结构照明显微镜 N-SIM
简要操作指南与注意事项

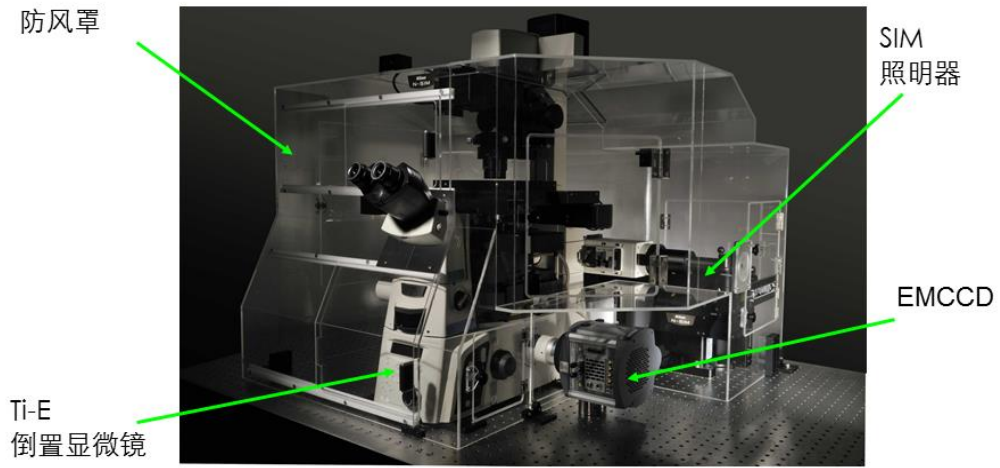**Nikon**

Super-resolution Microscope

N-SIM

Simple Operation Manual

N-SIM系统硬件组成

防风罩

SIM
照明器

EMCCD
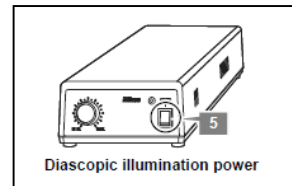
Ti-E
倒置显微镜

5激光底座，NIS-Elements软件

开关机流程

1. 根据观察需要开启长寿命金属卤化物灯控制盒（荧光光源）

   如需要明场观察或拍摄，还需打开白光灯控制盒

2. 根据拍摄需要打开各个激光器：

   <u>488nm/561nm 激光</u>：先打开后方 power 键，Set 值达到 100%后顺时针转动钥匙，Act 值达 100%即可

   <u>405nm/640nm 激光</u>：顺时针转动钥匙，指示灯亮起即可

3. 启动计算机

4. 打开 NIS-Elements AR 软件，选择 ANDOR with N-SIM 驱动

5. 活细胞拍摄时需调节温度到 37 度，打开 CO2 混匀开关，并将气瓶气阀打开到 0.1 刻度。注：固定样品拍摄时请勿更改温控装置温度。

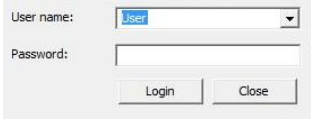关机时反向操作以上步骤即可（4—3—2--1）。注意：应先存储和传输数据，然后退出本人账号，将计算机安全关机。

