Correlative Microscopy Summer School

Correlative microscopy techniques have enabled us to unite the biochemistry and physics of complex systems with their spatial localization at varying length scales. Recent developments in sample preparation, labelling probes, instrumentation and image analysis have revealed functional insights of spatio-temporal processes with ultrastructural details. In this summer school, we have planned a series of talks from domain experts that will define the efficacy of the correlative imaging modalities. Followed by, the intensive hands-on workshops that will train the students to apply these cutting edge techniques to investigate the problem of interest. Lastly, we have introduced training sessions for data analysis, image processing, and machine learning tools to derive conclusions from the multiparametric data obtained from different data modalities.

1. Organising Committee (OC)

1.a. Main Contact of OC at EPFL : Prabhu Prasad Swain, Eveline Mayner
1.b. Main Contact of OC at ETH Zürich: Rajshikhar Gupta, Pia Lavriha
1.c. Supporting Prof. at EPFL: Prof. Georg Fantner
1.d. Supporting Prof. at ETH Zürich: Prof. G. V. Shivashankar, Prof. Volodymyr Korkhov
1.e. Application filed at EPFL

2. Target Audience

2.a. Number of participants: approx. 30.
2.b. Number of doctoral candidates and Master students thereof: Depending on applicants
2.c. Who consists of the targeted audience? Doctoral and master students of all institutes of EPFL/ETHZ.
2.d. Credits for the participation of ETH-domain doctoral candidates: 2 ECTS
2.e. Credits for the participation of ETH-domain Master students: 2 ECTS

3. Venue

EPFL Campus

4. Date

July 2023 – tentatively 10.07.23 to 14.07.23

5. Description of the summer school

This summer school aims to train current and incoming graduate students in cutting-edge correlative microscopy techniques. With this experience, students should be able to apply the knowledge in their own present and future projects. On Day 1, the students will be introduced to the program with a brief overview of correlative imaging modalities. Next, on Day 2, we will discuss the techniques combining super-resolution light (fluorescence) microscopy with electron microscopy and organise workshops to give the participants hands-on experience. On the third day, we will focus on correlative X-Ray tomography and visible light techniques, followed by the workshops on post processing X-Ray tomography data. On Day 4, we will introduce the advantages of combining atomic force microscopy with electron microscopy, followed by a demonstration. On Day 5 we will introduce novel tools of image processing, data analysis, data visualization and machine learning followed by a Hackathon.

Day 1 - Introduction

Objectives. An introduction to correlative microscopy techniques including its brief history, its vision and its importance in the scope of research. The participants would be introduced to a variety of complementary techniques along with its advantages and present challenges.

Skills learned. At the end of this first day, we expect participants to have an understanding of critical length scales and key complementary features pertaining to Correlative Microscopy Modalities. This should provide the participants with suffiction background for the upcoming talks that will be oriented towards the specific application, sample preparation, instrumentation and data analysis pertaining to the individual technique.

Day 2 - CLEM

Objectives. Highlight the advantages of combining light microscopy with electron microscopy. Both individual and correlative advantages will be presented, to demonstrate all the experimental steps between sample preparation, image acquisition, data analysis, and presentation.

Skills learned. The participants will learn how to choose from the variety of fluorescence and electron microscopy methods available and design a workflow to answer their specific research question. By the end of the day, they will know how to choose methods to combine for their research as well as important sample preparation considerations.

Workshops. There will be two workshop sessions during the afternoon of Day 2. In the first one, the BIOP team of the EPFL will provide the expertise on light microscopy modalities. In the second workshop, the participants will be introduced to various applications of electron microscope focusing on correlative workflows. There will be examples given of established workflows used extensively in correlative procedures and the participants will get an opportunity to discuss their ideas and application of relevant methods to their research projects.

Day 3 – Correlative Visible Light and X ray imaging methods

Objectives. Recent developments in the field of correlative light and Soft/Hard X-ray imaging techniques. Discuss the domain of individual Soft/Hard imaging modality. Discuss the workflow of sample preparation for correlative visible light and X-ray imaging, along with the specific examples of their application.

Skills learned. The participants will get a chance to interact with the domain's leading experts from the cutting-edge facilities developing Soft/Hard X Ray imaging methods. These interactions will potentiate long term collaborations among ETH Domain institutes and Large-scale research facilities developing cutting edge tools to visualize ultrastructural details of complex biological systems *in-situ*.

Events. There will be one workshop session during the afternoon of Day 3, followed by a meet-up event. During the workshop session, we will provide hand-on training to use the existing developments in CLEM methods for the analysis of Soft-X-Ray imaging data. In the meet-up event, the participants will be encouraged to interact with the domain experts to discuss their problem of interest.

Day 4 - AFM in SEM with LM

Objectives. The goal is to highlight the benefits of combining atomic force microscopy with electron microscopy and light microscopy. Both individual and correlative advantages will be presented, as a showcase for a journey from the instrumentation to combine these modalities, to the image acquisition, data analysis and data visualisation.

Skills learned. The participants will get insights into instrumentation and engineering challenges. They will get a unique representation of combining material properties with imaging in tomographic reconstructions.

Workshops. There will be two workshop sessions during the afternoon of Day 4. In the first one, the LBNI team of the EPFL will provide a demonstration of an AFM working inside an SEM. The experiments will show the image acquisition process and present the participants with various parametric properties that are available when using such a combination. It will be an immersive and valuable experience for the participants to move beyond the imaging aspect of correlative microscopy to the necessary instrumentation. The second workshop will involve imaging with AFM and LM to see cryosections and get correlative reconstructions.

