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3D PHASE: Phase microscopy and tomography – new approach to 3D measurements of biological and technological structures

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1. Introduction & background

In both biology and microtechnology a key for understating certain processes is to provide them with accurate characterization tools. In biology understanding of biological process is strongly gained by the ability of visualization and quantifying of living cells reaction [1]. On the other hand in technology it is crucial to facilitate design and control of manufacturing processes of phase photonics microstructures with measurements [2]. In this both seemingly apart areas phase information proved to be a key value (Fig.1).

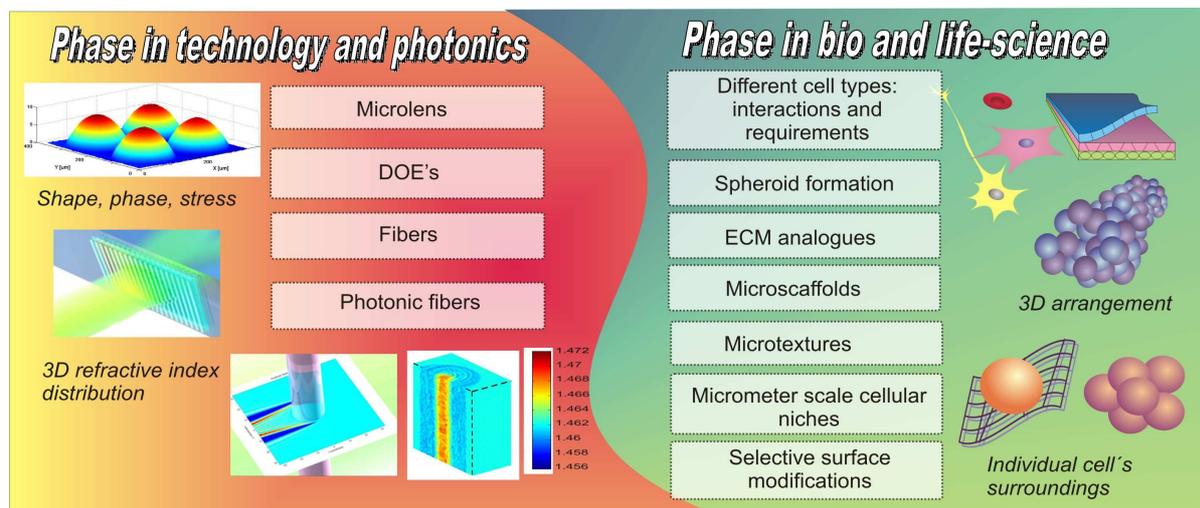


Fig. 1. Phase information in technology and biology [1,2].

In life sciences phase quantitative imaging with high resolution enables minimal invasive cell visualization and access to quantitative label-free spatial and temporal information on a cellular level [3]. Microoptical and micromechanical technologies progress towards higher levels of sophistication. Due to continuous miniaturization in manufacturing technology, the microsystems based on the combination of microtechnologies allows for miniaturization of classical, bulk instrumentation. In

bioanalytical applications especially designed and fabricated “smart” miniaturized tools (lab-on-a-chip or micro total analysis systems (μ TAS)) are required. These devices are essential for rapid and non-invasive investigations of biological objects, drug studies, speciation of toxic substances, early diagnosis and further medical treatment. In this domain the phase measurement techniques can gain new valuable information. In technology of microoptics the accurate phase information is also a key since it is determined by optical properties of microelements (refractive index distribution, shape) [4]. In general the most suited technique for quantitative, accurate phase determination is interferometry and especially its recent implementation through digital holographic microscopy [5]. It delivers integrated phase (averaged refractive index) as it passes through a specimens. Digital holographic microscope have been applied in the areas of biomedicine, life sciences or technology. The method is fast, accurate and with additional effort allows to study dynamic reactions in life sciences, technological processes of phase microstructures and functionalities of novel active photonics elements (e.g. electrostatically controlled microlenses or liquid crystal based elements). If optical diffraction tomography concept is implemented a 3D distribution of phase (refractive index) in microstructure is obtained. The technique requires several integrated phase measurements captured for different angular orientation of an object [6].

