



# FV10-ASW软件使用详解

# 宽场显微镜的基本组成部件



**HSD:高灵敏度检测器**

**SPD:常规检测器**



**扫描单元**



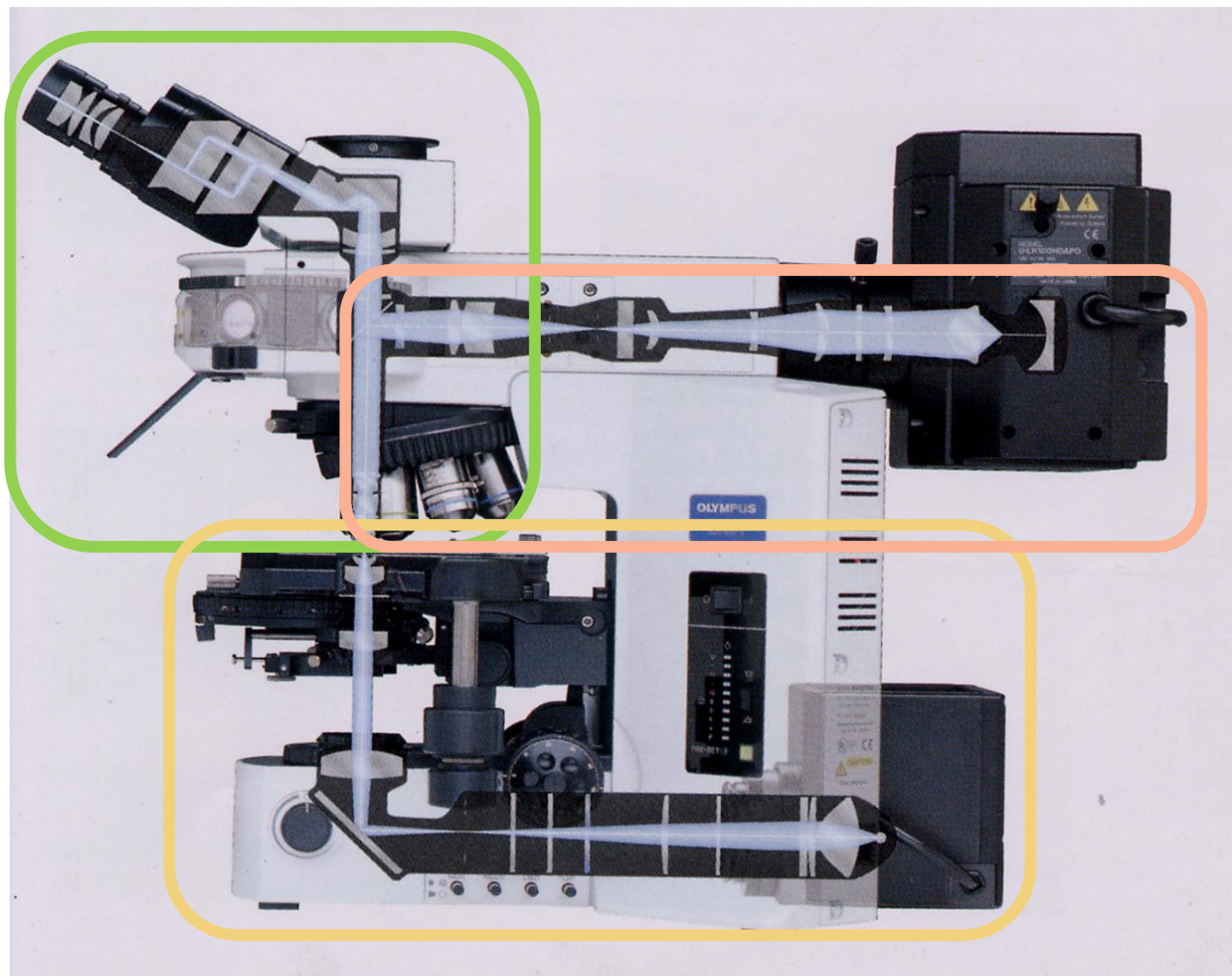
**TPC:微电脑**



**MCZ:调焦轴**



# 显微镜光路结构



成像光路结构

照明光路结构  
——落射光路  
EPI/reflected

照明光路结构  
——透射光路  
Transmitted

# 成像光路结构

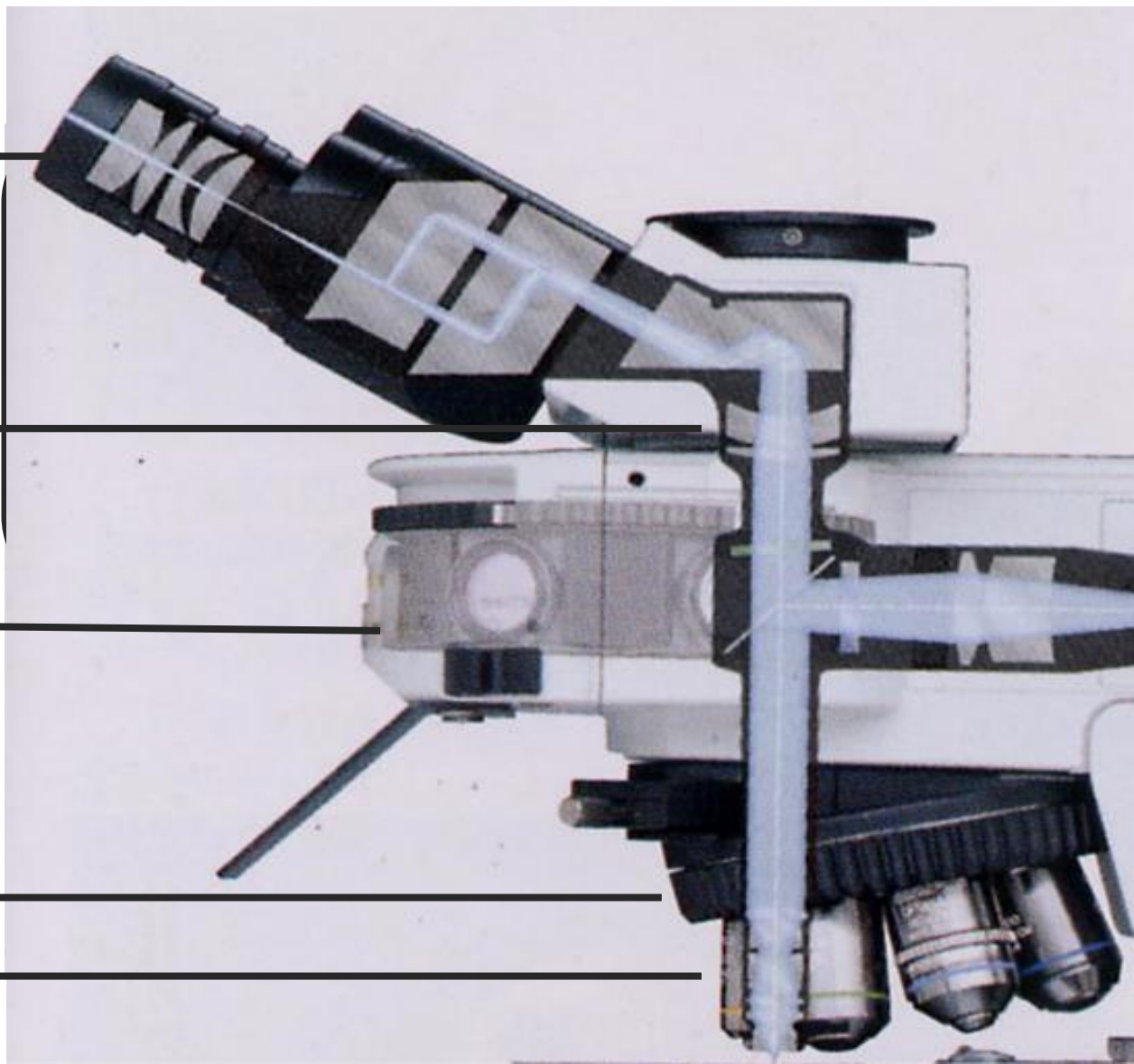
目镜

结像透镜

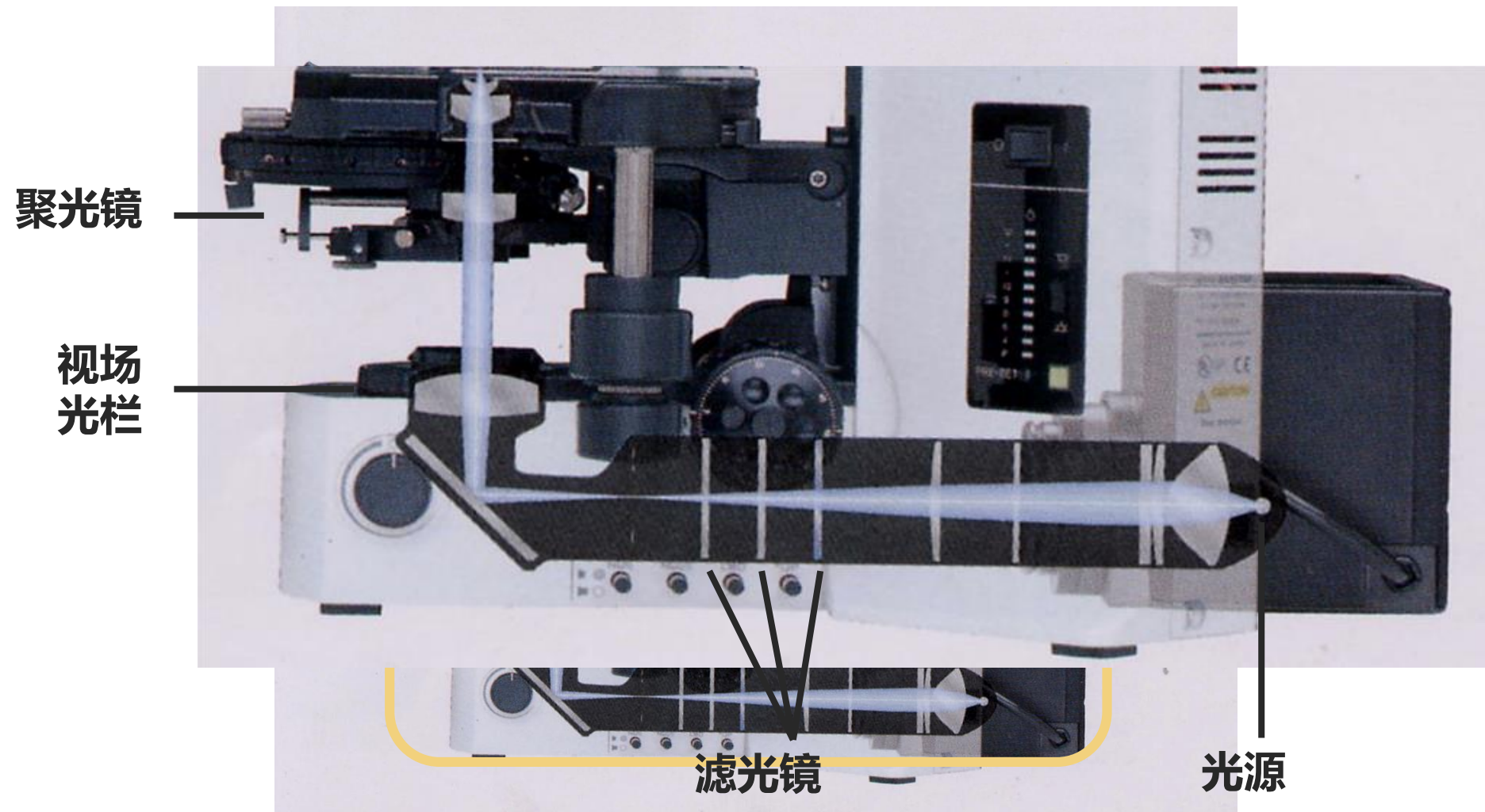
荧光镜转盘

物镜转盘

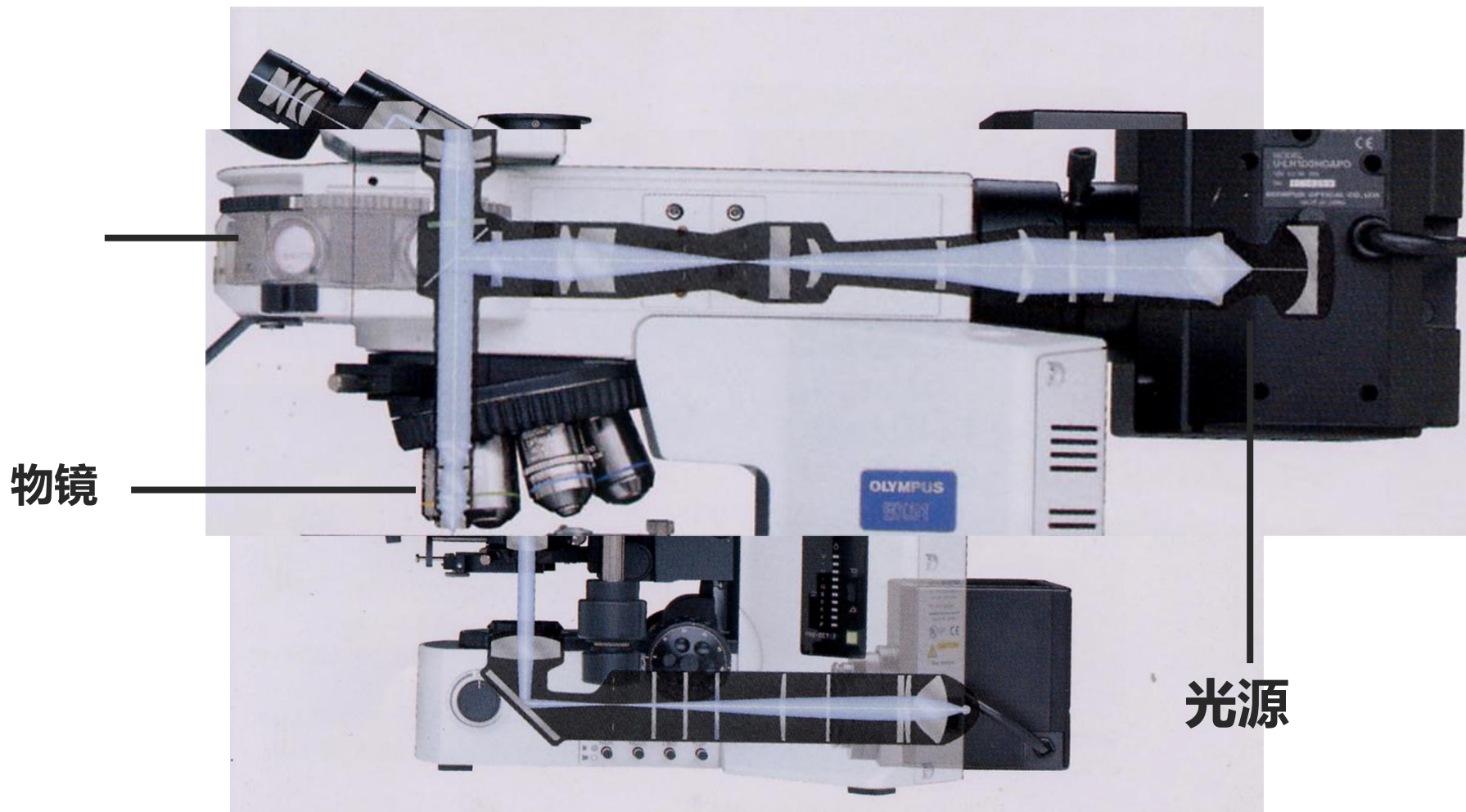
物镜



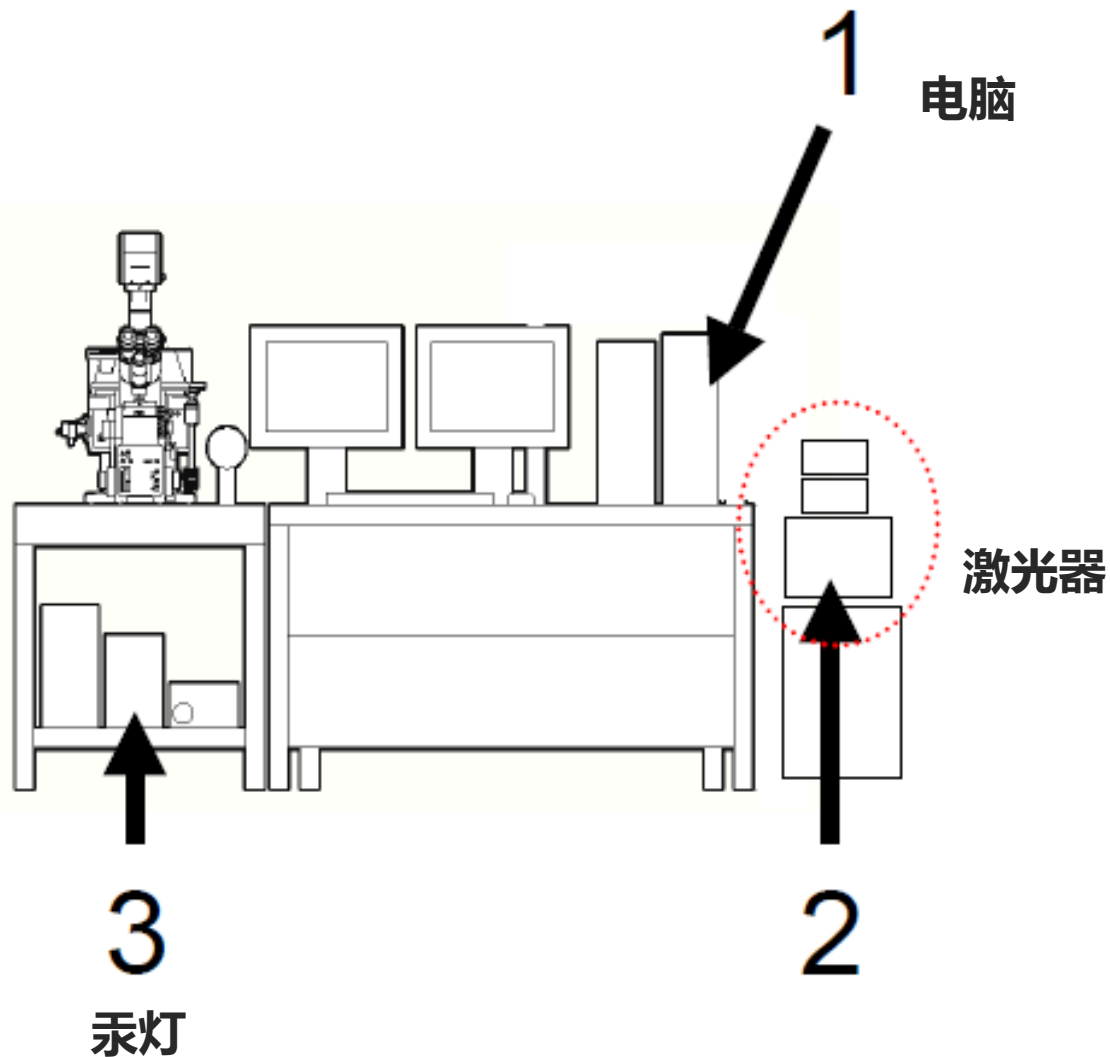
# 透射照明光路



# 落射/反射照明光路



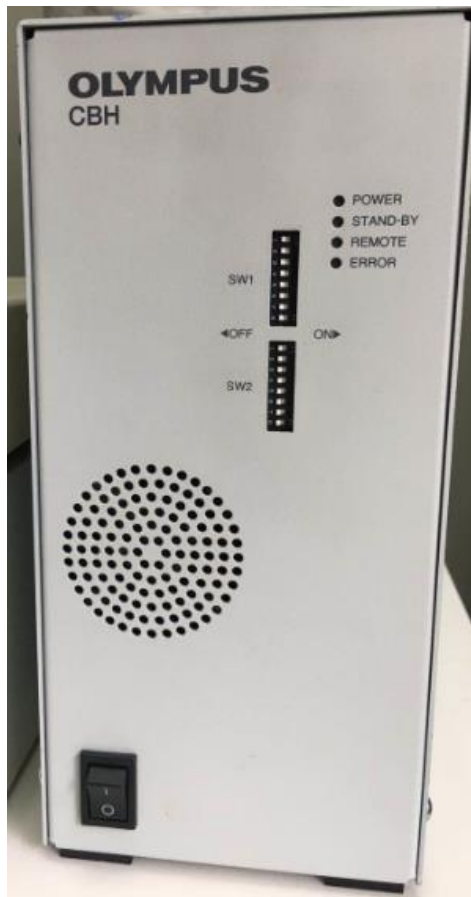
# 开机顺序



电脑—显微镜控制器—CBH—激光器—TPC—汞灯—软件



# 一定要按顺序开关的部件：CBH+TPC



开机：先CBH后TPC



背侧开关



关机：先TPC后CBH



# 激光器开关

开机：先开关再钥匙

关机：先钥匙再开关

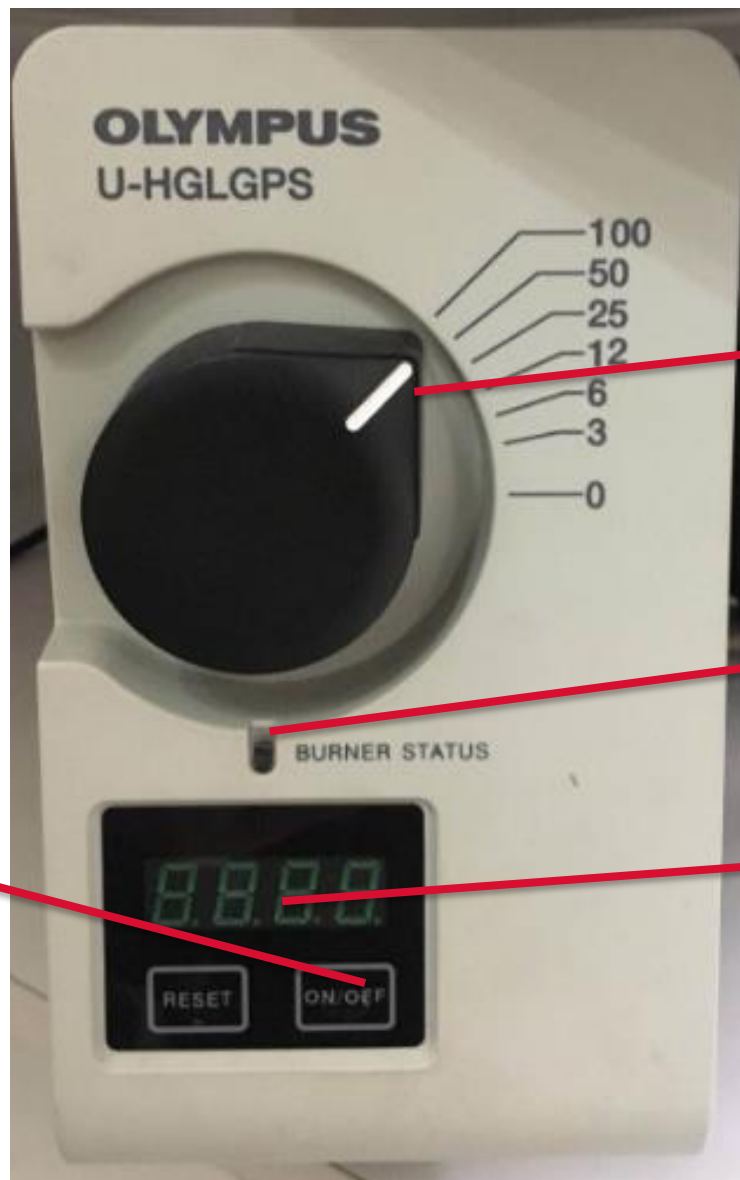


# 长寿命金属卤化物光源

建议：至少使用半小时以上再关机

开机：后方开关打开，短按on off键

关机：长按3s，倒计时300s结束后才可以关闭后方开关



转动调节  
光源强度

指示灯

数字显  
示屏

# 启动软件

Enter [User ID] and [Password], and then click the <OK> button.



FV10-ASW

Welcome to "FV10-ASW"

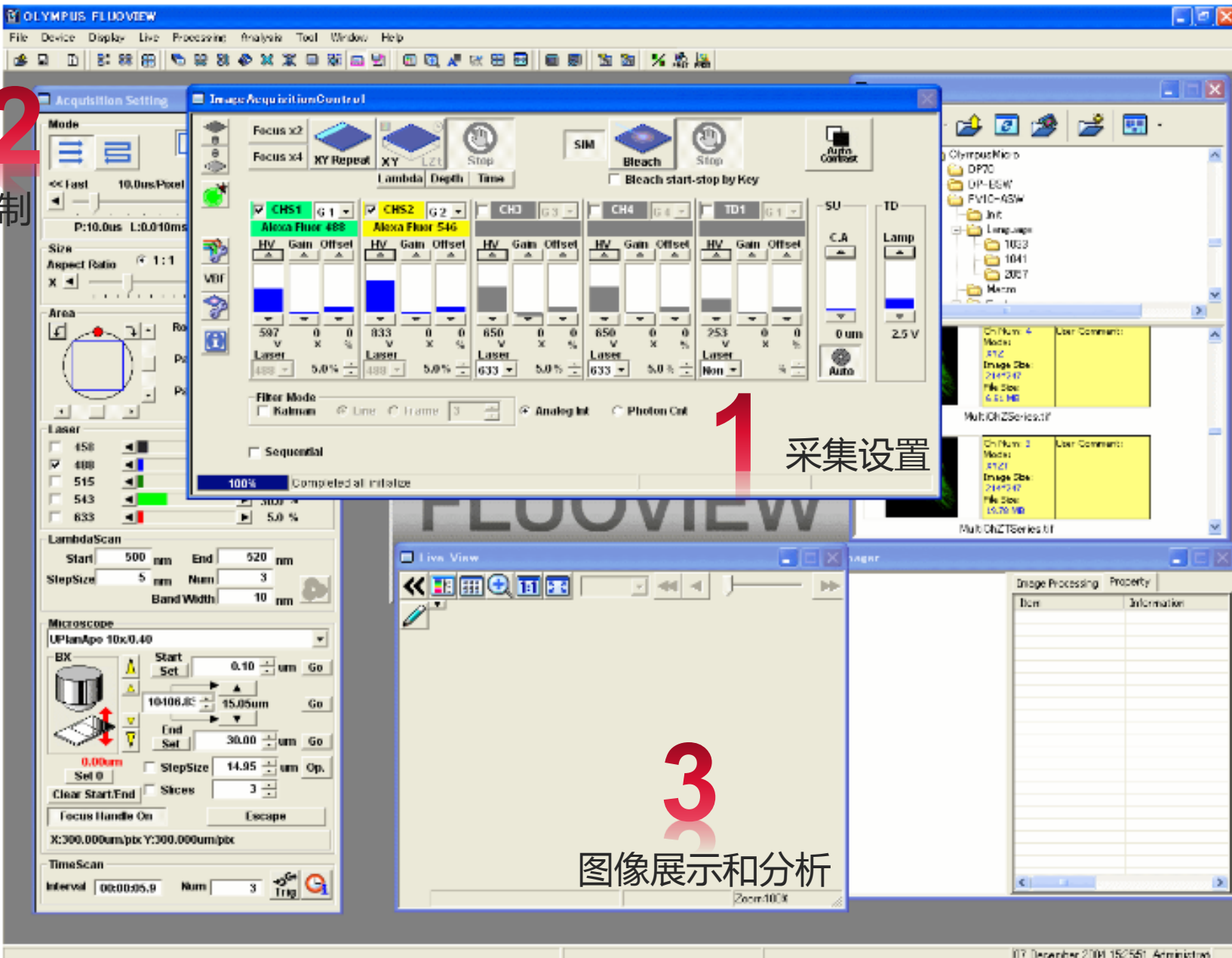
**OLYMPUS**

**FV10-ASW**

User ID: Adm **GUEST**

Password:

Buttons: Password, OK, Cancel



2  
硬件控制

1  
采集设置

3  
图像展示和分析

# 一、采集控制界面

Image Acquisition Control window when the compact scan unit is used

The screenshot displays the 'Image Acquisition Control' window. At the top, there are icons for Focus x2, Focus x4, XY Repeat, XY, LZ1, Stop, SIM LightPath, SIM Imaging, Bleach, and Auto Contrast. Below these are buttons for Lambda, Depth, Time, SR, Pause, and Bleach start-stop by Key. The main area is divided into six channel columns: CHS1 (Alexa Fluor 488), CHS2 (Alexa Fluor 568), CH3, CH4, TD1, and RXD1. Each channel has a grid of controls for HV, Gain, and Offset, along with a Laser dropdown and percentage. On the right, there are sliders for SU (C.A. at 50 um) and TR (Lamp at 5.9 V). At the bottom, there are sections for Filter Mode (Kalman, Line, Frame), Hard Disk Recording Setting (OFF), and Sequential acquisition. A status bar at the very bottom shows '100%' and 'Completed all initialize'.

