

• 基本操作(Basic Operation)

◦ 硬件(Hardware)

• 开机流程(Booting Process)

◦ 开机顺序(Booting Sequence)

- 首先打开设备电源，然后打开电脑，在听到设备滴声后打开采集软件，在温度稳定后开始采集
 - Firstly, turn on the power of the device, then turn on the computer, open the acquisition software after hearing the device drops, and start the acquisition after the temperature stabilizes

◦ 样本操作(Sample Operation)

- 样本载具选择(Sample carrier options)
 - 共聚焦支持多孔板、玻片、小皿等多种载具。需要注意的是，这些耗材都需要是共聚焦专用的，在物理特征上，首先他们的底部厚度需要低于17µm，其次需要是玻璃材质或者相似折射率材料。

Our confocal is compatible with a wide range of carriers, including multiwell plates, slides, and dishes. It is essential to note
 - that all of these consumables must be confocal-specific. In terms of physical characteristics, they must be less than 17 µm thick at the bottom and made of glass or a similar refractive index material.
- 适配器安装(Adapter Mounting)
 - 我们提供两种适配器，一种兼容各种类型的多孔板，另一种兼容玻片和共聚焦皿
 - We offer two types of adapters, one compatible with various types of multiwell plates and the other compatible with slides and confocal dishes.
- 样本移动(Move the sample)
 - 样本的移动，指的是它在坐标轴内进行X、Y、Z方向的移动。这里我们可以使用电动摇杆，也可以在软件上进行操作。额外需要掌握物镜的复位、不同步径的调节

The movement of the specimen refers to its movement within the axes in the X, Y and Z directions. Here we can use a
 - motorized joystick or we can control it through the software. In addition, the user must be able to perform operations such as resetting the objective lens, adjusting the unsynchronized diameter, etc.

• 关机流程(Shutdown Process)

◦ 样本操作(Sample Operation)

- 取走样本，并避免样品溅撒到设备表面。注意清洁。
 - Remove the sample and avoid spilling liquids on the surface of the device. Pay attention to cleanliness.

◦ 物镜(Object)

- 如果使用过油镜，关机前不要忘记擦拭
 - If you have used an oiled object, don't forget to wipe the objective lens before turning off the machine.

◦ 关机顺序(Shutdown Order)

- 关机的顺序和开机相反。取走样本后，先关闭软件，等待桌面软件Logo消失后，再关闭设备电源。
 - The order of shutting down the instrument is reversed from the order of turning on the instrument. After removing the sample, close the software first and wait for the desktop software logo to disappear before turning off the instrument.

◦ 软件(Fusion Software)

• 流程界面(Protocol Designer)

◦ 设定(Preferences)

- 图像采集原始文件保存路径的修改、查询(Modification and querying of the saving path of the original file for image acquisition)
 - 修改保存路径，在拍摄前先建立自己的专属文件夹。同时做好命名习惯，能方便找到自己的数据
 - Modify the save path and create your own dedicated folder before recording. Make sure you use a good naming convention so you can easily find your data.
- 文件后缀的选择(Selection of file suffix)
 - 后缀有通道名、日期、流程名、时间名、用户名可选。
 - The suffix has a channel name, date, process name, time name, and user name to choose from.
- 流程与组别的命名(Naming of Processes and Groups)
 - 流程名通常写上机者姓名缩写，方法名通常写改组别名字
 - The process name is usually written with the operator's initials, and the method name is usually written with the reorganization name.

◦ 通道(Channels)

- 通道的添加与删除(Adding and Removing Channels)
 - 在实验前确定自己的荧光通道，即激发光与发射光区间。选择合适的通道和光源
 - Determine your fluorescence channel, i.e., the excitation and emission light intervals, prior to the experiment. Choose the appropriate channel and light source
- 通道顺序的调整(Adjustment of the channel sequence)
 - 根据实际情况，选择荧光通道拍摄的顺序
 - Select the order in which the fluorescence channels are captured according to the actual situation.

