

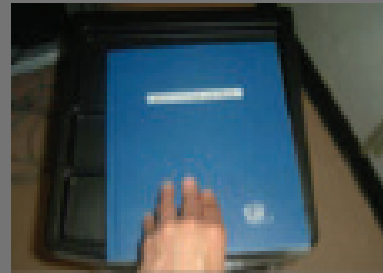
# How to Operate

- ① Turn on system
- ① Light-path adjust
- ① Observation
- ① Image Scan
- ① Z-section
- ① Time-lapse
- ① Dual Beam operation



# Turn on system

填寫登記簿



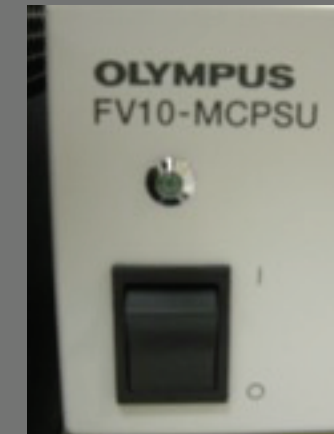
開啟AR雷射



開啟559雷射



開啟雷射總控制模組



開啟電動顯微鏡控制器



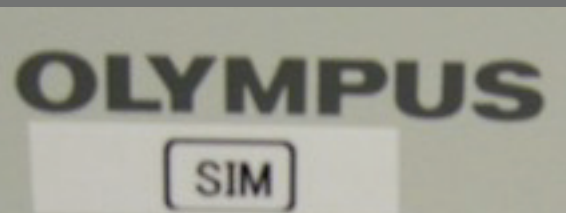
開啟螢光燈



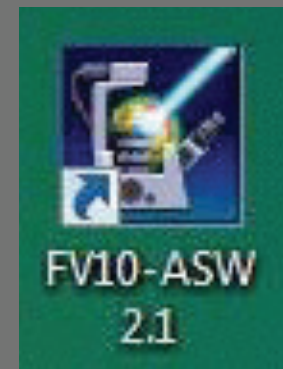
開啟電動載物臺控制器



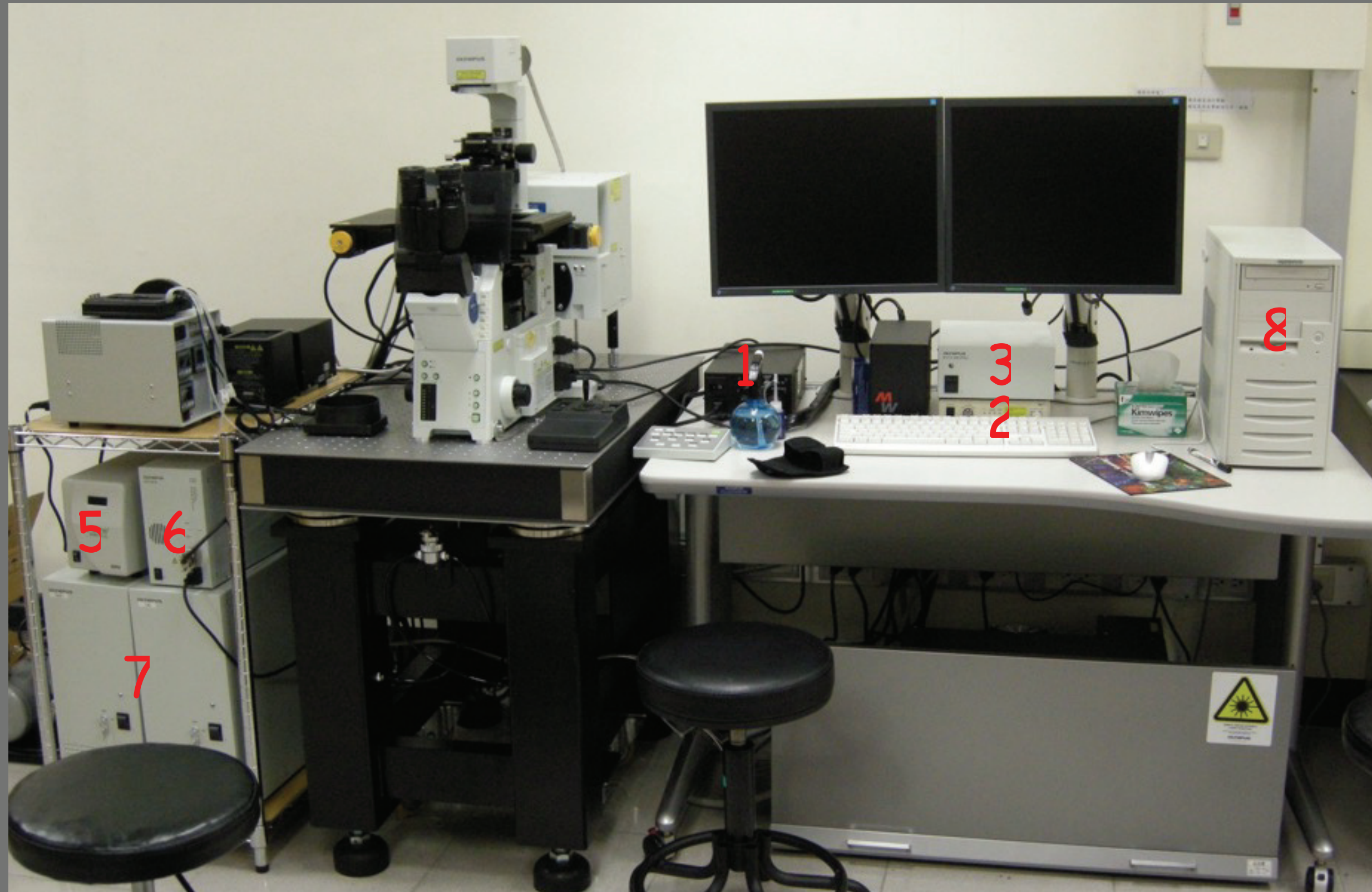
開啟掃描控制器



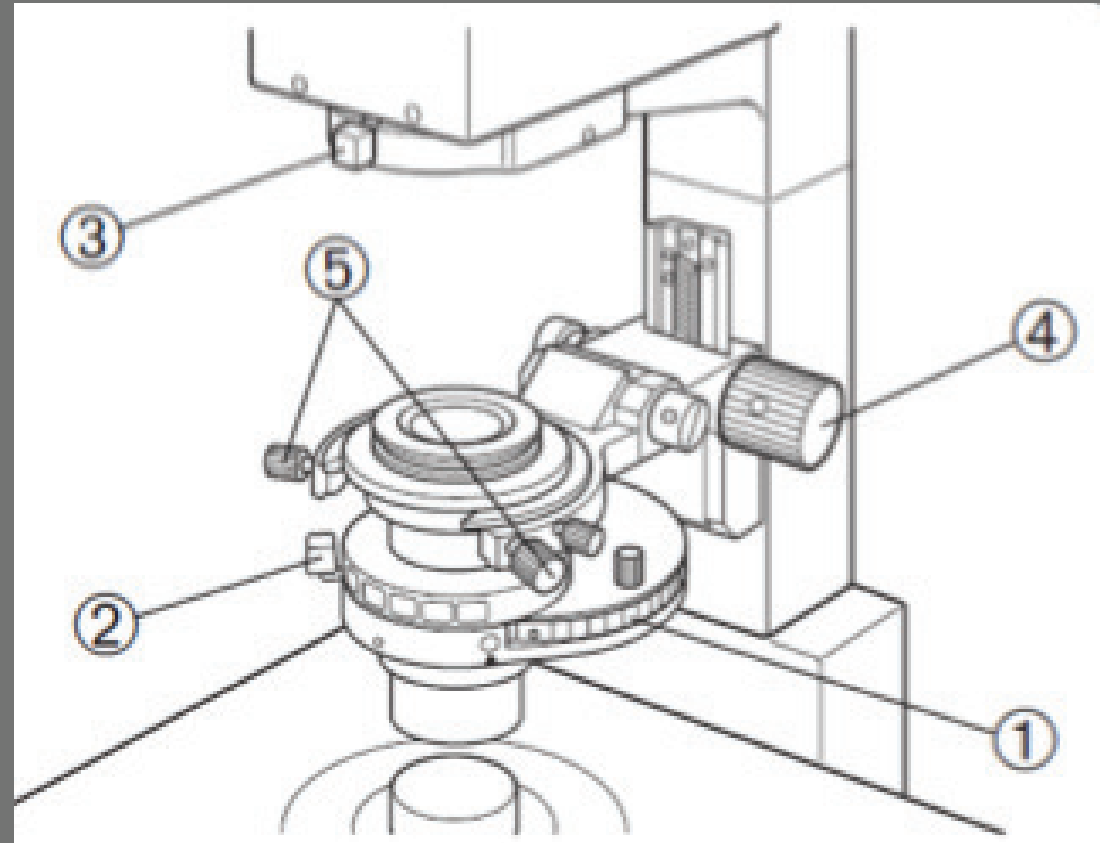
開啟電腦及軟體  
(Cleaning.....)



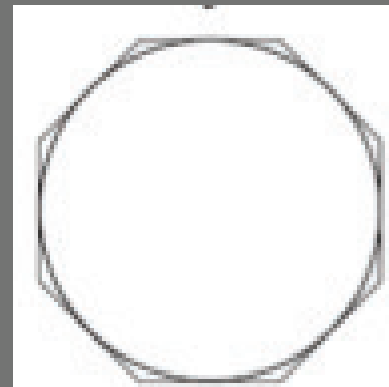
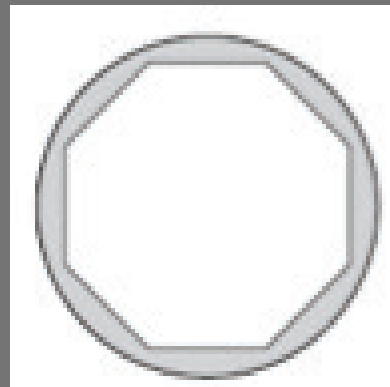
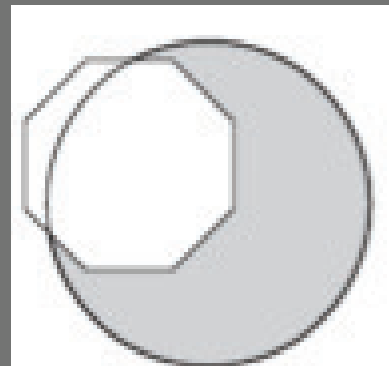
# Turn on system



# Light-path adjust



- 以10倍鏡頭找到焦距
- 將3關到最小
- 調整4到八角型最清晰
- 調整5光圈中心
- 將3開回最大原位



# SW Interface

The screenshot displays the Olympus Fluoview software interface with several key areas highlighted by red boxes:

- HW Setting:** Located on the left side, this panel includes controls for Mode, Speed (Fast/Slow), Laser power (405, 440, 488, 515, 568 nm), and ZDC settings.
- Image Acquire Setting:** Located in the top center, this panel includes Focus (x2, x4), XY Repeat, XY, Stop, and various acquisition parameters like Size, Aspect Ratio, and Area.
- Dual Beam Setting:** Located in the middle right, this panel includes Use Scanner, Mode, Speed, Laser power, and Wait Time (Frames, Lines, msec).
- Image Area:** Located on the far right, this panel shows a Live View window with a toolbar for navigation and zooming.

Other visible windows include Data Manager and Explorer, which shows a file tree structure for the current project.