**TD button**

**透射光观察**

The shutter of the transmitted illumination is opened/closed.



: The shutter is closed and the illumination light is not irradiated now.



: The shutter is opened and the illumination light is irradiated now.

**EPI button**

**荧光观察**

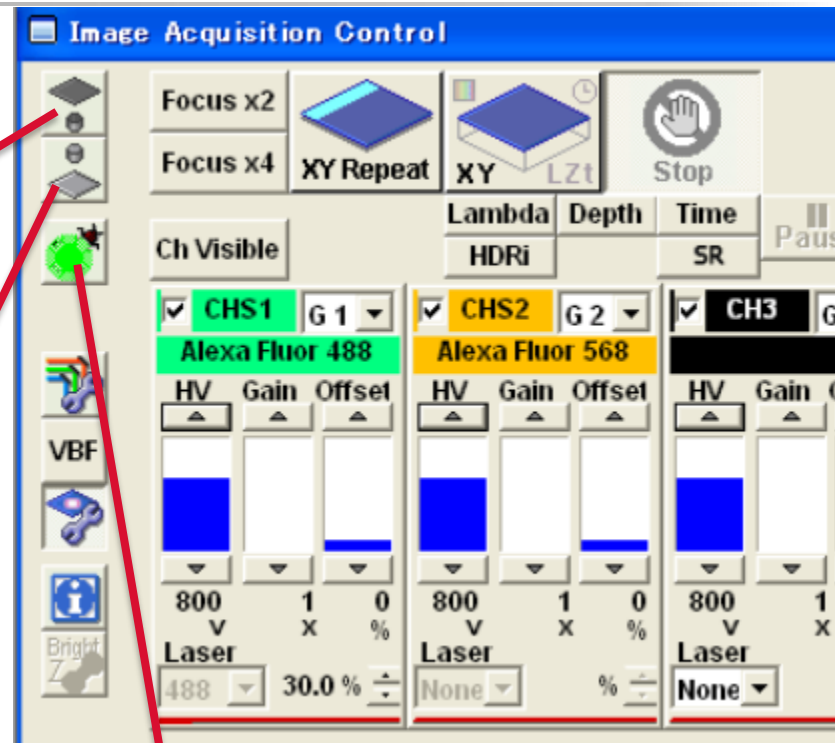
Change fluorescent mirror unit and the shutter of the reflected illumination is opened/closed.



: The shutter is closed and the illumination light is not irradiated now.



: The shutter is opened and the illumination light is irradiated now.



**Dye List**



Dye List (Fluorescent dye) can be set.

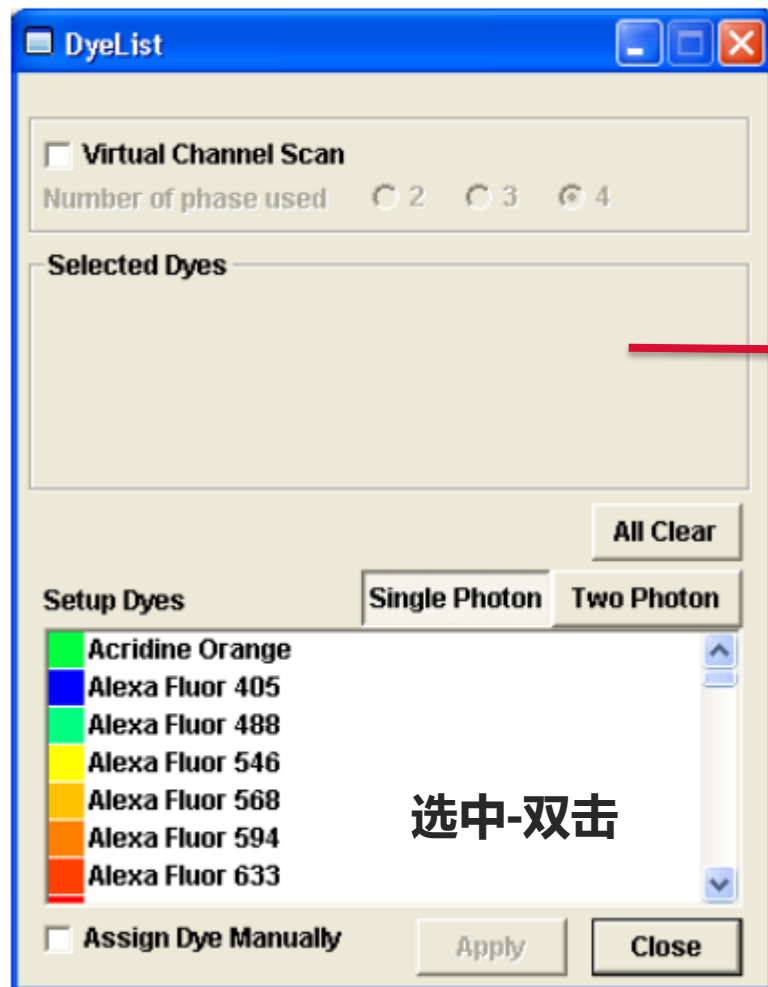
[▶ about Dye List](#)

Dye List button

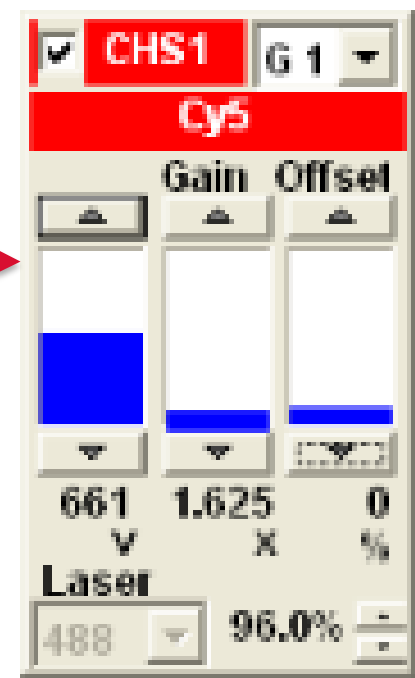


When this button is clicked, the [Dye List] window is displayed.

# 选染料



选择染料后自动分配光源光路检测器





# LightPath & Dyes button



Light path is set.

Stimulus Setting

UseScanner  
 None  Main  SIM

Mode  
 [Fast]  [Normal]  [Slow]

<< Fast 10.0us/Pixel Slow >>

P:10.0us L:0.010ms F:0.000s S:0.000s

Laser  
 405 [Slider] 52.0 %

StimulateStartSetting  
 Manual  Auto Stop 0.000 msec

Main Scanner Sync  
 ImageScan -> Activation  
 Activation -> ImageScan

Wait Time [Green bar]  
 ImageScan [Blue bar]  
 Activation [Red bar]

1000.000 msec

同步光刺激

Image Acquisition Control

Focus x2 Focus x4 XY Repeat XY LZt Stop

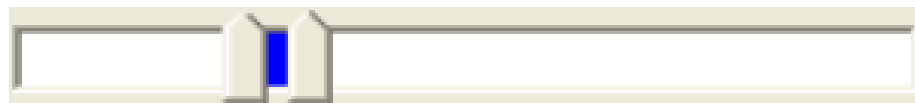
Ch Visible  
 CHS1 G 1  
 CHS2 G 2  
 CH3 G 3

Alexa Fluor 488 Alexa Fluor 568

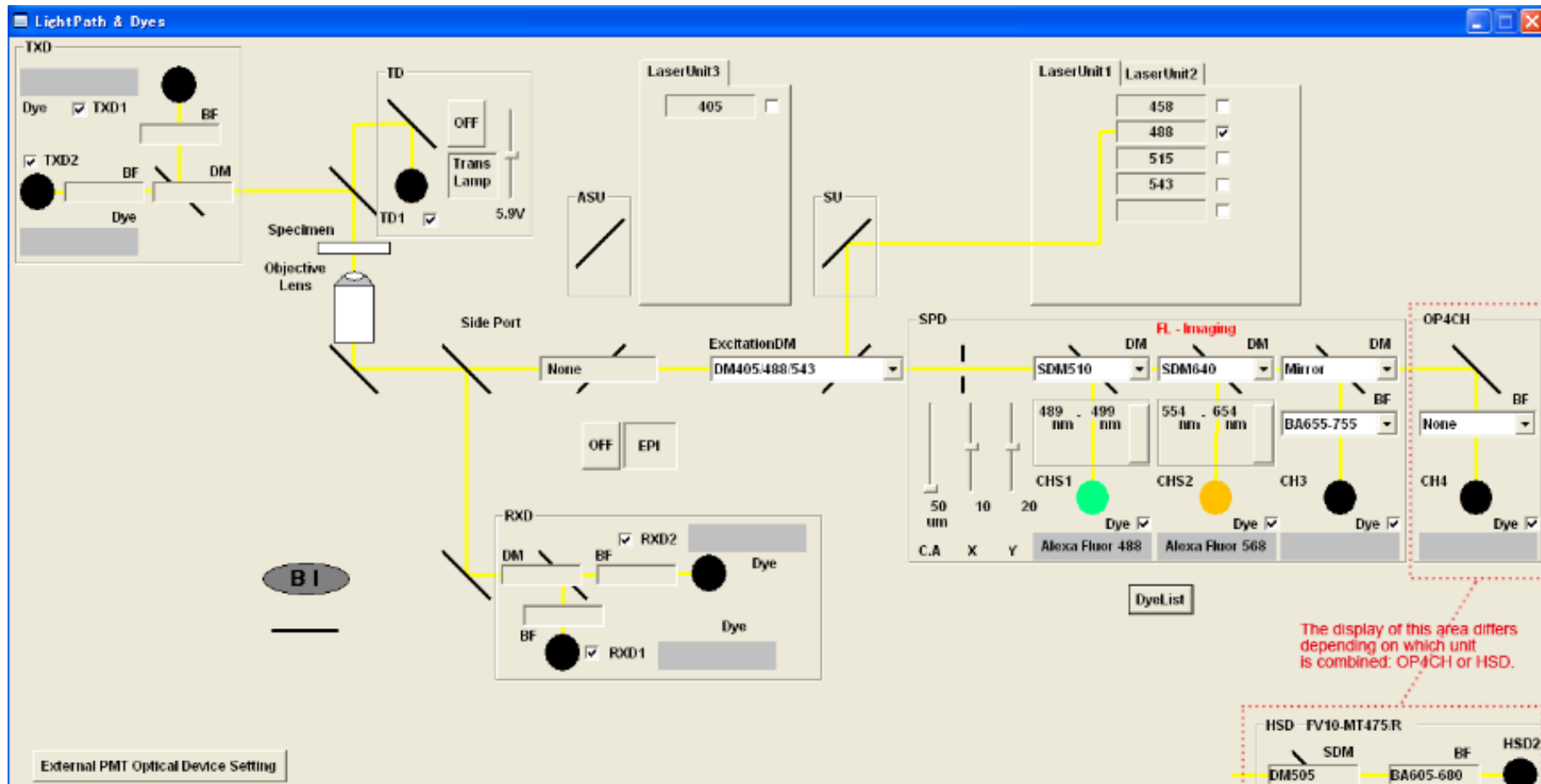
Channel	Wavelength (nm)	Power (%)
CHS1 (Alexa Fluor 488)	488	30.0
CHS2 (Alexa Fluor 568)	None	None
CH3	None	None

## VBF 检测器检测波段范围设定

Range of wavelength to be acquired in [CHS1] and [CHS2] can be selected.



# 光路图



## LightPath & Dyes button



Light path is set.

Information button



[Information] window displays the following information.

The Information window displays the following data:

Size	
<b>ZoomArea</b>	<b>ClipArea</b>
X 256 pix [1.000um]	X0728740 [0.256um]
Y 256 pix [1.000um]	Y 0 pix [0.256um]

Time	
OverScanArea	0.010ms
ImagingArea	0.000ms
( ActiveLine	2.560ms/Line )
( NonActiveLine	-2.550ms/Line )
RetraceArea	0.000ms
<b>PixelTime</b>	<b>10.0us/pix</b>
	0.000s/Frame
	0.000s/Series

Pixel Size	
PixelSize	Optical Resolution
X 1.000um/pix	300.000um/pix
Y 1.000um/pix	300.000um/pix
Z 0.002um/slice	300.000um/slice

The Image Acquisition Control window shows various acquisition parameters:

- Focus x2, Focus x4, XY Repeat, XY, LZ1, Stop
- Ch Visible: CHS1 (Alexa Fluor 488), CHS2 (Alexa Fluor 568), CH3
- Parameters for each channel: HV, Gain, Offset, Laser
- Buttons: VBF, Bright Z

