Faculty of Computer Science

Laboratory of Methods for
Big Data Analysis (LAMBDA)

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Mathematical theory of optimal processes in a common path interferometer using a diffraction phase microscope

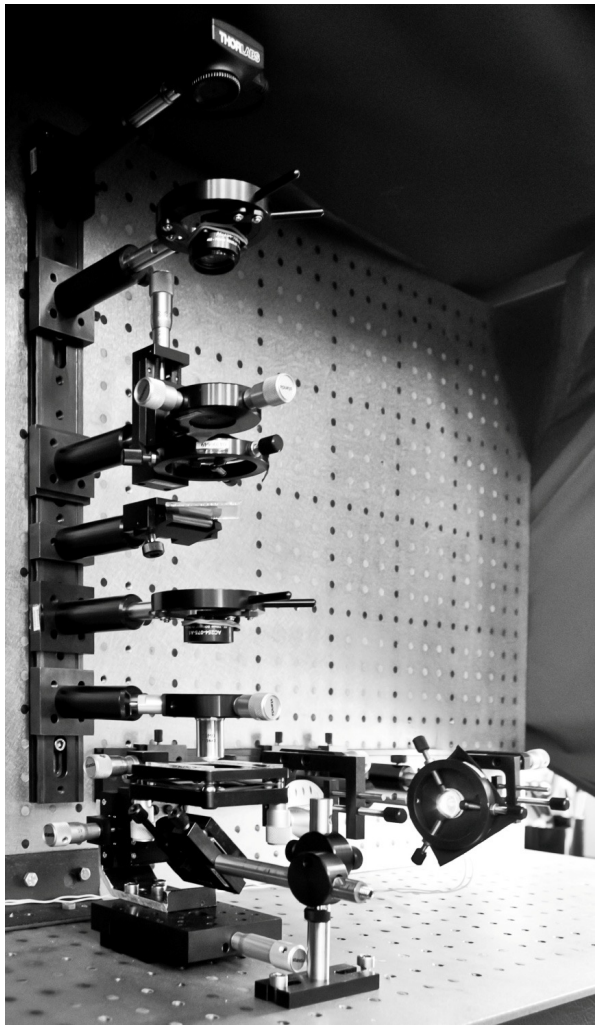
Natalia Talaikova
Researcher, HSE University
TG @ntalaikova

Tasks

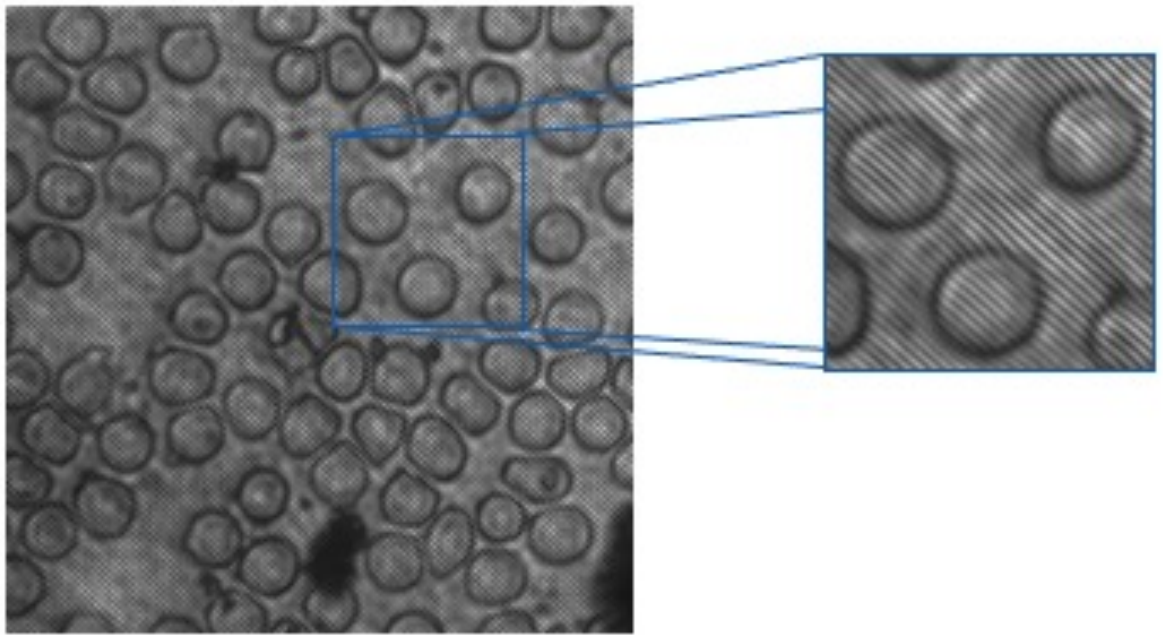
Quantitative phase visualization methods are currently being actively developed. We propose to consider one of the methods as a basis for the theory and practice of image formation analysis in the group of methods.

