DEVELOPMENT OF DIGITAL HOLOGRAPHIC MICROSCOPY IN MOLDOVA

Doctor of Physics and Mathematics **Elena ACHIMOVA**
Institute of Applied Physics of ASM

DEZVOLTAREA MICROSCOPIEI HOLOGRAFICE DIGITALE ÎN REPUBLICA MOLDOVA

Rezumat. Scopul proiectului HOLO este de a spori excelența științifică și capacitatea de inovare în microscopia holografică digitală a Institutului de Fizica Aplicată al Academiei de Științe a Moldovei, prin crearea unei rețele cu parteneri de twinning de înaltă calitate: Universitatea din Stuttgart, Universitatea de Tehnologie din Tampere și Intelligentsia Consultants. Proiectul cu durata de trei ani se bazează pe investigațiile desfășurate de către Institutul de Fizică Aplicată al AȘM și partenerii săi. Pentru a crește potențialul științific și capacitatea de inovare în microscopia holografică digitală, partenerii pun în aplicare o strategie științifică și inovativă axată pe două sub-teme:

1. Design și optimizarea elementelor optice difracționale pentru îmbunătățirea microscopiei holografice digitale;
2. Dezvoltarea algoritmilor avansați de procesare a imaginii pentru microscopia holografică digitală, folosind elemente optice difracționale.

Cuvinte-cheie: microscopie holografică digitală.

Summary. The overall aim of the HOLO project is to boost the scientific excellence and innovation capacity in digital holographic microscopy of the Institute of Applied Physics of the Academy of Sciences of Moldova by creating a network with the high-quality Twinning partners: Universität Stuttgart, Tampere University of Technology and Intelligentsia Consultants. To achieve this aim, the 3 year project builds upon the existing strong research and innovation base of IAP-ASM and its Twinning partners. To boost their scientific excellence and innovation capacity in digital holographic microscopy, the partners implement a science and innovation strategy focused on two sub-topics:

1. Design and optimization of diffractive optical elements to improve digital holographic microscopy;
2. Development of advanced image processing algorithms for digital holographic microscopy using diffractive optical elements.

Keywords: digital holographic microscopy.

In 2015 the Institute of Applied Physics (IAP) of Academy of Sciences of Moldova (ASM) has participated in „Horizon 2020 – the Framework Programme for Research and Innovation (2014-2020)" submitting the proposal to the call H2020-TWINN-2015, and has won the project "Boosting the scientific excellence and innovation capacity in digital holographic microscopy of the Institute of Applied Physics of the Academy of Sciences of Moldova – HOLO" (Grant Agreement number: 687328). The overall aim of the HOLO project is to boost the scientific excellence and innovation capacity in digital holographic microscopy of the IAP of ASM by creating a network with the high-quality Twinning partners: University of Stuttgart (Germany), Institute of Technical Optics, Tampere University of Technology (Finland), Signal Processing Department and Intelligentsia Consultants (Luxemburg). To achieve this aim, the 3 year project will build upon the existing strong research and innovation base of IAP-ASM, Laboratory of materials for photovoltaic and photonics and its Twinning partners. The total budget of the project is 1 million euro, more than half of that is Moldavian part. For the first time the Coordinator of the so large-scale European project is IAP-ASM. Such decision of EC is caused by the high fame of Moldova science in the world.

The ASM, in its role of the highest scientific forum of the country, is the only public institution representing of the national interests in the sphere of science and innovation. It is a plenipotentiary coordinator of scientific and innovation activity, scientific consultant of the public authorities of the Moldova Republic, has an autonomous statute and acts on the basis of self-administration principles. The IAP-ASM is one of the biggest institutes of the ASM. Among priorities of the IAP are the fundamental and applied investigations in physics of condensed matter: crystalline, non-crystalline and nanostructured materials, electronics and quantum optics, design of high technologies and multifunctional electronic, optoelectronic and photonic devices. The research profile of the Materials for Photovoltaic and Photonics Laboratory is „Physics and engineering of noncrystalline materials, photonic and optoelectronic devices".