采图指导
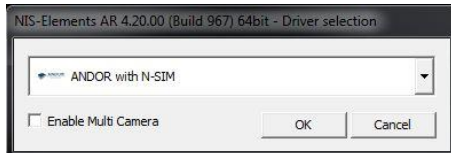
1. 开启计算机和显示器。

2. 根据需要开启所需激光、荧光光源和明场光源。

3. 使用 100X 油镜，滴加适量镜油，盖玻片向下放置样品。

注意：一般不需要更换镜头，更换镜头必须由管理员进行操作。

注：SIM 系统对热漂移敏感，样品放置后需稳定 15min 以上；

如果拍摄活细胞，温控装置温度调节至 37 度，稳定 3hr 以上，拍摄前开启 CO2 Mixture 开关，将 CO2 气阀打开，气压调节至 0.1~0.12。

4. 双击桌面上的 图标，直接点击 login ，选择 驱动选项，启动软件。
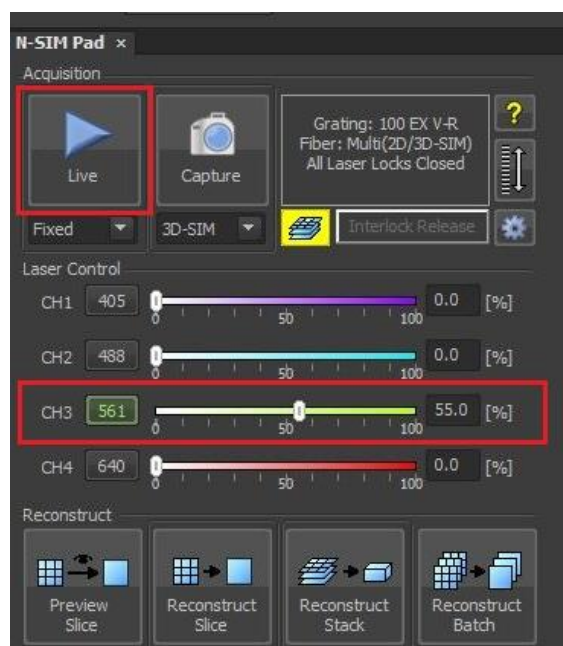
注：右侧为常用控制窗口，请勿关闭或更改位置。

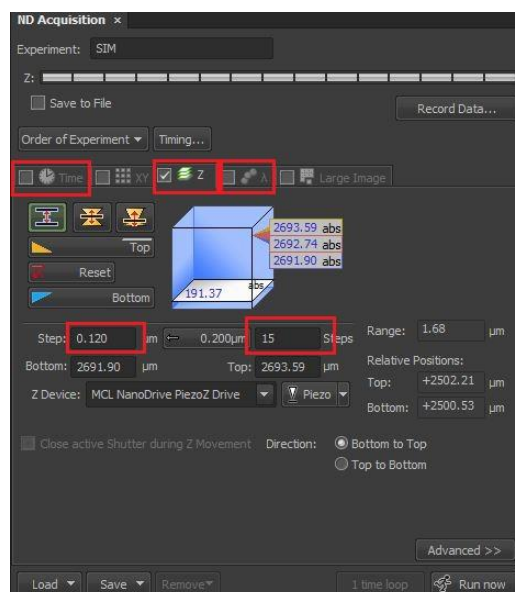**5.** 在预设光路界面（OC Panel），选择目镜明场观察模式或荧光观察模式（见下图），找到合适的焦面和视野。
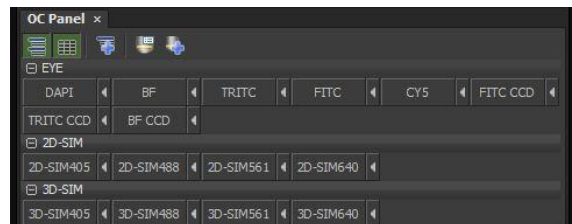
6. 设定拍摄条件：

在预设光路界面（OC Panel）选择特定波长的 2D-SIM 或 3D-SIM 拍摄光路；在 N-SIM 界面设定激光强度，注意激光强度增强易发生荧光漂白；在 DU-897 Settings 界面调节曝光时间；点击 Live 显示预览图像；观察荧光强度直方图（Histogram），调节激光强度和曝光时间使预览图像荧光值在 EMCCD 线性范围内（不超过 12000）。

注：一般原始信号强度为 4000-8000 即可重构出较好图像。

7. 设定拍摄程序：
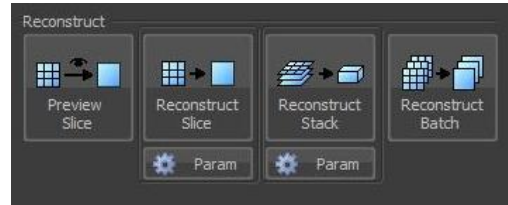
在 ND Acquisition 界面，设置多通道拍摄和多层拍摄范围，根据需要设定时序拍摄，以及多点：

8. 点击 Run now，开始拍摄；

9. 重构 SIM 图像：

点击 Reconstruct Stack，通过 Param 更改重构参数（一般采用默认参数）；

2D-SIM 用 Reconstruct Slice 重构；

符合 Stack 重构的原始数据 z 轴步进和层数如下表：

Number of steps and pitch sizes settable on the N-SIM Z-Stack window (magnification: 100x)