Even though interferometric and holographic techniques are fast and accurate they have a number of weaknesses shared by all interferometric devices: they require reference beam, high mechanical and environmental stability and coherent illumination. As the output a phase modulo 2π is delivered which requires in many cases complex unwrapping procedures. The measurement systems have complex construction, its resolution is limited by usage of coherent light and often cannot fulfill the functionality required especially in the case of 3D and 4D phase investigation. Therefore in this project we aim in development of a single beam phase microscopy and tomography (with and without imaging optics) with the phase recovery by the transport-of-intensity equation [7] extending significantly the recent capabilities of this technique. **As a result it is expected that the family of novel phase microscopes and tomographs will be developed and proved to have better functionalities for measurements of technological and biological microstructures of high interest to the research, industrial and medical communities.**

The projects area is compatible with the priority area of the Innovative Economy Operational Program namely with:

- **Techno** (Designing specialized systems, Mechatronics, New materials and technologies),
- **Bio** (Biotechnology and Bioengineering, New medical products and techniques).

2. State of the art

In techno and bio sciences optical visualization and fully or partially quantitative imaging is performed mainly by microscopy, optical coherence tomography (OCT), and interferometric techniques. Microscopy with phase contrast technique [8] or differential interference Nomarski technique [9] gives phase visualization. The OCT technique [10] builds 3D sample volume based on a known refractive index distribution. Among these only interferometric technique and especially digital holographic microscopy (DHM) provides high resolution quantitative phase imaging [11,12], which is highly required feature in both bio and techno science. In the digital holography interference fringes are recorded as an effect of interference of optically magnified object beam and reference wave. The image is then numerically reconstructed from a hologram providing accurate complex object beam i.e. information about its amplitude and phase. Let compare this device with conventional microscope. Conventional microscope has a simple construction (object beam is present only), it uses incoherent illumination, provides intensity images and phase can be visualized only. On the other hand interferometers have complicated design (additional reference beam has to be provided). They use highly coherent illumination limiting an imaging system resolution. High coherence gives measurements data disturbed by speckle noise of high contrast, the speckle effect is

additionally magnified by presence of reference beam, long optical path and large number of used elements. This additionally limits the system resolution. The additional disadvantage are aberrations that have to be compensated and high sensitivity to vibrations which limits out-of-laboratory applications of the system.

Concluding the above consideration, we state that a single object beam microscope combined with the capabilities of measuring an entire complex wave would be advantageous. There are two most attractive technical solutions for such apparatus: a point source DHM [13] and devices recovering phase from diffraction (defocused) images [14]. Both phase recovery solutions have yet number of problems that have to be solved prior to technique wide application.

The point source DHM works in in-line Gabor arrangement [15]. The alignment together with the developed algorithm provides images of high resolution. However the accuracy of phase reconstructions is questionable as the reconstructed phase is substantially distorted by a presence of unfiltered twin image and zero order. Technique uses an approximation, that object beam intensity and twin image are of known distributions. Nevertheless the method is attractive since it uses the only 'perfect' lens available i.e. an illumination is a spherical beam originating from a pinhole of size in the order of a wavelength.

Defocused images are often used in microscopy for phase objects visualization [8], slightly defocused images of phase objects corresponds to Laplacian of the phase. This concept is applied in the second technique for complex object beam recovery from a single beam only. The base for this technique is Transport of Intensity Equation (TIE), where a phase is recovered from measured axial changes of an object beam intensity. The equation was developed by Teague [16] and then the major technique progress is due to group of University of Melbourne [7] focused on X-Ray imaging problems. Their work resulted in the best numerical methods of direct phase recovery for TIE based systems [17]. The TIE is developed for Fresnel approximation and cannot be applied for high numerical aperture (NA) beam. Currently the high NA beam can be recovered from intensity only data using iterative methods [18]. Direct methods of phase recovery basing on TIE are very interesting in spite of theoretical and practical obstacles that have to be overcome for development of phase microscopy, that can deliver phase reconstructions of high accuracy for a wide range of objects.