# Observation

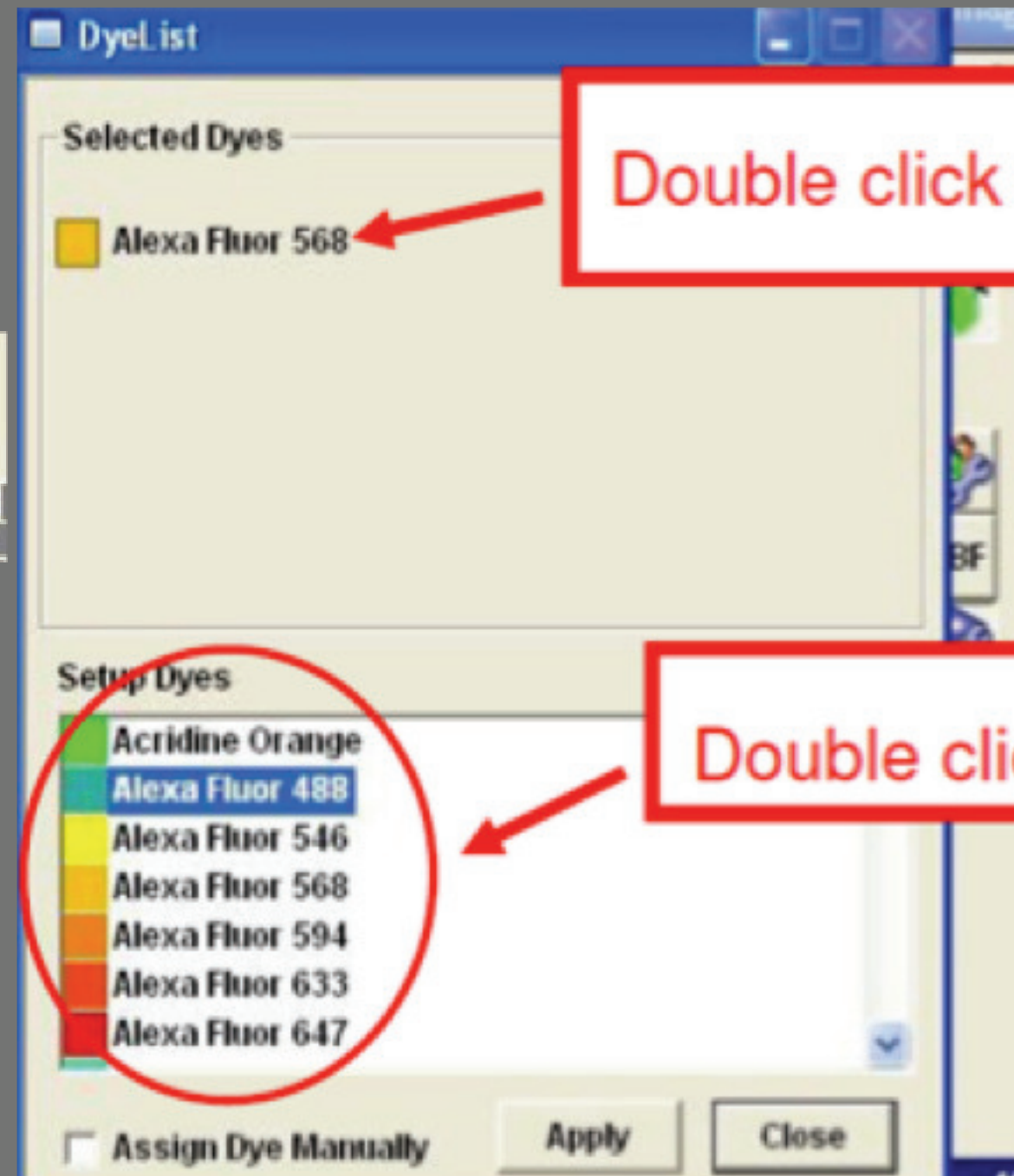
The screenshot shows the 'ImageAcquisition' software interface. Three red callout boxes with arrows point to specific controls:

- Click here for bright field**: Points to the 'VBF' (Bright Field) icon on the left sidebar.
- Click here for epi-fluorescence**: Points to the 'Focus' control area at the top of the interface.
- Bright field lamp intensity control**: Points to the 'Lamp' slider in the 'TD' (Teledyne) section, which is currently set to 41.

The interface includes several control panels:

- Focus xz**: Focus and Depth controls.
- Channels (CHS)**: CHS1 (Alexa Fluor 488), CHS2 (Alexa Fluor 568), CHS3, and TD1. Each channel has HV, Gain, and Offset sliders, and a Laser intensity control.
- SU (Shutter)**: C.A. (Camera) and Lamp intensity control.
- Filter Mode**: Kalman, Line, Frame (set to 2), Analog Int, and Photon Cnt.
- Sequential**: A checkbox at the bottom left.

# Image Scan



Double click to remove.

Double click to select.

# Image Scan

The screenshot shows the 'AcquisitionSetting' window with several key parameters highlighted by red boxes and arrows:

- scan mode:** A red box highlights the 'Fast' mode icon (three horizontal arrows) in the 'Mode' section.
- Scan speed:** A red box highlights the '8.0us/Pixel' value in the 'Mode' section.
- 512 x 512:** A red box highlights the '512 by 512' resolution value in the 'Size' section.
- zoom:** A red box highlights the 'Zoom' control (a vertical slider) in the 'Area' section.
- When setup is correct:** A red box highlights the 'Leach start-stop by Key' section in the bottom right.
- focus scan mode (fast scan):** A red box highlights the 'Laser' control in the bottom left.