Information中主要参考 pixel size信息

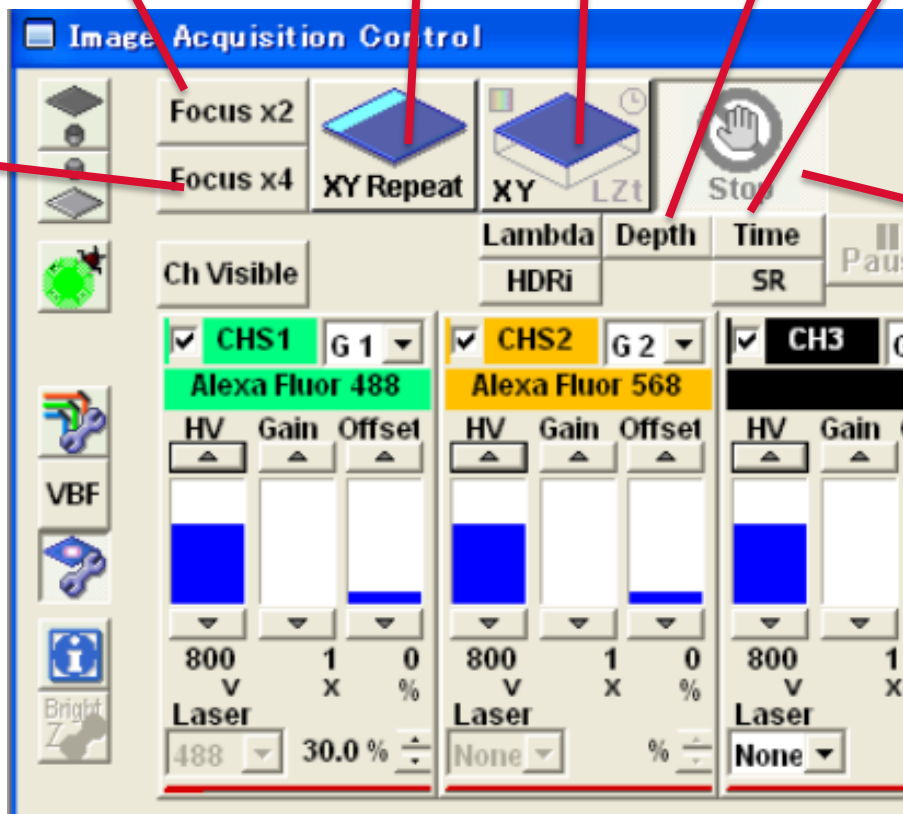
Pixel size=optical resolution/2

预览：1X速度

预览：2X速度，隔行扫描，快，图像质量差

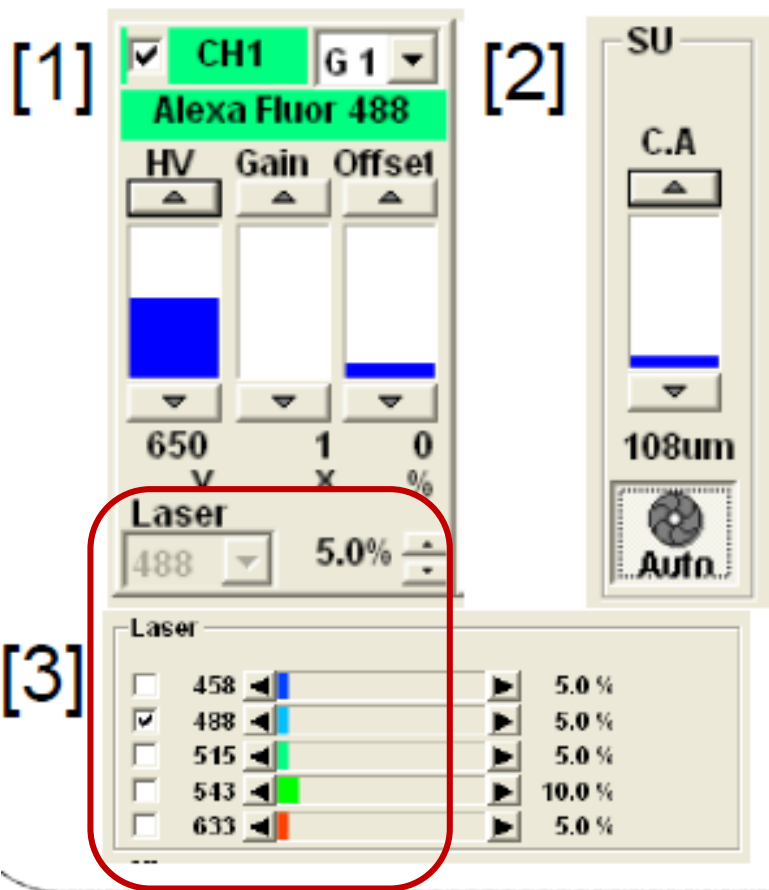
图像采集：XY  
XYZ XYT

预览：4X速度，隔3行扫描，更快，图像质量更差



停止预览

## 图像调节概述




- [1] 探测器的灵敏度调节 (HV)
- [2] 共聚焦的孔径大小调节 (C.A.)
- [3] 激光输出的调节 (Laser)

### 调节方法

(例: HV调节):

点击滑块, HV直接提高 (或降低) 到指定的位置.

点击此按钮  或者使用鼠标转轮进行微调.

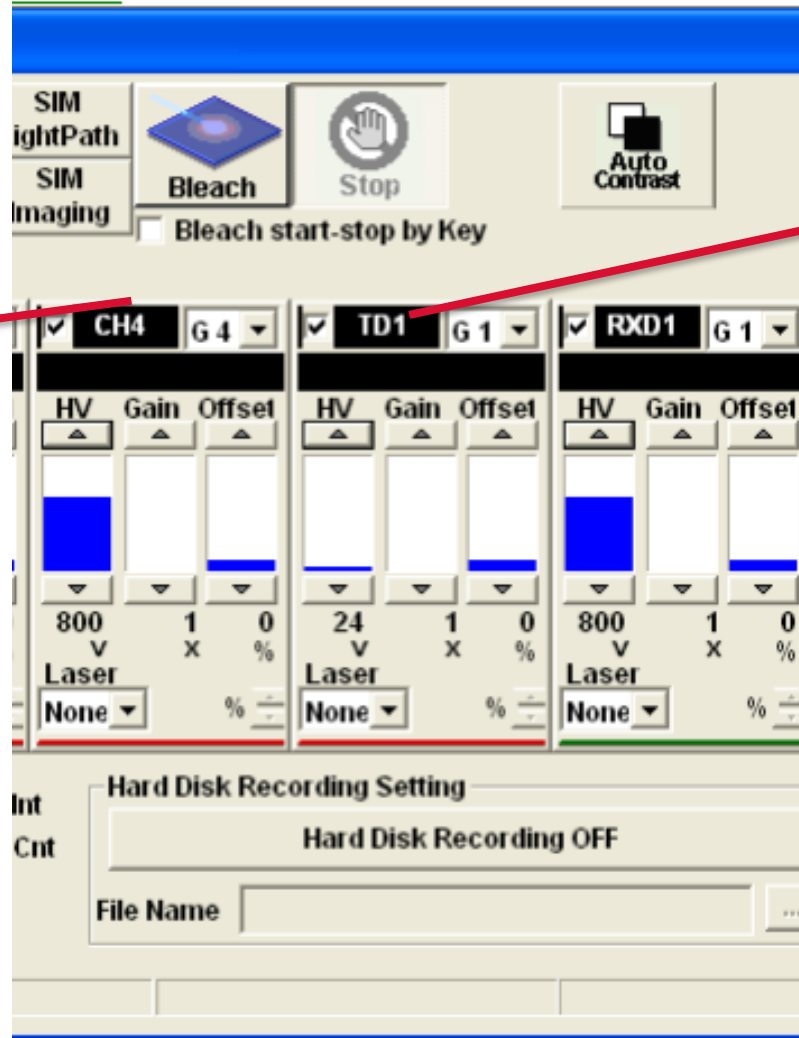
**HV: 电压值, 500-700之间**

**Gain: 数码放大, 背景和信号同步放大, 信噪比不变**

**Offset: 背景补偿, >1,背景变黑**

# TD通道

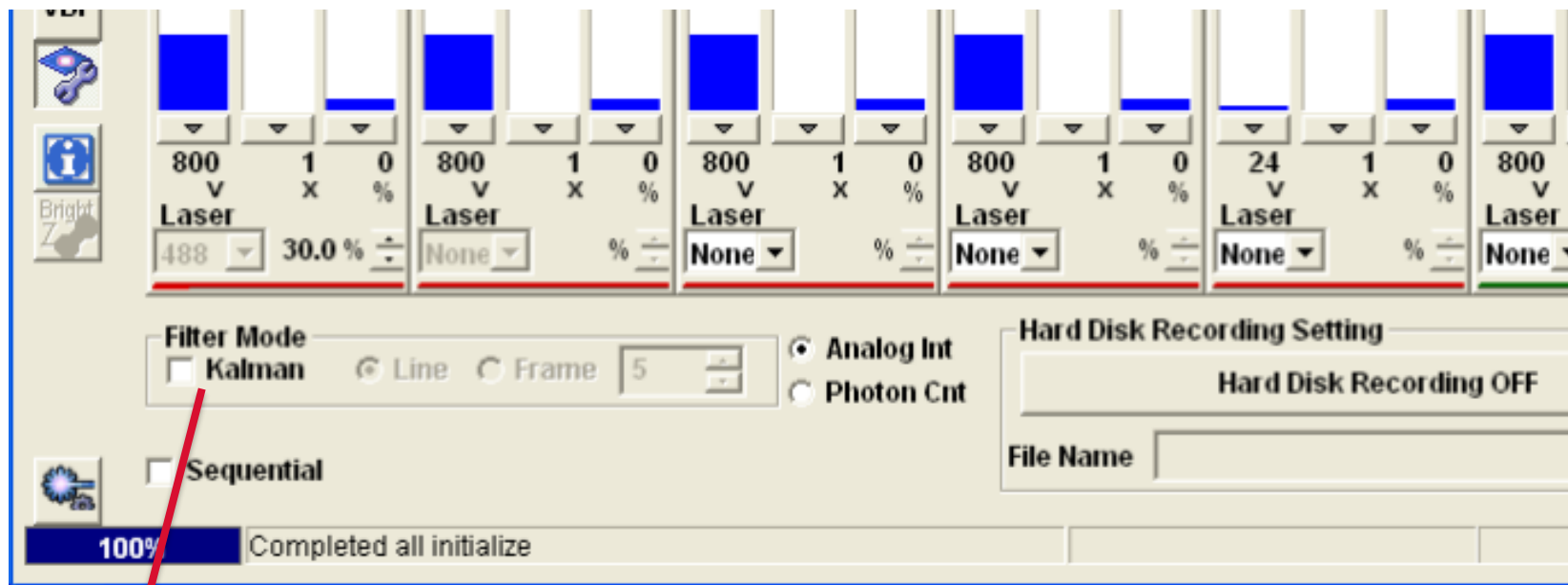
is used



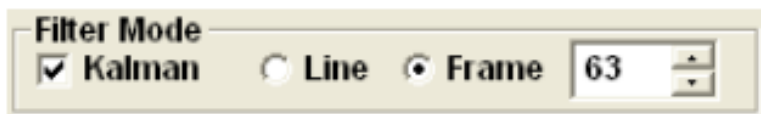
荧光通道

透射光通道

以laser为光源



**Filter mode**



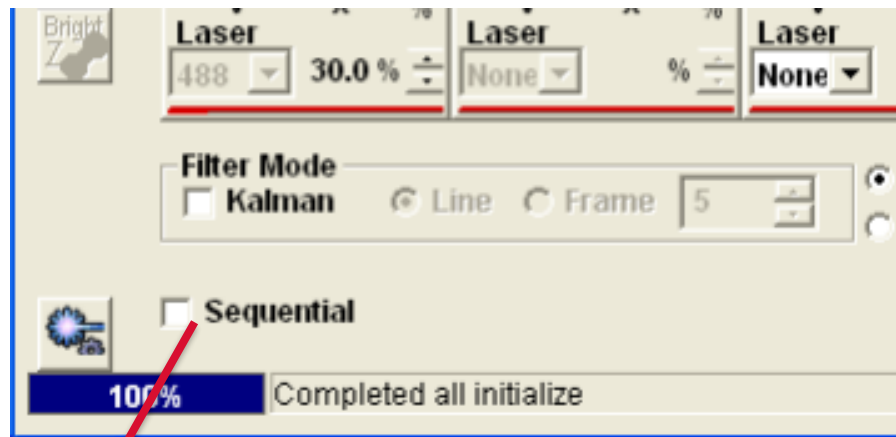
Execute Kalman operation. It acquires average of images by acquiring images for number of cycles specified. It is useful function to remove noise. Select either "Line" or "Frame" and then, enter number of integration in the text box.

**Kalman , 平均 , 降噪**

**Line : 线平均 , 推荐 , 不超过6次**

**Frame : 帧平均**

# Sequential



## Sequential check box

Sequential

When this check box is checked, sequential scan can be done.

Image of less cross-talk can be acquired by a way that image is acquired every fluorescence to observe.

- **Line** : Line sequential scan is executed.
- **Frame** : Frame sequential scan is executed.

将容易串色的通道分到不同group中，分开扫描

Group 1	Group 2	Group 3	Group 4	Group 5
Alexa Fluor 488	Mito Tracker	TD1		
Cy5				



## 二、硬件控制

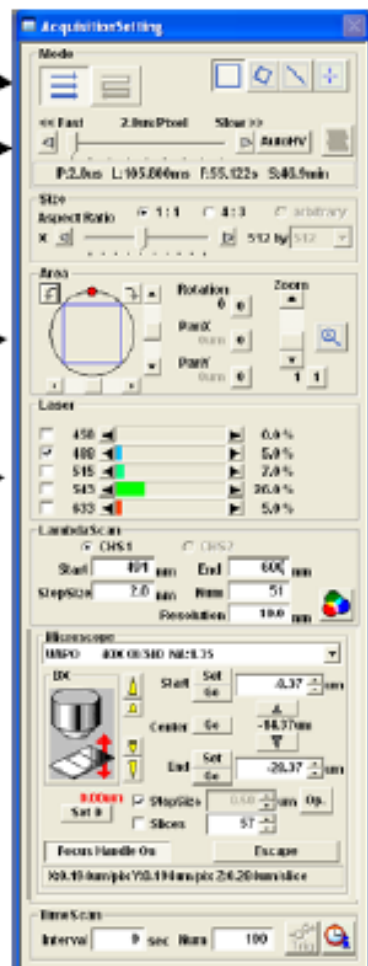
扫描模式

扫描速度

像素数

缩放和平移

激光输出的调节



Lambda扫描的设定

物镜

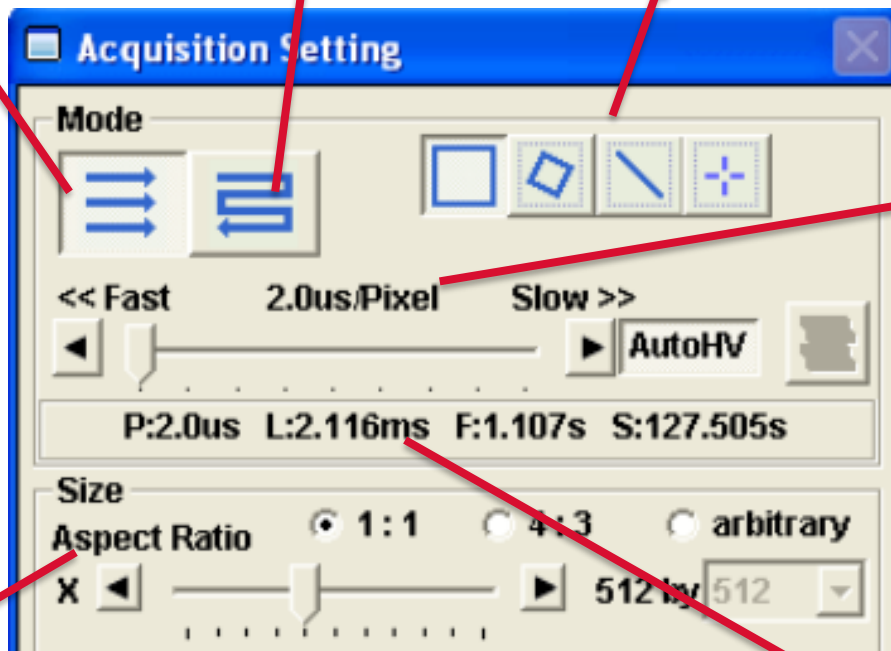
聚焦

时间间隔和时间计数  
(用于XYT或XT扫描)