The phase imaging techniques provide measurement of integrated (averaged) refractive index distribution. These measurements can be used for reconstruction of 3D refractive index distribution if the sample rotation is provided using concept of optical diffraction tomography (ODT)[19]. The object rotation can be replaced by a change of illumination wave direction. For tomographic reconstruction three algorithms are the most often used: hybrid filtered back projection method, filtered back propagation and method basing on ray optics and Bouguer formula [20]. There are many studies of ODT in both bio and techno areas [21,22]. TIE based phase recovery technique suites well for ODT, since in both TIE and ODT slow object phase variations are preferable. The first solution for TIE based tomography has been shown by Peele and Nugent in 2008 [23]. In principle a single beam phase microscope, especially lensless one, allows to build a compact and simple in design ODT system. However at the moment the technique is incapable of characterization of high contrast (high gradient and high phase difference) and high spatial frequency phase samples. Which are often met in biology and technology (e.g. photonic crystal fibers or cells with a complex intercellular structure).

3. Significance of the research, research objective and application

The main objective of the project is to provide such progress in the TIE based methods of an object phase recovery which would enable to design and develop a novel quantitative imaging of phase object instrumentation which bridge a conventional microscope with a digital holographic microscope.

This instrumentation will have a simple construction (object beam only) and will provide accurate measurement of entire complex wave. This solution will allow to addresses the next objective: **development of tomographic methods and instrumentation for determination of 3D ($n(x,y,z)$) and 4D ($n(x,y,z;t)$) refractive index distribution in a wide optical spectrum range.** This objective meets the needs of multidisciplinary science for accurate, reliable and simple way of investigations technological and biological samples.

There are three major breakthroughs and goals of the project:

- development of novel methods and algorithms capable to retrieve integrated and 3D phase distribution from a variety of technological and biological microobjects,
- design and development of high resolution and single beam phase microscope for wide range of samples (cooperation with VUB and CeBOP),
- design and development of compact ODT systems capable of 3D and 4D reconstructions (cooperation with MN2S and CeBOP).

The newly developed apparatus will serve within a project for:

- investigation of 2D, 3D and 4D (monitoring of techn. process and active photonics microoptics) refractive index distributions in technological elements (cooperation with VUB and MN2S),
- investigation of 2D, 3D and 4D refractive index distributions of cells and intercellular structures (both static and changing in time) (cooperation with CeBOP and VUB),
- development of new miniaturized tools for bioanalytical applications (cooperation with VUB).

4. Project structure and description

The project consists of two work packages: WP1 and WP2. Both of them include three main tasks focused at: theory/simulation), development/design/metrology of instrumentation and its applications in technological and bio- microstructures.

WP1: Novel methods of high resolution phase microscopy for biological and technological specimens.

The WP1 focuses on delivery of phase microscopy for high accuracy phase metrology and measurements of integrated (averaged) refractive index. There are number of theoretical, algorithmic and instrumental problems that have to be worked out during the project. In methodology of TIE we capture intensity in two parallel planes using a mechanical movement of detection sensor. Defocusing through mechanical movement is slow and inaccurate. We will explore defocusing by number of methods, for example, using different wavelengths, applying mirror on piezoelectric transducer or spatial phase light modulator. Our apparatus will be used in imaging of dynamics of living cells as well. For dynamic case we need to simultaneously capture object wave intensities in two defocused planes.

WP1 in tasks and specific goals.