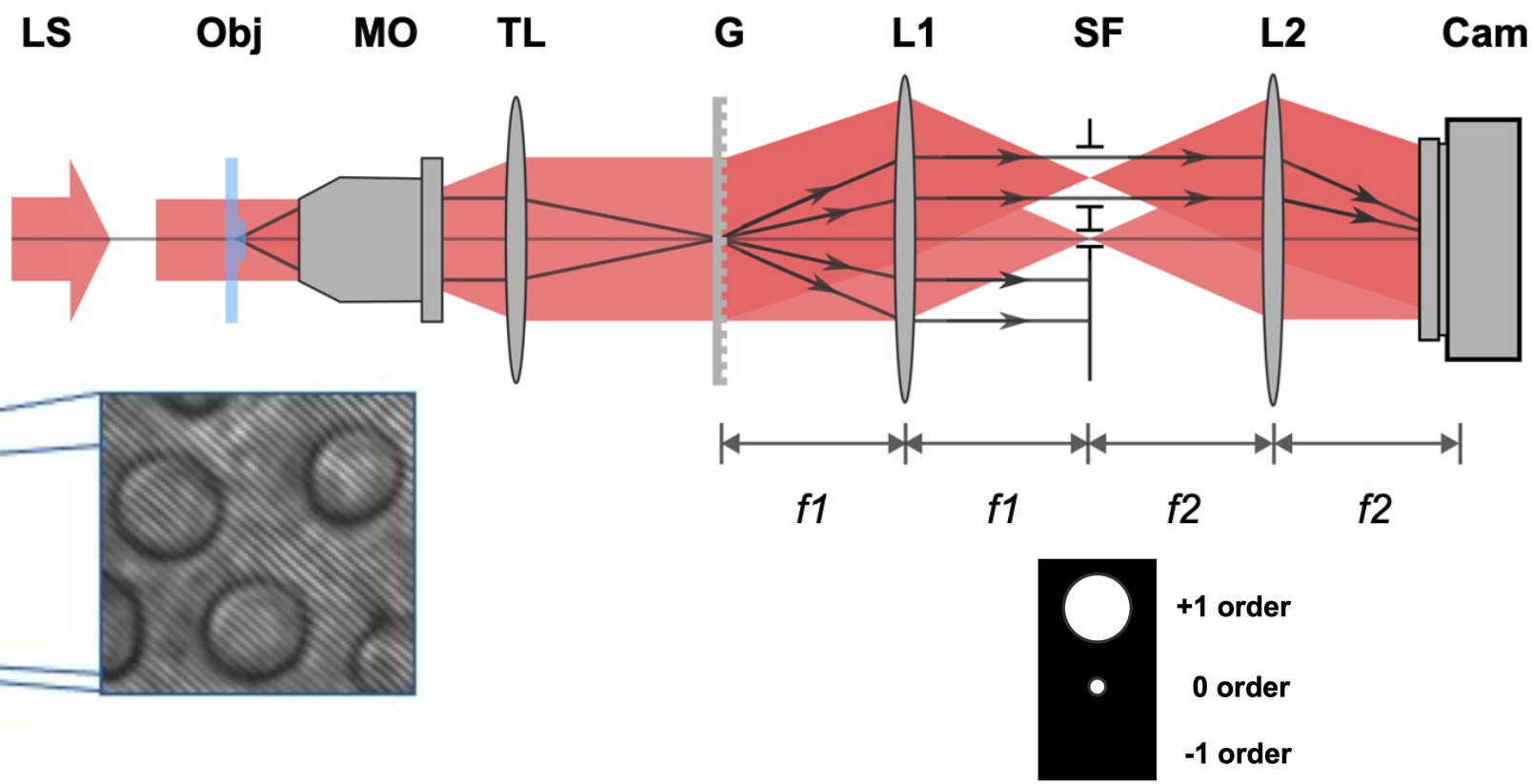
To receive a mathematical description of the image formation process in a diffraction phase microscope for:

- minimizing errors in calculating the parameters of objects;
- optimizing the technical parameters of the microscope;
- describing the theory of interferometers image formation



Diffraction phase microscope optical scheme

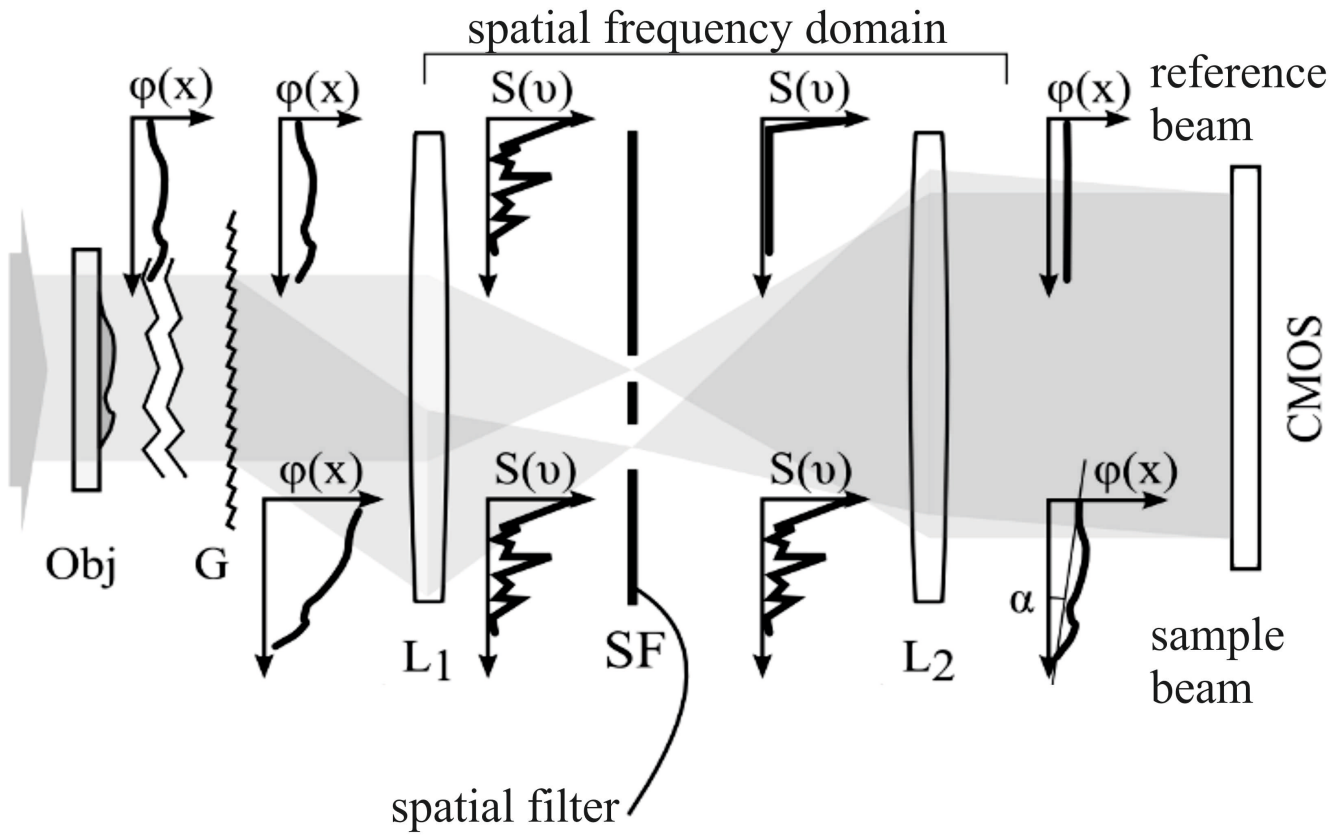




LS – light sourse, Obj – Object, MO – microobjective, TL.- tube lens, G – grating, L1,L2 – lenses, SF – spatial filter, Cam – CMOS detector

*NA Talaikova, VP Ryabukho. JPCS (2016)

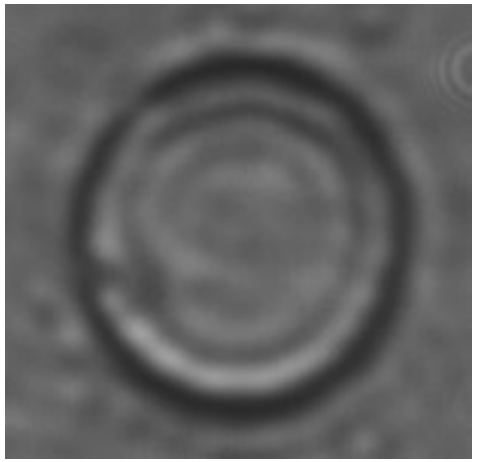
Principle of working diffraction phase module



A diffraction grating is placed at the microscope image plane to generate multiple diffraction orders. These diffraction orders are spatially separated in the Fourier plane, where a physical pinhole filter is located. This filter low-passes the 0th order diffracted beam to generate a reference beam and passes the 1st order diffracted beam without filtering to carry the signal from the sample. These two beams are then combined at the image plane through the second Fourier lens to form an interferogram at the detector.

