Report on the study trip of the PhD program Cutting Edge Microscopy to the Life Imaging Center (LIC) in Freiburg i. B., Germany, 2023

Written by
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The PhD students of the Cutting Edge Microscopy (CEM) PhD program were welcomed at the University of Freiburg by Dr. Roland Nitschke, director of the Life Imaging Center (LIC) and founder of the Microscopy and Image Analysis Platform (MIAP) network. The LIC is a central core facility of the Albert-Ludwigs-University in Freiburg that provides access to numerous microscopes as well as the expertise of experienced scientists. The LIC’s mission is to support research from experimental design and acquisition to data analysis. With a major focus on live cell imaging, the LIC infrastructure offers Biosafety level S2 and S1 environments, several conventional confocal microscopes, a 2-photon confocal microscope, a light sheet microscope, 3D-STED and more. The LIC helps users with image processing and analysis by providing computers loaded with the latest image analysis software. Besides, in collaboration with the MIAP network, Dr. Roland Nitschke and his team regularly organize trainings and events for researchers.

During the visit to their facility, we could learn about and observe different microscopes they regularly use and see novel applications as detailed further down.

One of the specialties of the LIC is the imaging of plants. In conventional microscopes, this has the major limitation that the living samples cannot be placed in their natural orientation, especially when imaging roots or developing sprouts. This comes as a result of the root’s growth being mainly driven by gravity and the body of the plant growing against gravity towards the light. In order to overcome this obstacle, they have developed a vertical LSM Airyscan microscope in the LIC. In this microscope setup, the platform where the sample is placed is vertical and the objective wheel lies horizontally. This new setup allows for imaging life plant samples in their natural orientation among other applications.

Figure 1. Vertical LSM Airyscan microscope.
Dr. Nitschke from the LIC has also participated in the foundation of QUAREP-LiMi, which is an initiative that aims to provide guidance and standardization for quality assessment (QA), quality control (QC), reproducibility, and metadata in light microscopy. QA is the process of evaluating the quality of the data obtained from a microscopy experiment. It involves assessing the accuracy and precision of the data, as well as identifying any sources of error or bias that may affect the results. The goal of QA is to ensure that the data obtained are of high quality and can be used for reliable and reproducible analysis. QC refers to a set of procedures that are used to ensure that the microscope and associated equipment are functioning properly and producing reliable and accurate data. This includes regular calibration, maintenance, and verification of performance, as well as tracking and reporting of any errors or deviations from expected results. Reproducibility is the ability to obtain the same results from an experiment, using the same methodology, equipment, and conditions. It is an essential component of scientific research, as it allows for the validation of findings and the replication of experiments. Metadata refers to the information that describes the microscopy experiments and the data obtained from them. This includes information about the sample, the microscope used, the imaging parameters, and the data processing and analysis procedures. Metadata is critical for ensuring the reproducibility and transparency of microscopy experiments and data analysis. The QUAREP-LiMi initiative aims to provide guidelines and best practices for QA, QC, reproducibility, and metadata in light microscopy, with the goal of improving the quality and reliability of microscopy data and promoting transparency and reproducibility in scientific research.

In another talk, we were shown the possibilities of 2-photon lithography by Dan Son, a chemistry PhD student. This technique, also known as multiphoton direct laser writing, allows for the precise printing of 3D objects with a resolution below 100nm. Part of her work involves creating 3D microstructures on which cells, in her case osteoma cells, can grow. She uses a hydrogel containing monomers that, if enough energy reaches a point in the liquid monomer cube, polymerize by C-H insertion crosslinking. With this method, she was able to produce woodpile-like structures for the cells to grow on. However, the printed structure is relatively soft given its young’s modulus and therefore unfit for implants. When assessing the cell growth on the structures she noticed strong autofluorescence in every channel. This was overcome by immersing the structure in Sudan Black B.

Moreover, we also had the chance to see the systematic research they are conducting on the analysis of nuclear dyes (Hoechst) with the CellDiscoverer 7 from Zeiss. CellDiscoverer 7 is an automated live cell imaging platform that combines both wide-field microscopy and laser scanning microscopy (LSM 900 Airy Scan 2), thereby allowing faster acquisition by high...
framerate with better resolution. In this practical session, cells were stained with different Hoechst dyes (33342, 33258, 34580...) in 6-well cell culture dishes. The wide-field imaging mode grants the ability to navigate and locate the regions of interest not by eyepiece but through a camera. Phase contrast images taken in this setup can be merged with fluorescent signals later. Frame position in tile mode could be automatically transitioned within the same well. Once a region of interest has been identified, the LSM microscope is used by scanning with a water-immersed objective supported by an automatic pumping/washing system. By time-series spectral imaging, the dynamic change of Hoechst dyes over time in the medium was recorded in an automatic fashion. For analysis, smaller frames in tile mode were auto-stitched into a whole image and the fluorescent intensity of each dye was auto-thresholded and auto-fit to their spectral curves. Based on these parameters (signal intensity, spectral curve, exponential decay by time series), this experiment provided a comprehensive comparison of dye properties and dynamic changes in cell medium over time of different nuclear dyes.

In addition to the visit to the LIC, we also joined a tour to explore and enjoy the beautiful city of Freiburg i. B, with its many medieval buildings in the city center. We were lucky enough to visit on a sunny day so that we could experience a town filled with public life. Moreover, our hosts from the LIC did not only show us their facility but also took the time to show us their favorite restaurants. For the warm welcome, the time invested as well as for the fun dinner and lunch full of exchange we would like to cordially thank the LICs team around Dr. Nitschke. Overall, we thoroughly enjoyed the scientific as well as the social part of our study trip and despite some short-term changes in the train schedule, most of us could make it to Freiburg i.B. and back in time.