Day 5 – Correlative data analysis and visualisation

Objectives. The goal is to provide the participants with awareness of the challenges faced in the software sector to combine two different methods of microscopy, to introduce them to these challenges, and to demonstrate how to tackle this problem with modern image processing methods.

Skills learned. The participants will get insights into software development and programming challenges. They will get a unique experience of solving problems with the pioneers of image analysis research.

Workshops. There will be a hackathon which will serve as part of the evaluation criteria for the participants. It will be an exciting experience in tackling image reconstruction and correlation challenges.

7. List of attached supporting letters

(Provisionary) supporting letters from:

- Prof. Georg Fantner

- Dr. Arne Seitz

- Prof. GV Shivashankar

- Prof. Dr. Volodymyr Korkhov

- Dr. Marcos Penedo Garcia

- Dr. Gabor Csucs

8. List of Potential Speakers

Day 1 - Introduction

- Arne Seitz, EPFL BIOP facility head, will introduce super-resolution microscopy and its advantages and the benefits of combining it with EM.

- Alexander Myasnikov, Head of the DCI Lausanne will give an introduction to cryoEM facilities in DCI .

- Dr. Volodymyr Korkhov, group leader at PSI and professor at ETHZ, will give an introduction on application of CLEM techniques in *in situ* structural studies.

- Dr. Julia Mahamid, group leader at the Structural and Computational Biology Unit in Heidelberg at European Molecular Biology Laboratory (EMBL) will give a talk on cryo-CLEM to resolve protein complexes in their native environment at atomic resolution.

- Carolyn Larabell, Director of the National Center for X-ray Tomography at University of California San Francisco, A pioneer of Soft-X-Ray microtomography for subcellular components.

- Harald Hess/Jennifer Lippincott-Schwarz, Head at Janelia campus, A pioneer in microscopy They will talk about challenges in correlative workflows.

- Dr. Kay Grünewald, Professor and University of Hamburg, will give a talk on methodological considerations in development of protocols for cryo-CLEM.

Day 2 - (cryo) Correlative Light and Electron Microscopy

- Prof. Dr. Wanda Kukulski, professor at University of Bern, will give an introduction on correlative light and electron microscopy workflows using samples embedded in plastic or vitrified samples.

- Dr. Martin Pilhofer, group leader and professor at ETHZ, will give a talk on using CLEM for targeted data collection in complex samples.

- Dr. Jose Maria Mateos Melero, research associate at the Center for Microscopy and Image Analysis at University of Zurich, is an expert on super-resolution light microscopy techniques and their implementation in correlative workflows, involving transmission and scanning electron microscopy.

Day 3 – Correlative Visible Light and X ray imaging methods

- Dr. Eva Pereiro, Beam line scientist at ALBA Synchrotron, Spain has standardized protocols for correlative Visible Light and Soft-X-Ray tomography techniques. She will give a talk highlighting critical steps during sample preparation for establishing correlative imaging workflows.

- Prof. Dr. Sarah Köster, a group leader of the Cellular Biophysics group at the University of Göttingen, Germany has established sample preparation and imaging workflows for hard X-ray holography, X-ray scanning diffraction and super resolution microscopy to visualize cellular organelles. She will introduce the participants with cutting edge developments in the application of hard X-Ray imaging methods to visualize biological samples.

- Dr. Maria Harkiolaki, principal beamline scientist at Diamond Light Source, UK, has established correlative super-resolution 3D-SIM and soft X-Ray tomography. She will provide the participants with a comprehensive workflow on multimodal image overlaying strategies to colocalize features from the two techniques.

- Dr. Ana Diaz, Senior Scientist at cSAXS beamline at Swiss Light Source at the Paul Scherrer Institut, has contributed towards the implementation of hard X-ray ptychography at the cSAXS beamline, in particular for ptychographic tomography. In her talk she will provide the participants with a brief overview of data acquisition and reconstruction strategies to visualize the data obtained from ptychographic tomography.

Day 4 - AFM with SEM and LM

- Prof. Georg Fantner, the group leader of the LBNI, EPFL, works with challenging instrumentation projects towards method development for different measurement techniques. He is a pioneer in open science projects. He will give a talk on AFM in SEM and LM correlative use for multiparametric data acquisition.

- Dr. Sergei Sekatski, EPFL, has been working on combining AFM with FM and LM. He will give the basic insights into the complementarity of these techniques.

- Dr. Marcos Penedo Garcia, Scientist at EPFL, He works with AFM nanotomography combining AFM with FIB SEM and LM. He will give a talk about novel strategies to facilitate such correlative measurements.

Day 5 - Correlative image analysis

-   Dr. Daniel Sage, EPFL, Head of Software Development at Biomedical Imaging Group, pioneered the methods of deconvolution used in light and electron microscopy. He will talk about adaptive software for combining and getting the most out of the datasets.

- Dr. Perrine Paul-Gilloteaux, CNRS Research Engineer at University of Nantes, works with correlative multimodal image acquisition and analysis. She will talk about challenges in designing software for correlative data acquisition and analysis.

- Correscopy, a company based in Poland, case by case solver of merging correlative datasets in AFM, SEM. This company would give a perspective towards challenges based on my users and how they approach the problem.