Diffraction phase microscope principle

Image of red blood cell obtained using **optical microscope**



Interference picture (**diffraction phase module**)

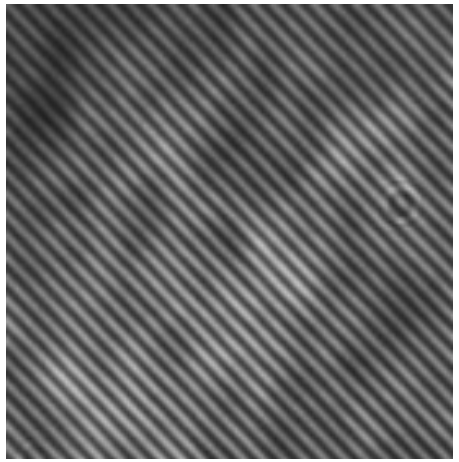
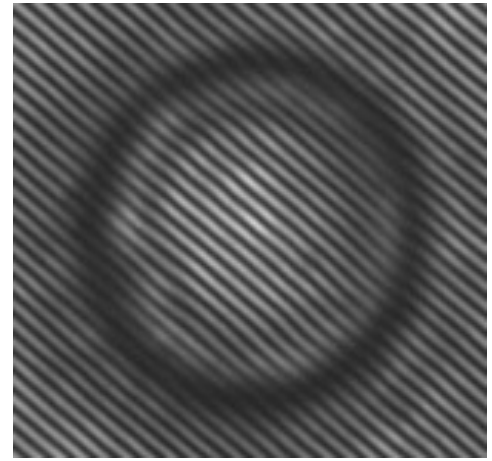
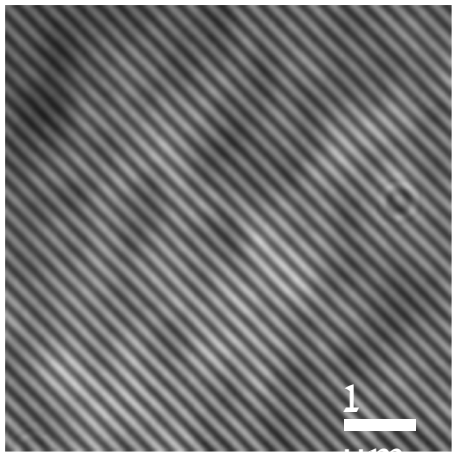


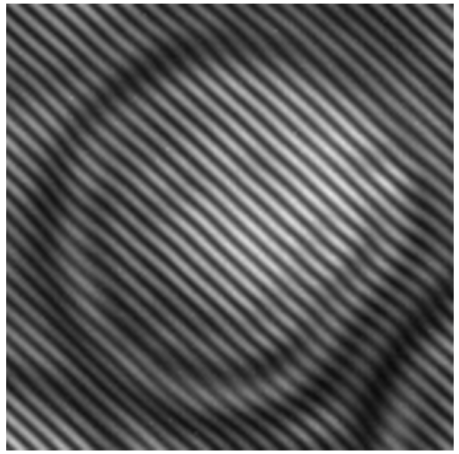
Image of red blood cell obtained using **diffraction phase microscope**



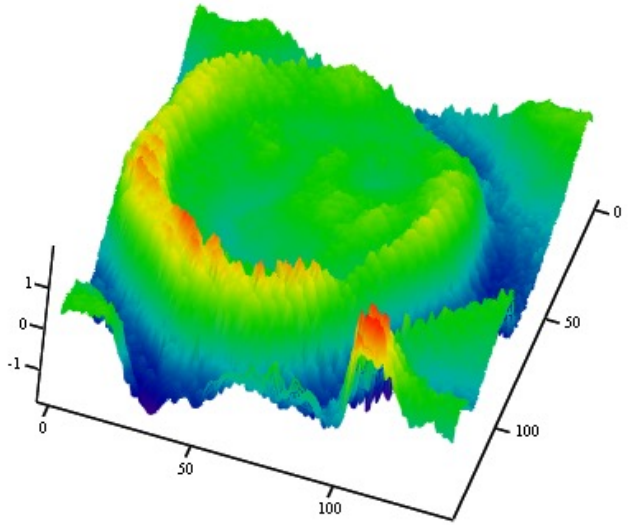
Interference pattern



Glass



Red blood cell (Phase object)

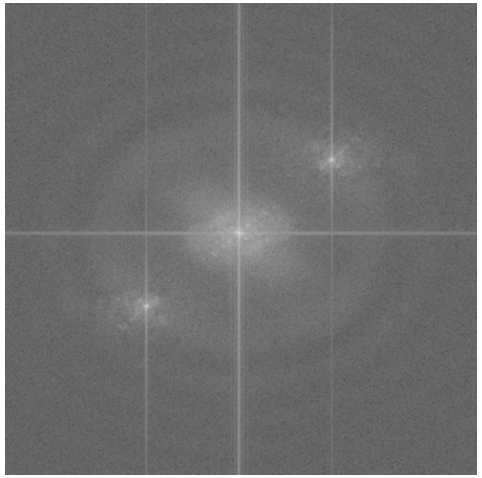


Now we know that we have 2 waves, we know the intensities of each, we know the period of the diffraction grating. From here we can find the phase delay at each point in the image as in the picture on the right

