Amplification of speckle-microscope signal by using gold nanoparticle stable of contents

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TABLE OF CONTENTS

1. Abstract
2. Introduction
3. Materials and methods
   3.1. Detection of intensity fluctuations, scattered by moving bacterial cells.
   3.2. Set-up of speckle-microscope
4. Results
   4.2. Dependence of speckle pattern structure on the position of speckles observation plane in a speckle-microscope
   4.3. Dependence of the spatial structure of speckles on the number of scatterers
   4.4. Simulation of the process of a speckle formation in the case of scattering of strongly focused laser beam on single bacteria, surrounded by a set of nanoparticles, connected to the cell via CT-Mab-Au complex
   4.5. Experimental study of speckles with a small number of scatterers forming at the scattering by the samples, containing combined “chlamydia trachomatis bacterial cell”-“monoclonal antibody”-“gold nanoparticles” system
      4.5.1. Sample preparation
      4.5.2. Scanning of samples, containing combined “Chlamydia trachomatis bacterial cell”-“monoclonal antibody”-“gold nanoparticle” system with a strongly focused laser beam
5. Discussion
6. Acknowledgments
7. References

1. ABSTRACT

Speckle-microscopy is used for the detection of Chlamydia trachomatis in aqueous suspensions or on slides. Monoclonal antibody tagged with gold nanoparticles that form biospeckles have also been used with a small number of scatterers. By devising a prototype of laser scanning speckle-microscope, we now demonstrate that the signal of speckle microscope can be significantly amplified in the presence of gold nanoparticles. This enhancement of signal emitted from gold nanoparticles offers a better approach to the detection of C. trachomatis.

2. INTRODUCTION

Strongly focused laser beams are widely used in different high resolution measuring devices
Detection of *C. trachomatis* by speckle-microscopy

for biomedical applications and surface characterization, such as confocal, Doppler or scanning microscopes. Speckle-microscopy is a modern direction of microscopy. The first papers devoted to speckle-microscopy were published by the authors of the present paper in the late 90s of the last century (1, 2). Speckle-microscopy is based on the diffraction of strongly focused Gaussian beam on a moving random object. When focused, a laser beam is scattered by random surface or from a thin layer of optically-inhomogeneous medium, the diffraction picture contains only a few speckles of a large size: the so-called, speckles with a small number of scatterers (1-8) or small-N speckles (9) are formed in this case. Such speckles possess specific statistical characteristics. Earlier, the first-order statistics of speckles, which are formed at the diffraction of focused coherent beam from non-Gaussian phase screen, has been successfully analyzed by S. Yu. Kuzmin et al. (7). In particular, it has been shown that statistics of speckles with a small number of scatterers usually is not the Gaussian one (5, 7-8). These speckles may be classified as statistically inhomogeneous random fields (10). New characteristics of manifestations provided by the Doppler effect may be observed in their dynamics. As it has been found, (10-12), the spectrum of intensity fluctuations of scattered light contains a high-frequency peak even in the absence of a subsidiary reference wave. The frequency position of Doppler peak is defined not only by the angle of observation and velocity of scattering microflow, but also by the number of scatterers as well. Statistical properties of the non-Gaussian speckles with a small number of scatterers, in general, with the applications to high-resolution microflow measurements, also have been analyzed in several papers (13-18). Characteristics of speckled speckles (19), which are obtained at the diffraction of focused coherent beams, have been considered in the paper (20). Relatively recently, statistical analysis of the unique properties of these speckles (the first order statistics) has been presented in reference (21). Specific correlation properties of speckles with a small number of scatterers have been scrutinized in reference (22).

In (23) the principles of laser scanning microscopy of rough surfaces have been considered. The salience of speckle-microscopy of bioflow in the smallest microvessel has been discussed in (24-25). General output characteristics of speckle microscope have been studied (26). It is important to note that very recently speckle-microscopy has been applied (27-29) in the investigation of a unique biological object – *Chlamydia trachomatis* (CT), the causative agent of chlamydial genital infection that is known as one of the greatest challenges for both Public Human and Animal Health worldwide. A specific feature of these bacterial cells is that they can live only inside other mammalian cells of larger size, for example epithelial cells.