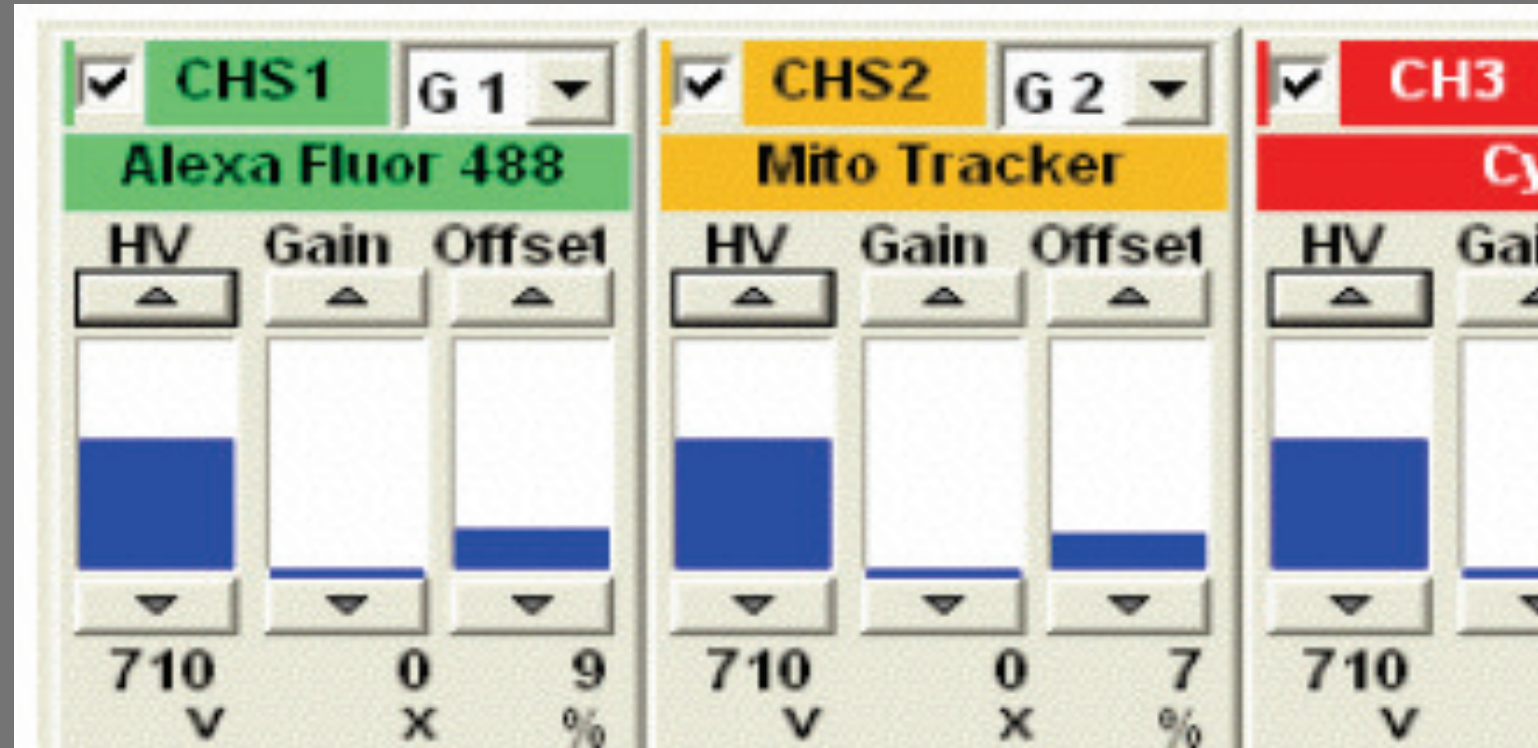
Other visible parameters include: Aspect Ratio (1:1), PanX (65 um), PanY (-2 um), Rotation (0), and various control buttons like 'Leach', 'Stop', 'C.A', and 'Lamp'.



# Image Scan

The screenshot shows the 'ImageAcquisitionControl' software interface. The top toolbar contains several icons: 'Focus x2', 'Focus x4', 'XY Repeat', 'XY', 'LZ', and 'Stop'. The 'Stop' icon is circled in red, with a red box containing the text 'Click stop when setup is correct'. The 'XY Repeat' icon is also circled in red, with a red box containing the text 'Repeat image for adjusting focus'. The 'Focus x2' icon is circled in red, with a red box containing the text 'focus scan mode (fast scan)'. The 'XY' icon is circled in red, with a red box containing the text 'Click here to start.'. The interface also shows various control panels for channels (CHS1, CHS2, CH3, TD1), gain, and other parameters like 'Lambda', 'Depth', 'Time', 'Bleach', 'SU', and 'TD'. The 'SU' parameter is set to '80 um' and 'TD' is set to '41 %'. The 'Dye List' shows 'Alexa' and 'HV'.

# Image Scan

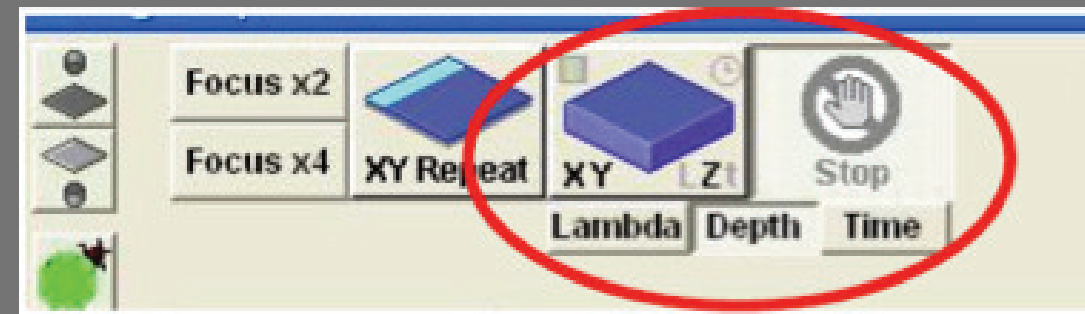


**HV** = PMT 電壓，增加 HV 會提高檢測敏感度，但圖像噪音也會隨之增加

**Gain** = 後期信號放大，在圖像信號極低的情況下可以適當調節

**Offset** = 影像位準調整，Offset 越高，圖像背景變暗，但一些位準以下的螢光信號也可能被同時扣除

# Z-Section



A detailed software control panel for Z-sectioning. The panel includes a 3D schematic of a microscope objective and a sample, with a red double-headed arrow indicating a 20.81um distance. The main control area has several input fields and buttons:

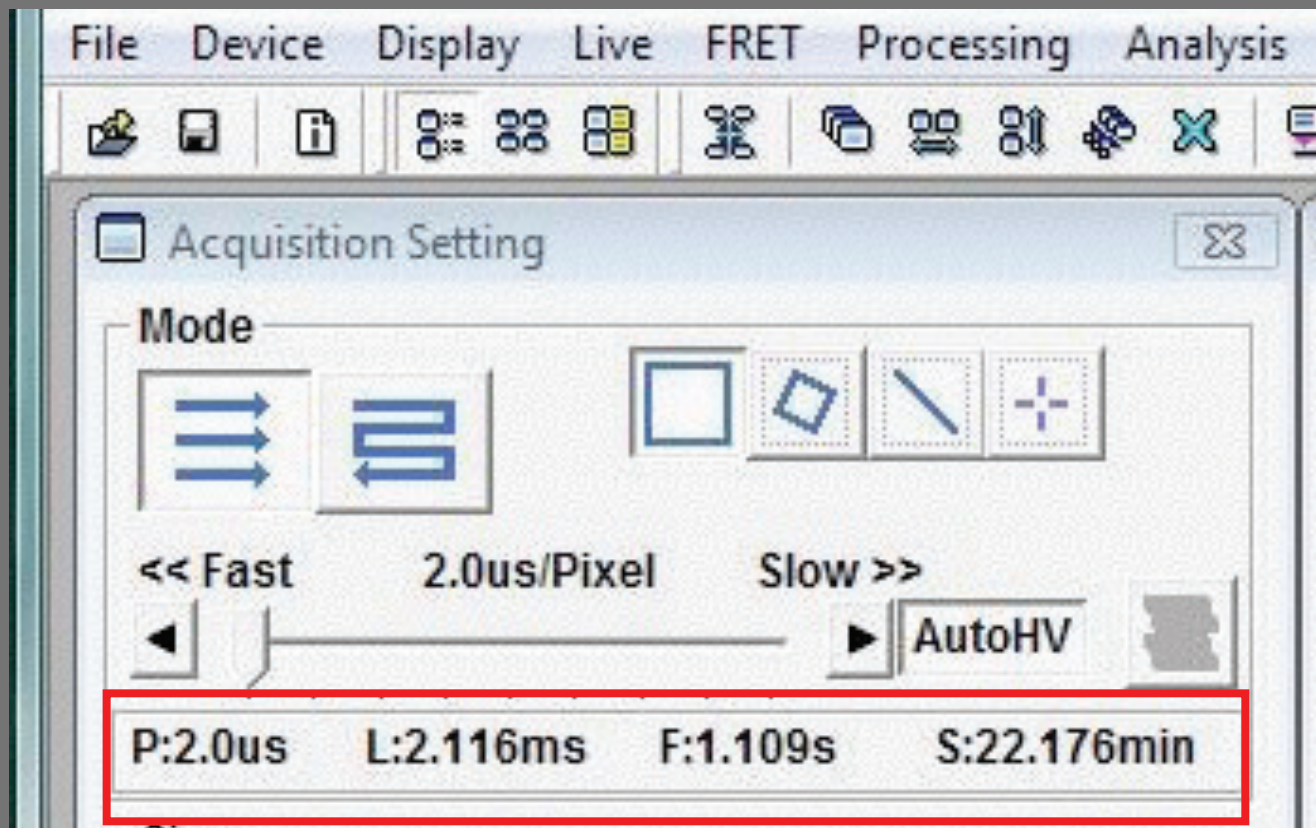
- Start Set:** -39.78 um (circled in red)
- StepSize:** 0.01 um (circled in red)
- End Set:** -29.34 um (circled in red)
- Slices:** 13 (circled in red)

Other controls include 'Go' buttons, 'Clear Start/End', 'Focus Handle On', and 'Escape'. Three callout boxes with Chinese text point to specific fields:

- Callout 1: 找到上平面後按 set (Point to Start Set)
- Callout 2: 找到下平面後按 set (Point to End Set)
- Callout 3: 設定掃描間隔或掃描張數 (Point to StepSize)

At the bottom, the current coordinates are displayed: X:0.504um Y:0.504um Z:8.038um.