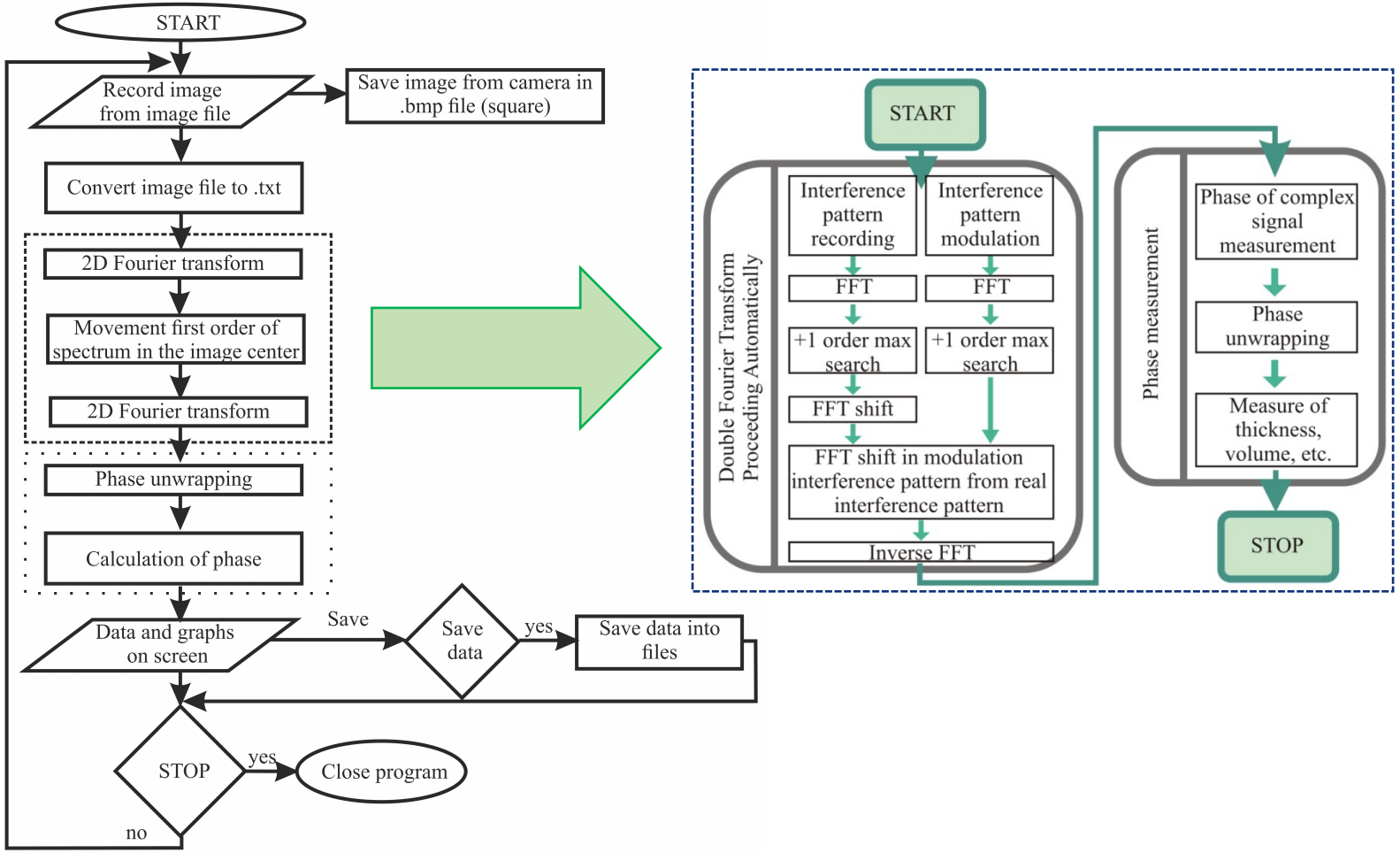
$$I(x;y) = I_0(x;y) + I_R(x;y) + 2\sqrt{I_0 I_R}\cos\left(\frac{2\pi}{d}x - \frac{2\pi}{d}y + \varphi(x;y)\right),$$

$I_0(x;y)$- object field, $I_R(x;y)$ - reference field , d – grating period,
$\varphi(x, y)$ - the phase shift for the wave propagation through the object

Software algorithm



Fourier spectrum

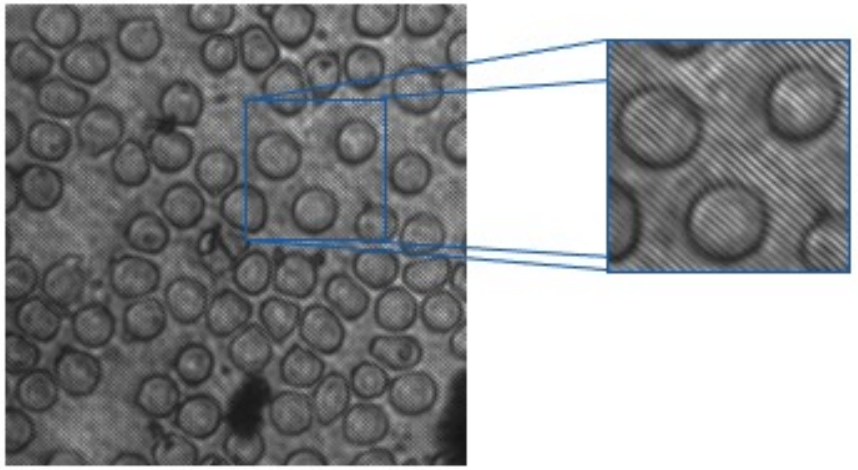


Scheme of the developed software

Subprogram. Description of automatic double Fourier transform algorithm. FFT; Fast Fourier Transform

Interference pattern

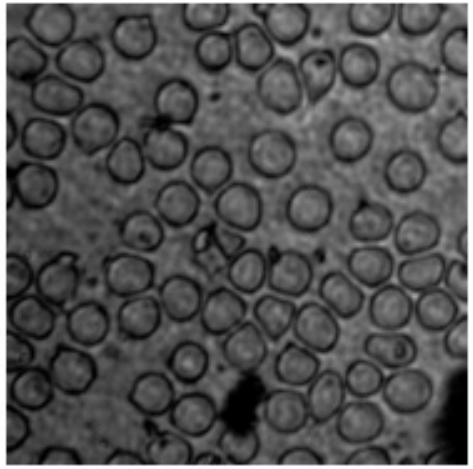
a)



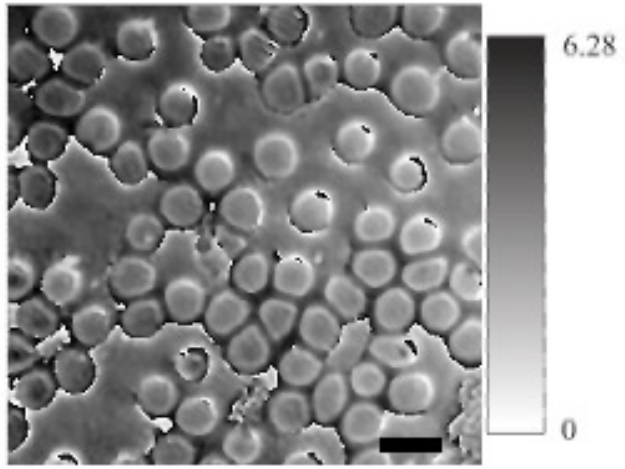
$$I(x;y) = I_0(x;y) + I_R(x;y) + 2\sqrt{I_0 I_R}\cos\left(\frac{2\pi}{d}x - \frac{2\pi}{d}y + \varphi(x;y)\right),$$