3. MATERIALS AND METHODS

3.1. Detection of intensity fluctuations, scattered by moving bacterial cells.

Speckle fluctuations are recorded by photodetector from McCoy cells, HeLa cells etc. Specific output characteristics of speckle-microscope with the implication to the scanning of *C. trachomatis* bacteria inclusions inside the human epithelial cells have been analyzed (27). In the citing papers (27, 28), it has been shown that: (i) in the scanning of a thin sample of cellular suspension fixed between two glasses the influence of Brownian motion of CT Elementary Bodies (here as CT cells) on the formation of output signal of speckle-microscopy is negligible and (ii) the signal from scanning CT cells can be reliably and stably detected. But signal-to-noise ratio of output signal of speckle-microscope is not so high and the useful part of output signal generated by scattering of light from CT cell should be enhanced.

In this paper the amplification of output signal of laser scanning speckle-microscope using gold nanoparticles has been attempted. Clearly, the formation of speckle patterns in the speckle-microscope in the case of presence of scattering nanoparticles requires additional very detailed and thoughtful investigation. A combined "*C. trachomatis* bacterial cell"-"monoclonal antibody"-"gold nanoparticles" system has been used as a complicated scattering element in the case of formation of biospeckles with a small number of scatterers. Specific optical model of scattering of strongly focused laser beam on *C. trachomatis*
Detection of *C. trachomatis* by speckle-microscopy

bacteria with set of connected gold nanoparticles has been developed. The processes of light scattering on such complexes have been simulated. It has been convincingly demonstrated, both theoretically and experimentally, that the usage of gold nanoparticles is a powerful way to amplify output signal of speckle-microscope. A prototype of laser scanning speckle-microscope for the detection of *C. trachomatis* bacteria has been designed and tested.

3.2. Set-up of speckle-microscope

The optical scheme of speckle-microscope is described (26, 28). A beam of semiconductor laser KLM (Kantegir, Russia) with 5 mW power and 650 nm wavelength is focused into a small spot on the analyzed object using a microobjective with the magnification of 60x and the numerical aperture NA=0.75 (LOMO, Russia). Biological sample is fixed on the motorized linear stage (Standa, Lithuania). The presence of chlamydial agent was preliminary confirmed with both PCR and Direct Immunofluorescent Assay (DFA). Speckle fluctuations are recorded by photodetector PDA-10 (Thorlabs, USA), connected to data acquisition card (National Instruments, USA). A general view of scanning speckle-microscope and scanning unit sample (with CT bacterial cell, fixed on the microscope slide) on the speckle-microscope, microscope slide with CT Elementary Bodies and CT cells, fixed on the glass is demonstrated (28). The optical model of scattering focused laser beam on the "*C. trachomatis* bacterial cell"-"monoclonal antibody"-"gold nanoparticle" system.

The optical model, used in computer simulation is shown in Figure 1. Let us consider the scattering of strongly focused laser beam on a single bacteria surrounded by a set of nanoparticles connected to the cell via the CT-Mab-Au complex. The main scattering element in such a system is a nanoparticle with the diameter of 20 nm. The number of nanoparticles enfolded in the CT cell can vary in range from unity to one hundred and obeys to the Poissonian statistics. It is assumed that the process of diffraction is described by the Mie theory. So, in the far zone of diffraction each nanoparticle can be considered as a point source, producing spherical wave. Thus, the forming speckle-pattern is the result of interference of several spherical waves, the number of contributions exactly equals the number of illuminated particles.

4. RESULTS

4.1. Computer simulation of the process of speckle formation in the speckle-microscope. Influence of interference effects on the formation of speckles in speckle-microscope

Partially developed speckles with very low intensity produced by the scattering of light on nanoparticles interfere with high-intensity weakly-disturbed (specular) diverging component. Evidently, that contrast of speckles will tend to zero in the center of a diffraction picture, where the intensity of a

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*Figure 1. The optical model of scattering of focused laser beam on the "*C. trachomatis* bacterial cell"-"monoclonal antibody"-"gold nanoparticles" system (CT-Mab-Au complex): A) microobjective, forming strongly focused Gaussian beam; B) Gaussian beam, illuminating *C. trachomatis* bacterial cell; C) *C. trachomatis* cell connected with a set of monoclonal antibodies, labeled by 20 nm gold nanoparticles; D) speckles with a small number of scatterers; E) photoreceiver (shifted from optical axis to peripheral area of speckle-pattern).*
specular component is essentially higher than the intensity of light diffracted on nanoparticles. But, if the observation point is shifted from the optical axis to the edge of a weakly-scattered Gaussian beam, the speckle contrast of resulting speckle pattern may exceed unity. This happens because the intensity of specular (diverging) beam tends to zero, but the speckle component is essential in the case of isotropic scattering by small particles.