Step / Pitch	7	8 to 15	16 to 31	32 to 63	64 to 127	128 to 255	256 to 511
0.03 μm	No	No	Yes	Yes	Yes	Yes	Yes
0.06 μm	No	Yes	Yes	Yes	Yes	Yes	Yes
0.12 μm	Yes	Yes	Yes	Yes	Yes	Yes	Yes
0.24 μm	Yes	Yes	Yes	Yes	Yes	Yes	No
0.48 μm	Yes	Yes	Yes	Yes	Yes	No	No
0.96 μm	Yes	Yes	Yes	Yes	No	No	No
1.92 μm	Yes	Yes	Yes	No	No	No	No
3.84 μm	Yes	Yes	No	No	No	No	No
7.68 μm	Yes	No	No	No	No	No	No

图像观察与输出

1. 图像观察：

通过调节 LUT (look-up table)来调节图像的对比度和亮度，观察重构后的 SIM 图像，可以在单层观察/三维观察/最大投影等模式直接切换
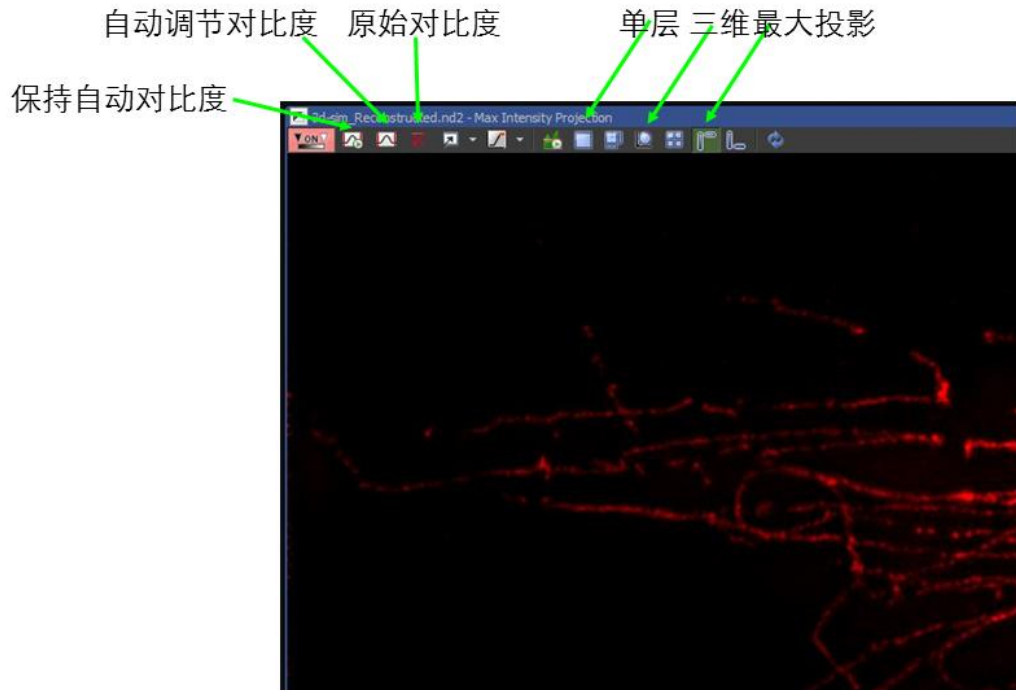
重构后 LUT 调节：

2. 数据导出

输出单层 Tiff 图像时，点击 File-Export-ND to Tiff，输出图像；

输出多层叠加后的最大投影图像时，先点击最大投影 ，点击

，使图像 100%显示；Edit-Create Full View Snapshot（快捷键
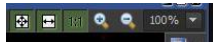
Shift+Z）产生最大投影的截图，将截图存储为 Tiff 格式即可。

3. 数据传输

复制需要拷贝的数据，打开桌面的 FTP 快捷方式，将数据粘贴到 FTP 本实验
室文件夹目录下，传输完成后即可从 126 工作站使用 U 盘/移动硬盘等移动存
储设备拷贝数据。

N-SIM 实验方法参考

Methods for N-SIM:

3D-SIM images were acquired/recorded/performed on an N-SIM imaging system (Nikon) equipped with a 100X/1.49 NA oil-immersion objective (Nikon) and four laser beams (405, 488,561 and 640). Images stacks with 0.12/0.24/0.48 μm interval were acquired and computationally reconstructed to generate super-resolution optical serial sections with two-fold extended resolution in both xy and z directions/in all axes. The reconstructed images were further processed for maximum-intensity projections and/or 3D-rendering with NIS-Elements AR 4.20.00 (Nikon).