$I_0(x;y)$ object field, $I_R(x;y)$ reference field , d – grating period, $\varphi(x, y)$ - the phase shift for the wave propagation through the object

b)

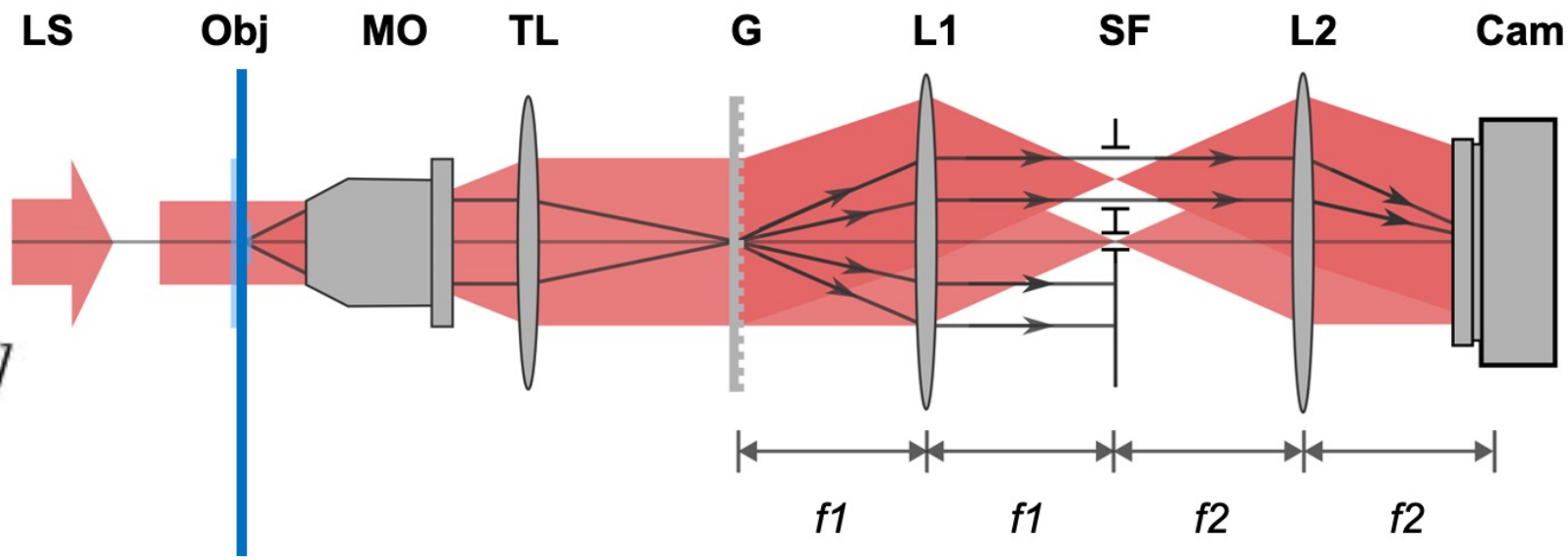


c)



a) Interference pattern recorded using a diffraction phase microscope;
b) Amplitude image;
c) Reconstructed three-dimensional image of a blood smear over the entire field of view of the microscope,
Scale bar on image is 10 μm.

Theory of image formation in DPM



$$E(x, y;t) = a(x, y)\cos[\omega t - \varphi(x,y)]$$

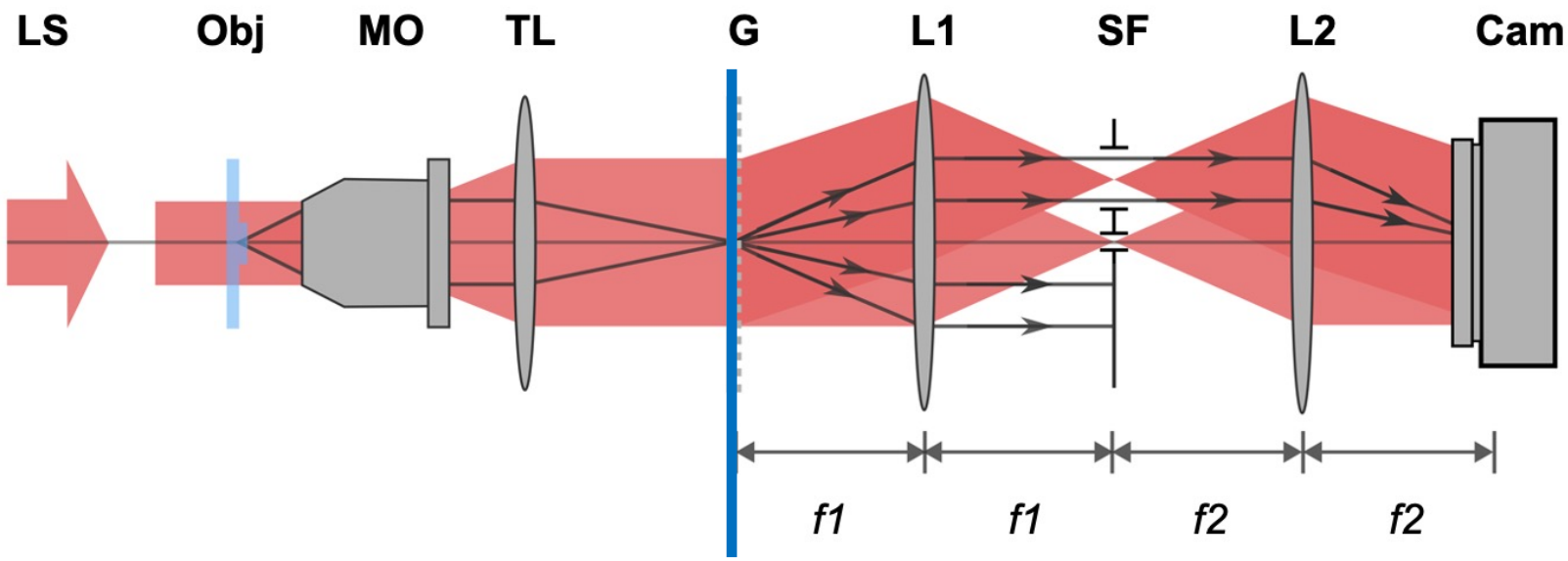
$$s^0(x,y) = a_0 * \exp[i(k_x x + k_y y)]$$

$$f_{obj}(x,y) = \begin{cases} 0, -\frac{p}{2} > x; \\ P, -p/2 \le x \le p/2; \\ 0, \frac{p}{2} > x. \end{cases}$$

$$f_{Obj} = s^0(x,y) * f_{obj}(x,y)$$

Let's consider a plane monochromatic wave.
We write its complex amplitude as s0(x,y) and represent our object as a step function

