Project:

Fast Life Cell Structured Illumination Microscopy (FastFibreSIM)

Technological key words:

Optical fibres and fibre components; Fast Life Cell Structured Illumination Microscopy; Superresolution fluorescence microscopy; structured illumination microscopy (SIM); live cell imaging

Industrial sectors addressed:

high-end microscopy and microscopy systems, 3D fluorescence light imaging, Optical superresolution microscopes, instrumentation for optical instruments

Total project costs:

1.200.000 Euros

Partners' descriptions:

- **Leibniz Institute of Photonic Technology e.V. (IPHT)**, Jena, Germany, public research organization, develops technological solutions for life sciences and medicine. The microscopy department has strong expertise in system and algorithm development for structured illumination microscopy.
  In **FastFibreSIM** IPHT will develop robust image processing algorithms for artefact-free reconstruction of super-resolution structured illumination microscopy images of live, moving sample.
  Web: www.ipht-jena.de

- **Carl Zeiss Microscopy GmbH (ZEISS)**, Jena, Germany, industry. As a world leading manufacturer of both light and electron microscopes, Carl Zeiss manufactures compound light microscopes as well as a diverse range of fluorescence optical sectioning systems, electron and ion microscopes. In **FastFibreSIM** ZEISS will be responsible for the system design of the final pre-commercial demonstrator.
  Web: www.microscopy.zeiss.com

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- **Fibotec Fiberoptics GmbH (FIBOTEC)**, Meiningen, Germany, SME. Fibotec develops and manufactures optronic, and particularly fibre-optical subsystems and furthermore is development partner and private label supplier for complete systems. In **FastFibreSIM,** Fibotec will investigate the illumination system for the SIM microscope.
  Web: www.fibotec.com

- **Cairn Research Ltd (CAIRN),** Kent, UK, SME, is a scientific instruments manufacturer with an established track record and IP in image splitter technology for projecting multi wavelength, polarization or focal plane information onto single or multiple sensors. In **FastFibreSIM** CAIRN will develop new image splitting technology adapted to the requirements for the SIM microscope for simultaneous imaging of several colour channels on a single camera chip.
  Web: www.cairn-research.co.uk

Project abstract:

Fluorescence microscopy is an essential technology for current research in the biological sciences. **Superresolution fluorescence microscopy** in the far field has been developed recently and has opened the door to new biological insights [1]. So far however, when imaging live cells, none of the recent developments could establish itself as a generally accepted standard tool in life sciences in terms of speed or live cell viability. By introducing the Elyra-S platform, Carl Zeiss Microscopy GmbH brought a product to the market, which allows for imaging biological samples, prepared by standard protocols, via structured illumination microscopy (SIM) at a significantly improved resolution.
In this project, a transnational consortium (Germany, UK) aims to improve the imaging speed of the SIM microscopy significantly to allow live cell imaging. This new microscope will enable **life cell imaging** of fast processes under physiological conditions. We will accelerate the imaging speed by the development of a fast and robust fiber optic illumination pattern generator, new SIM algorithms and an integrated image splitter, especially adapted to the microscope. The system is designed to allow use in biological laboratories and

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preclinical environments. It will be sensitive and non-invasive enough to not disturb the biological processes under investigation.

State of the art for 3D live cell superresolution microscopy based on structured illumination is described in Shao et al [2]. However, the system is restricted to only one wavelength within a selected band. This is too restrictive for a generally accepted commercial product.

There is a **market need** for high-resolution microscopy systems, with the capability to image fast enough to enable monitoring specific labeled proteins and organelles at 100nm resolution. Such information yield insights into dynamic processes in the living cell.

[1] Heintzmann R.; Cremer C.: Laterally Modulated Excitation Microscopy: Improvement of resolution by using a diffraction grating; In Proceedings of SPIE, Vol. 3568 (1998)
[2] Shao L.; Kner P.; Hesper E.; Gustafsson Mats G. L: Super-resolution 3D-microscopy of live whole cells using structured illumination; In Nature Methods, Vol. 8 (2011)

Expected results and exploitation plan:

- We will develop new algorithms adapted to visualizing a number of processes in living cells.
- A fiber-based SIM system will be realized, which will be robust enough to achieve superresolution at the demanding environmental conditions of life-cell imaging (25-37°C, 70-100% relative humidity).
- Excitation and emission will be flexible enough to allow the user the acquisition of 2-4 color channels simultaneously using common stains. The wavelength range should cover the usually applicable dyes (400nm-660nm).
- The fiber-based system will be fast and gentle enough to not disturb the biological processes under investigation.
- The applicative relevance will be enhanced by an integrated image splitter with a shutter-based crosstalk-reduction technology to simultaneously image at two or more different wavelengths on the same camera chip. Thereby 2 or more fluorescently labeled proteins and their interaction can be visualized in the living cell.

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