



如何利用共聚焦显微镜拍出自己想要的图片

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Zeiss LSM 880 with Airyscan

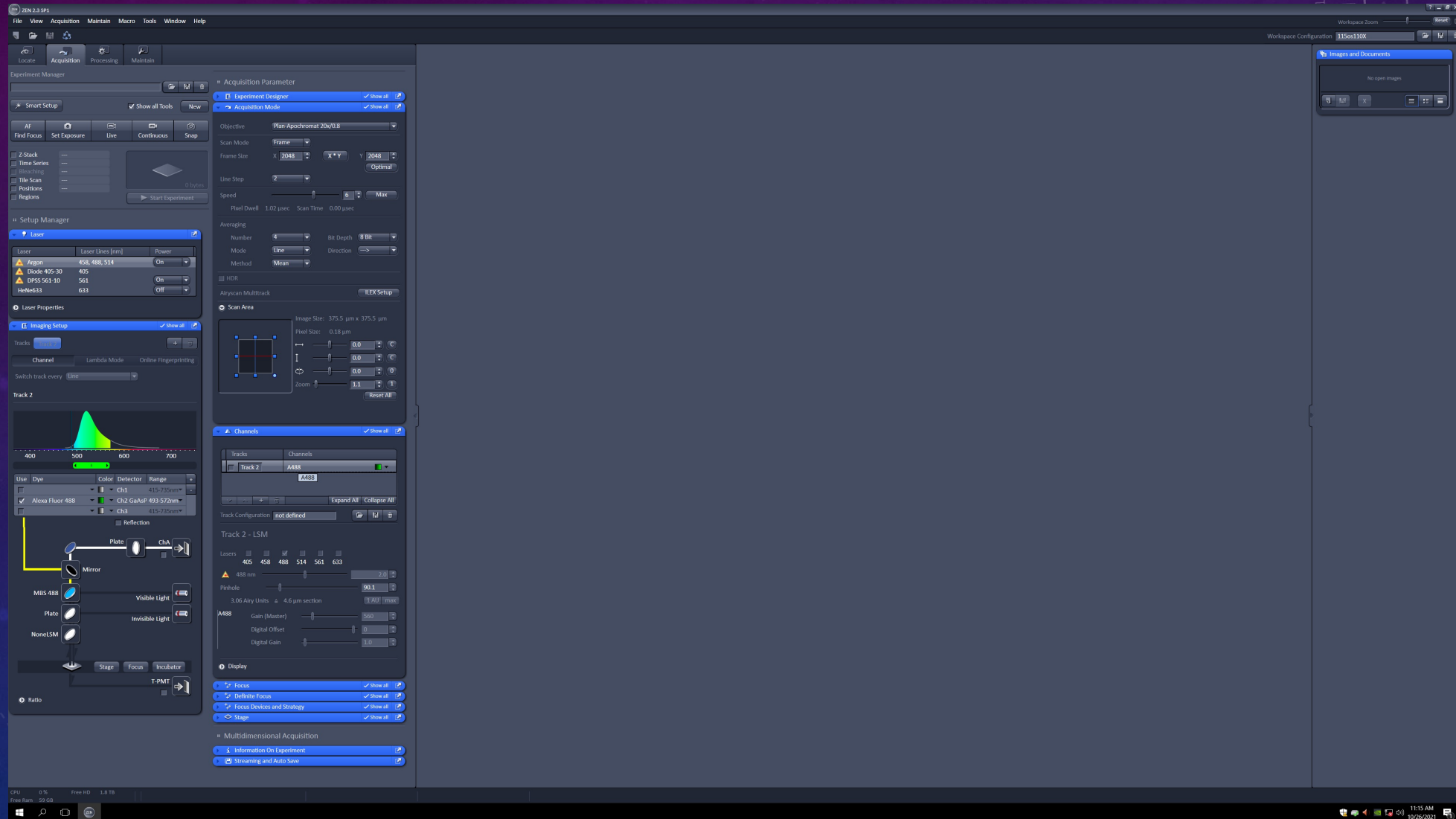


激光共聚焦显微镜的设置



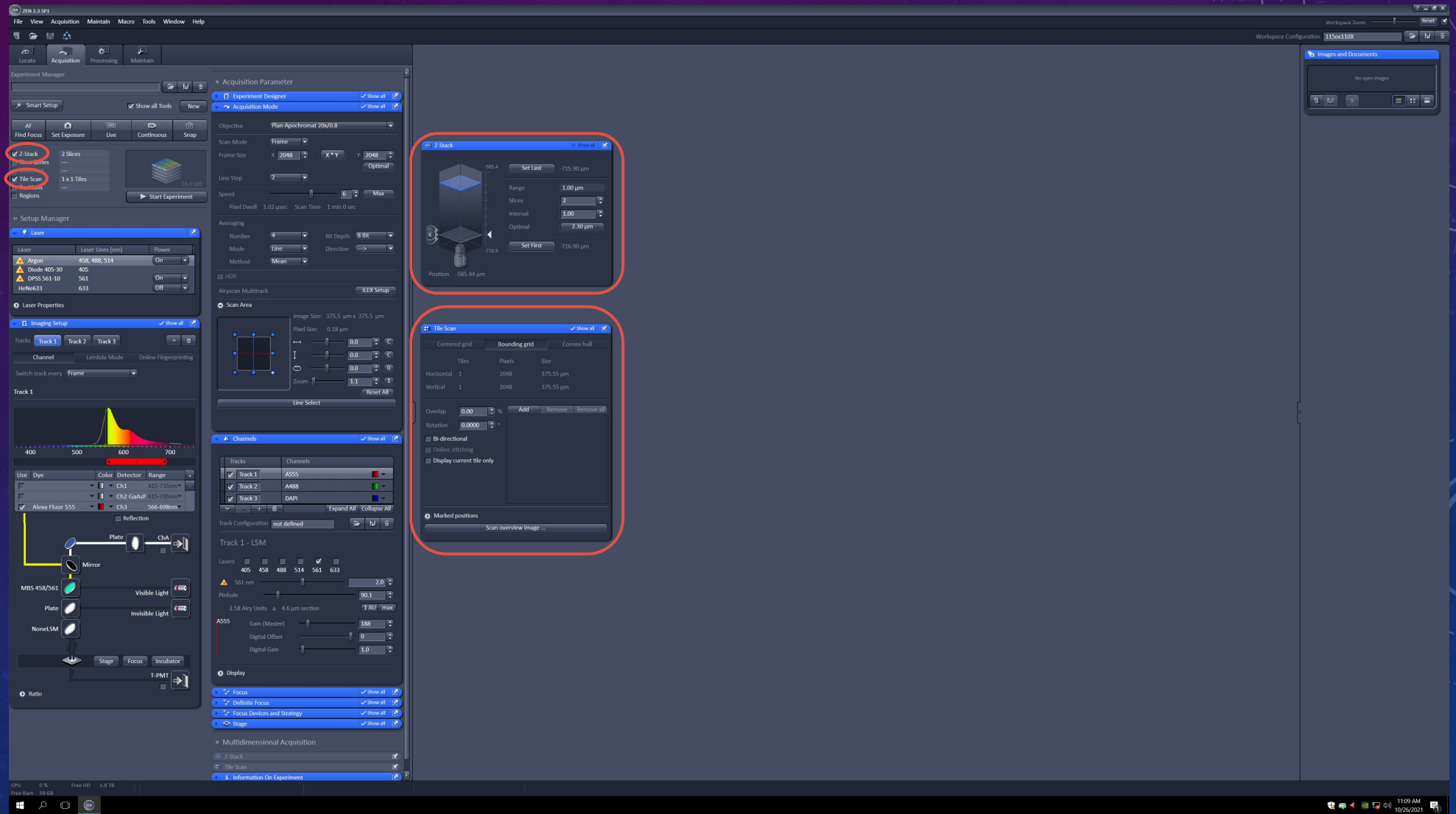
免疫荧光切片的制备

Zeiss LSM 880 with Airyscan

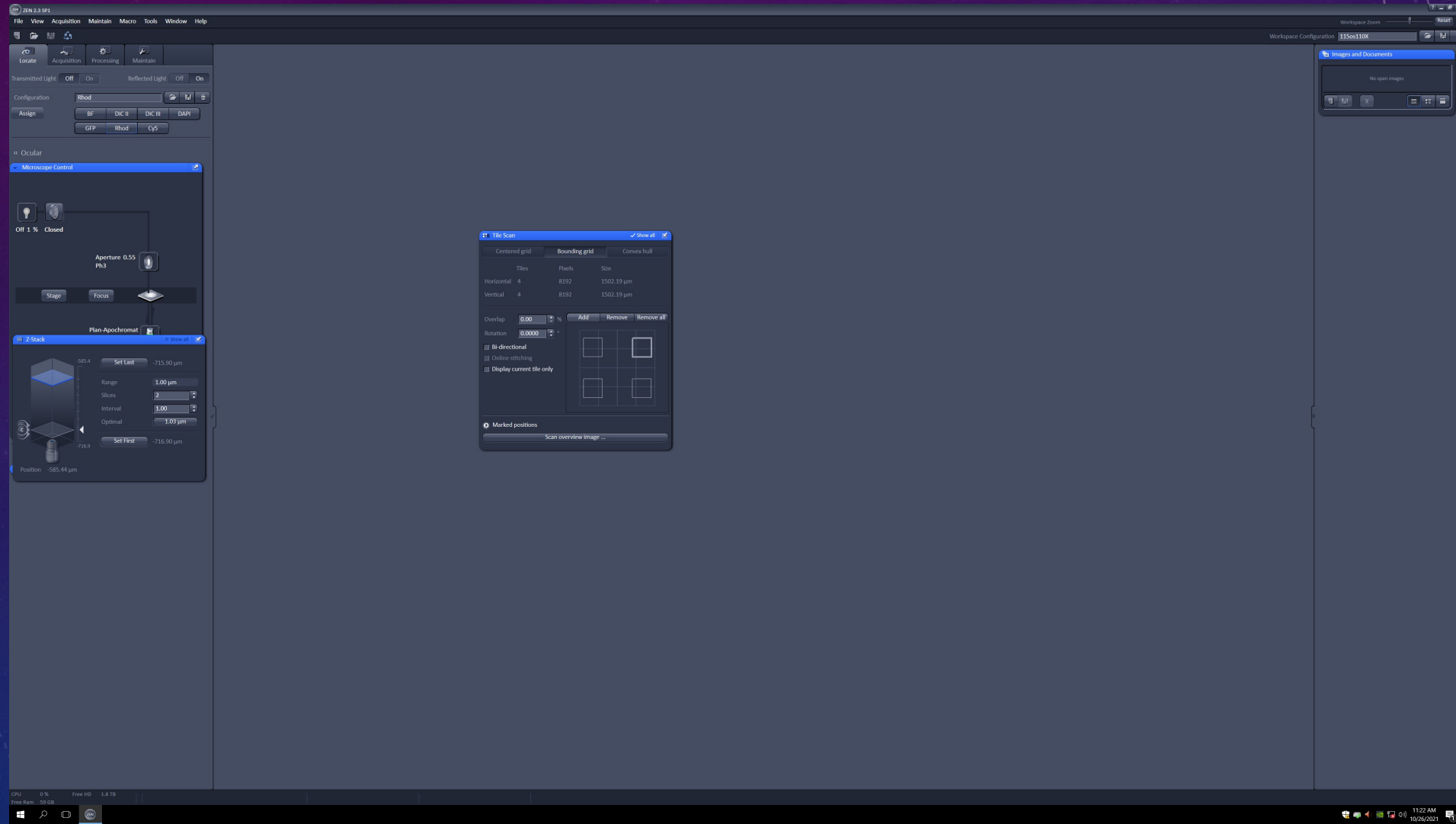


Zeiss LSM 880 with Airyscan

- ☒ Z-Stack
- ☐ Time Series
- ☐ Bleaching
- ☒ Tile Scan
- ☐ Positions
- ☐ Regions