Theory of image formation in DPM

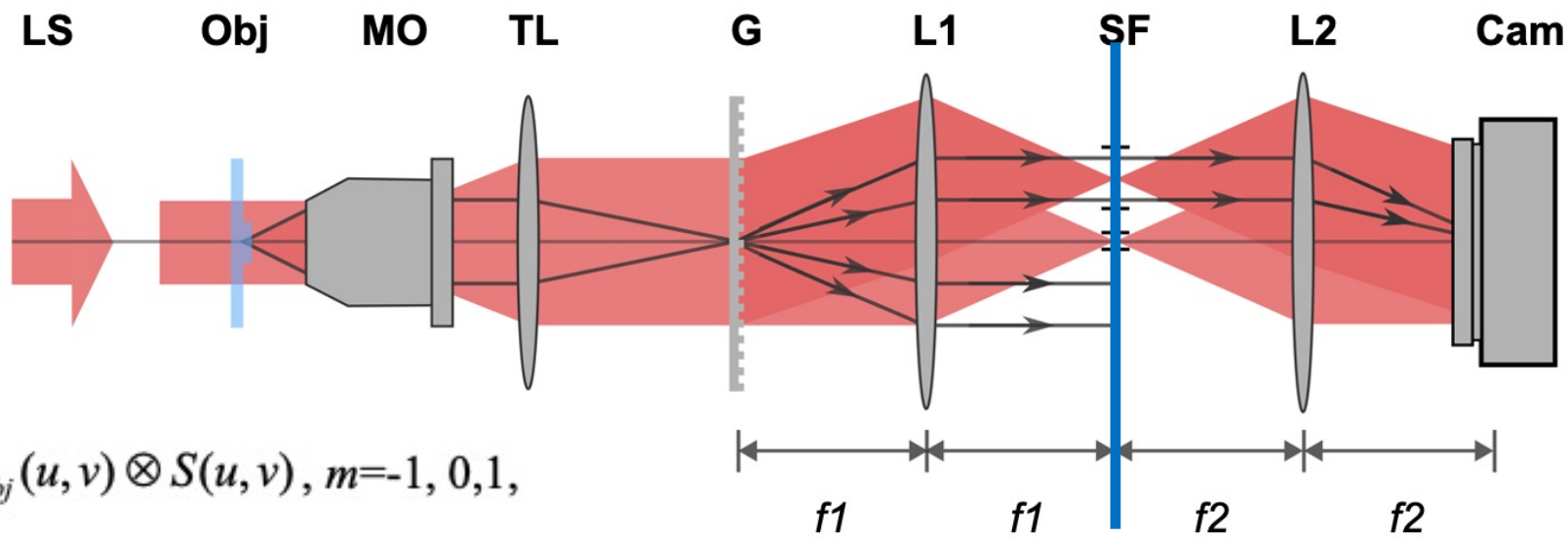


$$f_{IP}(x,y) = f_{Obj}(x,y) \cdot s(x,y) \cdot g(x,y)\,,$$

where $f_{Obj}(x,y)$ is a complex amplitude of magnified object field, $s(x,y)$ is a complex amplitude of magnified illumination field LS, $g(x,y)$ is transmission function of diffraction grade

*AL Kalyanov et al. Proc. SPIE (2015)

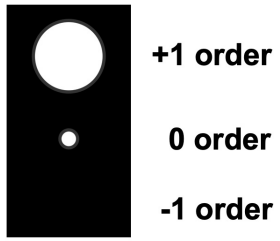
Theory of image formation in DPM



$$F_{IP}(u,v)=\sum_{m=-1}^{1}\delta(u-2\pi m/d,v)\otimes F_{Obj}(u,v)\otimes S(u,v),\ m=-1,0,1,$$

$$b(x,y)=p_{b_0}(x,y)+p_{b_1}(x-\lambda f_{L_1}/d,y),\qquad b_1\geq 2\frac{f_{L_1}NA}{1.22M},$$

where $p_{b_0}(x,y)=\begin{cases}1, \text{if } x^2+y^2\leq b_0^2,\\ 0, \text{if } x^2+y^2>b_0^2;\end{cases}$ and $p_{b_1}(x,y)=\begin{cases}1, \text{if } x^2+y^2\leq b_0^2,\\ 0, \text{if } x^2+y^2>b_0^2;\end{cases}$.



where NA is a microobjective numerical aperture, M is a microscope magnification and f_{L_1} is a focal distance of lens L2

*AL Kalyanov et al. Proc. SPIE (2015)

Correlation function for Fourier plane

Correlation function calculate as:

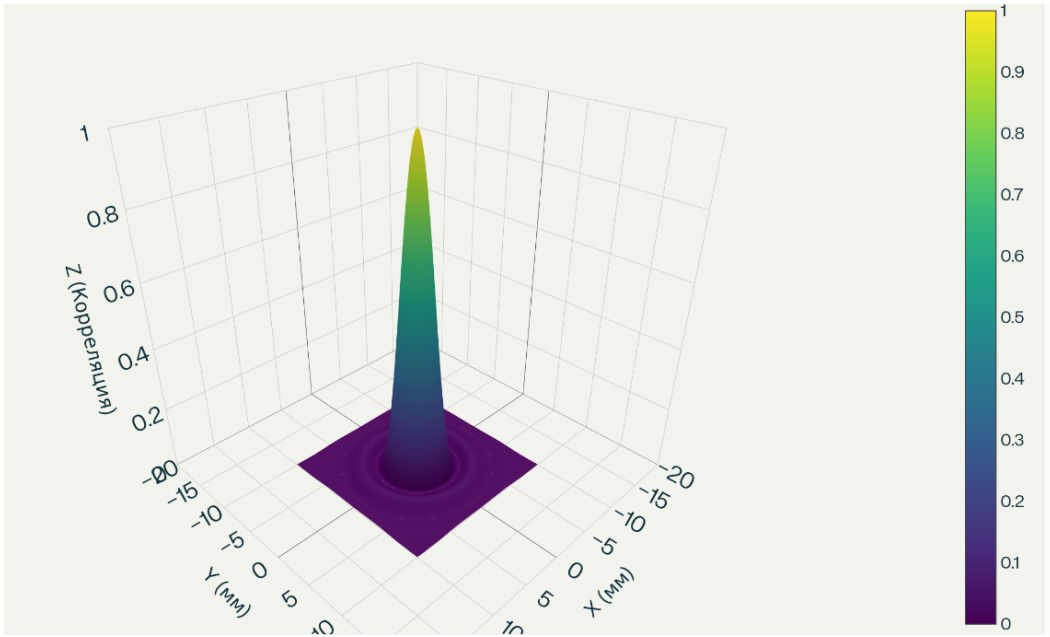
$$\Gamma(\xi_1,\xi_2)=\int_{-\infty}^{\infty}\int_{-\infty}^{\infty} I_0\cdot\gamma(x,x')\cdot P(x)\cdot P(x')\cdot t(x)\cdot t(x')\cdot e^{i\frac{2\pi}{\lambda f}(\xi_1 x-\xi_2 x')}dxdx'.$$