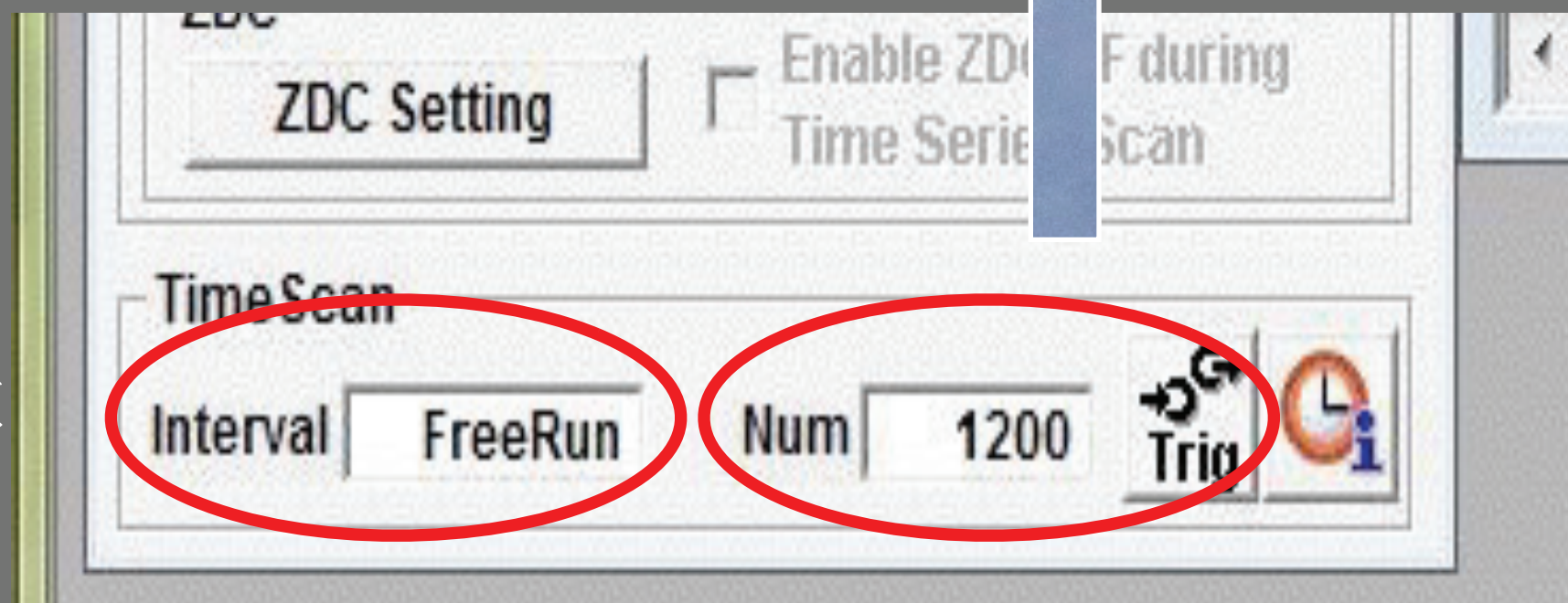
# Time Lapse



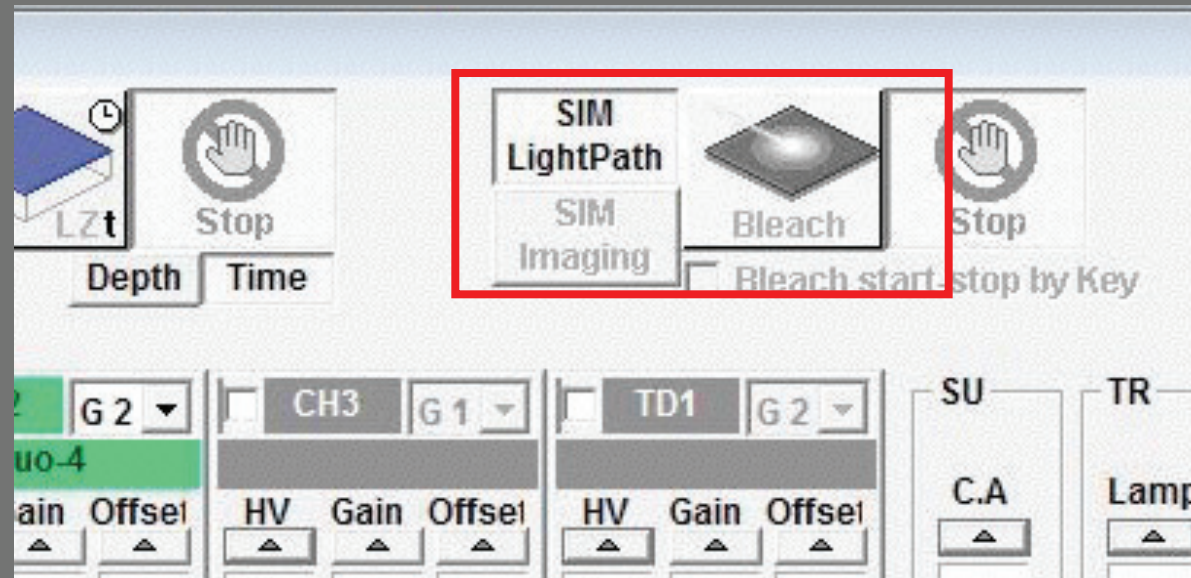
重複次數



時間間隔



# Dual Beam Operation



The 'Stimulus Setting' dialog box is shown, detailing the configuration for the SIM scanner. The 'Use Scanner' section has 'SIM' selected. The 'Mode' section shows a red circle around the SIM icon. The 'Laser' section is set to 405 nm at 50.0% intensity. The 'Stimulate Start Setting' section is set to 'Main Scanner Sync' with 'ImageScan --> Activation' selected. A timing diagram shows 'Wait Time' (green bar), 'Image Scan' (blue bar), and 'Activation' (red bar) with a 1000.000 msec interval. The 'Wait Time' is configured as 6 Frames + 250 Lines + 0.000 msec. A 'Close' button is at the bottom right.