During many years the team of the laboratory carries out the study of optical sensors and fibers, holography, holographic interferometry, new phenomena concerning photo-induced changes, in chalcogenide glasses and polymers, as well as elaboration of holographic high resolution registration media and holographic information technologies. In the last few years laboratory developed new technology for deposition of multinanolayers from chalcogenide glasses and photopolymers. Thanks to nano thickness of layers the new unusual and useful for applications properties of the glasses films were discovered.

Specifically, polarization sensitive layers demonstrate direct surface relief formation of diffraction grating under laser illumination. Diffraction efficiencies of the diffraction grating reach out for about 40% at direct one-step holographic recording. Holographic laser microscopy and elaborated nanotechnology are most in demand technologies. The synergy of the successful results in above technological and scientific fields succeeded to achieve the EU project in frame of HORIZON- 2020.

The other partners involved in the HOLO project are follows.

The University of Stuttgart was founded in 1829 and is situated in the middle of a highly dynamic economic region known for its technological excellence. With more than 150 institutes, about 10 faculties and more than 20,000 students, the main emphasis of the university is on engineering and the natural sciences. The University of Stuttgart ranks as one of Germany's most successful research universities and is a member of TU 9, a group of the leading technical universities in Germany. Notably, the Institut für Technische Optik has over 50 full-time and part-time scientists involved in a wide range of optics research including 3D surface metrology; active optical systems; interferometry and diffractive optics, coherent optical metrology, and biomedical optics.

Tampere University of Technology was ranked a Top 100 university under 50 years old in 2014/2015 by the Times Higher Education. The university combines a well-establish tradition of research in natural sciences and engineering with close links to industry and business. It believes that technology is the key to addressing global challenges. Its 2000 staff caters for over 10.000 students and around 1.500 foreign nationals either work or pursue study at the university. The Department of Signal Processing has over 180 people teaching and conducting research on audio, images, video and biological signals, and hardware and software systems related with them.

Intelligentsia is well-experienced in providing high-quality training services to support science, technology and innovation in the public and private sectors. Notably, the company has worked on over 20 FP7 and H2020 projects with Central and Eastern European research institutes concerning the development of centers of excellence, integration into European Research Agency, and technology transfer (e.g. FP7 CEOSeR, FP7 NANOSENS, FP7 IPERA, H2020 INTELUM and H2020 HEALTH-TECH).

CIENTIFIC AND TECHNICAL OBJECTIVES OF HOLO PROJECT

The overall concept of the HOLO project and its impacts, objective, events is captured in the following figure 1:

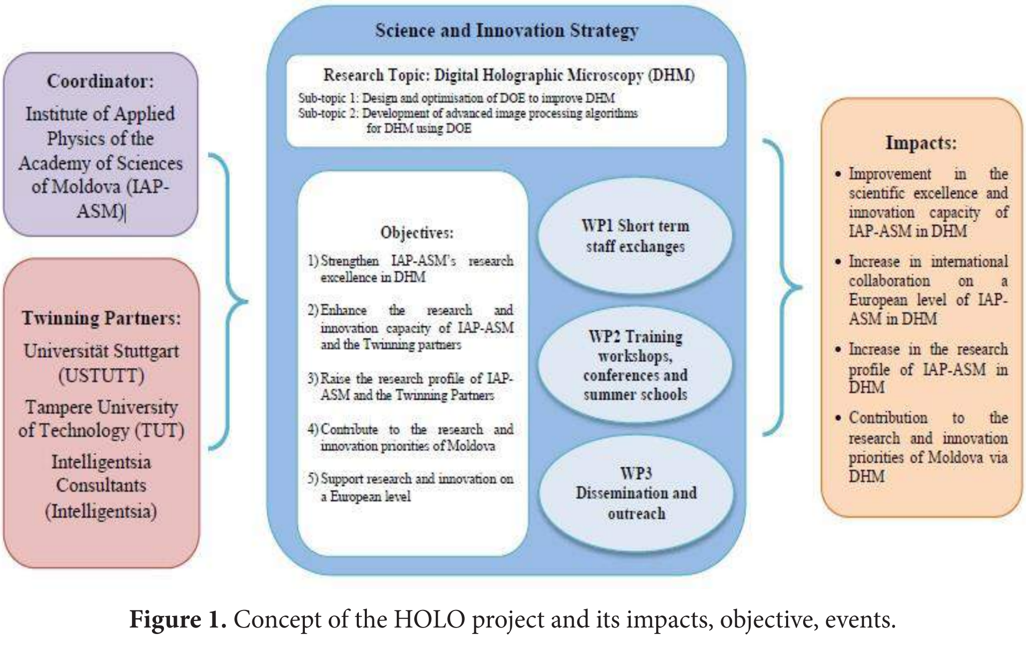

Figure 1. Concept of the HOLO project and its impacts, objective, events.

The objective is to strengthen IAP-ASM's research excellence in digital holographic microscopy (DHM) by focusing efforts on two sub-topics with the support of Twinning partners Universität Stuttgart, Germany and Tampere University of Technology, Finland:

1. Design and optimization of diffractive optical elements (DOE) to improve digital holographic microscopy (DHM);

2. Development of advanced image processing algorithms for digital holographic microscopy (DHM) using diffractive optical elements (DOE).

DHM is digital holography applied to microscopy [1, pp. 398-403]. DHM distinguishes itself from other microscopy methods by recording not only the intensity image of an object, but also the phase of the wavefront coming from the object. The light wave front information originating from the object is digitally recorded as a hologram, from which a computer calculates the object image by using a numerical reconstruction algorithm. Thus, the image forming lens in traditional microscopy is supplemented by a computer algorithm to correct some aberrations. In this way, 3D DHM is a modern concept based on intensive computational data processing.

DHM systems typically comprise of the following features:

- Hardware (holographic optical set-up and video camera for 3D image acquisition) and software algorithms (digital image processing for extraction of desired information about test samples);
- Measurement of surface features (topology, morphology and displacement) and internal surface features (thickness and refractive index) using light wave front information;
- Digital reconstruction of 3D information of the object using optical processing and image analysis.

The use of DHM techniques is well-established in many industrial applications. Furthermore, over the past decade, the techniques have become increasingly applied to other fields - such as biophotonics, life sciences and medicine - since they offer several compelling advantages over other imaging methods:

- Non-destructive imaging,
- Marker-free,
- "Full-field" imaging (no scanning required),
- Quantitative phase recovery (important for imaging transparent cells),
- Numerical refocusing (image focus can be changed without additional scanning),
- Simultaneous online monitoring.

DHM enables the recovery of quantitative phase images from living biological sample dynamics with interferometric accuracy using just a single recorded digital hologram [2, pp. 1-9; 6, pp. 2-24]. The optical phase shift induced by a sample on a transmitted/reflected wavefront can be regarded as a powerful endogenous contrast media (i.e. inherent to the sample) as it contains information about both the thickness and refractive index of the sample [3, pp. 1-7; 4, pp. 456-468].

To extract meaningful information about such biological samples, advanced image processing algorithms can be developed based on advanced Sparse Phase and Amplitude Reconstruction (SPAR) techniques.

IAP-ASM develops state-of-the-art diffractive optical elements (DOE) for DHM to further enhance the image-quality of biological tissue and optoelectronic components [5, pp. 380-386; 7, p. 286]. Conventional optical components – such as lenses, mirrors, and prisms - exploit refraction and reflection. However, DOE utilise diffraction which provides the following advantages:

- Can perform more than one function; for example, have multiple focal points, corresponding to multiple lenses on a single element. Also, can be designed for use with multiple wavelengths,
- Much lighter and occupy less space than refractive and reflective optical elements,
- More easily embedded in different microscope optical set-ups.

The DOE are used together with a phase-shifting interferometer in the DHM. The interferometer helps to eliminate errors from autocorrelation and twin image terms. Lithography and micropatterning techniques are of paramount importance for the implementation of innovative high-precision optical devices. In this respect, IAP-ASM has spatial light modulator (SLM) and scanning electron microscope (SEM) facilities to design and record DOE into photosensitive structures.