Where ξ ,- spatial coordinates, I_0 - light intensity, *P(x)* – rectangle function, *t(x)* – object propagate function, *exp (x)* describes wave structure and direction

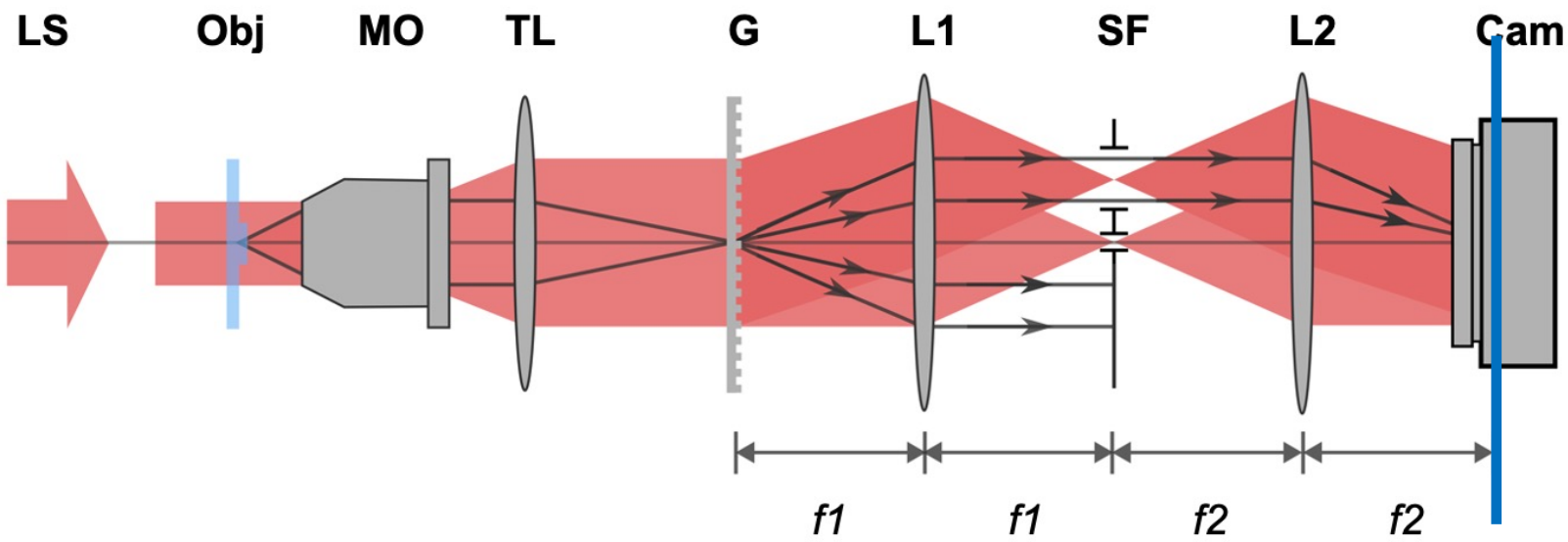
After calculation we get the following result

$$\Gamma(\xi_1;\xi_2)=I_0\cdot b\sin c\left[\frac{\pi b}{\lambda f}(\xi_1-\xi_2)\right].$$

where b – aperture size



Theory of image formation in DPM



As a result, we obtain the field intensity distribution in the plane of the detector matrix as:

$$f_{FP}(x,y) = F_{IP}(\kappa x,\kappa y)\cdot b(x,y) =$$
$$= p_{b_0}(x,y)\cdot\left(\delta(\kappa x,\kappa y)\otimes F_{Obj}(\kappa x,\kappa y)\otimes S(\kappa x,\kappa y)\right)+$$
$$+ p_{b_1}(x-\lambda f_{L_1}/d\,,y)\cdot\left(\delta(\kappa(x-\lambda f_{L_1}/d),\kappa y)\otimes F_{Obj}(\kappa x,\kappa y)\otimes S(\kappa x,\kappa y)\right)$$

*AL Kalyanov et al. Proc. SPIE (2015)