Stimulus Setting

Use Scanner  
 None  Main  SIM

Mode  
 SIM

<< Fast 10.0us/Pixel Slow >>

P:10.0us L:10.000ms F:0.010s S:0.010s

Laser  
 405 50.0 %

Stimulate Start Setting  
 Manual  Auto Stop 1 Frame

Main Scanner Sync  
 ImageScan --> Activation  
 Activation --> ImageScan

Wait Time  
Image Scan  
Activation

1000.000 msec

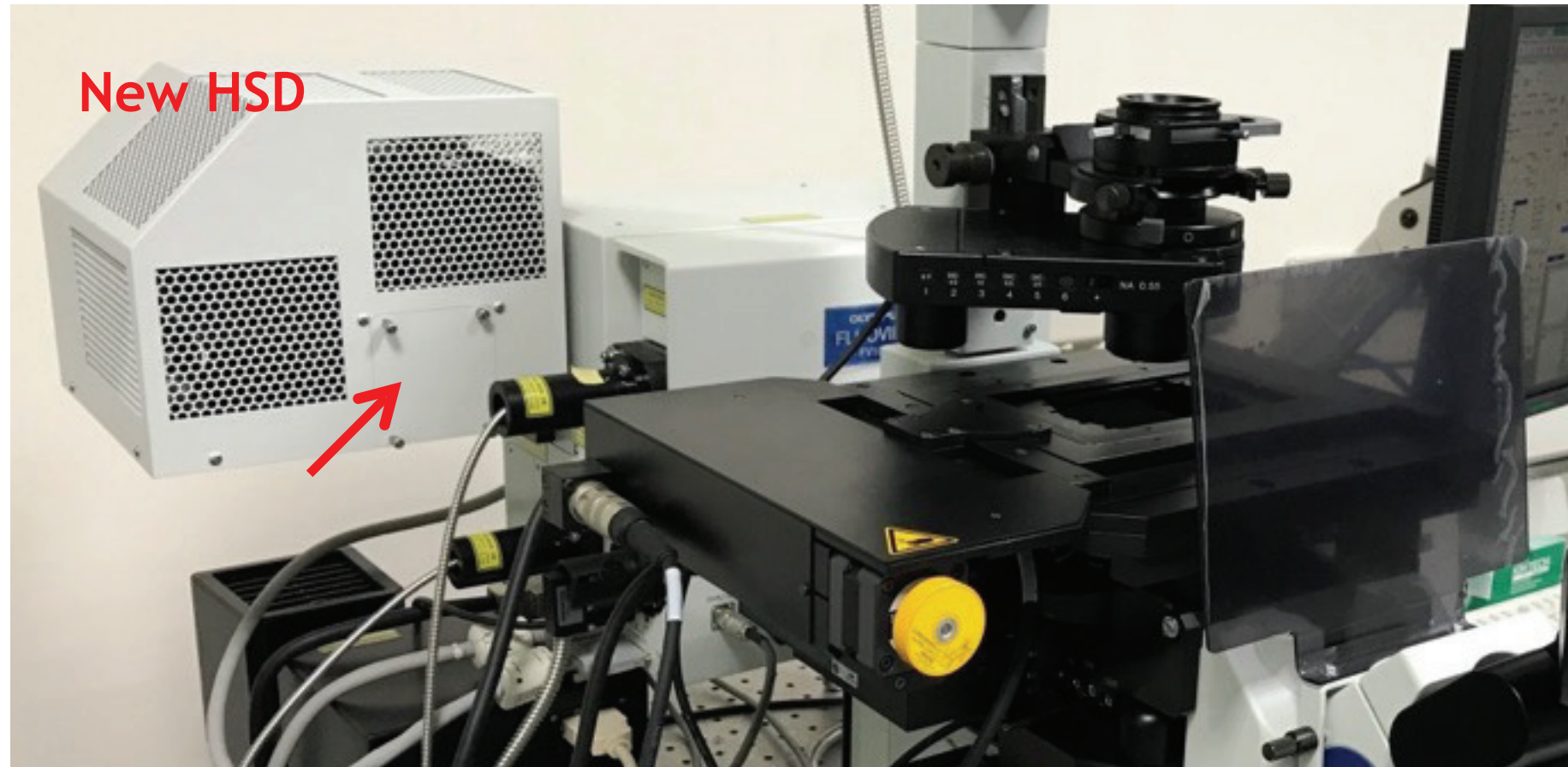
Wait Time  
6 + 250 + 0.000  
Frames Lines msec

Close

OLYMPUS

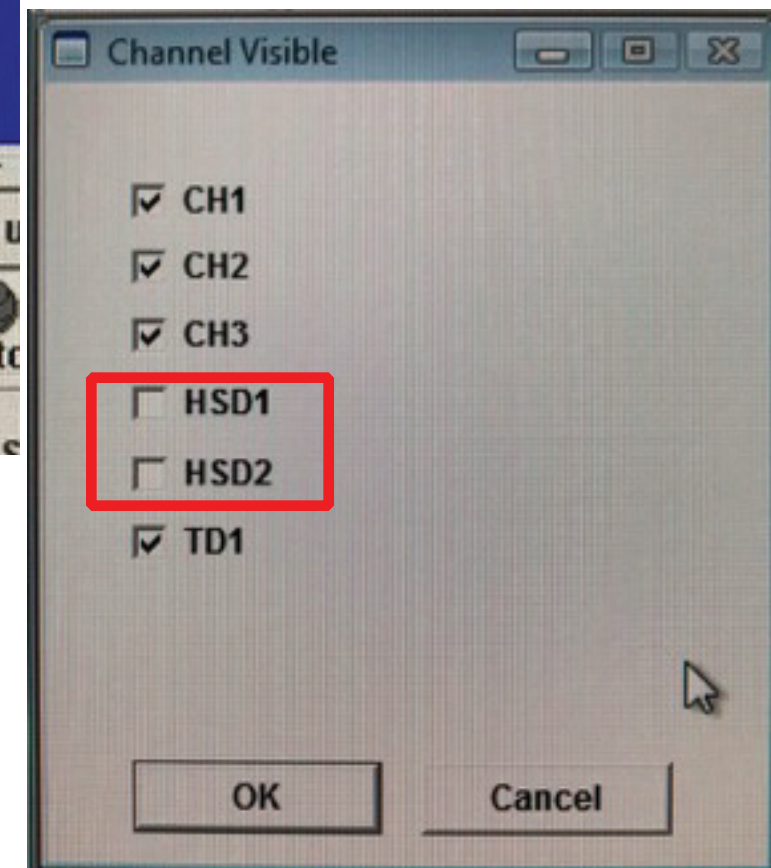
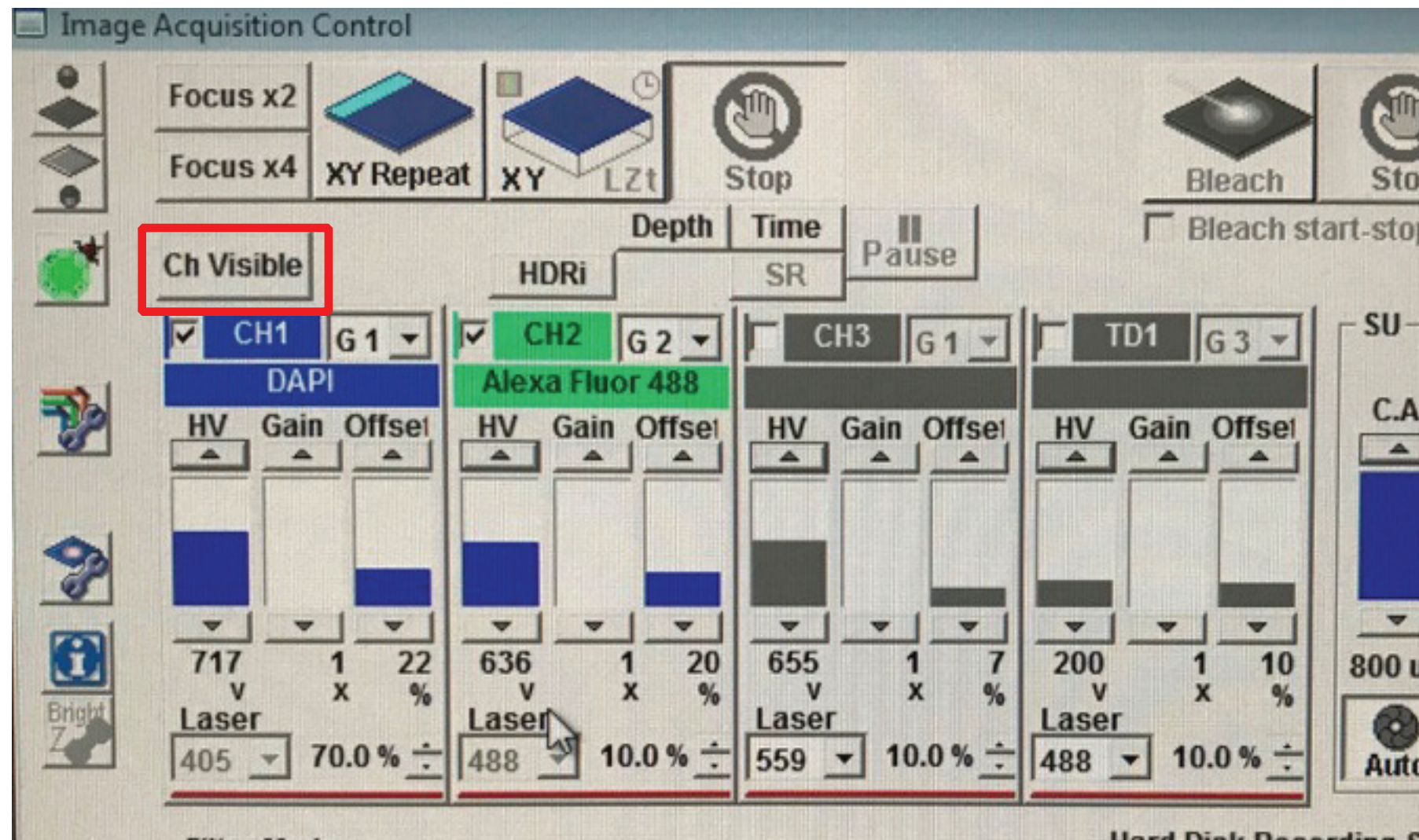
Our Vision, Our Future