So, interferential processes play an essential role in speckle formation in a speckle-microscope and need to be treated very carefully. As it was already mentioned, speckle contrast typically may take very low values. So, in this paper, with the purpose of enhancing speckle contrast for better observability of features of speckle-patterns, intensities in all speckles have been renormalized into the range from 0 to 255 levels of greyscale intensity.

Normalized intensity speckle-patterns are shown in Figure 2. The radius of the sphere on which nanoparticles were fixed was 1500 nm (the radius of this test sphere is 10 times larger than the radius of a CT cell). The distance between waist beam plane and observation plane was z=1.5 mm. The number of scatterers (i.e. number of gold nanoparticles, fixed on the cell) was N=100.

As it can be seen from Figure 2 the speckle patterns forming in the case of a small number of scatterers with and without reference wave are practically identical. The correlation coefficient between images presented in Figure 2A and Figure 2B equals 0.995.

4.2. Dependence of speckle pattern structure on the position of speckles observation plane in a speckle-microscope

Speckle-structures with a small number of scatterers, simulated for different positions of the observation plane, are show in Figure 3. In this figure, z is the distance between the observation plane and the waist beam plane of focused Gaussian beam. As it can be seen from Figure 3, all the images are absolutely identical, and the correlation coefficient between all images equals 1. This means that it does not matter in which plane the photoreceiver is placed.

4.3. Dependence of the spatial structure of speckles on the number of scatterers

The small collection of speckles (12 speckle-patterns) forming in the case of 10 scatterers is shown in Figure 4. The collection of speckles (12 speckle-patterns) forming in the case of 50 scatterers is shown in Figure 5. Small statistics of the speckles forming in the case of 100 scatterers is shown in Figure 6. The comparison of the structures of the
Detection of *C. trachomatis* by speckle-microscopy

Figure 3. Speckle-structures with a small number of scatterers simulated for different positions of the observation plane: A) z=1.5 mm; B) z=3 mm; C) z=7 mm; D) z=15 mm.

Figure 4. Seria of speckles forming in the case of 10 scatterers, carrying sphere radius is 1500 nm, z=15 mm.

speckle-patterns formed at different numbers of scatterers (at least, within the range [10; 100] allows us to make the conclusion, that the number of speckles does not depend on the number of illuminated nanoparticles in the probing volume.

4.4. Simulation of the process of a speckle formation in the case of scattering of strongly focused laser beam on single bacteria, surrounded by a set of nanoparticles, connected to the cell via CT-Mab-Au complex

As it was already mentioned, the size of CT bacteria is much smaller than the size of carrying spherical particle considered in Sections 4.1-4.3. The results of simulation of focused Gaussian beam on real CT bacteria (with the typical radius, of 150 nm only) are presented in Figure 7 and Figure 8. Because of smaller distances between nanoparticles fixed to bacteria the variation of optical paths from nanoparticle to each observation point is also smaller. This leads to the reduction in speckle contrast, so forming speckles with a small number of scatterers become partially-developed. The case of scattering on 50 gold nanoparticles connected at random to a sphere is demonstrated in Figure 7; the case of scattering on 100 gold nanoparticles is shown in Figure 8. Observation has been carried out on peripheral areas of speckle patterns.
As it can be clearly seen from Figure 7 and Figure 8, a change in the realizations of scatterers immediately produces very weak, but visible changes in speckle structure. The value of destructions in speckle structure very weakly depends on the number of scatterers.
Detection of *C. trachomatis* by speckle-microscopy

**Figure 7.** Seria of the speckles forming in the case of 50 scatterers, carrying sphere radius is 150 nm (the size of *C. trachomatis* cell), $z=15$ mm.

**Figure 8.** Seria of the speckles forming in the case of 100 scatterers, carrying sphere radius is 150 nm (the size of *C. trachomatis* cell), $z=15$ mm.
4.5. Experimental study of speckles with a small number of scatterers forming at the scattering by the samples, containing combined “chlamydia trachomatis bacterial cell”-“monoclonal antibody”-“gold nanoparticles” system

4.5.1. Sample preparation

The blood serum with the presence of CT cells confirmed by the commercial kit ChlamyScan was used as the clinical sample. The sample was concentrated by centrifugation at 13000 rpm for 60 min, the supernatant was discharged and the sediment was diluted with the DMEM supplemented with HEPES and L-glutamine.