By using IAP-ASM's DOE the morphology and integral refractive index of an object is calculated directly from the quantitative phase signal of its diffraction pattern. Furthermore, IAP-ASM's DOE enable the 3D surface and intrinsic microstructure of biological tissue to be analyzed, which otherwise appear transparent to visible light. The positions of diffractive objects can be identified by analyzing the change of diffracted wave according to propagation. Moreover, it is possible to solve the angular spectrum of optical wave by numerical reconstruction in the spatial frequency domain.

As a rule, biological samples possess very complex structures and layer-like morphology, which hinder achieving the theoretical optical resolution. The

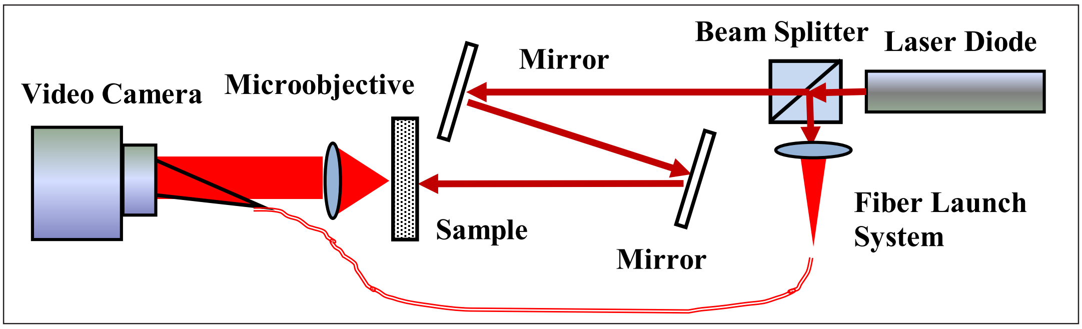

Figure 2. The optical scheme of DHM. Single Mode Fiber as a Reference Beam.

most important obstacle here is the diffraction on biological specimen structure. A compact DHM for the investigation of biological and technical samples is available at IAP-ASM presented in figure 2 [8].

To boost the scientific excellence and innovation capacity in digital holographic microscopy, the HOLO partners implement a science and innovation strategy focused on design of diffractive optical elements (DOE) and development of advanced image processing algorithms. In figure 3 we present the optical setup for recording holograms on multinanolayers from chalcogenide glasses As2S3-Se elaborated by Moldavian team. The Spatial Light Modulator is used as modulating media for recording scene.

Local Least Square method (LLS) based on a sparse representation for both amplitude and phase of the object gives significant reconstruction enhancement from digital holograms recorded in Digital Holographic Microscopy. The main feature of the proposed algorithm is a good ability for noise filtration due to the original formulation of the problem taking into account the presence of noise in the recorded intensity distribution and the sparse phase reconstruction approach with the data-adaptive block-matching 3D technique. Tampere team's Matlab algorithm realization greatly simplifies simulations and experimental data reconstructions for DHM using DOE. The theory and implementation are presented to research staff of IAP-ASM.

IAP-ASM hosted the 8th International Conference on Materials Science and Condensed Matter Physics (13-16 September 2016), a biennial international gathering for academics and research-oriented practitioners in the areas of physics and electrochemistry. The HOLO project was co-organizer of the conference and summer school. The conference was attended by 300 participants from Azerbaijan, Armenia, Belarus, Czech Republic, Finland, France, Germany, Italy, Japan, Latvia, Lithuania, Luxemburg, Netherlands, Poland, Romania, Russia, Spain, Ukraine, Hungary and Moldova.