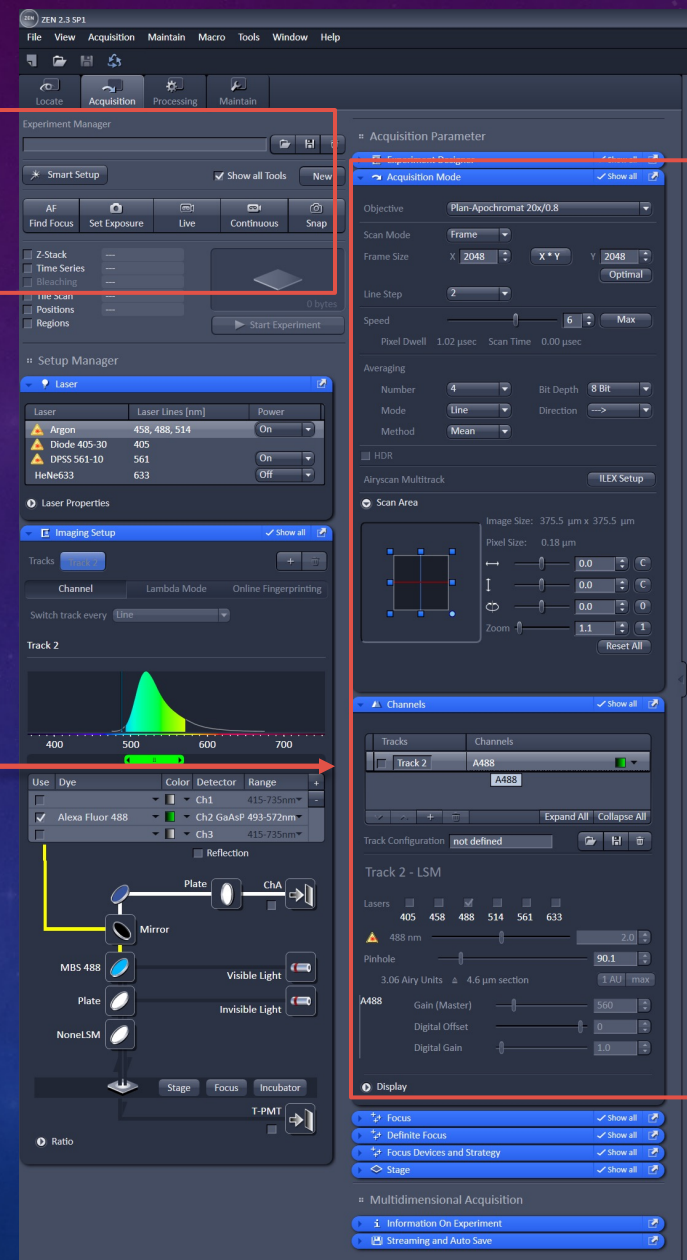
Zeiss LSM 880 with Airyscan



Zeiss LSM 880 with Airyscan

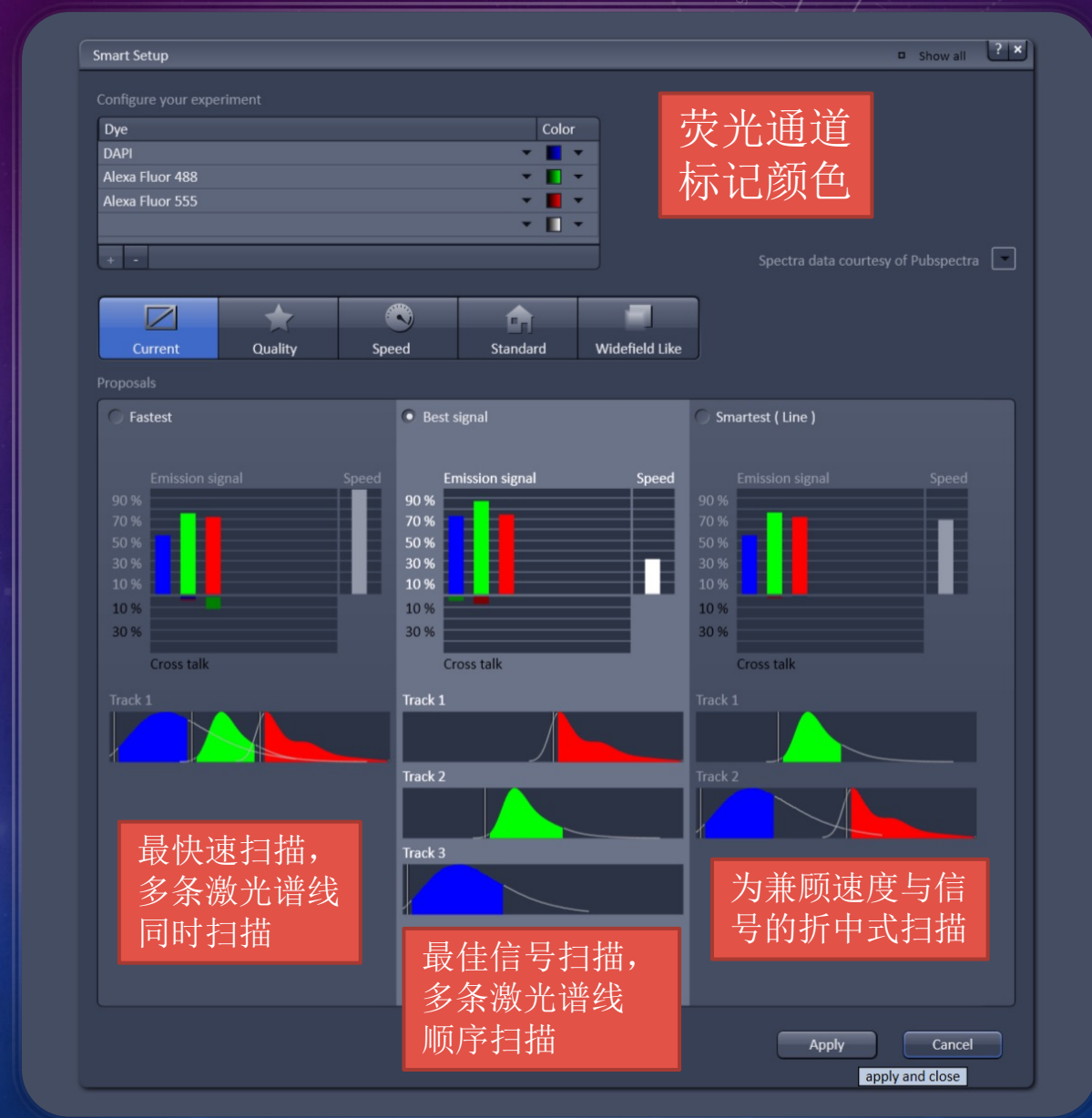
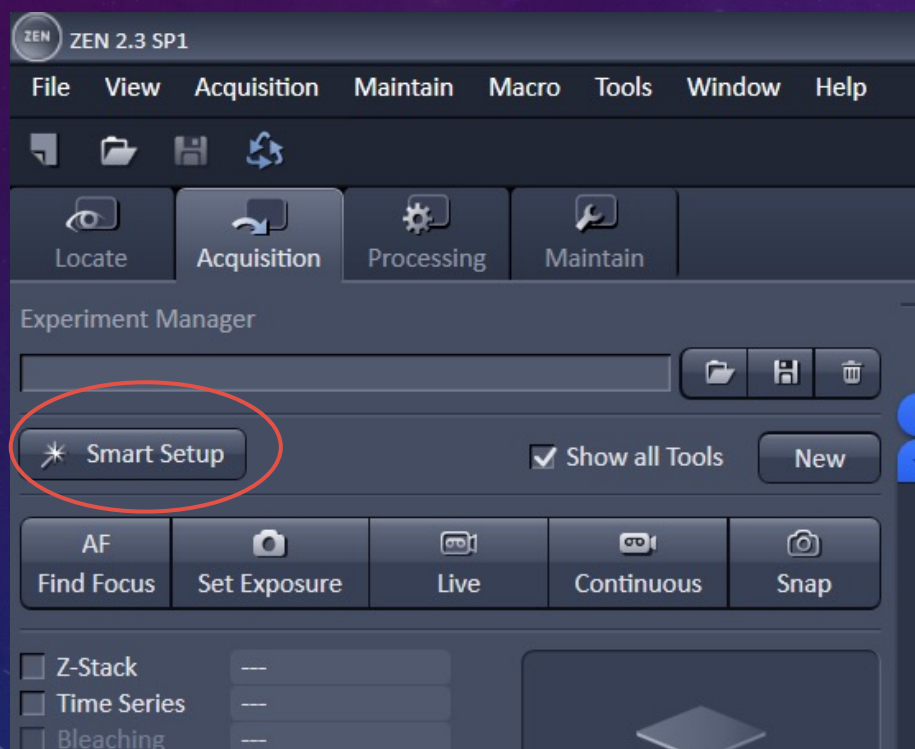
光路的设置