单向扫描，推荐

双向扫描，速度快

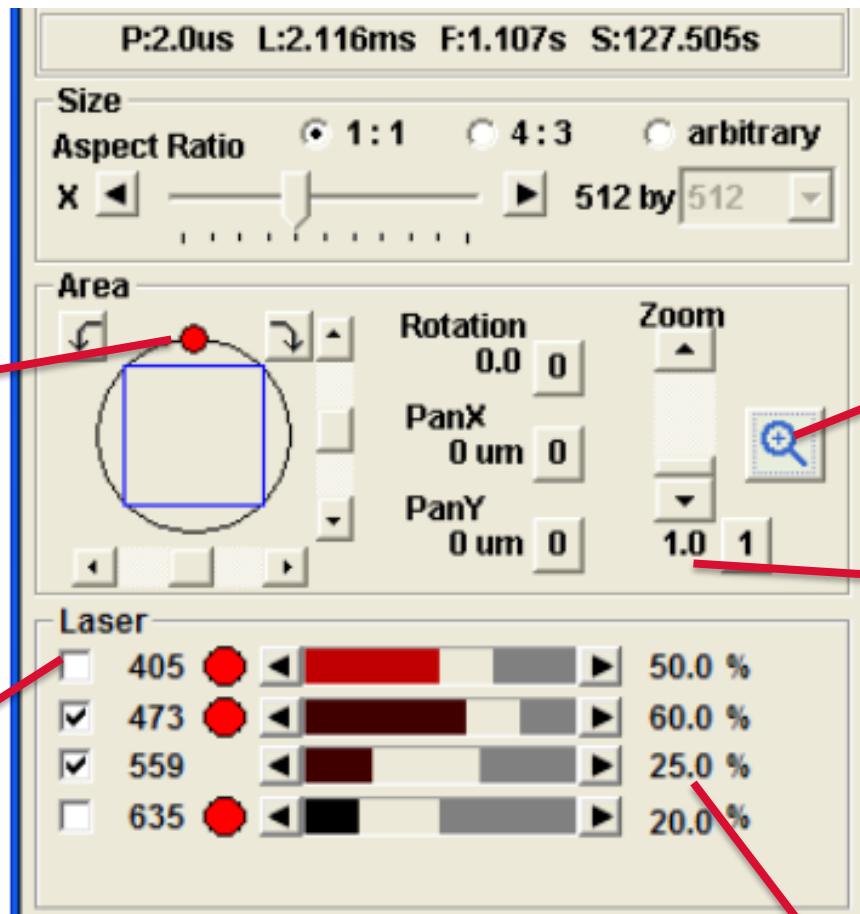
设定扫描区域



扫描速度，每个像素点扫描时间，推荐4或8，不建议超过10。数值越大，光毒性越大。

图像大小，建议512\*512，至少预览使用512\*512

- P: Time required to acquire 1 pixel
- L: Time required to acquire 1 line
- F: Time required to acquire 1 frame
- S: Time required to acquire 1 series



红点：调节扫描头角度

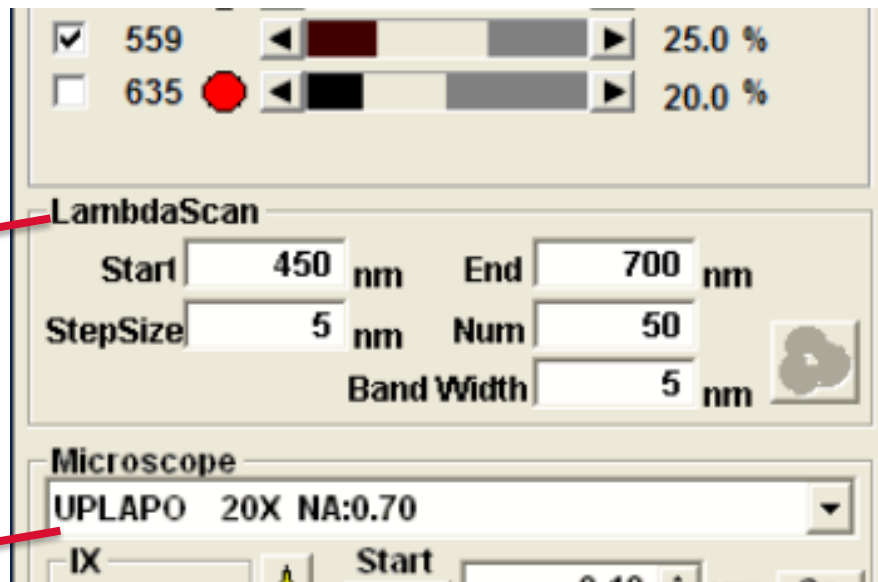
选中，鼠标左键在窗口中绘画区域进行ZOOM

Zoom倍率，参考info中数值

勾选控制laser是否出光

Laser强度，鼠标滚轮或拖动滑块

Pixel Size		
	PixelSize	Optical Resolution
X	1.000um/pix	300.000um/pix
Y	1.000um/pix	300.000um/pix
Z	0.002um/slice	300.000um/slice



光谱扫描

切换物镜

**步进** StepSize  nm (StepSize) text box: Interval between wavelength that starts acquisition and wavelength that starts next acquisition is set. Minimum unit programmable is of 1.0nm.

Num  (Number) text box: Number of frames is set.

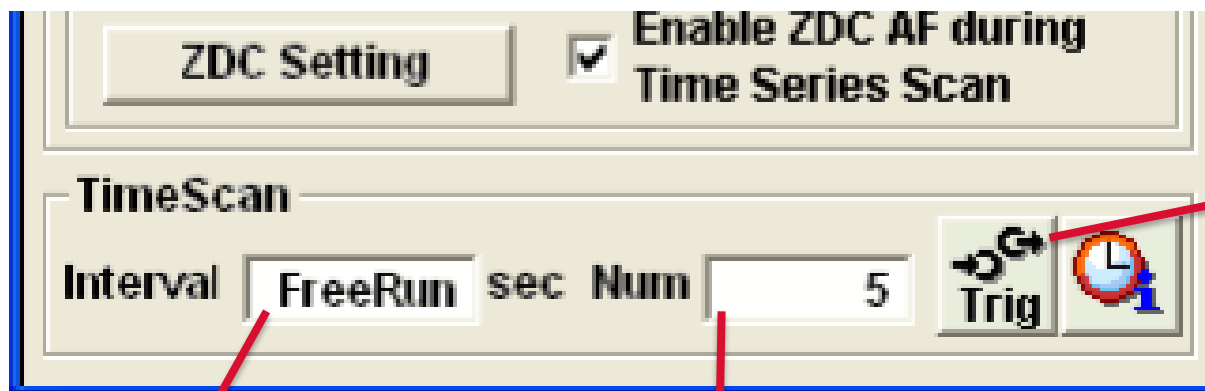
**带宽** Band Width  nm (Band Width) text box: Range of wavelength to acquire at one frame is set.

# XYZ scan

The screenshot shows the 'Microscope' control panel with the following settings and annotations:

- Microscope:** UPLAPO 20X NA:0.70
- IX:** Includes focus adjustment buttons (up/down triangles) and a red double-headed arrow. Annotation: **上下调焦按钮** (Up/Down focus buttons).
- Start Set:** 0.10 um. Annotation: **设置开始采集的位置** (Set start acquisition position).
- End Set:** 30.00 um. Annotation: **设置结束采集的位置** (Set end acquisition position).
- StepSize:** 14.95 um. Annotation: **理论推荐步进值** (Theoretical recommended step size).
- Slices:** 3. Annotation: **步进, 两层之间的距离** (Step, distance between two layers).
- Buttons:** 'Focus Handle On' (Annotation: **防止硬件调焦轴误触** - Prevent hardware focus axis misoperation), 'Escape', and 'Fine'.
- ZDC:** 'ZDC Setting' button (Annotation: **Z轴防漂移系统** - Z-axis drift prevention system) and 'Enable ZDC AF during Time Series Scan' (checked).
- XY LzLz:** A button with a 3D cube icon. Annotation: **Depth=Z采集选项** (Depth=Z acquisition option).
- Bottom Panel:** Includes 'Lambda', 'Depth', 'Time', 'HDMI', and 'SR' buttons. A red arrow points to the 'Depth' button.

# Time scan



外触发设备

两帧之间间隔时间，手动输入X秒，freerun代表无间隔拍摄

时间序列总帧数

Time选用



### 三、图像展示和分析处理

LUT:调节显示

ROI tool

单击切换显示或隐藏

The screenshot shows the 'Live View' window of the software. It features a top toolbar with icons for LUT (Look Up Table) adjustment, ROI tool, and display mode switching. A central area displays a microscope image with a green ROI box. A callout box on the right provides detailed information about the display mode buttons.

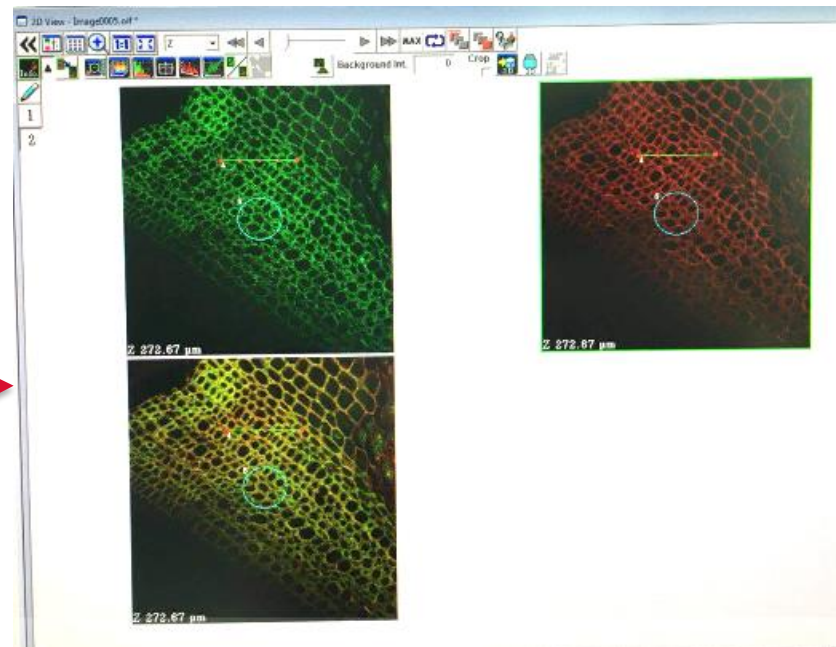
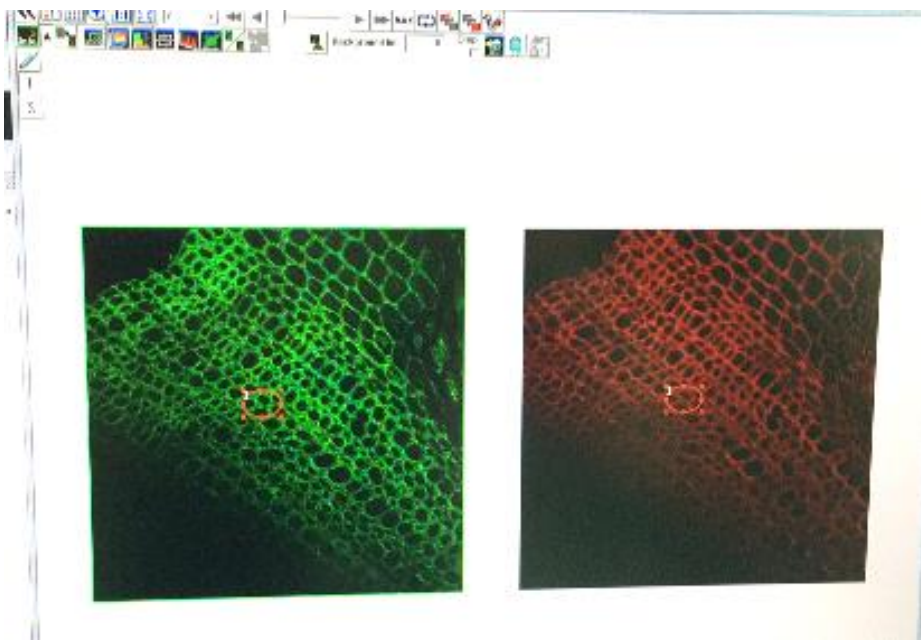
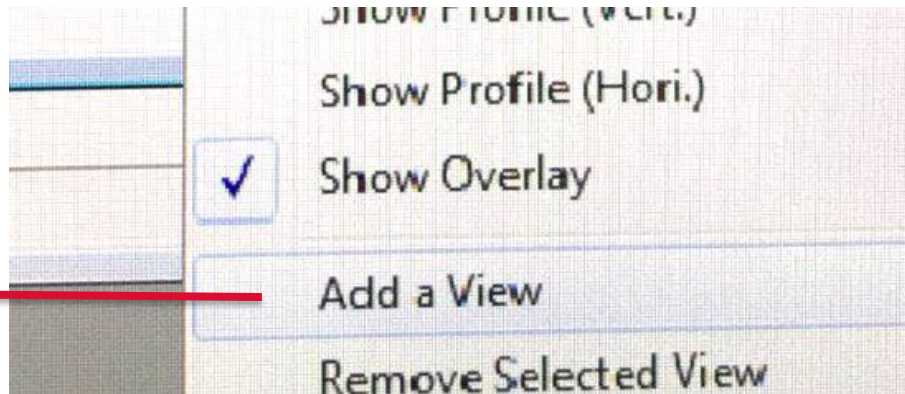
**Single/Three Planes/Tile button**

-  (Single) button: Image can be made in one display.
-  (Three planes) button: Section displays are added to one display and 3 Plain displays can be made.
-  (Tile) button: Tiled display can be made.

