Many scientific, industrial and biological applications benefit from the knowledge of the phase information, which allows revealing hidden features of various objects. The phase data can be accessed with quantitative phase imaging (QPI) techniques, enabling visualization of unknown biological processes and development of advanced nano- and micro-technologies. The pioneering work in the field of QPI was the phase contrast microscope developed by Frits Zernike in the early 1930s. The Zernike's invention was a major breakthrough in optical microcopy, as it enabled converting the phase shifts in light passing through a transparent sample into observable brightness changes. Nevertheless, a significant drawback of the Zernike phase contrast microscope was inability to provide quantitative results, which prevented major breakthroughs in science and technology. What made the difference was the advent of Interferometry and later Holography. Nowadays, perhaps the most promising and versatile tool of QPI is a technique stemming from powerful combination of holographic, bright field microscopy and computer science. The technique, called digital holographic microscopy (DHM), enables quantitative and accurate phase distribution determination with impressive submicron resolution. Although DHM can give an impression of three-dimensionality of an object, it does not provide truly 3D imaging. This is due to the limited spatial frequency support provided by this method. In other words, the information capacity of a single DHM measurement is too poor to provide a valuable access to the 3D internal structure of a sample. For this reason, DHM is often called a 2½D imaging technique. The project proposal is based on the concept of enlarging the spatial frequency support of DHM by utilizing dynamic modulation of an illumination wavefront. The proposed modification will enable increasing the general capabilities of conventional DHM and allow for quantitative 3D imaging. In the project the 3D QPI will be realized with digital tomographic microscope (DHTM), where multiple holograms are taken with DHM with dynamic modulation of illumination wavefront, and then the numerical tomographic procedure is applied. The unlocked 3D imaging potential of DHM will be an answer to the growing needs of microscale inspection and study in technology and life sciences, which requires measurements of micro shape topography and 3D distribution of refractive index.
QPI realized with DHM has enormous potential. The great power of this method is perhaps best demonstrated by several key developments important for this proposal, including:
- extended focus imaging (EFI), in which a single hologram is used to obtain a sharp image of object features located at various depths,
- autofocusing methods allowing for automatic detection of in-focus plane,
- exact methods of absolute shape calculation,
- possibility of 3D imaging of refractive index distribution.

Beside the great potential, there are still severe and principal obstacles limiting the DHM applications. Addressing these challenges is one of main objectives of this proposal. The most pressing problems in DHM are: low accuracy of the EFI; insufficient accuracy of autofocusing methods and its dependence on a sample properties; accuracy of shape measurement limited by the precision of the autofocusing methods; requirement for realizing EFI or shape measurement relatively to an accurately localized imaging plane; very small unambiguous measurement range (UMR) corresponding to the optical wavelength. Another project challenge is related to realization of the tomographic concept of 3D QPI. This task is technically very demanding as it requires capturing large amount of data, usually 100 – 1000 holograms, for various modulation of an illumination wavefront. Moreover, the required alternation of the illumination conditions should be precise and fast, which makes the task very demanding. These two aspects represent a bottle neck for broad application of 3D QPI.
The project proposal presents solutions to all of the above challanges and opens a new exciting perspective for application of QPI. The declared advancements of the project will be achieved through a novel concept of a DHM, which makes full use of its information capacity due to dynamic modulation of the illuminating wavefront and is supported by several novel theoretical solutions of recovery of 3D/4D objects information, which is encoded in a set of holograms with different modulation of the illuminating wavefront.
This solution will allow opening new existing perspectives of application of DHM for imaging and shape measurement:
- new methods for measurement of topographies with large steps of height;
- new automatic focusing methods with improved accuracy and extended focusing range basing on three different principles: frequency support extension, analysis of phase interdependence between captured holograms, construction of an artificial partially coherent hologram;
- new non-paraxial shape and thickness measurement methods without the need for a reference measurement plane;

In terms of tomographic technique, the proposed solution has two main advantages - simplified construction and fast acquisition. These two crucial aspects may potentially allow for an increase in the availability of the techniques for 3D imaging of microstructures and dynamic processes. The project will provide enhancements of DHTM by proposing novel and complete tomographic solutions (systems + algorithms) with exceptional parameters addressing the mentioned problems of 3D imaging:
- multiplexed tomographic solution for 4D imaging of dynamic objects capable of high quality tomographic reconstruction for minimal number of captured projection images,
- tomographic approach based on transverse scanning of spherical wave illumination allowing for simple and inexpensive technical realization.

For the transverse scanning approach, a novel dedicated diffraction-based tomographic algorithm for spherical wave scanning will be developed. The challenge of the dynamic 4D imaging will be addressed by developing new iterative tomographic reconstruction algorithms capable of high quality 3D tomographic reconstruction with minimal number of captured projection images. To minimize the effect of missing frequency components we plan to apply two major approaches: 1) 3D deconvolution and 2) iterative algorithm employing accurate 3D scattering approaches. For this purpose, novel, fast and accurate scattering simulation methods for 3D refractive index distributions will be developed.