图像扫描参数设置



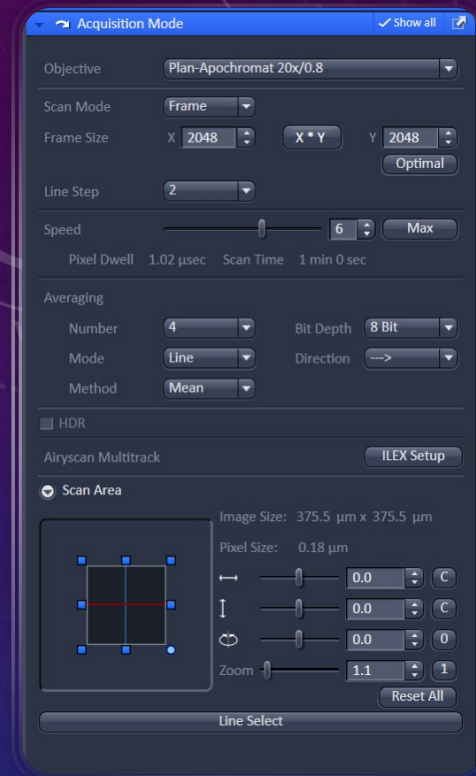
Zeiss LSM 880 with Airyscan

光路设置: Fastest、Best signal、Best compromise

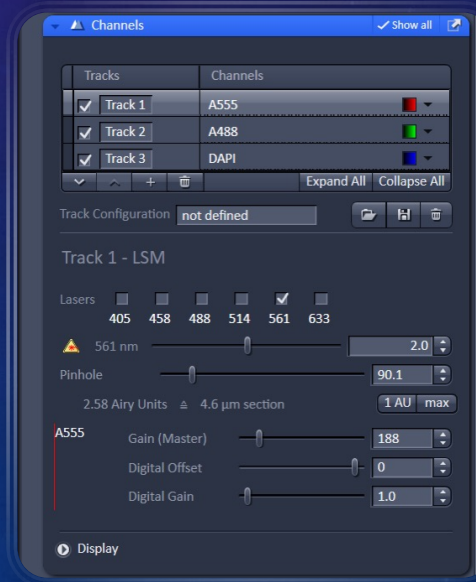


ZEISS LSM 880 WITH AIRYSCAN

图像扫描参数设置



图像采集模式



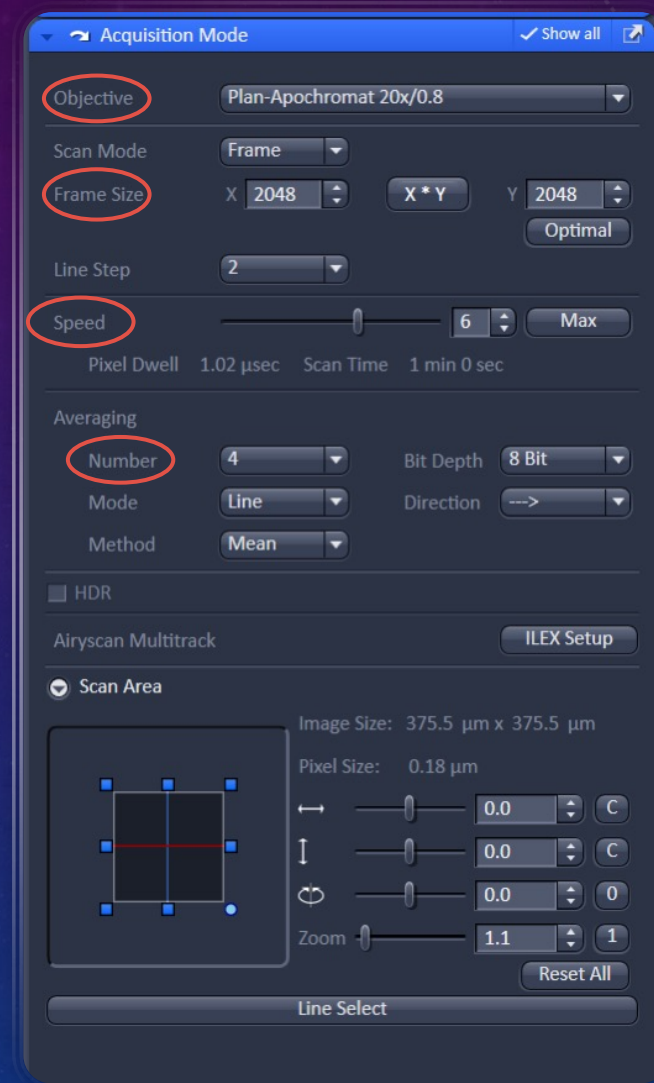
每个通道精细调节

