TUTORIAL ON STRUCTURED ILLUMINATION MICROSCOPY

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Structured Illumination Microscopy (SIM) improves resolution in all three spatial dimensions (3D) and also has optical sectioning capability [1]. This workshop will give an introduction to the practical application of SIM in biological research. It will cover both theoretical and practical aspects of the technique itself and give advice on how to plan a super-resolution imaging project. We will discuss specimen mounting, how to choose proper acquisition parameters and SIM data reconstruction.

SIM and other super-resolution microscopy techniques are relatively new and still finding their way to useful application in Biological research [2]. Several commercial SIM systems exist: DeltaVision OMX, Zeiss Elyra and Nikon N-SIM. Academic imaging facilities play an important role both in providing access to these instruments but also in providing training and education for users. Commercial SIM microscopes come with their own data reconstruction software. Free, open-source software is also available to complement the commercial programs [3]. Careful examination of reconstructed SIM data is always necessary. Artifacts can be introduced in the reconstructed images if the raw data is too noisy, if there is sample drift or photobleaching in the data set, or if the wrong input parameters are used. We will discuss how to avoid these pitfalls by carefully examining the data.

Classical SIM improves resolution by a factor of two compared to wide-field microscopy. There is no hard limit to the resolution obtainable by SIM if a non-linearity is introduced, but in practice resolution is limited by the signal strength available from the specimen and the noise present [4].

References;

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