可参考文献中的方法描述：

3D-SIM images were recorded using electron-multiplying CCD (charge-coupled device) cameras (Photometrics) on a DeltaVision OMX V3 imaging system (GE Healthcare) equipped with a ×100/1.40 NA oil-immersion objective (Olympus) and three laser beams (405, 488 and 593 nm). Immunofluorescence images of Alexa Fluor 647 were acquired with deconvolution microscopy. To obtain optimal images, immersion oils with refractive indices of 1.512 and 1.520 were used for U2OS cells on glass coverslips and MTECs on Transwell polyester membrane inserts, respectively. The microscope was routinely calibrated with 100 nm fluorescent spheres to calculate both the lateral and axial limits of image resolution. Serial z-stack sectioning was carried out at 125 nm intervals. Images were reconstructed with the softWoRx 5.0 software package (GE Healthcare). The following parameters were set: channel-specific optical transfer functions, Wiener filter 0.001000, discard negative intensity background (post-processing), drift correction with respect to the first channel and custom K0 guess angles for camera positions. The pixel registration was corrected to be less than 1 pixel for all channels using 100 nm TetraSpeck beads (Life Technologies). The reconstructed images were further processed for maximum-intensity projections with softWoRx 5.0. 3D rendering was produced by using Imaris 7.6.0 (Bitplane).

All the antibodies used are listed in Supplementary Table 2.

Wide-field and structured illumination microscopy

Cells were fixed in methanol for 5 min at −20°C. Antibody incubations and washings were performed as described previously (Meraldi et al., 1999). Super-resolution 3D-SIM imaging was performed on a DeltaVision OMX V3 system (Applied Precision) equipped with a 100×/1.40 NA PlanApo oil immersion objective (Olympus), and 405, 488 and 592 nm diode lasers and Cascade II:512 EMCCD cameras (Photometrics). Exciting light was directed through a movable optical grating to generate a fine-striped interference pattern on the sample plane. Emitted fluorescence light was directed through a set of appropriate dichroic beam splitter and emission filter according to its wavelength range onto three cameras. Image stacks of typically 4 µm height with 15 images per plane (5 phases, 3 angles) and a z-distance of 0.125 µm were acquired and computationally reconstructed to generate super-resolution optical serial sections with two-fold extended resolution in all three axes. Color channels were carefully aligned using alignment parameter from control measurements with 0.5 µm diameter multi-spectral fluorescent beads (Life technologies). Images were acquired in the sequential imaging mode to rule out bleed-through (supplementary material Fig. S1C). SI reconstruction and image processing was performed with the SoftWoRx 3.7 imaging software package (Applied Precision).

3D-SIM. NRK cells were plated on glass coverslips for 24 h and starved for 4 h. After immunostaining, 3D-images were acquired on an N-SIM using a ×100 oil-immersion lens (numerical aperture 1.49) by Z-stack scanning at 0.2 or 0.4 µm intervals. Laser lines at 488 and 561 nm were used for excitation using Nikon's N-SIM microscopy system, which can achieve a resolution of 100 nm along the x–y axis and 300 nm along the z axis. The instrument resolution is verified by 100 nm fluorescent spheres according to the Nikon procedure after installation.

使用注意事项

1.本机实行先预约后使用，多次迟到或爽约将被取消预约权限。

2.网上预约机时必须使用自己的账户，不得擅自使用他人账户预约

按照预约时间使用仪器，并如实登记实际使用时间。

4．在管理员工作时段使用仪器的用户，在实验结束后必须通知管理

员；凡在管理员非工作时间段使用仪器的用户，在实验结束后必须

与下一个用户进行交接后方可离开。如确定下一个用户不能按时

使用或取消实验，则需按照仪器操作规定清理并关闭相关设备。

注：如与下一用户使用时间间隔半小时以上，需关闭荧光光源和激

光器。

4.每天最后一个使用仪器的用户需按照规定关闭仪器及照明，按照仪

器管理员的要求完成仪器整理工作，如擦拭镜头等，并锁好门窗。

5.用户在使用仪器过程中，应遵守中心有关规定并听从仪器管理员的

安排，尊重管理员的工作。

6.数据必须保存在指定位置，文件夹名称需标明日期，不得随意保存。

7.实验数据及时备份，中心仅负责保留一个月。

8.数据使用 FTP 传输后，到 126 房间使用 U 盘或移动硬盘进行拷贝。

9.未经同意请勿私自使用用来采集实验数据的计算机的 USB 接口，以

免计算机感染病毒影响他人使用。