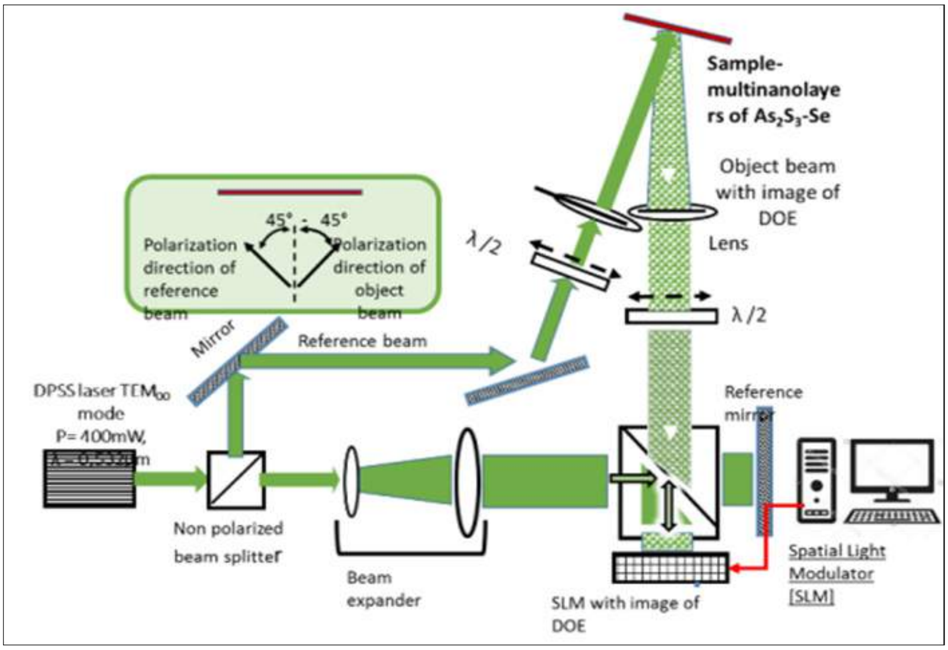

Figure 2. The optical setup for recording holograms.

REFERENCES

1. Achimova E. Development of Digital Holographic Microscope for 3D Sensing of Biological Surface Morphology. In IFBME Proc. 3rd Int. Conf. on Nanotechnologies and Biomedical Engineering, V. Sontea, I. Tiginyanu (Eds.) Vol. 55, © Springer Science+Business Media Singapore 2016.

2. Stronski A., Achimova E. Design of new functional materials based on chalcogenide glasses, polymers via modification and nanocomposite techniques. Sviridov Readings. Iss. 11. Minsk, 2015.

3. Stronski A., Achimova E., Paiuk, O., Meshalkin A., Abashkin V., Lytvyn O., Sergeev S., Prisacar A., Triduh G. Holographic and e-beam image recording in $Ge_5As_{37}S_{58}$–Se nanomultilayer structures. Nanoscale Research Letters, 2016, 11(39).

4. Achimova E. Direct Surface Relief Formation in Nanomultilayers Based on Chalcogenide Glasses: A Review. Surface Engineering and Applied Electrochemistry, 2016, Vol. 52, No. 5, pp. 456–468. © Allerton Press, Inc., 2016.

5. Abaskin V., Achimova E., Meshalkin A., Prisacar A., Triduh G., Vlcek M., Loghina L., Voynarovich I. Investigation of Structural Features of As2S3-Se Multilayer Nanostructure by Raman Spectroscopy. Surface Engineering and Applied Electrochemistry, 2016, Vol. 52, No. 4, pp. 380-386. © Allerton Press, Inc.

6. Achimova E., Stronski A. Functional structures based on chalcogenide glasses obtained via nanocomposite techniques and their applications. Open Access House of Science and Technology (OAHOST), 2017, Vol. 3, No. 28.

7. Stronski A., Achimova E., Paiuk O., Meshalkin A., Prisacar A., Triduh G., Oleksenko P. and Lytvyn P. Direct Magnetic Relief Recording Using $As_{40}S_{60}$: Mn–Se Nanocomposite Multilayer Structures. Nanoscale Research Letters, 2017, Vol. 12.

8. Cazac V., Meshalkin A., Abashkin V., Achimova A., Katkovnik V., Shevkunov I., Claus D. and Pedrini G. Surface relief and refractive index gratings patterned in chalcogenide glasses and studied by off-axis digital holography. Applied Optics, Vol. 57, Iss. 5, 2018.

Ludmila Șevcenco. *Domnișoara cu pasărea și peștele*,
2010, batik, mătase, 110 × 90 cm