Title	**Fast Volumetric imaging for Live cell nanoscopy: bringing super-resolution to life.**
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State of the art: The diffraction limit that has long restrained the observation of biological systems is being currently outpaced by new approaches combining optics and photophysical control of fluorescent emitters. Super-localization microscopy (dSTORM/PALM/PAINT), by constraining excited fluorophores to emit at different times, enables localization of molecules within 10nm lateral precision. **NanoBio** team proposes novel methods to enrich single molecule imaging, in particular for 3D imaging with new optical concepts (Nat. Photonics 2015,2021[1,2], Nat. Commun. 2019[3]) enabling in-depth localization of molecules in complex samples (organoid, embryos). Part of these developments have been achieved in close collaboration with **Abbelight**, a French SME founded in 2016, which focuses on the development of super-resolution add-on transforming inverted microscopes into nanoscopes.
While single-molecule localization microscopy (SMLM) provides high spatial resolution, the actual challenge is to address live cell imaging where biological events occur on timescales exceeding the temporal resolution of SMLM, leading to blurred or inaccurate image reconstructions. We proposed an interdisciplinary PhD project, where a new optical implementation for excitation/detection of the fluorescence emission will be developed to permit volumetric imaging, along with the implementation of fast deep learning-based processing workflow. The final goal of these developments is to address biological questions, in particular on cancer cell migration into surrounding tissues during metastasis.

Objectives and Methodology: With our synergistic approach, we are aiming at addressing existing challenges in acquisition speed and 3D resolution, to enable observations of live cell processes with nanoscopic precision. Benefiting from the competitive research environment of supervisors, and the training and network provided by LIGHTinPARIS, the PhD candidate will be central for the development of pioneering instrument combining optical and image processing innovations.

- Adapting the nanoscope to live imaging: we propose to further develop single shot volumetric imaging, in particular we will investigate the use of inclined excitation which permits to increase the observed volume without increasing the background which is mandatory for localization process, but also the implementation of alternative detection similar to Oblique Plane Microscopy (OPM)
- Increasing acquisition speed: by combining the volumetric imaging capability of the OPM with deep learning algorithms, both the number of acquired frames and the needed signal to noise ratio will be reduced. Using our bank of images, machine learning methods will be deployed to generate "synthetic frames" and boost the reconstruction of imaged structures, without compromising the desired 3D resolution.
- Applications : after calibration steps on reference samples, the new setup will be applied to key biological questions with close collaborators in biology, to decipher mechanism at the nanoscale such as the role of various proteins in cancer migration.

Complementarity of supervision: The PhD candidate will be embedded in an interdisciplinary and innovative academic and industrial environment. Both supervisors demonstrated an excellent track of research and applicative achievements (publications, patents, funded grants and awards). The collaboration between ISMO and Abbelight has been lately reinforced by the creation of Nanolife, common laboratory dedicated to providing nanoscopic solutions for live cell imaging. The proposed PhD project fully aligns with the framework of existing collaboration and will benefit from expertise in super-resolution fluorescence imaging and computational science. Demonstrating its active commitment to provide solutions with strong economic and societal impacts, Abbelight will ensure exploitation of joint research results, reinforcing its portfolio of products with biomedical applications. Building on its close collaborations with academia, Abbelight has the capability to rapidly translate research innovations into commercial products used worlwide, as exemplified by Spectral demixing (Friedl K et al. *Cell Rep*

Methods. 2023[4]) or ASTER (Mau et al. Nat.Comm 2021[5]) which has been developed during a previous co- supervised PhD, for which Adrien Mau was awarded a Prize from the Doctoral Program.

Ressources: The PhD will be co-hosted by Abbelight and ISMO. **Abbelight** is a leading 3D super-resolution microscopy company providing SAFe Evolutive system for automated sample preparation, imaging tools and Neo SAFe software. With 55 staff, it operates from HQ in the South of Paris for R&D, manufacturing, and quality control. The PhD candidate will be hosted at Abbelight's R&D department composed of 4 sections: photonics, mechanics, computer science (imaging software development and machine learning) and biophysics. The candidate will benefit from dedicated 300m^2 premises enabling access to cutting-edge microscopes (i.e. TIRF, SMLM...), mechanical workshop with 3D printing technology, and to the biological laboratory for sample preparation. The **NanoBio team** is developing patented optical solutions to improve 3D SMLM, such as intrinsic supercritical angle fluorescence or structured excitation for precise in-depth imaging with ModLoc. ISMO will provide access to 100 m^2 lab spaces for optical development, imaging facility, Biosafety Level (BSL) 1 and 2 labs and access to common support resources for electronic and mechanical realizations.

3-i Dimension : Since its creation, Abbelight translates into its commercialized solutions an entire imaging pipeline, from sample preparation kits, through proprietary optical system, to developed image acquisition, and image analysis software. The PhD project proposed together with NanoBio (ISMO) subscribes to this strategy, by providing the candidate with a pattern of complementary interdisciplinary skillset. Moreover, this project arises as natural continuation of fruitful long-lasting collaboration. Joining forces of academic and industrial research and innovation actors enables us to successfully translate groundbreaking research results, published in high impact journals (Nature Photonics, Nature Communications), into cutting-edge commercial solutions, putting us at the forefront of existing and emerging markets in microscopy and nanoscopy. In addition to the LIGHTinPARIS training activities, PhD candidate will benefit from international network of expert collaborators of Abbelight and ISMO. Short-term visits in partners laboratories (e.g. Politecnico di Milano, IGC Lisbon) will be organized to advance his knowledge and develop new skills.

Qualifications: **Valentina Caorsi** is ABBELIGHT's R&D Imaging director, leading a team of 20 professionals in optics, mechanics, and informatics, and coordinating national and international partnership projects (e.g. ANR 3D-SuperES, EIC-Transtion NanoSCAN, MSCA-DN NEXTSCREEN). With a PhD in Physics and >20 years' experience in microscopy, she specialized in advanced fluorescence imaging techniques, project management and product development. Through her intersectoral career (King's College London, Institut Curie, Abbelight), she successfully co-supervised 15 Master, 6 PhD students, and co-supervises 2 ongoing PhD projects (academic-industrial national funding CIFRE, and MSCA-DN). **Sandrine Lévêque-Fort** is CNRS Research Director with a PhD in physics and >20 years' experience in microscopy, advanced fluorescence imaging, FLIM and super resolution. Authored 90 publications and 7 patents. 2020 Irene Joliot-Curie prize winner for microscopy achievement and technology transfer to ABBELIGHT (co-founder and Scientific Advisor), and "Chevalier de l'Ordre National du Mérite" awarded in 2022 by the French president. Leading an interdisciplinary NanoBio team at the intersection of chemistry-physics-biology, she supervised 20 Master, 15 PhD thesis and 3 ongoing, with 2 being awarded by National prize (Chancellerie des universités) and 1 by the EDOM doctoral program. She's currently involved in various funding program in particular EIC RTSuperES, EIC Nanoscan, and she's leading the ERC project TimeNanolive for translation of SMLM towards live cell imaging. Both supervisors will dedicate 10% of their time to the project.

References:

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