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扫描图像参数设置

图像采集模式

- Objective
- Size
- Speed
- Number

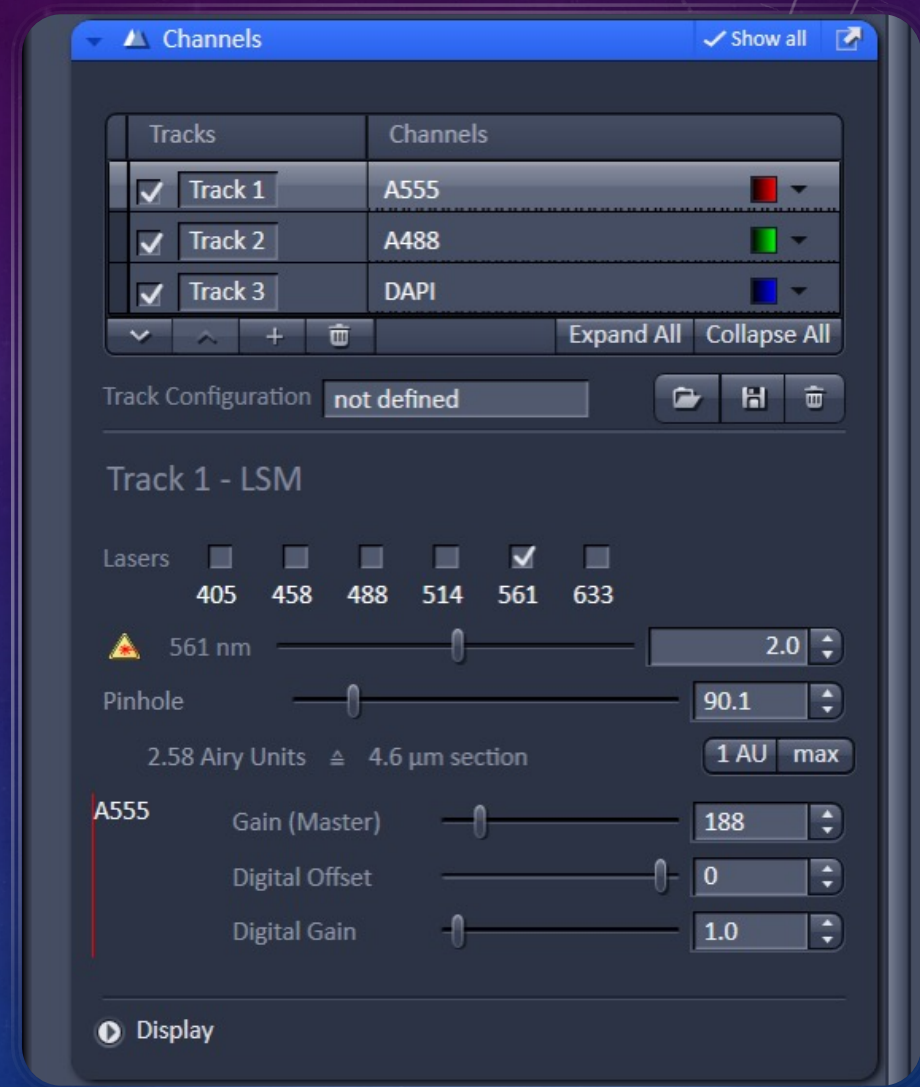


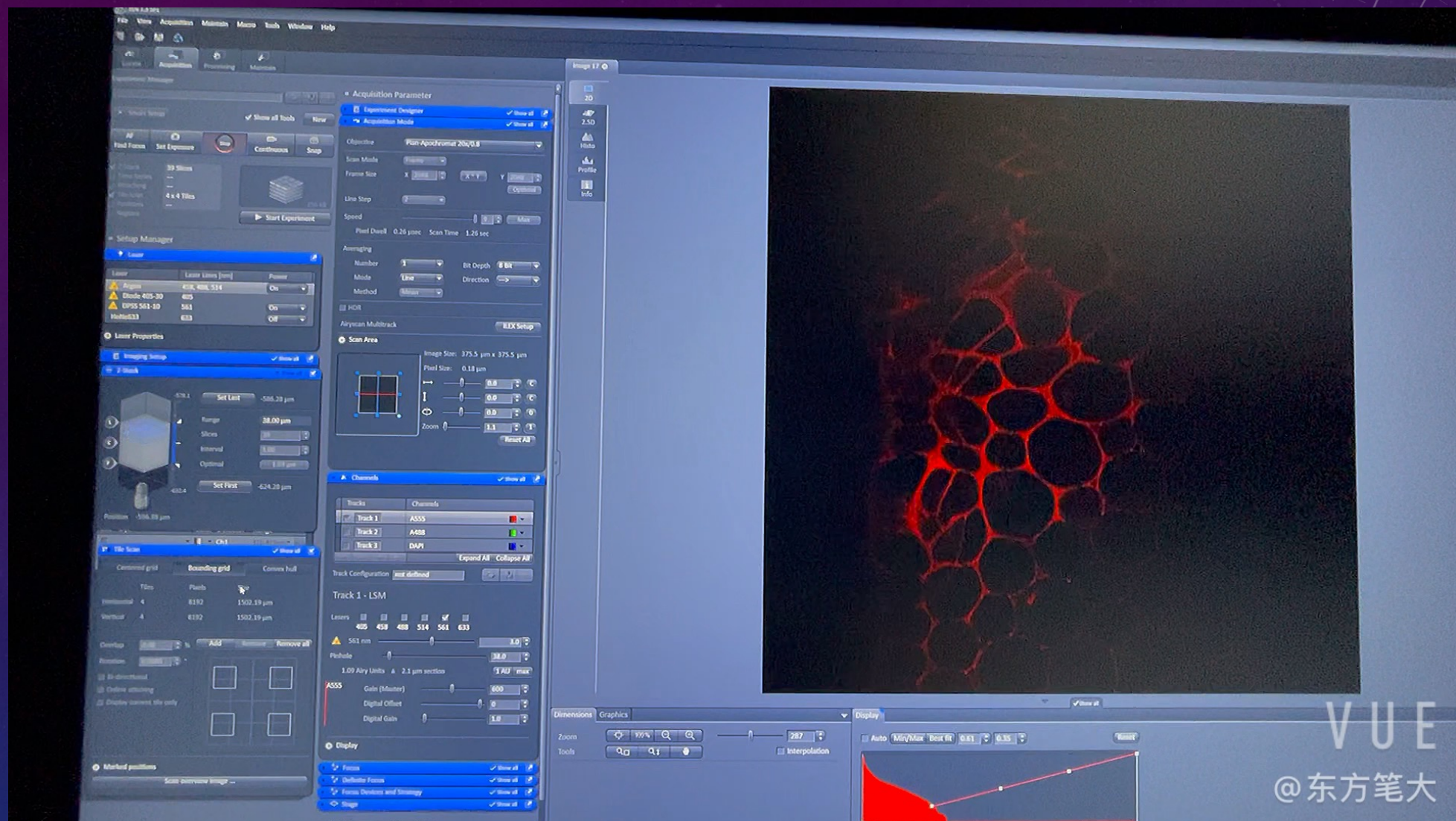
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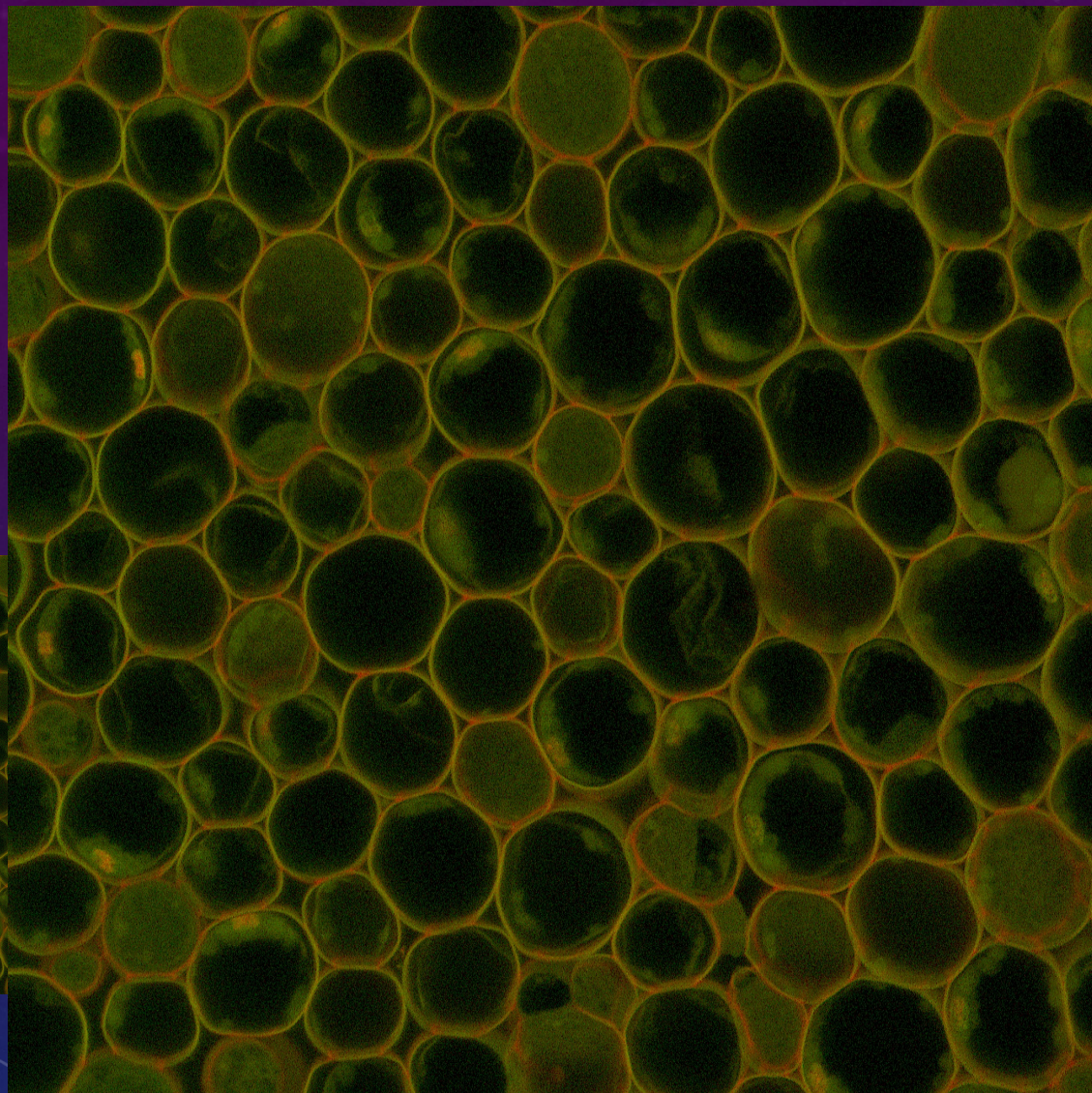
图像扫描参数设置

