Nikon N-SIM-E Demo Sample Preparation

As you are likely aware of, SIM is a technique that projects a grid-like illumination pattern onto the sample. For generating an N-SIM image a series of widefield images are captured while moving and rotating the illumination grid. From this series of images the super-resolution image is calculated or reconstructed. SIM can theoretically improve the resolution by a factor of 2x compared to widefield imaging.

During the demo, we will use a CFI SR Apochromat TIRF 100x oil objective, with a NA of 1.49 and a working distance of 120 µm. The following techniques will be demonstrated:

3D-SIM: features XYZ-resolution improvement and can be used to image at focus depths up to 10 µm from the coverslip. The N-SIM-E system provides illumination with 488, 560 and 640 nm laser lines.

An important aspect of SIM techniques is sample preparation. To achieve the best super-resolution results, the wide-field image quality must be optimal. This imposes some requirements on the samples and sample preparation. If you are willing to use your own samples for training (I will provide bead and test samples), please kindly follow the following suggestions:

- Use 170 um thick high quality glass coverslips
- Clean the coverslips very well before mounting
- Mount the coverslips levelled on the slide using a spacer
- Mount only one sample per microscope slide
- Fresh samples image the best
- Use petroleum ether to clean the objective lens and the coverslip (this is critical)
- Best results are achieved when the refraction index of the mounting medium and the immersion oil match (ideally, use Prolong Diamond).