T.1	(Y0-Y3) Theory and simulations of a single beam phase microscope
T.1.1	(Y0-Y3) Development of new theory and algorithms of phase recovery from single beam propagation (problems related to wide angle field and sample properties)
T.1.2	(Y0-Y1.5) Study of partial coherence requirements for single beam phase microscope
T.1.3	(Y0-Y1.5) Research on new algorithms and techniques for defocus (temporal, spatial, spectral)
T.1.4	(Y1-Y3) New theory and algorithms for high divergence-structured light illumination (single and multiple point sources)
T.1.5	(Y1-Y3) Study of sampling issue (super resolution) of technique of phase recovery from defocused diffraction images
T.2	(Y1-Y4) Development, design and metrology of a single beam phase microscope
T.2.1	(Y1-Y3) Design and laboratory development of single beam phase microscopy apparatus
T.2.2	(Y2.5-Y4) Metrological study of phase microscopy at certified models
T.3	(Y2-Y4) Phase microscopy applications
T.3.1	(Y2-Y4) Phase microscopy application for technological elements
T.3.2	(Y2-Y4) Phase microscopy application for biological samples

Team members within WP1 for entire 4 year project duration:

- 1 *postDoc position* (T1)
- 2 *PhD students* (one – T1, second – T2 and T3.1)
- 0.5 *PhD student* (WP1 T3.2)
- 2 *students per each year* (e.g. DOE based defocus technique, Multiwavelength illumination and defocus technique, accuracy assessment of TIE-based algorithms).

WP2: Novel methods of optical tomography for biological and technological specimens

The WP2 goal is a development of novel instrumentation for phase measurements of 3D and 4D refractive index distributions for transparent or semitransparent micro structures. We are going to develop two instruments: one for characterization of static structures and second for dynamic ones (bio samples). Both instrumentation will apply phase microscopy and algorithms developed in WP1. Tomographic system for static samples measurement is a phase microscope with a sample attached to a rotating mechanism or in configuration allowing to change direction of illumination beam. Application of lensless phase microscope gives a very simple design when compared with digital holography [24] and it can be easily miniaturized. However there is a very important challenge and limitation of ODT systems. They are capable of characterization of low contrast and low spatial frequency phase distributions only [25]. An important class of objects in both biology and technology characterizes phase distribution of high gradient, high phase differences and high frequency. Such a samples cannot be characterized now (e.g. photonic crystal fibers). We will therefore put an effort to overcome this limitations.

WP2 in tasks and specific goals.

T.1	(Y0-Y3.5) Theory, algorithms and simulations of ODT system
T.1.1	(Y0-Y3) Development of theory and algorithms for tomographic algorithm of phase recovery
T.1.2	(Y0-Y3) Simulations of ODT system and optimization of its algorithms and design
T.1.3	(Y2-Y3.5) Research on new algorithms and implementation of efficient algorithm for dynamic phase reconstruction
T.2	(Y1-Y4) Development, design and metrology of ODT system
T.2.1	(Y1-Y3) Development of tomographic instrumentation for static structures
T.2.2	(Y1-Y3.5) Development of compact tomographic instrumentation for dynamic structures.
T.2.3	(Y2-Y4) Metrological study of both tomographs at standardized (master) elements
T.3	(Y2.5-4) ODT applications
T.3.1	(Y2.5-Y4) ODT application for technological elements
T.3.2	(Y2.5-Y4) ODT application for biological samples

Team members within WP2:

- 1 *postDoc position* (T.1 and T.2)
- 2 *PhD students* (one - T.1, second T.2)
- 0.5 *PhD student* (WP1 T3.2 and WP2 T3.2)
- 2 *students per each year* (e.g. Design and control of rotational actuator for ODT system, ODT with fiber optic flow cells for bioanalytical applications).

5. Researchers and research environment

WUT members of the research team from :

1. Photonics Engineering Division, Inst. of Micromechanics & Photonics, WUT_IMiF (optical metrology, technological applications):

- **prof. dr hab. inż. Małgorzata Kujawińska** - team leader, responsible for phase microscope and tomography concepts, measurement methodology, investigations of technological structures and future applications and implementations,

- **dr inż. Tomasz Kozacki** - responsible for theoretical and numerical work on phase recovery for phase microscope, new tomographic algorithms for high contrast and high frequency phase structures, simulations,

- **dr inż. Michał Józwik** - responsible for development of instrumentation and metrological studies.