Size: 256x256 x,y=(252,2) Int.

# 增加一个视图窗口

图像上右键-add a view





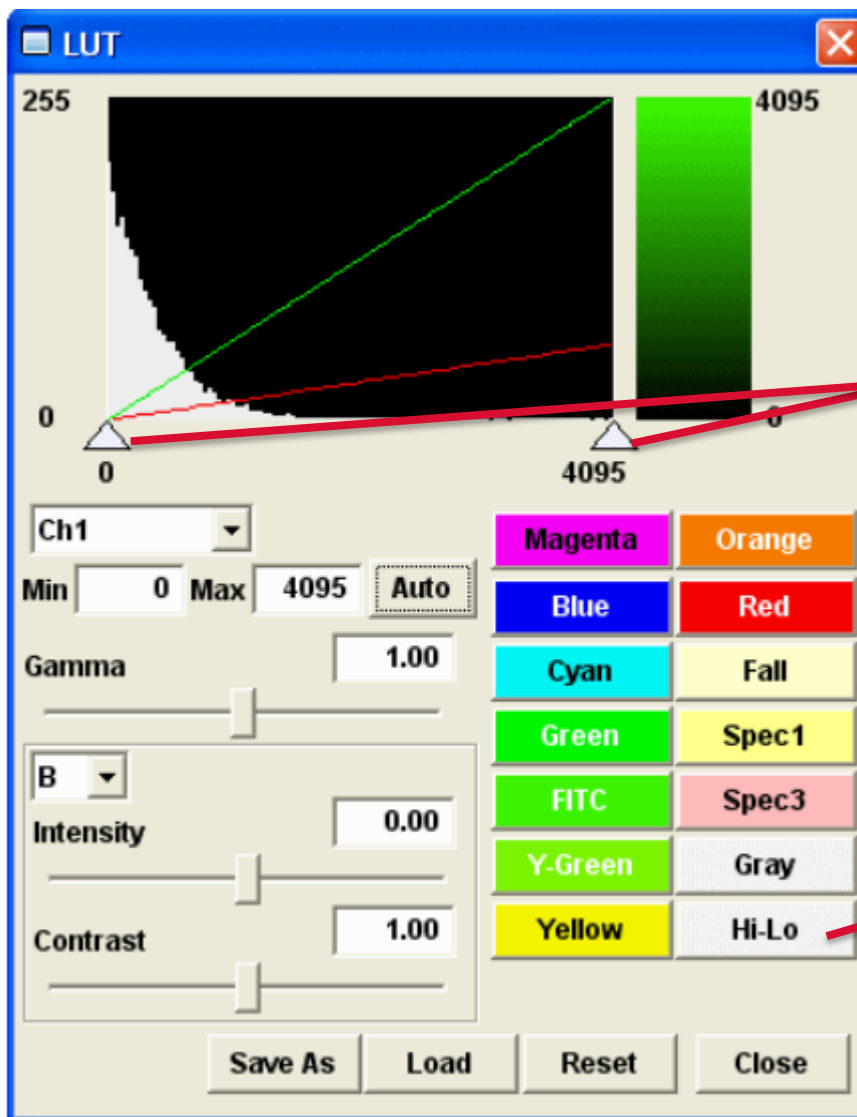
# LUT:调节显示

荧光图像：本质是灰度图，无颜色，为便于识别，添加伪彩色

显微图像采集原则：

不可过曝 不可过曝 不可过曝！

保存原始格式！




调亮度  
对比度

伪彩色色板

过曝指示模式，过曝像素显示为红色

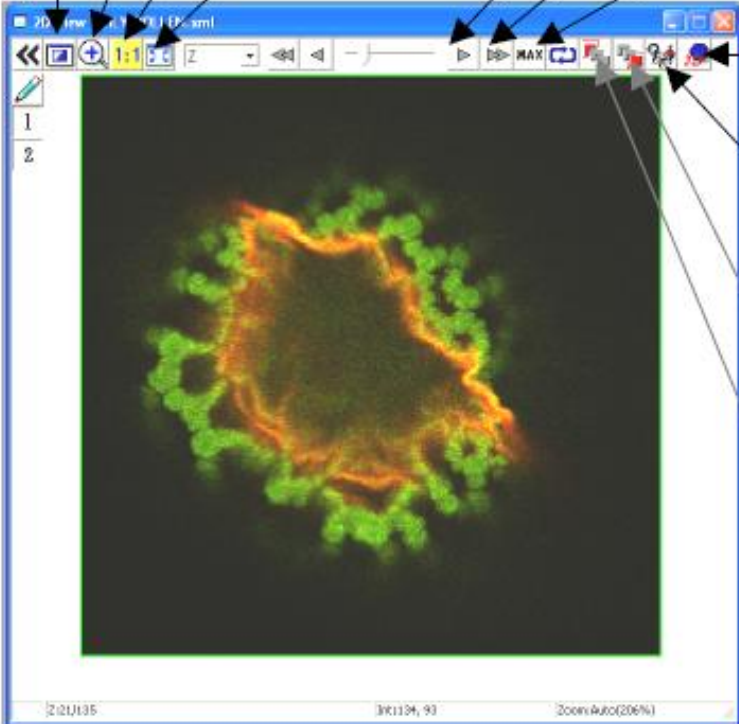
Ctrl+H

# ROI tool

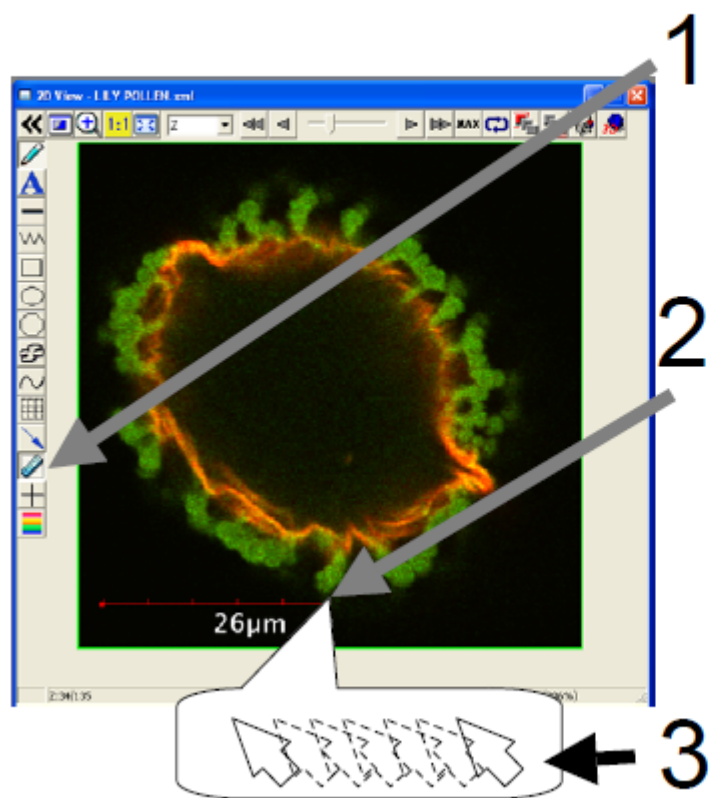
点击按钮  转换显示模式 显示状态的切换  
 放大 1:1显示 调整窗口的大小 帧的播放 动画 播放速度  
 3D重建 投影的切换 选择结束帧 选择开始帧

文本  
 不同形状的ROI区域  
 网格  
 箭头  
 比例尺  
 点  
 颜色条

1 Ch1 display  
 2 Ch2 display




# 图像分析 (比例尺的使用)



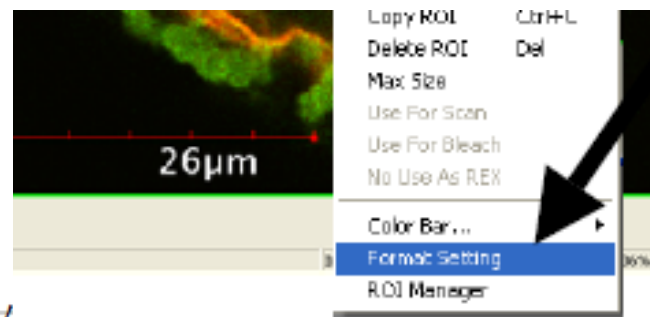
1. 点击按钮



2. 点击图像的同时, 拖放此比例尺到一个特定的位置.

3. 点击手柄的左端或右

改变比例尺的大小



更改比例尺属性

# 2D Control Panel

图像平铺展示逻辑

剖线工具，测量  
线段上的灰度值

**2D Control Panel**

**Stepping**

Z  / 18

T  / 10

L  / 10

A  /

**Tile**

**Automatic setting**

Z	T	Z	L	L	T	Z	Ch	Ch	T	Ch	L
Ch	Z	T	L								

**Manual setting**

Column  Z Intvl.

Row  T Intvl.

L Intvl.

Columns: 10 Rows: 2

**Multi Plane View**

Axis   Need Projection and Merge in Tile Mode

**Profile**

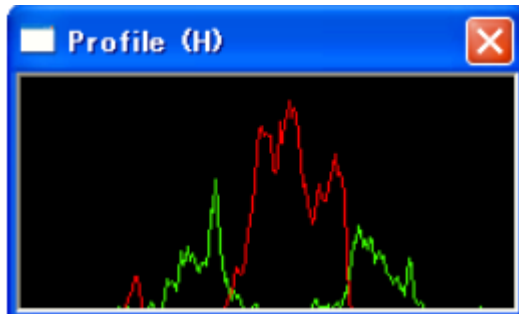
ON

**Live View**

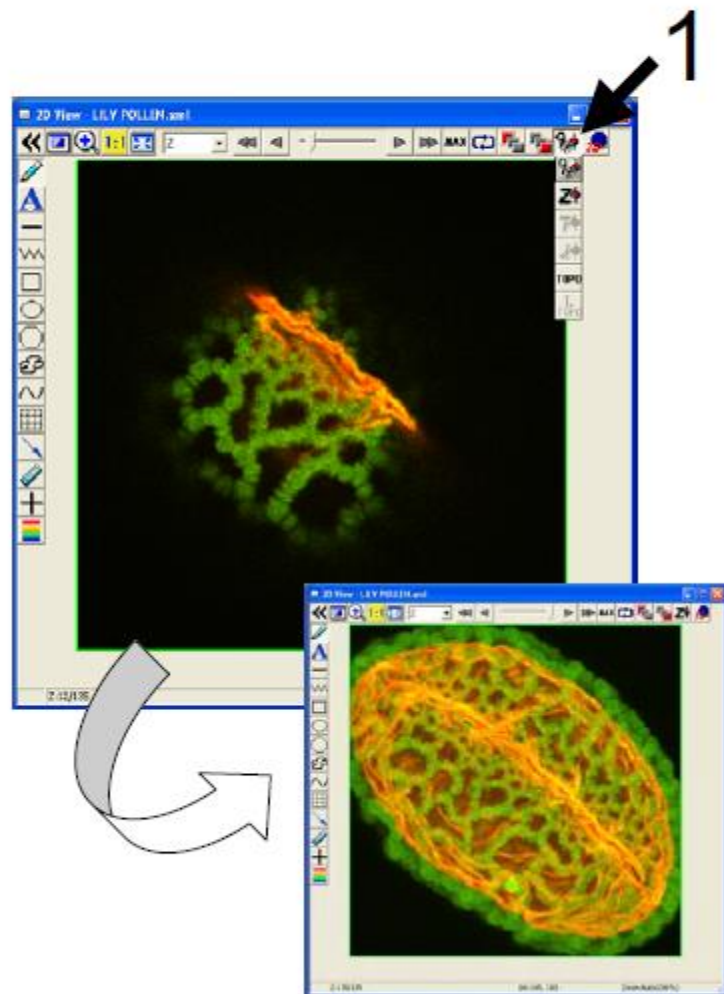
1

2

Size: 256x256 x,y=(252,2) Int:8






## 图像分析 (3D图像的叠加)



## 最大亮度投影

1. 点击按钮  并选择  .

-  (X intensity projection)
-  (Y intensity projection)
-  (Z intensity projection)

# 图像分析工具

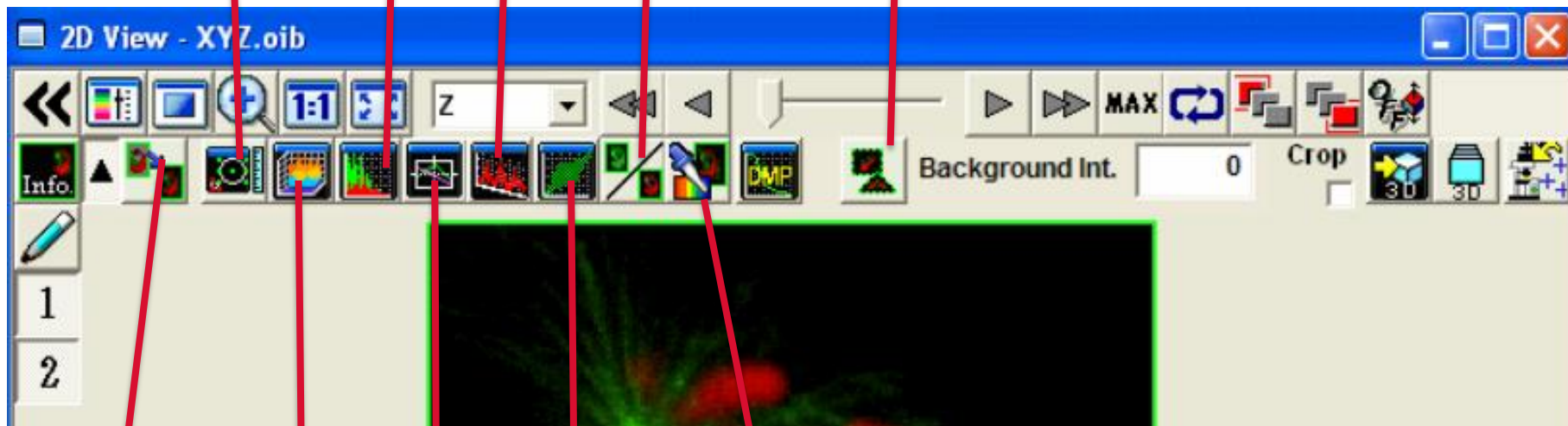
测量

序列图剖面

histogram

ratio

截图



亮度剖面

共定位

光谱拆分

序列图亮度分析

背景校正

Average in ROI  Background image

# 测量前提：图像上有ROI

当前活动ROI测量值

全部ROI 测量值

ROI汇总统计值

Region Measurement - Sample\_XYZ.TIF Z:7 T:0 L:0 ROI:3

Image: Sample\_XYZ.TIF Measure All ROIs

Measure	ROI No. 3	Statistics	Ch.1	Ch.2
CenterX	21.039	Integration	624129.000	2275653.000
CenterY	37.521	Average	480.099	1750.502
Area	97.144	Max	3692.000	4016.000
Perimeter	34.943	Min	0.000	0.000
		Range	3692.000	4016.000
		StdDev	429.250	1135.886
		3StdDev	1287.751	3407.657

