Workshop on Single Molecule-based Super-resolution Microscopy: from Acquisition to Analysis, from Theory to Applications

PROGRAM:

Day 1 (21. Sep)

8:30-8:45 Opening remarks (Auditorium 1, BBB)

8:45-9:15 Lecture: Technical features of Nikon Eclipse Ti2-E (Dominik Frei, Inter Instruments AS, Oslo, Norway, Auditorium 1, BBB)

9:15-9:45 Lecture: Introduction to TIRF and Super-resolution microscopy (Hege Dale, Molecular Imaging Center, UiB, Auditorium 1, BBB)

9:45-10:15 coffee break

10:15-11:30 Lecture: Principles, labeling strategies, and applications of Single-Molecule Localization Microscopy (Hongyu ZHANG, Department of Biomedicine, UiB, Auditorium 4, BBB)

11:30-12:30 Lunch

12:30 -14:30 Hands-on session: Single particle tracking of surface AMPA receptors (Hongyu ZHANG, 6th floor microscope room 6c129bA, BBB)

14:30-15:30 ZOOM lecture: Translation dynamics of single mRNAs in live cells (Robert SINGER, HHMI's Janelia Research Campus)

https://uib.zoom.us/j/67510326276?pwd=bGRqekFBYVRHZVVzUko4OGtnc2Y2dz09

Meeting ID: 675 1032 6276 Password: 7RMsHRWX

15:30-18:00 Analysis of single-particle tracking images (Hongyu ZHANG, 7th floor Seminar room 7A129aY and 7A132aY, BBB)

Objectives of the day:

1. To understand general strategies for SMLM DNA/RNA/protein labeling and applications.

1. To learn how to track surface proteins at the single molecule level using quantum dots (sample preparation, acquisition, and analysis). Here we will use AMPAR tracking as an example. Similar methods can be used to track NMDA receptors, metabotropic glutamate receptors, dopamine receptors, Glycine Receptors, and GABA receptors, and other surface proteins.
2. Advantages: endogenous protein tracking. Disadvantages: low throughput; limited to surface protein tracking.

Day 2 (22. Sep)

8:30-9:00 Summary of knowledge of the previous day and outlook of the day (Hongyu ZHANG, Auditorium 4, BBB)

9:00-10:00 Lecture: Imaging the Accessible Genome at the nanometer scale (James Zhe LIU, HHMI's Janelia Research Campus) (Auditorium 4, BBB)

10:00-10:30 coffee break

10:30-11:30 Lecture: Direct visualization of dynamic molecular exchange at the interface between organelles (Christopher OBARA, HHMI's Janelia Research Campus) (Auditorium 4, BBB)

11:30-12:30 Lunch

12:30-18:00 Joint Hands-on sessions:
Single-particle tracking of Histone H2B (fused with HaloTag) in UO2S cells and analysis
Single-particle tracking of membrane proteins using photoactivation and temporal stability analysis. (James Zhe LIU, Christopher OBARA, Hongyu ZHANG, 6th floor microscope room 6c129bA, BBB)

Objectives of the day:

1. To learn how to track intracellular proteins by genetic fusion of HALO tag to the target protein and synthetic fluorescent ligands.
2. To learn how to track intracellular protein by genetic fusion of a photoswitchable//photoactivatable protein to the target protein.

Here we will use HaloTagged Histone H2B and a variety of membrane-associated proteins that are associated with the nucleus in U2-OS cells, but these methods can be potentially used for diverse intracellular and surface proteins in cell lines or neurons. HaloTag needs to be used in combination with cell-permeable and cell-impermeable fluorescent dyes, similar to SNAP and FIAsH tags. Advantages: high throughput; applicable to diverse intracellular and surface proteins. Disadvantages: Recombinant protein tracking; overexpression may create artifacts.

Day 3 (23. Sep)

8:30-9:00 Summary of knowledge of the previous day and outlook of the day (Hongyu ZHANG, Auditorium 4, BBB)

9:00-11:30 Joint analysis and comparison of Single-particle trajectories in the nucleus and nuclear envelope. (James Zhe LIU, Christopher OBARA, 7th floor Seminar room 7A129aY and 7A132aY, BBB)

11:30-12:30 Lunch

12:30-18:00 Flexible time for imaging/analysis/discussion depending on the needs of participants (James Zhe LIU, Christopher OBARA, Hongyu ZHANG, 7th floor Seminar room 7A129aY and 7A132aY, BBB).

Objectives of the day:

To learn how to analyze single-particle tracking images and make comparisons between the behavior of molecules in the plasma membrane, nucleus, and nuclear-associated membranes. We will pay special attention to ways that known parameters of the system can be used to extract additional resolution by informing the analysis parameters.