Chlamydiae were cultivated in the McCoy cell monolayer, the most appropriate cell line for growing zoonotic bacteria. To assess the bacteria viability and to cumulate the bacterial biomass McCoy cell monolayers were infected with a suspension of CT cells. Briefly, the medium was carefully removed from the wells containing the McCoy cell monolayer and 100μl of concentrated and vortexed clinical sample were added in each well of 96-wells plate. The plate was centrifuged at 2500 rpm, 37 °C for 60 min and then incubated in CO2-enriched conditions for 48-72 hours.

After the incubation, the medium was removed, and infected cells were detached by Trypsin-Versene solution (3:1) and collected in cryogenic vials. The cells were destroyed by multiple cycles of deep freezing (up to -80 °C) and defreezing followed by the disruption with glass beads for 3-4 min. Then the disrupted cells were transferred to a clean tube and centrifuged at 1000 rpm for 10 min. The supernatant was collected and used for the next CT passage. After three-passage of the CT culture procedure the bacterial biomass was concentrated by centrifugation at 15000 rpm for 1 hour. Then the supernatant was discharged and the sediment was diluted with the sterile Physiological saline.

4.5.2. Scanning of samples, containing combined “Chlamydia trachomatis bacterial cell”-“monoclonal antibody”-“gold nanoparticle” system with a strongly focused laser beam

The results of scanning a motionless sample containing only CT cells (without immune complexes of chlamydial Mab with Au) are shown in Figure 9. This signal can be considered as a pure noise of measuring set-up. No peaks generated as the result of light scattering by Mab labelled with Au can be found in the output signal. The results of scanning CT cells, which do not bind with Mab and Au, are demonstrated in Figure 10. Again, no peaks produced by laser light scattering by CT-Mab-Au are observable. Seria of examples of output signal forming in the case of scanning CT cells connected with Mab and Au are demonstrated in Figure 11. Narrow peaks produced by laser light scattering by CT-Mab-Au, are clearly detectable in the output signal of a speckle-microscope.

Seria of three smoothed spectra obtained for three independent realizations of output signal of scanning microscope are shown in Figure 12. The
Detection of *C. trachomatis* by speckle-microscopy

Figure 10. Scanning of CT cells, which do not bind with Mab and Au.

Figure 11. Series from three realizations of output signals of a scanning speckle-microscope in the case of scanning of CT cells, connected with Mab and Au.

shapes of spectra are very similar. This means that the presence of single peaks in the output signal of a speckle microscope cannot be defined using usual spectral analysis.

5. DISCUSSION

In the present paper, the reasons of decreasing of the contrast of partially developed
Detection of *C. trachomatis* by speckle-microscopy

Speckles, forming at the scattering of strongly focused laser beams on the CT cells, have been analyzed for the first time. The contrast of biospeckles goes down as a result of manifestation of interference effects due to appearance of unscattered component in resulting speckle pattern. But, at the same time, speckle contrast may essentially increase at the shift of observation point from the center to the edge of diffraction picture and, may achieve the value of 1. However, as a rule, speckle contrast takes very low values.

The main aim of this paper was to enlarge the contrast of biospeckles. It has been done by using of metal nanoparticles. As it has been shown on the base of theoretical and experimental investigations, the level of scattering of strongly focused Gaussian beam on bacteria of CT, connected with gold nanoparticles via CT-Mab-Au complex, is essentially increased. This leads to the amplification of output signal of scanning speckle-microscope, applied for the detection of CT cells.

As it has been demonstrated in this paper, speckle-structures, forming as a result of scattering of strongly focused Gaussian beam on a set of gold nanoparticles, are clearly observable in a speckle-microscope. Figure 4-8 are the main theoretical results of this paper. These pictures indicate that the signal of a speckle-microscope enhanced by the CT-Mab-Au complex scan be reliably detected.

It is clearly demonstrated, that in the case when CT cells are connected with Mab labelled with gold nanoparticles the output signal of a scanning speckle-microscope is essentially enhanced. The appearance of peaks corresponds to the moment of scanning of immune nanocomplex CT-Mab-Au with a strongly focused laser beam and is clearly observed in the experiments. It opens the way to the detection of a single CT bacterial cell in the analyzing samples. Potentially, the proposed technique can be used for the fast 2D scanning of clinical samples and automatic recognition of pathogenic cells.

6. ACKNOWLEDGMENTS

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Detection of C. trachomatis by speckle-microscopy

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Detection of *C. trachomatis* by speckle-microscopy

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