每个通道精细调节

- 激光强度：强度高信号强噪点高易淬灭
- Pinhole：越接近1AU越好
- Master Gain：Gain越小越好
- Digital Offset：越接近0越好
- Digital Gain：1.0~1.2

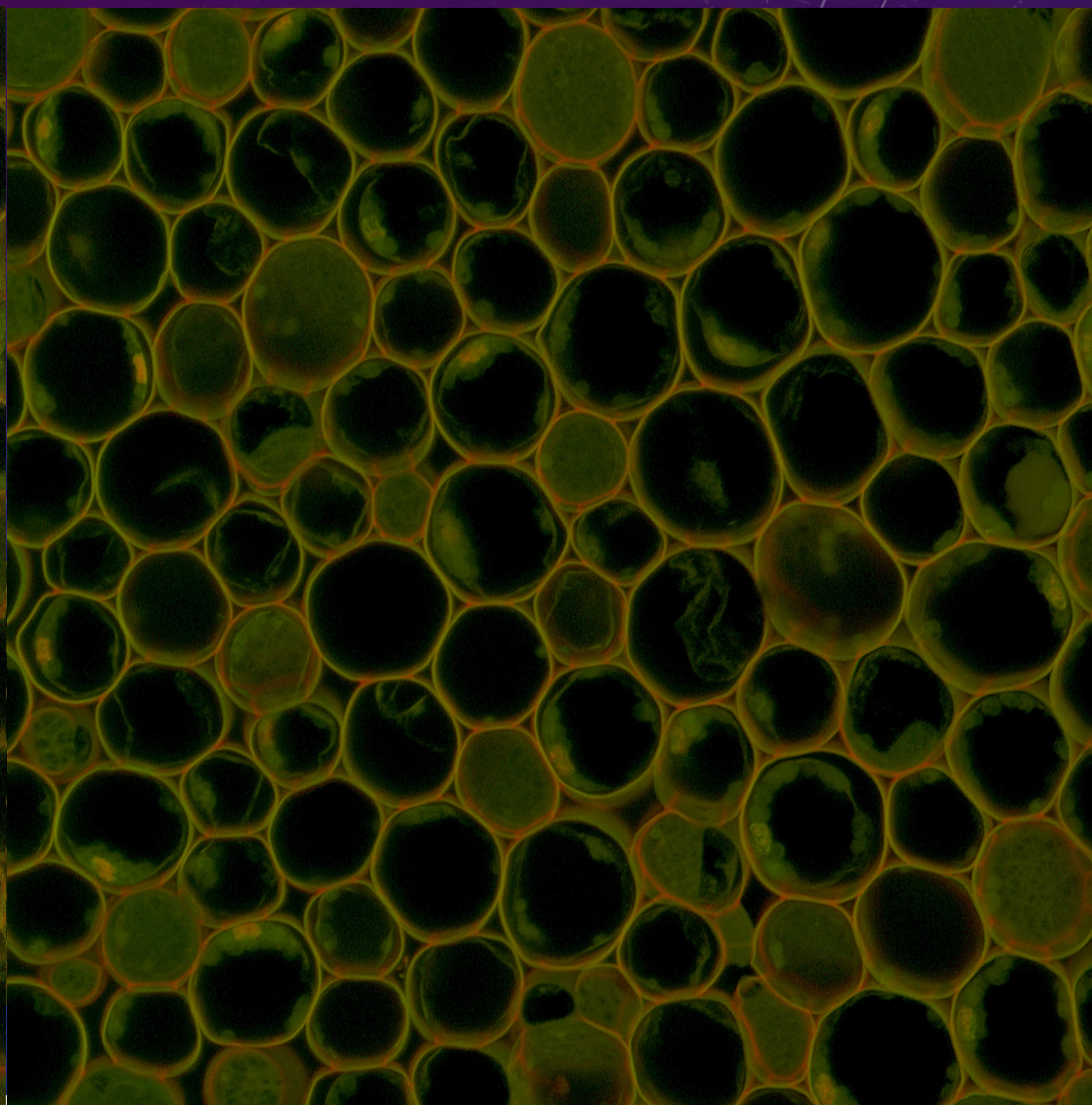






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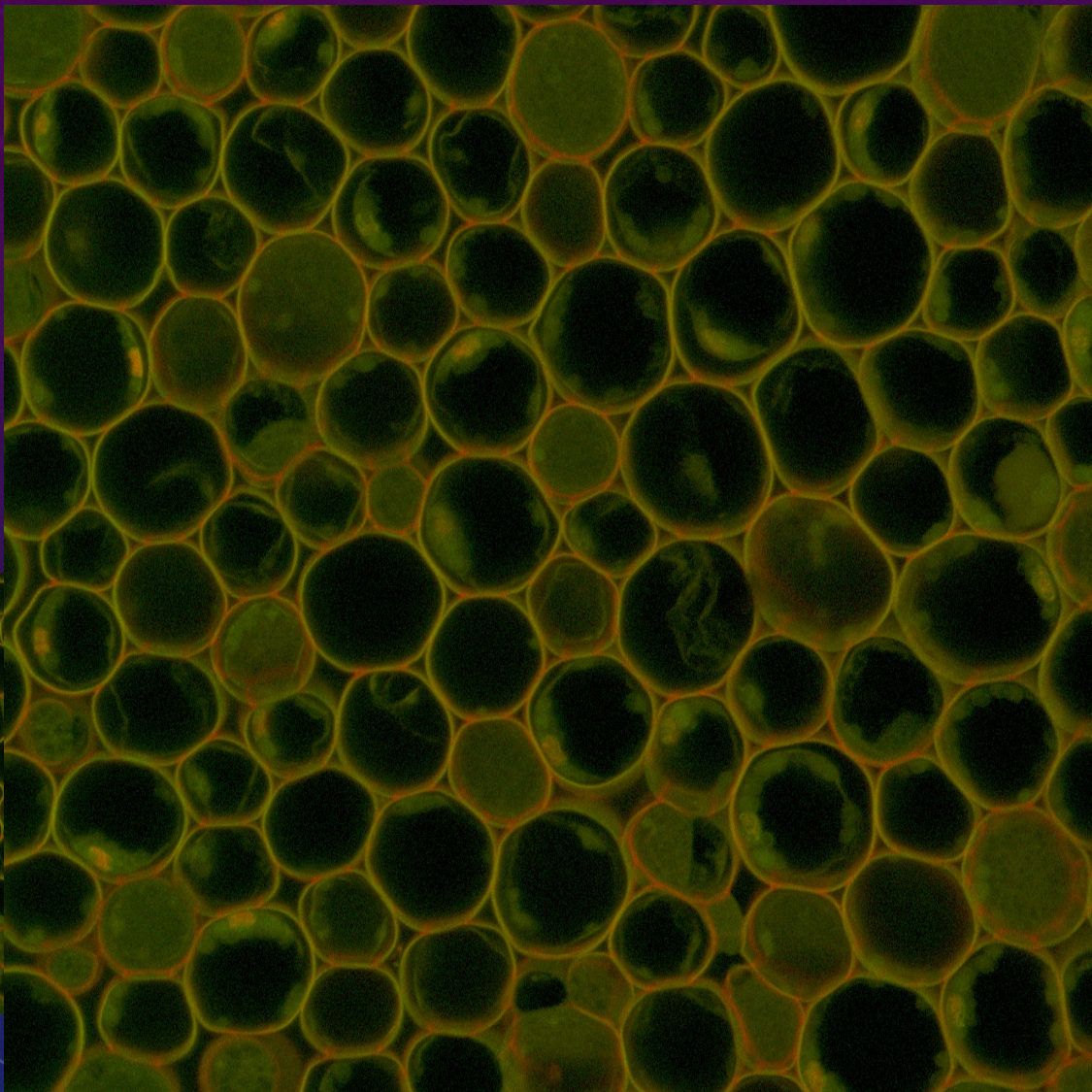
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00:48:05

