.4±
3.6	20.02.2±
2.5	16.01.8±
1.6	5.00.4±

As evident from the data in Table 2, when the pH of the solution changes from acidic to alkaline, albumin aggregation is observed near the isoelectric point, followed by de-aggregation with further increase in acidity. Thus, the proposed hardware and software system allows not only to detect particle sizes the size of particles, but also to observe their variation.

To confirm that this method can be applied to studying the composition of real biological fluids, the distribution of particle size in serum was measured in [24]. The result is shown in Fig. 4.

It is known that the sizes of particles in serum are different for different types of proteins. For example, albumin and amino acids have particle sizes from 1 to 10 nm, globulins from 11 to 30 nm; high-density lipoproteins and low-molecular circulating immune complexes have sizes from 31 to 70 nm; high-molecular circulating immune complexes have sizes greater than 150 nm [25].

Thus, we can divide the obtained size distribution into separate groups of proteins, analyzing their relative concentrations and tracing the dynamics of cluster formation in case of certain effects, and determine some important diagnostic parameters [26, 27]. Since the sizes of proteins in circulating immune complexes are drastically different from the sizes of other components, we can draw certain conclusions about the state of human immune system in terms of the size distribution and relative concentration of circulating immune complexes [24].

All of the results given above were obtained assuming that the scattering particles were approximately spherical. We used a solution of the magnetic fluid Fe_3O_4 to assess non-spherical particles. Magnetic fluid in equilibrium consists of an aqueous suspension of particles with diameters of about 10 nm [28]; however, it was found that magnetic particles lose equilibrium upon dilution, forming clusters of elongated ellipsoidal shape. The magnetic fluid in our experiments was diluted to a concentration of 0.15 mg/ml and tested using the proposed hardware and software system.

The translational and rotational diffusion coefficients for the agglomerates of magnetic particles and for single nanoparticles were calculated using the obtained values of the exponents G for polarized and depolarized components of scattered light. The diameters of the ellipsoids in two orthogonal sections d_b and d_a were calculated by Eqs. (10). We ultimately concluded that the nanoparticles were non-spherical judging from the calculated aspect ratio $\varepsilon = d_b/d_a$ (Table 3). The obtained data show that single nanoparticles predominantly have shapes close to spherical; this is confirmed by the results of scanning electron microscopy [29], while their aggregates have more elongated ellipsoidal shapes. Similar data were also obtained using other methods but the exact dimensions were not calculated. The size ranges given in Table 3 indicate that the given magnetic fluid is polydisperse and describe the sizes

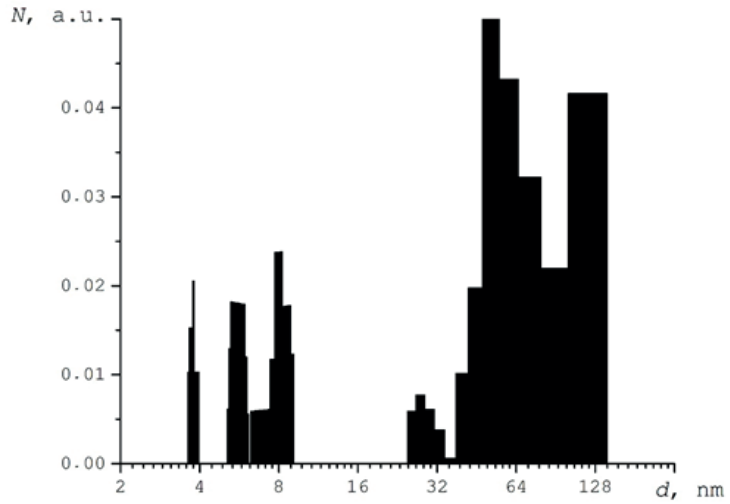

Fig. 4. Experimentally obtained particle size distribution in blood serum

and shapes of the clusters forming, which complicates analysis related to determining the shapes of nanoparticles. At the same time, the obtained result indicates that the classical spherical approximation cannot be applied to study of aggregates in magnetic fluids.

Table 3

Calculated sizes of magnetic particles and their aggregates

Parameter	Value	
	Aggregates	Single particles
D_T, (μm)²/s	2.8–3.5	58–80
D_R, s^{-1}	700–1060	(4–6)·10^5
d_a, nm	73–94	4.1–5.0
d_b, nm	48–70	3.5–4.7
ε	0.50–0.96	0.85–1.00

Notation: d_a, d_b *are* the diameters of ellipsoids in two orthogonal sections, D_T is the translational diffusion coefficient; D_R is the rotational diffusion coefficient; $\varepsilon = d_b/d_a$

Conclusion

We have introduced a modified method of laser correlation spectroscopy and a hardware and software system developed based on this method, making it possible to detect the sizes of individual molecules and nanoparticles, as well as the dynamics of their clusterization in liquid media, including in blood serum [30]. The algorithm for solving the inverse problem of laser correlation spectroscopy described in this paper allows to calculate the sizes of polydisperse particles with an error not exceeding 6%. The modification proposed for the scheme of the laser correlation spectrometer and the approaches used to analyze the experimental data made it possible to determine the longitudinal and transverse dimensions of non-spherical nanoparticles in polydisperse solutions for the first time ever.

Testing the developed hardware and software system, we have proved that the accuracy with which dimensions are measured is not inferior to the commercially available spectrometers (Zetasizer Nano ZS and Photocor) for single-component solutions [4] and is considerably better that the known equivalents for multicomponent solutions.

The study was financially supported by the Innovation Promotion Fund (contract no. 13463GU/2018 of 20/07/2018).

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Received 31.03.2019, accepted 24.04.2019.

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Статья поступила в редакцию 31.03.2019, принята к публикации 24.04.2019.

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