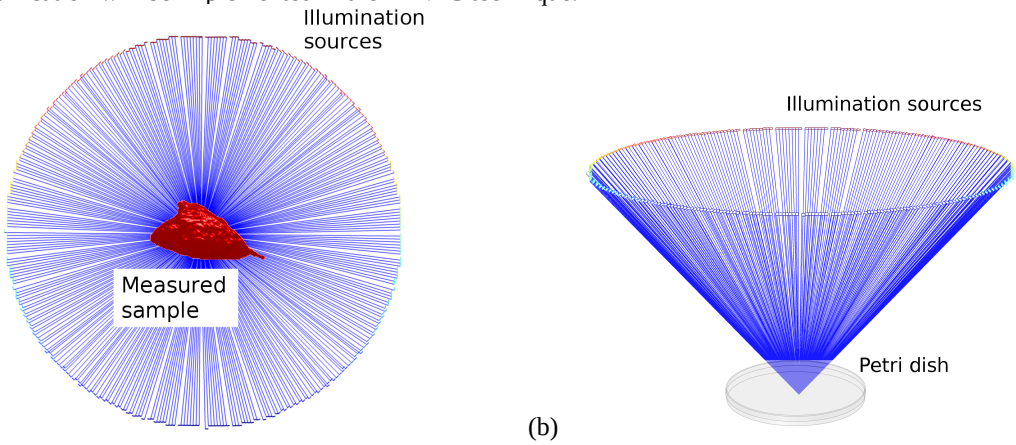
For years an analysis of single biological cells that are subject to different pharmacological substances has constituted the basis for the development of medicine and pharmacology. Until recently, the basic method used for observation of biological cells was classic optical microscopy, where a measured sample is observed visually through the microscopic objective. This method provides only approximated information about how different cell structures absorb light. Over time, information about absorption only was insufficient. There was a need for visualization of cell thickness and distribution of refractive index. The refractive index of a medium tells us, how fast light travels through a medium, compared to the speed of light in vacuum. Since different cell structures have different values of refractive index (light travels through these structures with different speed), the visualization of refractive index distribution allows for analysis of geometry and structure of cell organelles, like cytoplasm, nucleus, vacuole and others. Additionally, a deviation from a standard refractive index value may inform about cancerous cell alterations, infection, inflammation mediated processes, toxicity effects, etc. One of the first devices that allowed visualization of refractive index was optical microscope with Zernike phase contrast. A user received two-dimensional, integrated information about thickness of a sample and average refractive index values along the illuminating light rays that passed through the sample. It was thus impossible to learn about the refractive index value of a certain cell structure. What is more, this was qualitative data (in contrast to quantitative data, qualitative data cannot be assessed numerically, only visually). This problem has been partially solved by holographic microscopy – a technique, where a sample is illuminated by a laser source. Laser beam, after passing through a sample is recorded by a camera and saved in computer memory. Then, the recorded image is numerically reconstructed, and integrated refractive index distribution of a sample is obtained. At first it seems, that this technique allows obtaining quantitative data. However, the result is very difficult to interpret. What is more, the information about refractive index and object thickness is mixed. In order to separate this information from each other, a new technique, called optical tomography, was developed. Here, a sample is illuminated by a laser source, just like it was in holographic microscopy. But additionally, the sample is rotated after each projection has been recorded by a camera. Then, special reconstruction algorithms are utilized to combine this set of projections into a three-dimensional refractive index distribution. This process is quite similar to the one that is taking place in medical CT scanner. The difference is the source used: in medical CT scanner, x-ray source is utilized. This analysis results in quantitative data. In order to obtain correct three-dimensional reconstruction, it is necessary to rotate a sample by 360° during projection acquisition process. Since it would be very difficult to rotate a biological cell itself, it is usually inserted into a glass tube (capillary) which is then rotated. This complicated the measurement process. The objective of this project is to:

- Develop a new method, called DTVIC (Diffraction-based Total Variation Iterative Constraint), for investigation of biological cells. The new technique will be based on my past achievements in the field of optical tomography. This will allow reconstructing three-dimensional refractive index distribution of biological cells that are placed in a Petri dish, on which these cells are usually grown. There will be no need to insert a cell into a glass capillary, which will make the measurement process much quicker. What is more, the sample (cell in a Petri dish) will be stationary and the illumination beam will be rotated.
- Analyze, from which direction it is best to illuminate the sample, to obtain the best-quality reconstruction of refractive index. Since the samples will be placed in Petri dishes, it will be impossible to illuminate them within a 360°. Instead, it will be necessary to rotate the illumination beam within a cone, like presented in Fig. 1. Within this cone, a number of illumination scenarios are possible, like illuminating a cell from points in space that lie on an element of a cone or are evenly distributed within the base of this cone.
- Develop an algorithm to calculate three-dimensional refractive index distribution from projections, that will be acquired within a limited angular range (a cone). Standard algorithms require projections that are captured within 360°. Otherwise, they provide refractive index reconstructions that are erroneous. Development of a new method will allow compensating this limited angular range of projections.
- Take into account the difference between x-ray and laser light in a new DTVIC method. Standard methods assume that rays travel in straight lines through an object measured. This assumption is reasonable for x-ray sources. In the case of laser source however, it leads to significant errors in reconstructions. To avoid these errors, it is necessary to modify the standard approach. This modification will be implemented in the DTVIC technique.



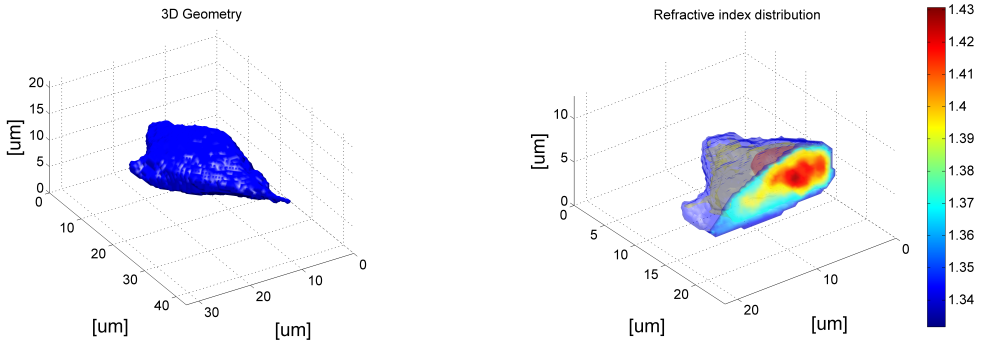
(a) (b)

Fig.1. Visualization of illumination directions. Blue lines represent direction of illumination: (a) in the case of classic tomography, where a cell is illuminated within 360°, and illumination directions are in one plane, and (b) in the case of limited angle tomography, where a cell is placed in a Petri dish and is illuminated from points in space that lie inside the cone.

The project involves basic research on precise understanding of the image formation process in optical tomography, where light is generated by a laser (which means, that we may no longer assume that light rays travel in straight lines through a sample) and where object projections were acquired in a limited angular range only.

I decided to carry out this study, because combination of optical tomography with tomography, where projections are acquired within a limited angular range only, with a thorough analysis of illumination source distribution, will be a milestone in optical metrology of biological microstructures. Achieving the objectives specified in the project will allow for a faster and more reliable

investigation of biological cells that are grown in Petri dishes. My past study proved that these objectives can be fulfilled. I have already developed a technique, called TVIC, on which I will base my research within this project. This technique does not take into account the fact that light rays do not travel in straight line through an object. However, it partially compensates the fact, that projections were acquired within a limited angular range. Initial tests of this technique, conducted on C2C12 cells (mouse muscle cell) and on HeLa cells (cervical cancer cell) prove, that it is possible to obtain a high-quality three-dimensional distribution of refractive index. An example of a reconstruction is presented in Fig. 2.



(a) (b)

Fig. 2. Reconstruction of a C2C12 cell calculated with TVIC algorithm: (a) a reconstruction of three-dimensional geometry and (b) reconstruction of three-dimensional refractive index distribution presented for 1 cross-section through a sample.