01:24:18

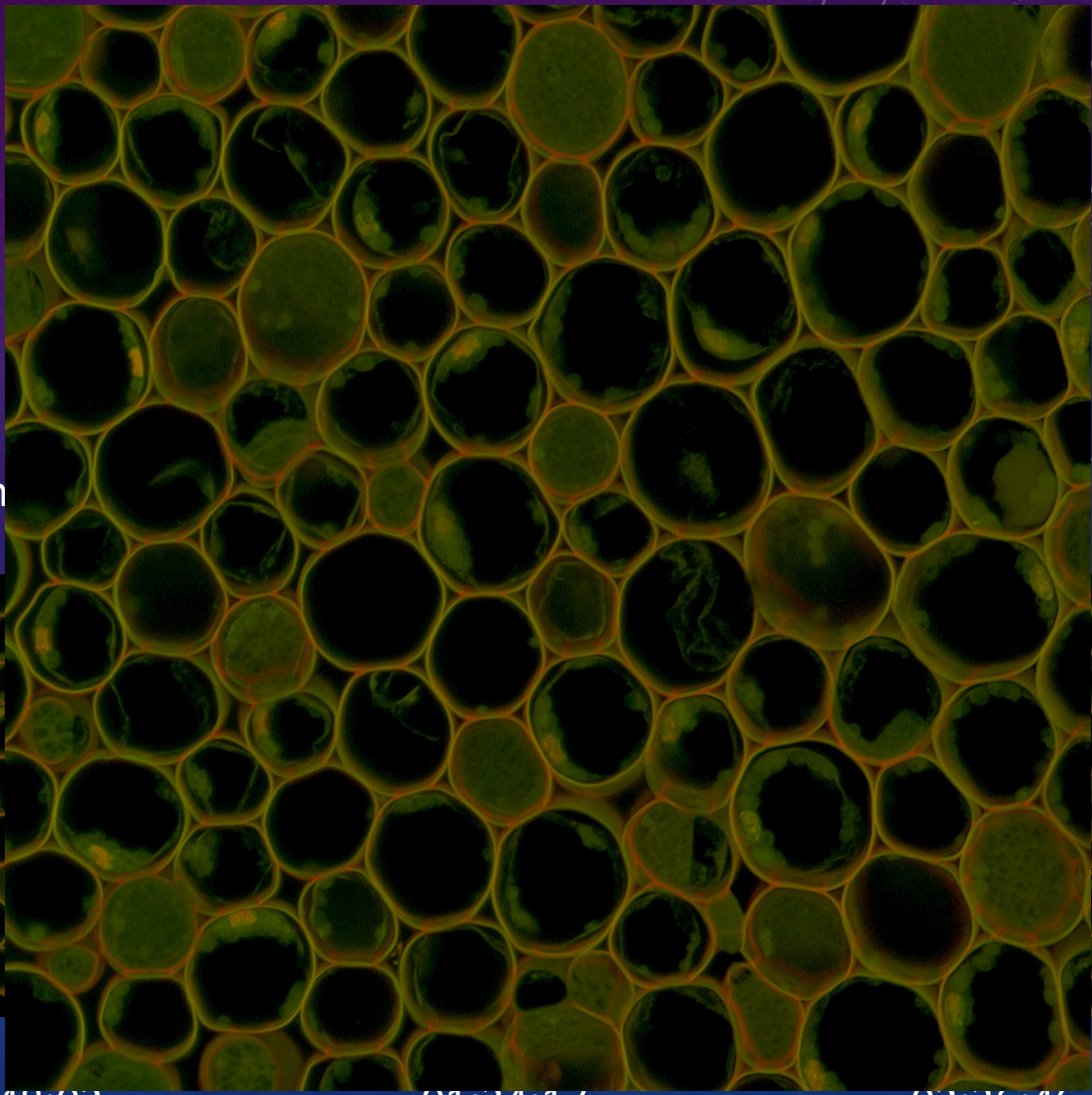
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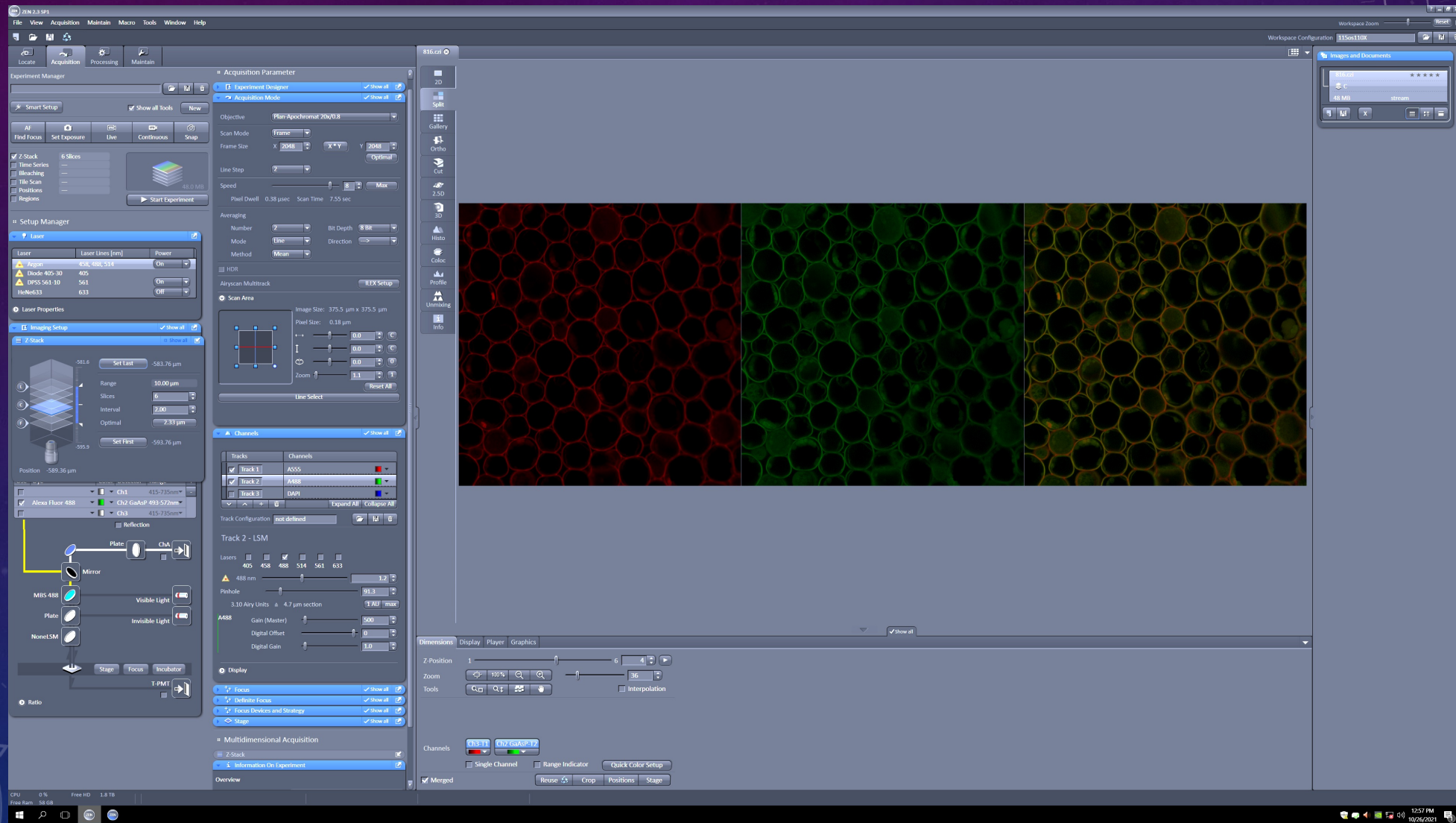
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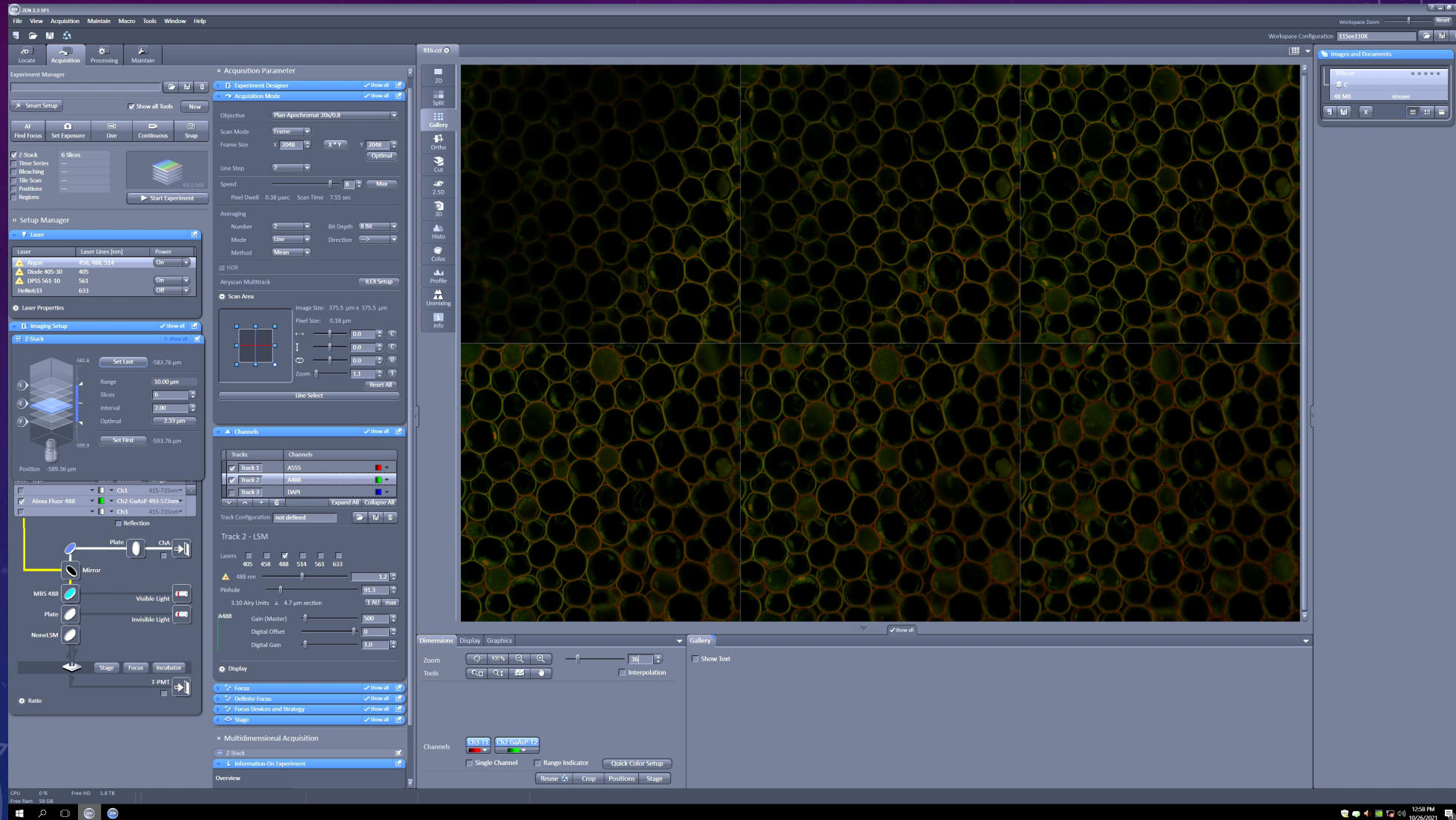
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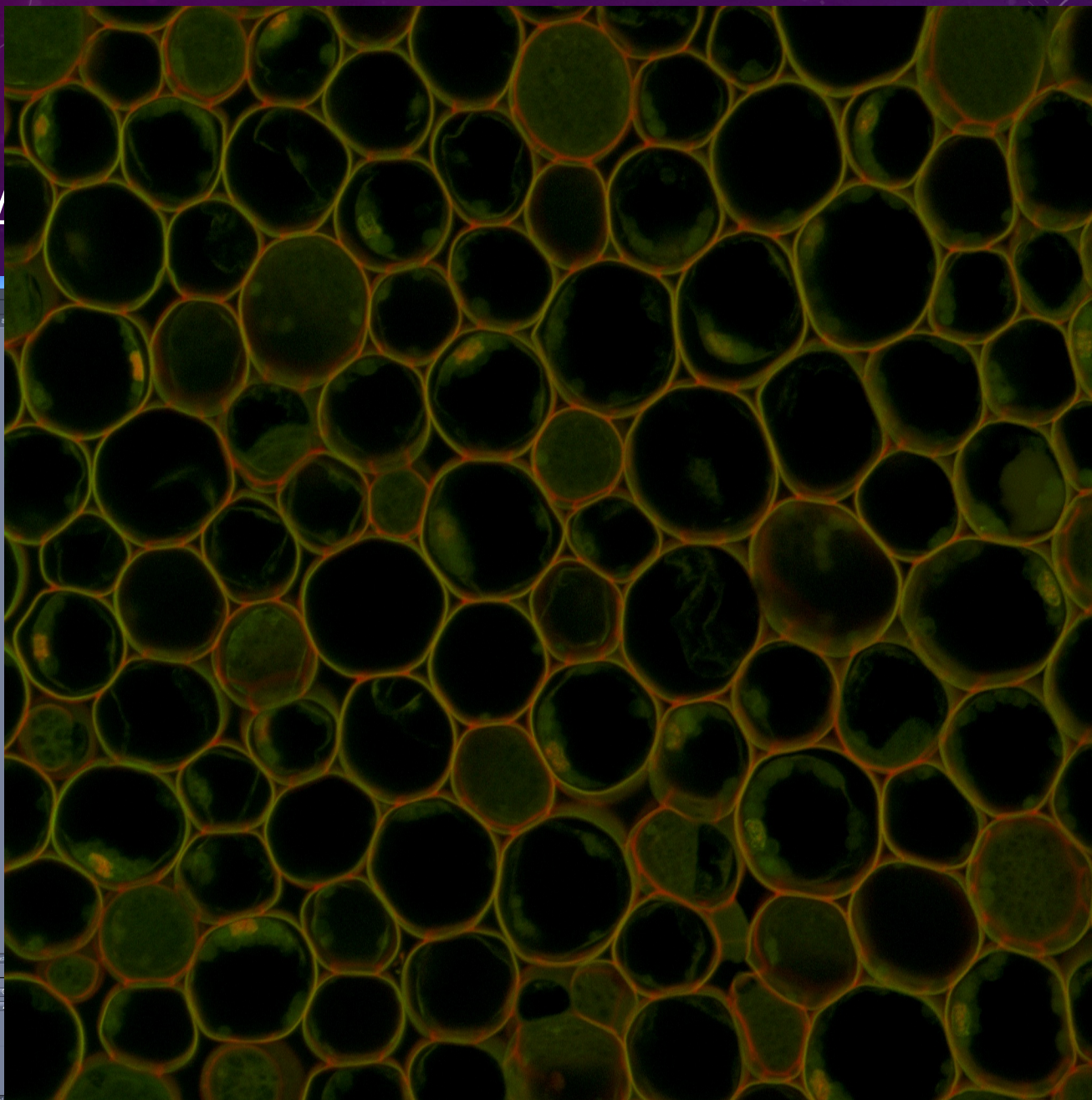
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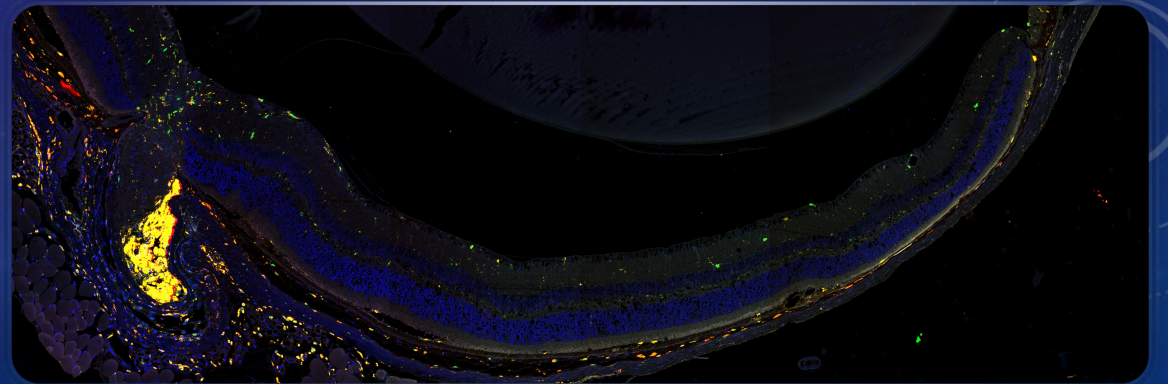
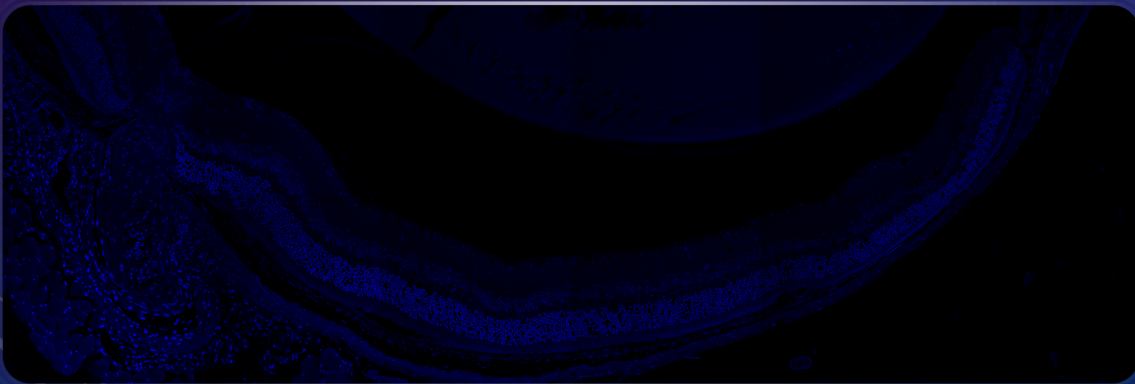
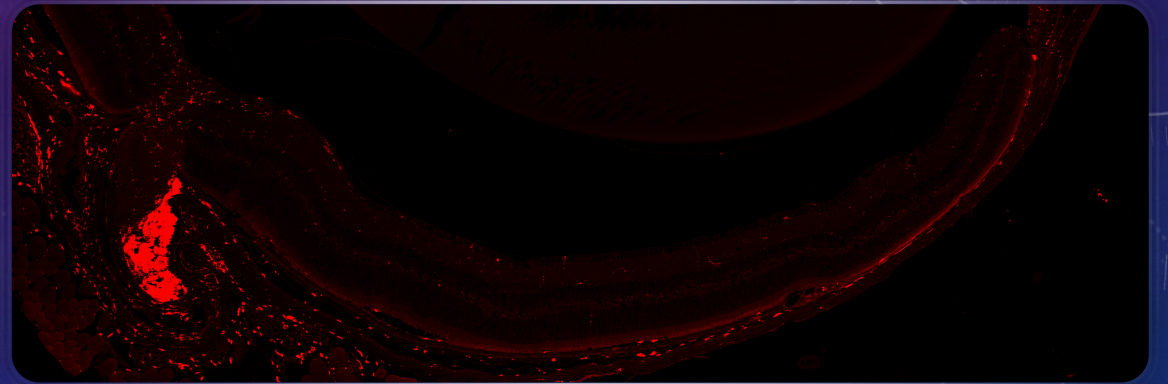
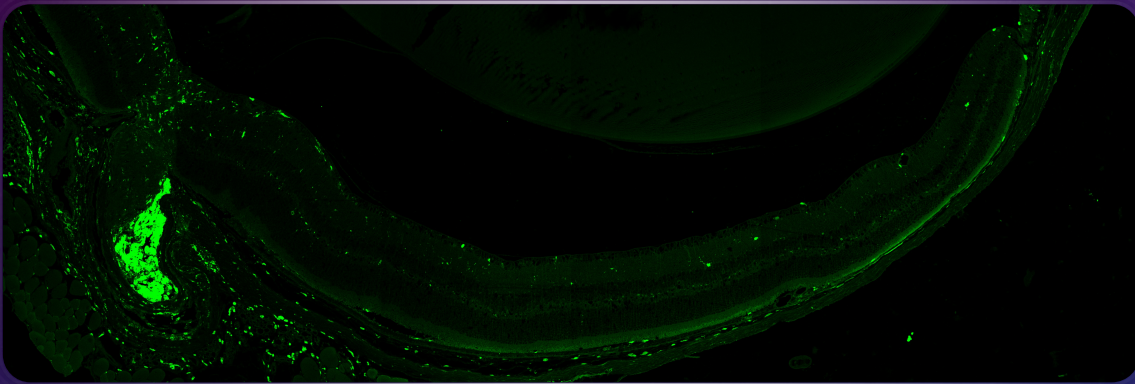
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The screenshot displays the Zen software interface. The top menu bar includes