2. Department of Microbioanalytics, The Faculty of Chemistry, WUT_DMb (μ TAS, biological applications):

- **prof. dr hab. inż. Zbigniew Brzózka** - responsible for bioanalytical applications of developed measurements systems.

WUT_IMiF laboratories are equipped into wide range of commercial and in-house interferometry and digital holography systems for MO investigations (incl. unique interferometric and elastooptics tomographs). It has also the optical workshop for manufacturing of demonstrators and several commercial and in-house developed software for optical design and beam propagation simulations.

WUT_DMb laboratories are equipped into full technology chain for μ TAS development and manufacturing as well as the access to biological material and tools for its manipulation.

6. International cooperation

Department of Applied Physics and Photonics (TONA) at Vrije Universiteit Brussel (VUB) (<http://tona.vub.ac.be/Tona/>) specializes in full technology chain of micro-optics from optical design and modeling (powerful computer cluster equipped with a variety of professional and in-house software) through a unique rapid prototyping technology for the fabrication of high aspect ratio plastic micro-optical components and the micro-optical measurement and nano-instrumentation facilities which host a unique collection of high-end instrumentation for the quantitative characterization of micro- and nano- optical components and structures. They also have photonics research labs which feature state-of-the-art high-precision optical and opto-mechanical components, opto-electronic and photonic measurement equipment, lasers, and a variety of high-end optical sources, dedicated to built proof-of-concept demonstrators. VUB-TONA is the partner in European NoE Photonics4Life. The scientific and educational cooperation with VUB-TONA is very successful and dates back to 1997 including cooperation in EU NoE NEMO and Acces Center for Microoptics ACTMOST.

Center for Biomedical Optics and Photonics (CeBOP), University of Münster, (<http://www.cebop.uni-muenster.de>) specializes in quantitative phase contrast imaging for high resolution and minimal invasive life cell analysis including investigations of dynamic processes in cells. They have extended expertise in both biological sciences (direct cooperation with Medizinischen Fakultät der Westfälischen Wilhelms-Universität) and optical metrology. For many years they have applied holographic and ESPI techniques for imaging and quantitative analysis of biostructures. Since 2004 the main CeBOP focus is on digital holography and digital holographic interferometry enhancements, new methods and their applications in biomedicine. As the result they had gained extended and widely internationally recognized expertise in the field as well as laboratories have the state-of-the-art equipment for biomaterial manipulation, its imaging and investigations. Prof. Gert von Bally is the coordinator of the European NoE Photonics4Life.

Département MN2S (Micro Nano Sciences & Systèmes), FEMTO-ST, Université de Franche-Comté (<http://www.femto-st.fr>). FEMTO-ST is a joint research unit which is affiliated with the French National Centre of Scientific Research (CNRS), the University of Franche-Comté (UFC), the National School of Mechanical Engineering and Microtechnology (ENSMM), and the Belfort-Montbéliard University of Technology (UTBM). Département MN2S (Micro Nano Sciences & Systèmes) is a member of FEMTO-ST. The research activities of Dr C. Gorecki's MOEMS group focus on the development of miniature optical sensors made by using batch-fabrication processes (silicon technology). This activity combines the integrated optics with micromechanical structures and microoptics, offering new integration potentials for sensing applications and also atomic clock applications. MN2S FEMTO-ST is also well-known for its competences in the fields of physics, electronics devices and systems, nanosciences, and, on the other hand for its competence and its know-how in micro-manufacturing, integrated and assembled microsystems. The scientific cooperation between WUT and FEMTO-ST is very successful and dates back to 1990.

WUT_IMiF has excellent cooperation with most of the European institutions working in microoptics, optical metrology. Additionally through the European Erasmus Mundus Masters in optics and Technology (partnership with London Imperial College, Delft Technical Univ., Inst. d'Optique in Paris, Fredrik Schiller Univ. in Jena) we have access to big international pool of the students in the field of optics and photonics.

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