Image Info  
Current  
Zpos :7  
Tpos :0  
Lpos :0

Add  
 Auto

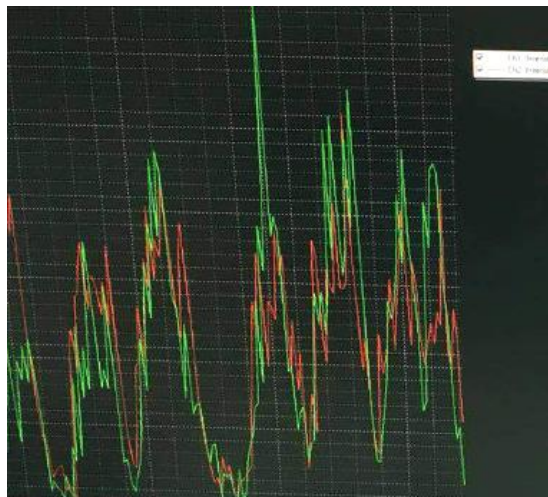
Display Table(Zpos:7,Tpos:0,Lpos:0)

ROI	CenterX [μm]	CenterY [μm]	Area [μm <sup>2</sup> ]	Perimeter [μm]	Radius [μm]	Diameter [μm]	Inte
1	36.449	26.533	216.853	52.202	8.308	16.616	3297
2	16.348	14.874	100.754	35.892			1668
3	21.039	37.521	97.144	34.943			624

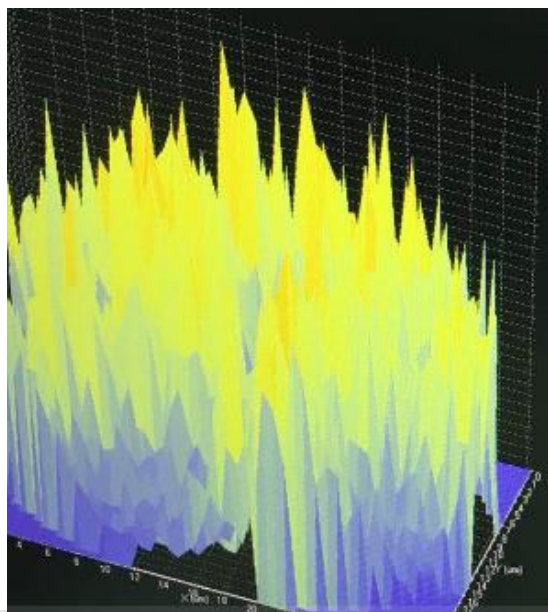
Count	3	3	3	3	1	1
Average	24.612	26.309	138.250	41.012	8.308	16.616
Max	36.449	37.521	216.853	52.202	8.308	16.616
Min	16.348	14.874	97.144	34.943	8.308	16.616
Range	20.101	22.647	119.709	17.259	0.000	0.000
StdDev	10.516	11.325	68.096	9.702	0.000	0.000
3StdDev	31.548	33.975	204.287	29.107	0.000	0.000

Save Histogram Clear Close

# 亮度剖面

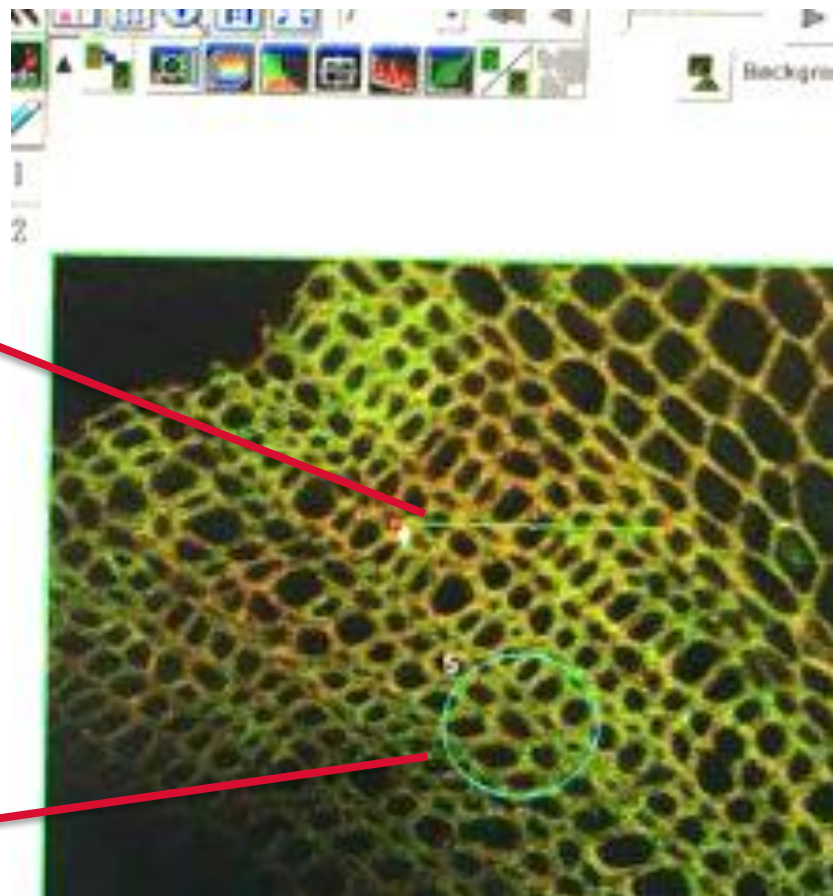


线ROI



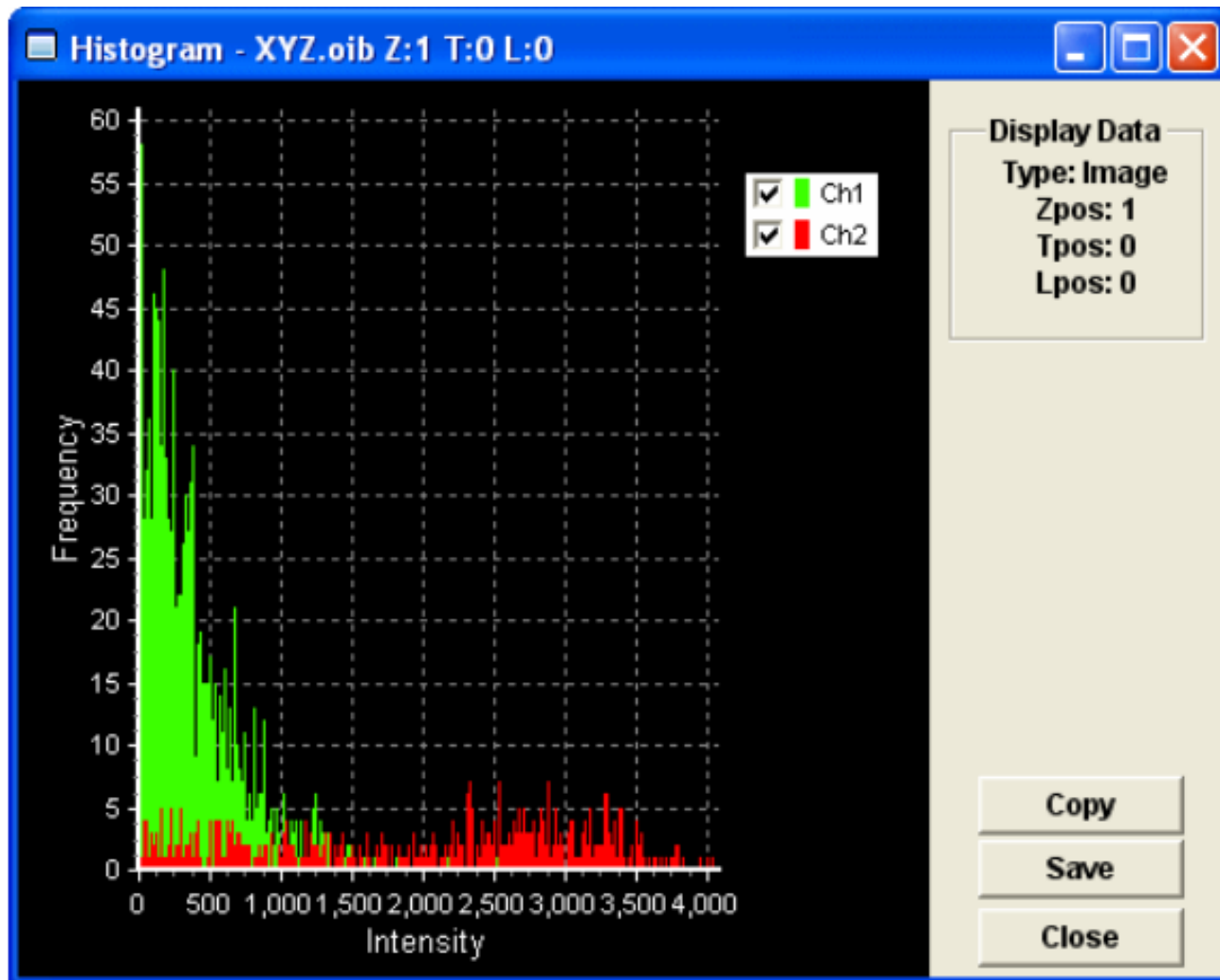
形状ROI

高度代表灰阶值





# histogram

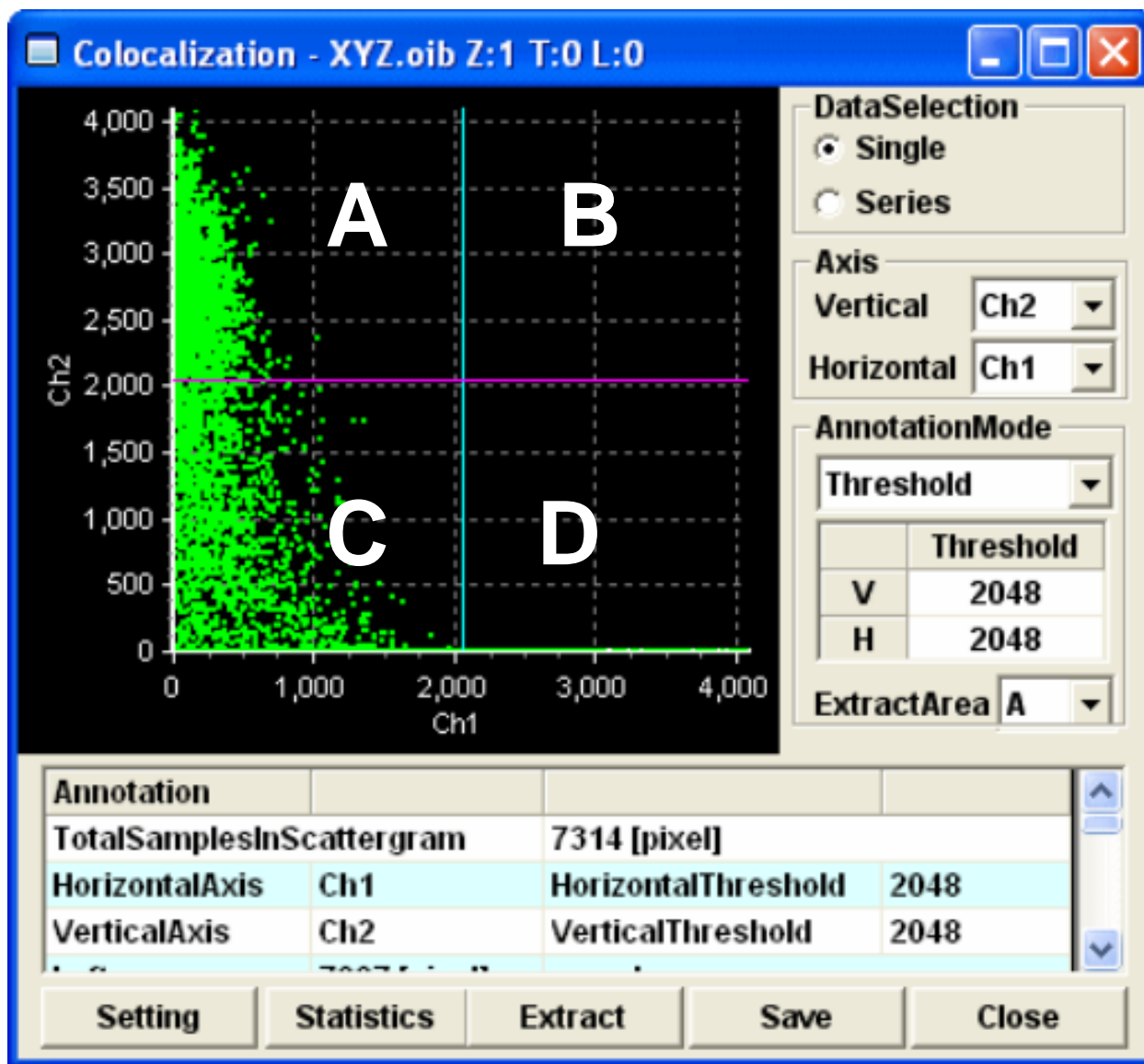


直观展示图像中亮暗信号的占比

# 共定位分析

设定阈值

信号在B象限分布情况



# 共定位分析结果

$R_r$ , 共定位系数

$R_o$ , 重叠系数

**Colocalization Statistics** ✕

**Statistics by Region** Thresh.  Thresh.

ROI	Area (pixels^2)	Pearson's Coeff.	Overlap	Overlap Index 1	Overlap Index 2	Coloc. Index 1	Coloc. Index 2
1	549	-0.27975	0.1541	0.0050487	4.7036	0	0
2	5935	-0.447	0.38099	3.6402	0.039876	0.41368	0

