



## Practicals

Practical sessions will be run by experts in their respective fields, marine biologists and passionate microscopists. They will be organised as parallel sessions that attendees will be able to choose from, on both April 10 and 11 in the afternoon.

## Practical #1 - From the ocean to the microscope: sample treatment and observation of plankton diversity

Ian Probert, Diana Sarno, Pierre Kostyrka

This practical aims to show how to manipulate, observe and identify plankton samples (phytoplankton and zooplankton). Fresh plankton samples will be processed (filtration, concentration) to fractionate by size and manual manipulation will be presented to isolate single plankton organisms. Discussions will cover the different methods to observe, preserve and identify plankton. In parallel, high throughput quantitative imaging using the [planktoscope](#) will be used on the plankton samples to rapidly image the plankton community followed by automated taxonomic identification (EcoTaxa).

## Practical #2 - High-throughput phenomics: automated microscopy-based strategies to isolate single plankton species from mixed communities

Tina Wiegand, Mike Bonadonna, Francis Smet, Aliaksandr Halavatyi, Rainer Pepperkok.

In this practical we will demonstrate an automated pipeline to identify, mark and isolate single microorganisms from complex mixtures. The pipeline includes (i) imaging samples at high resolution and identifying phenotypes/species by online image analysis; (ii) marking target phenotypes by selectively switching on photoactivatable fluorophores stably interacting with all microorganisms and (iii) isolating the photoactivated cells by using a large particle sorter.

Imaging, phenotype recognition and photoactivation steps are automated by using *Adaptive Feedback Microscopy*, which includes confocal imaging, AI-based phenotype recognition and photoactivation without the need for any online supervision.

Downstream applications include but might not be limited to multi-omics comparison and culturing of isolated species.

## Practical #3 - Sample preparation workflows for cryo EM and correlative volume EM

Benoit Gallet, Paulina Cherek, Giovanna Benvenuto and Paolo Ronchi, Chandni Bhickta

This practical will show how to prepare diverse marine organisms for electron microscopy (EM), making use of state of the art cryo immobilization techniques such as plunge freezing and high pressure freezing. We will further discuss the downstream processing to generate samples that can be imaged by cryo-EM or by room temperature (volume)EM.

## Practical #4 - High-throughput imaging of microbial biodiversity using adaptive feedback microscopy

Sebastien Colin, Aliaksandr Halavatyi, Joanna Zukowska

We will demonstrate the method for automated confocal imaging of a broad diversity of unicellular organisms ranging in size from 5  $\mu\text{m}$  to 200 $\mu\text{m}$ . Cells are fixed and stained for visualising nuclei, membranes, chlorophyll and outer-surface by means of 3D-fluorescence microscopy. An adaptive feedback microscopy will be used, to set up a fully automated imaging pipeline with a high throughput. The workflow includes a low-resolution imaging of the entire preparation, the automated processing of these images for localising and selecting cells of interest, and subsequent automated triggering of slower 3D high-resolution acquisitions at relevant positions.

## Practical #6 - Gotta Catch 'Em all: Quantitative image-enabled cell sorting

Mike Bonadonna, Francis Smet, Flora Vincent, Johan Decelle

With this practical, we aim to showcase the next generation of image-based cell sorting, that tremendously expands our ability to study both model and non-model marine organisms, combining high resolution molecular, morphological, and physiological downstream analyses.

## Practical #8 - Ultrastructure expansion microscopy on plankton

Paul Guichard, Virginie Hamel, Gautam Dey, Hiral Shah, Omayya Dudin, Marine Oliveta

This course will cover the fundamental principles of Ultrastructure Expansion Microscopy (U-ExM). This method uses the isotropic expansion of hydrogel-embedded biological samples, which helps in achieving super-resolution imaging with standard microscopes and increases cellular accessibility to antibodies and dyes. Participants will gain hands-on experience in preparing ExM gels, executing the expansion process, mounting gels, and imaging with confocal microscopy. This practical course aims to provide students with the basic skills and knowledge needed to use U-ExM in studying plankton.

## Practical #9 - Label-free imaging of marine organisms using a mobile optical coherence microscopy platform

Samuel Davis, Robert Prevedel

Optical coherence microscopy is a label-free imaging modality that operates similar to ultrasound but based on light. OCM obtains label-free, cross-sectional images of microstructure in biological systems with high optical resolution by measuring the echo time delay of backscattered light. In this workshop, we aim to showcase a custom built, mobile OCM imaging platform that can be utilised for mapping and screening a variety of samples, such as the morphological diversity found in marine organisms (such as sponges, plankton, etc.) but also other marine sample types. We will work together with the conference organizers to ensure a wide range of different samples to be imaged and showcased on our mobile OCM..

## Practical #5 & 10 - A sea full of voxels

Dieter Lauer, Wolf Heusermann

A.I. algorithms can be trained to recognize and classify various marine organisms and structures in microscopic images, allowing for quicker and more accurate analysis. However, there are still challenges to overcome, such as the need for large amounts of high-quality training data and the access to low- or no-code machine learning tools for scientists in the field.

In this practical we welcome you on board our journey from automated microscopy, over to high resolution 3D image data processing, to how we can leverage A.I. for multi-dimensional image segmentation and analysis. Take a deep breath and dare to train your own DNN.

## Practical #7 and 11 - Flamingo: A compact, shareable light-sheet microscope for imaging live, fixed, and expanded samples.

Mette Handberg-Thorsager

Light sheet microscopy has become the new standard for fast and gentle 3D fluorescence imaging in living specimens. Still today, many scientists struggle to gain access to a powerful light-sheet microscope tailored to their needs. To give more researchers the opportunity to utilize this powerful technology for their research, we developed Flamingo, a custom-built, compact, and shareable light-sheet microscope.

In this hands-on practical, we will introduce the Flamingo microscope and teach you the basics of fluorescence light sheet imaging. We will describe a typical workflow from sample embedding in fluorinated ethylene propylene (FEP) tubes to imaging in the Flamingo, using live, fixed, or expanded samples up to 2 mm long. Among others, we will examine fixed larvae of the worm *Platynereis dumerilii*, a marine model system for the study of evolution and developmental biology.

## Practical #12 - Ocean on a table top - long term scale free tracking microscopy (Gravity machine) in ecological context

Hannah Rosenblatt, Manu Prakash

Most biological systems are stripped away from the ecological context and environment they call home - in a desire to study them in closer detail including sub cellular imaging. Sharing stories from field expeditions (from Arctic to Antarctic), we will first establish the importance of mapping ecological landscapes plankton call home. Next we will share an array of tools including AI driven tracking microscopes that mimic these environments on a table top - bringing the ocean to the lab. We will train participants on modular microscopes that can be re-configured rapidly (Cephla) for a range of experiments from high resolution imaging of freely swimming microbes to sedimentation dynamics of marine snow or open-source high throughput plankton imaging. We will discuss how to optimize environmental parameters and establish a “virtual reality” framework for these experiments - at the scale of a single cell. We will also cover practical tricks for volumetric PIV imaging, data analysis and cell density measurements. Time permitting, the practical course will also cover theory and modeling of cell behavior in context of a vertically stratified ecosystem.