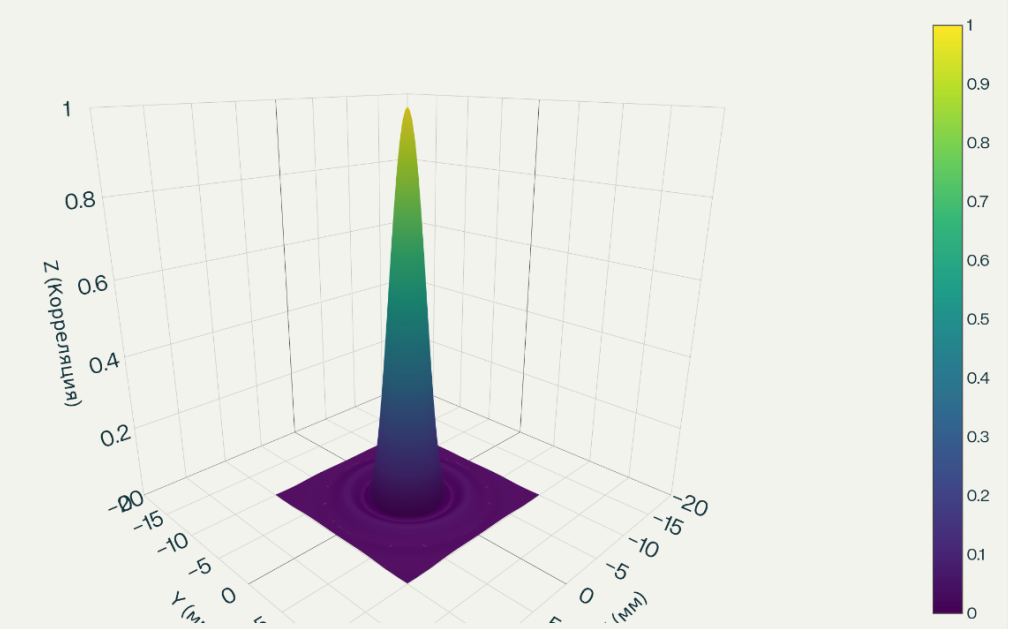
Correlation function for Detector plane

$$\Gamma(x;y) = c_0\left[\frac{2sinc\left(\frac{\pi b}{\lambda f_2}\sqrt{x^2+y^2}\right)}{\frac{\pi b}{\lambda f_2}\sqrt{x^2+y^2}}\right]^2 \times p_{b_0} + c_1\left[\frac{2sinc\left(\frac{\pi b}{\lambda f_2}\sqrt{\left(x^2-\left(\lambda f_{L_1}/d\right)^2\right)+\left(y^2-\left(\lambda f_{L_1}/d\right)^2\right)}\right)}{\frac{\pi b}{\lambda f_2}\sqrt{\left(x^2-\left(\lambda f_{L_1}/d\right)^2\right)+\left(y^2-\left(\lambda f_{L_1}/d\right)^2\right)}}\right]^2$$

where c0 and c1 – zero and first order intensities, respectively

As a result, we obtained

- a shift in the correlation maximum toward the first order
- from the formula, the discrepancy in intensity of both orders is obvious



Experimental results

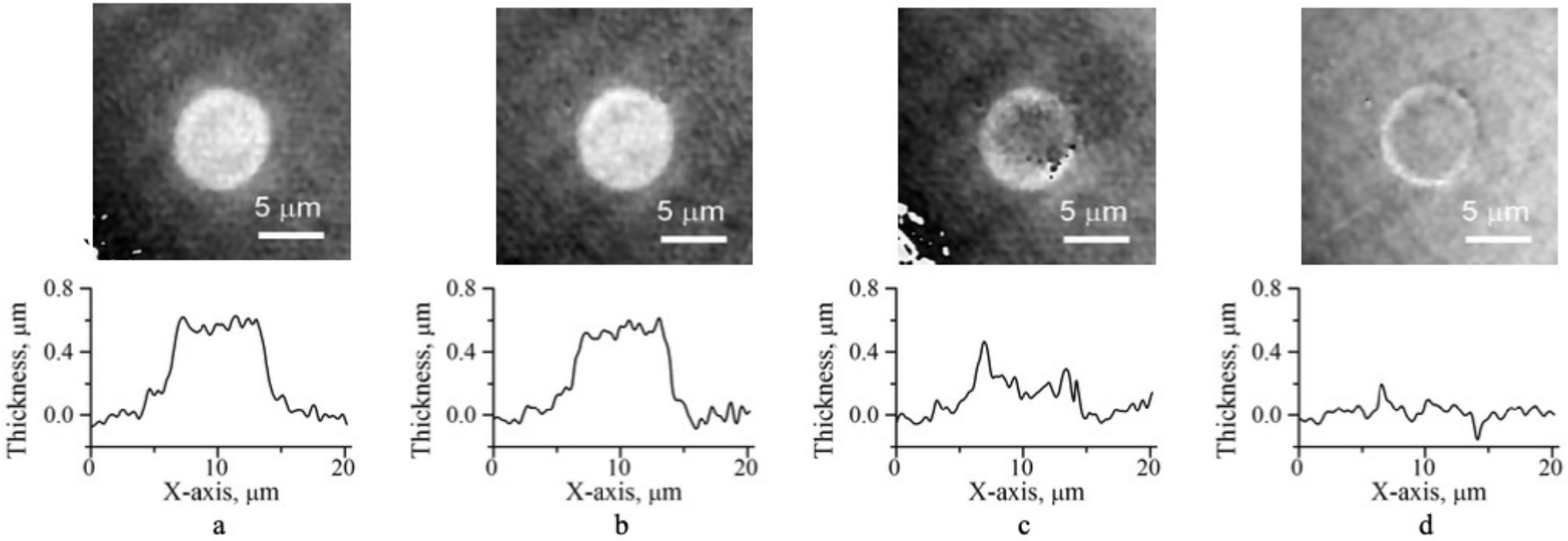


Figure 3. Phase maps and profiles of red blood cell captured with different spatial filter sizes: 25 μm (a), 120 μm (b), 180 μm (c) and 500 μm (d). The profiles are reconstructed along central line. Thickness of the cell is calculated from its optical thickness, refractive index of it is assumed to be 1.65

*AL Kalyanov et al. Proc. SPIE (2015)

Conclusion and Future work

- We have obtained explicit formulas for constructing the correlation function of images in an DPM and it may be applied for any types of interference microscopes
- The modeling results are consistent with our and other scientific groups (C. Edwards et al., 2014) experimental results

For further research we plan to consider different shapes of objects, types of sources and lighting schemes, as well as different types and shapes of diffraction gratings and filters.

For example, various research groups are now using window filters, as well as automatic matrix liquid-crystal structures, which may introduce additional phase delay

Acknowledgments

1. Государственное задание, Разработка теоретических основ и методов генеративного искусственного интеллекта и их приложение к неоднородным доменным областям, № ФИ-2025-19

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1. РФФИ, «Изучение механизмов регуляции сосудистого тонуса при различных физиологических состояниях организма с применением оптических, математических и компьютерных методов», № 12- 02-31204 мол_а
2. Федеральная целевая программа «Научные и научно-педагогические кадры инновационной России» на 2009-2013 гг.: «Разработка новых фотонных технологий анализа биофизических процессов в живых организмах на субклеточном, клеточном и тканевом уровнях для задач неинвазивной и минимально-инвазивной диагностики и терапии», № 02.740.11.0879
3. Федеральная целевая программа «Научные и научно-педагогические кадры инновационной России» на 2009-2013 гг.: «Развитие физических основ и приложений высокоразрешающей томографической и поляризационной микроскопии для субмикроннного анализа объемной структуры объектов технического и биологического происхождения», № 14.B37.21.0728
4. Грант Президента РФ для государственной поддержки ведущих научных школ РФ «Исследование оптических и биофизических свойств биологических тканей и жидкостей, направленное на создание фундаментальных основ оптической медицинской диагностики и лазерной терапии, включая point-of-care медицину» НШ-703.2014.2

Thank you for attention!


HSE UNIVERSITY
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