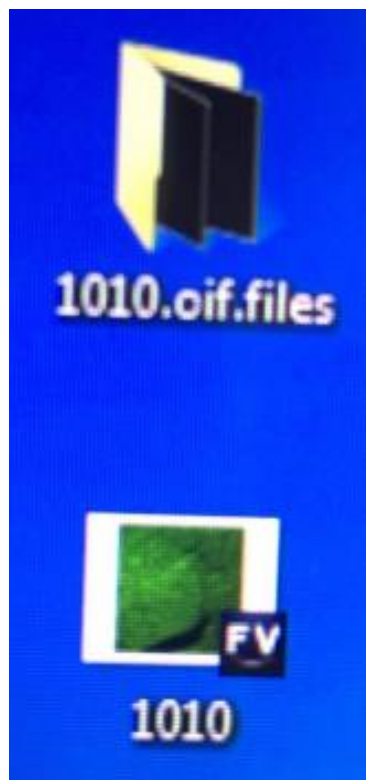
**Cumulative Statistics**

	Area (pixels^2)	Pearson's Coeff.	Overlap	Overlap Index 1	Overlap Index 2	Coloc. Index 1	Coloc. Index 2
Count	2	2	2	2	2	2	2
Average	3242	-0.36337	0.26755	1.8226	2.3718	0.20684	0
Max	5935	-0.27975	0.38099	3.6402	4.7036	0.41368	0
Min	549	-0.447	0.1541	0.0050487	0.039876	0	0
Range	5386	0.16725	0.22689	3.6351	4.6638	0.41368	0
StdDev.	2693	0.083625	0.11345	1.8176	2.3319	0.20684	0
3StdDev.	8079	0.25087	0.34034	5.4527	6.9956	0.62052	0

# 图像保存

OLYMPUS默认格式：.OIB .OIF，原始格式包含全部拍摄信息，可直接调用参数

OIF格式为 图标+文件=一份图像文件

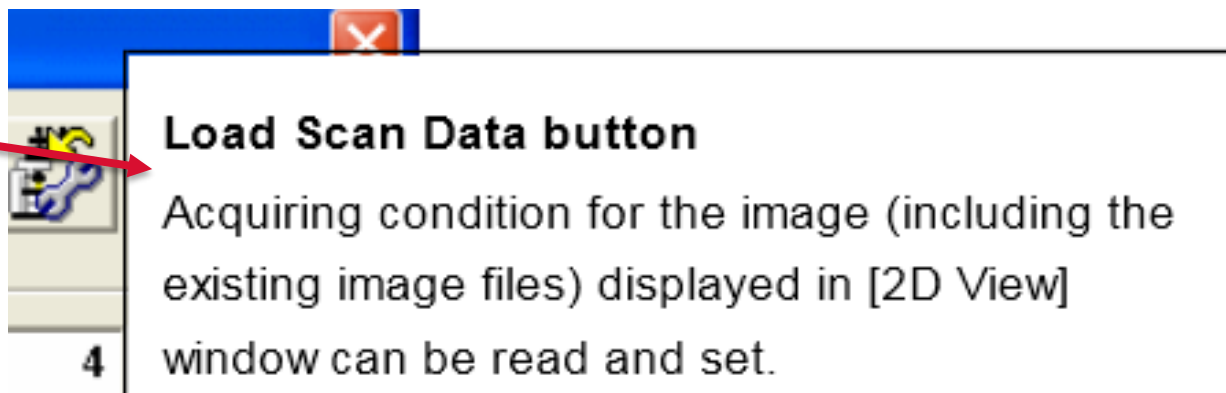
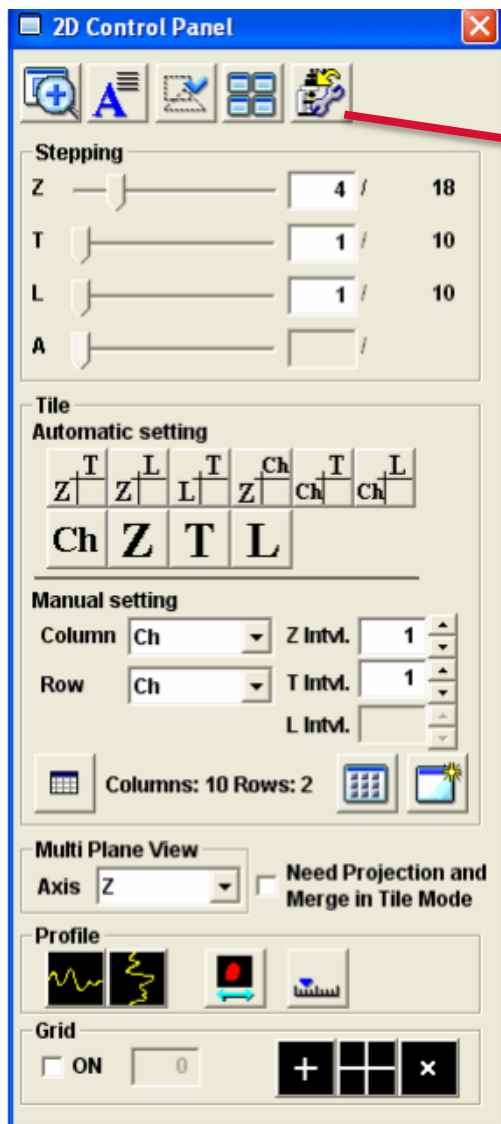


**Data Manager**

Image Processing applied Properties

Item	Information
[General]	
Path	F:\SampleImages\ImageData\
Name	Sample_XYZT.TIF *
Scan Mode	XYZT
Date	19/07/1996 13:32:13.000
System Name	FluoView
System Version	Version 4.3.14, w/ TIEMPO
[Image]	
Primary Dimensions	X * Y
Image Size	214 * 247 [Pixel]
Image Size(Unit Converted)	57.353498 [um] * 66.197729 [um]
Bits / Pixel	12 [bits]
[Dimensions]	
X Dimension *	214, 0.0 - 57.085491 [um], 0.268007 [um/Pixel]
Y Dimension *	247, 0.0 - 65.929722 [um], 0.268007 [um/Pixel]
Channel Dimension	2, 1.0 - 2.0 [Ch]
Z Dimension	16, 0.0 - 7.5 [um]
T Dimension	8, 0.0 - 0.007 [s]

# 调用图像采集参数



调用已采集图像的拍摄参数，应用于当前实验采集，保证实验的可比性

# Export

A:图像位深, 保存位置

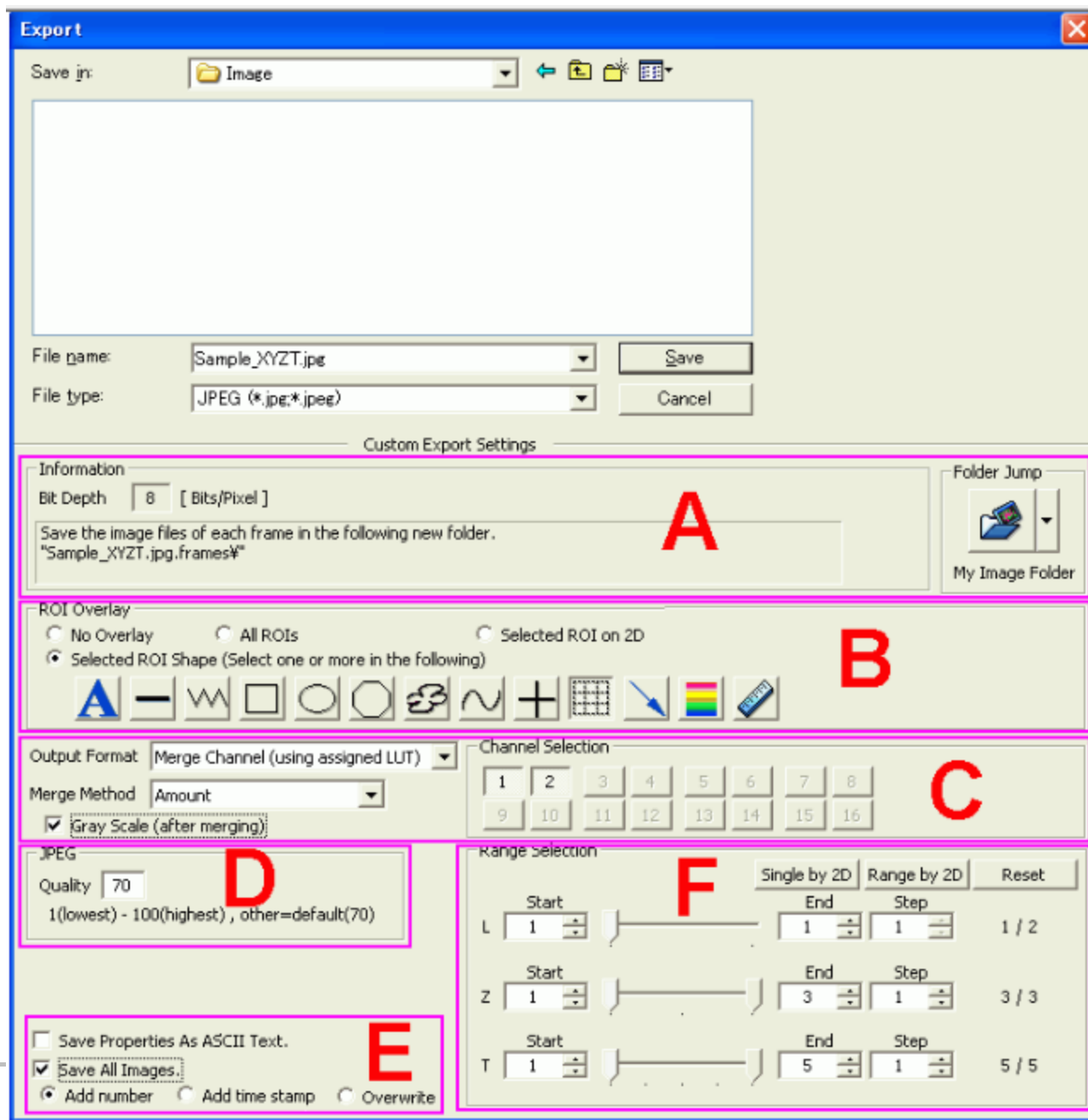
B:ROI叠加区域, 选中的ROI将向下合并图层

C: 导出格式、通道选择

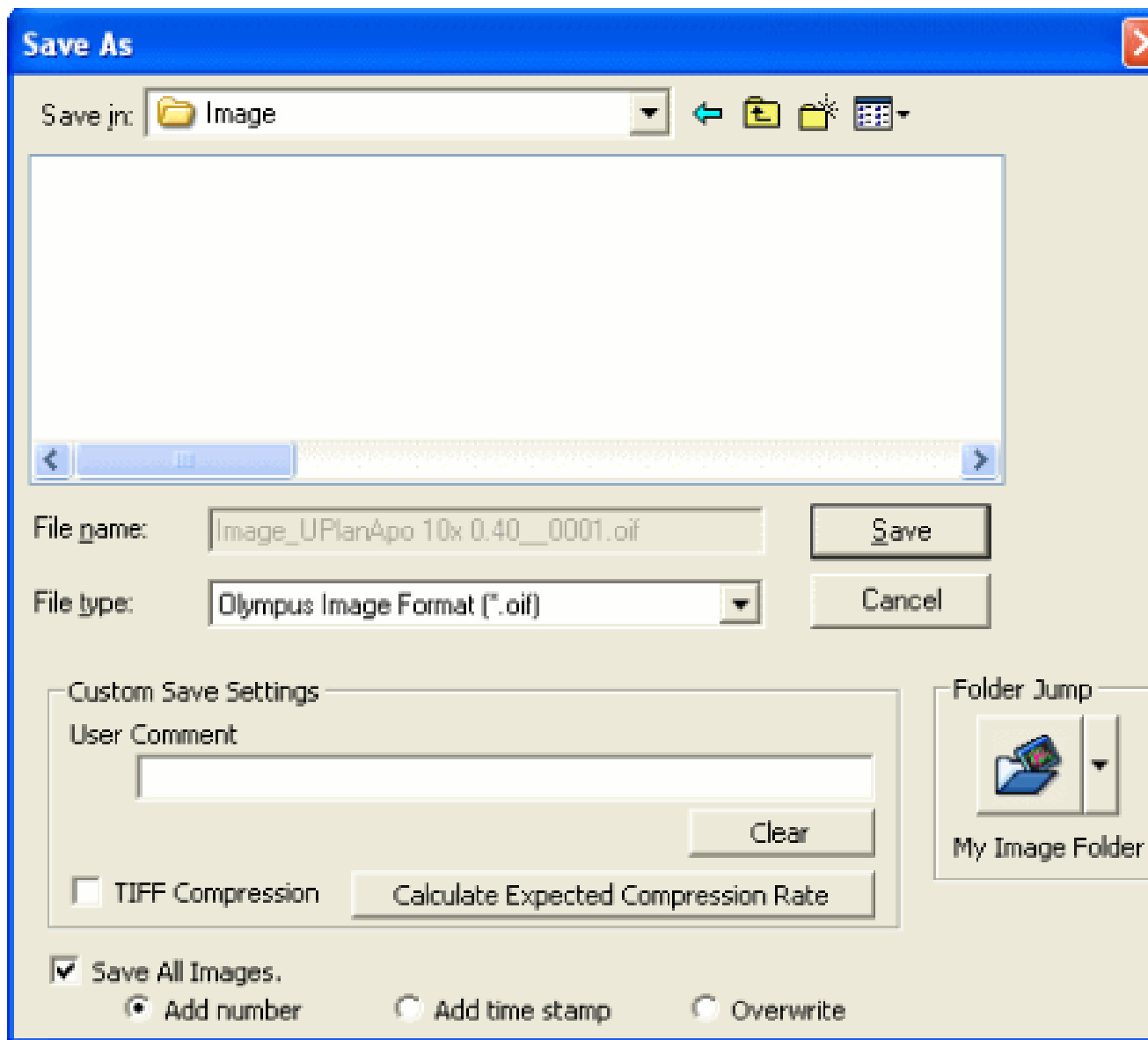
D:图像质量

E: 批量选项

F: 选维工具区



# Save as



**OLYMPUS**

Your Vision, Our Future



**谢谢！**