File, Edit, View, Acquisition, Graphics, Tools, Window, and Help. The main window is divided into several panels. On the left, the 'Processing' panel shows the 'Function: Orthogonal Projection' and a list of methods: Rotate 2D, Orthogonal Projection (selected), Image Export, Stitching, and Airyscan Processing. Below this is a 'Search' bar and a list of methods: Geometric, Change Orientation, Channel Alignment, Channel Alignment (Extended), Color-coded Projection, Image Overlay, Mirror, and Orthogonal Projection. The 'Method Parameters' panel on the right shows settings for 'Orthogonal Projection', including 'Projection Plane' (Frontal XY), 'Method' (Maximum), and 'Input' (Stitch). The 'Input' section shows a preview of the stitched image. The bottom status bar indicates 'Settings: 1 pixel (measured)' and 'System Information: Info'.

[illegible]

C57BL/6j Retina---20 × 0.8 Z-stack+Tile-Scan



免疫荧光切片的制备

- 石蜡切片 结构整齐
- 免疫荧光染片
 - a) 环保脱蜡液脱蜡, 10-15min $\times$ 3次, 晾干切片
 - b) 95%乙醇1-2min $\times$ 3次, 75%乙醇1-2min $\times$ 1次, H₂O 2min $\times$ 1次
 - c) 柠檬酸钠热修复, 98 °C, 40min, 冷却至室温
 - d) PBS 5min $\times$ 3次
 - e) H₂O₂ 20min, PBS 5min $\times$ 3次
 - f) 0.3% Triton X100 20min, PBS 5min $\times$ 3次
 - g) 山羊血清封闭, 1h, RT
 - h) 一抗孵育, 4 °C, 过夜
 - i) 室温平衡30min-1h, PBS 5min $\times$ 3次
 - j) 二抗孵育, 40min, RT, PBS 5min $\times$ 3次
 - k) 含DAPI的抗荧光淬灭封片剂封片, 4 °C避光保存

Thank you