◦ 流程(Protocol)

- 时间序列(Time series)
 - 时间序列成像通常与活细胞成像相结合，我们的共聚焦借助于活细胞工作站设备，可用于毒理学、药理学、发育学等方向的研究分析。
 - Time series imaging is usually combined with live cell imaging, and our confocal with the help of live cell workstation equipment can be used for research and analysis in the direction of toxicology, pharmacology, development and so on.
- 层扫(Z Scan)
 - 掌握两种层扫的设定方法。挑选层扫合适的步径。
 - Master the two ways to set up a Z scan. Select the appropriate step size for the Z scan.
- 多点点采集(Multi-position)
- 扫片(Montage)
 - 矩阵(Tiles)
 - 矩阵扫片前是无法预览最终拼图的大小的，但是操作简便
 - There is no way to preview the size of the final puzzle before Montage-Tiles, but it is easy to do.
 - 围栏(perimeter)
 - 围栏扫片，通过几个点可以大致确定扫片的形状，且摆脱了矩阵拼图长和宽的上限
 - Montage-Perimeter, where the shape of the sweep can be roughly determined by a few points, and get rid of the upper limit on the length and width of Montage-Tiles
- 图像处理(Image Processing Options)
 - 超分辨(Super Resolution)
 - 超分辨成像在提高分辨率的同时，耗时也显著增加，所以通常用于亚细胞器的单张图像采集
 - Super-resolution imaging is significantly more time-consuming while increasing resolution, so it is usually used for single image acquisition of subcellular organelles
 - 拼图(Stiching)
 - 掌握在流程中设置自动拼图，以及掌握手动拼图
 - Proficiency in setting up automated stitching in the Protocol, as well as proficiency in manual stitching
 - 反卷积(Deconvolution)
 - 同上
 - As above
- 流程执行(Acquisition Protocol)
 - 在执行前最好检查一下流程是否有问题。如果拍摄过程出现问题，掌握如何暂停或者取消拍摄
 - It's a good idea to check if there are any problems with the process before executing it. Master how to pause or cancel the shoot if there is a problem with the process

• 参数界面(parameters)

◦ 实时影像(Live)

- 在实时影像打开的阶段是无法预览过去拍摄的图像的，此外打开机器盖子的时候，建议提前关闭实时影像。最后实时影像建议不要长时间打开。
 - It is not possible to preview images taken in the past while the live image is open, and it is recommended to close the live image before opening the machine cover. Finally, it is not recommended to leave the live image on for a long period of time.

◦ 物镜选择(Selection of Objective Lenses)

- 不同的物镜，由于其放大倍数、工作距离、数值孔径的不同，适用于不同应用场景。选择最佳的物镜或者组合。
 - Different lenses have different magnifications, working distances and numerical apertures for different applications. Choose the best lens or lens combination.

◦ 光源参数(Parameters of light source)

- 激光强度(Laser)
 - 由于转盘的技术优越性，激光强度从1%-100%都是推荐值。具体根据荧光实际强度去调整
 - Due to the technical superiority of the turntable, laser intensity from 1%-100% are recommended values. The actual intensity of the fluorescence is adjusted according to the actual intensity of the fluorescence.
- 曝光时间(Exposure)
 - 曝光时间在低倍镜下建议100ms以内。在高倍镜下，如果荧光太弱可以适当倍比延长。如果曝光太长，则会极大拉长拍摄总时间。因此同上，根据样本实际荧光强度，主观判断最优值。

Exposure time is recommended to be within 100ms at low magnification. At high magnification, if the fluorescence is too
 - weak, it can be extended accordingly. If the exposure time is too long, the total acquisition time will be greatly prolonged. Therefore, as above, the optimal value is subjectively judged according to the actual fluorescence intensity of the specimen.

◦ 坐标参数(Coordinate parameters)

- XY移动(Navigation)
 - 学会摇杆以及软件进行XY移动的方法。同时学会切换XY和Z轴的控制面板。
 - Learn how to make XY movements with the joystick as well as the software. Also learn to switch the control panel for XY and Z axes.
- 对焦(Focus)
 - 自动对焦(AutoFocus)
 - 自动对焦适用于除1号和5号位的物镜。
 - Find Coverslip applies to objective lenses except for positions 1 and 5.
 - 手动找焦(Manual Focus)
 - 手动找焦的技巧，通常我们切换到EPI或者明场光源进行粗调，然后再切回共聚焦光源进行细调。
 - In the case of manual focus finding techniques, it is typical to switch to an EPI or bright field light source for the initial coarse adjustments, before switching back to a confocal light source for the finer adjustments.

◦ 快照(Snapshot)

- 单通道快照(current channel snap)
- 多通道快照(protocol channel snap)
- 单通道扫片快照(specimen overview)

• 成像结果的基本操作(Basic operation of the imaging results)

◦ 调整对比度(Adjusting Contrast)

- 自动对比度(Auto Contrast)
 - 在找焦的时候，自动对比度建议关闭。否则很难感知到图像明暗变化
 - It is advisable to disable the Auto Contrast function when attempting to identify the focal point of an image. Otherwise, it is challenging to discern alterations in the image's luminosity and darkness.
- 手动对比度(Display using Logarithmic scale)
 - 最大值 (Max Value)
 - 相当于增减明暗中间的级数。Max值越大，明暗分级越多，而样本相对整体图像则会越暗。

This is equivalent to an increase or decrease in the number of intermediate levels of light and dark. A larger Max value
 - results in a greater gradation of light and dark, which in turn produces a darker sample in comparison to the overall image.
 - 最小值 (Min Value)
 - 相当于调节阈值功能，把低于该数值的背景去除。该数字越大，背景越黑。

This function is analogous to the Adjustment Threshold function, which eliminates the background below a specified value. As the value increases, the background becomes progressively darker.
 - 伽马值 (Gamma Value)
 - 对图像明暗区域的线性调节
 - Linear adjustment of dark and light areas of an image

◦ 多维展示(Multi-dimensional display)

- 普通(Normal)
 - 普通模式下图像是2D的，可以选择多通道Merge或者单通道。可以选择特定XYZT下的某一个视野
 - The image is 2D in Normal Mode, and you can select Multi-Channel Merge or Single Channel. You can select a certain field of view under a specific XYZT
- 2D投影(2D Projection)
 - 通过叠加强压缩，将层扫的图片压缩成一张，可以有效解决样本不平或者荧光太弱带来的困难
 - The difficulties caused by uneven samples or too weak fluorescence can be effectively solved by overlay compression, which compresses the layer-sweeping images into a single one
- 通道拆分(Channels)
 - 该功能适用于展示或者比较
 - This function is suitable for displaying or comparing
- 通道拆分+2D投影(2D Projection&Channels)
 - 同上
 - as above
- 3D普通模式(3D Normal)
 - 通过层扫得到的三维立体图像，层扫步径越小则图像越平滑清晰
 - 3D stereo image obtained by layer scanning, the smaller the layer scanning step, the smoother and clearer the image.
- 3D渲染模式(Blend)
 - 渲染后视觉效果更酷炫的3D图像
 - Rendered 3D images with cooler visual effects

◦ 数据(Raw Data)

- 保存(Save)
 - 数据的保存可以通过Fusion保存，也可以通过Imaris保存
 - Data can be saved either through Fusion or through Imaris
- 格式转换(ImarisFileConverter)
 - 我们可以通过Imaris将其它显微镜格式的数据结果转换成IMS进行分析，也可以反其道行之
 - We can use Imaris to convert data results from other microscope formats into IMS for analysis, or we can do it the other way around!
- 分析(analysis)
 - Imaris软件的学习和使用
 - Learning and using Imaris software