# HSD 的使用



1. 開關機請依照原本的方式即可。
2. 更換濾片請將紅色箭頭所指處的外殼卸下 (四顆螺絲，手轉即可)→放鬆濾片匣固定螺絲後將濾片匣取出→更換濾片匣→鎖緊濾片匣固定螺絲後蓋上外殼，完成更換。

# 選取HSD 偵測器



若是開啟軟體之後看不到 HSD 的選項，請選擇 Ch Visible，選取 HSD1 及 HSD2 即可看到 HSD1 及 HSD2。

Focus x2 Focus x4 XY Repeat XY LZ1 Stop Bleach Stop Auto Contrast

Ch Visible HDRi Depth Time SR Pause  Bleach start-stop by Key

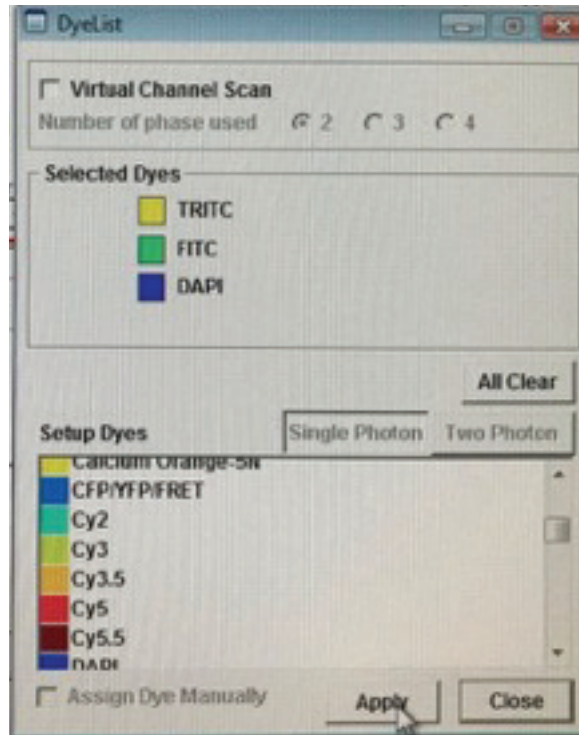
CH1	CH2	CH3	HSD1	HSD2	TD1
DAPI	Alexa Fluor 488				
HV Gain Offset	HV Gain Offset	HV Gain Offset	HV Gain Offset	HV Gain Offset	HV Gain Offset
717 1 22	636 1 20	655 1 7	730 1 10	690 1 6	200 1 10
V X %	V X %	V X %	V X %	V X %	V X %
Laser	Laser	Laser	Laser	Laser	Laser
405 70.0 %	488 10.0 %	559 10.0 %	488 10.0 %	405 70.0 %	488 10.0 %

Hard Disk ... Settings

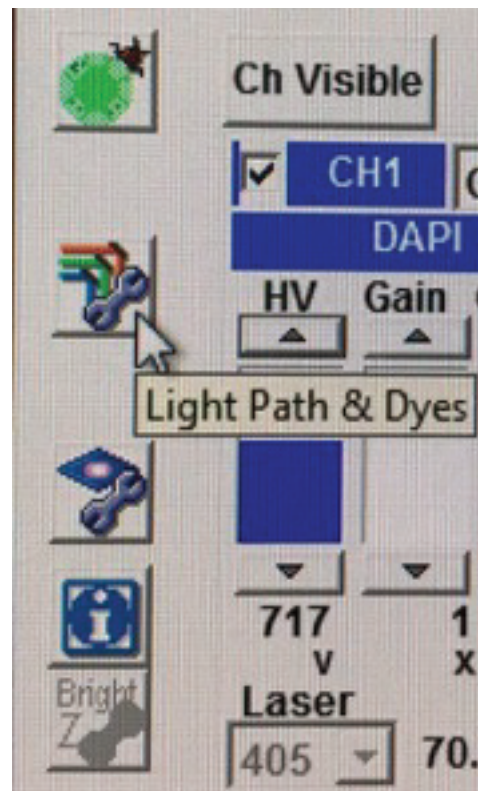




# HSD 光路設定

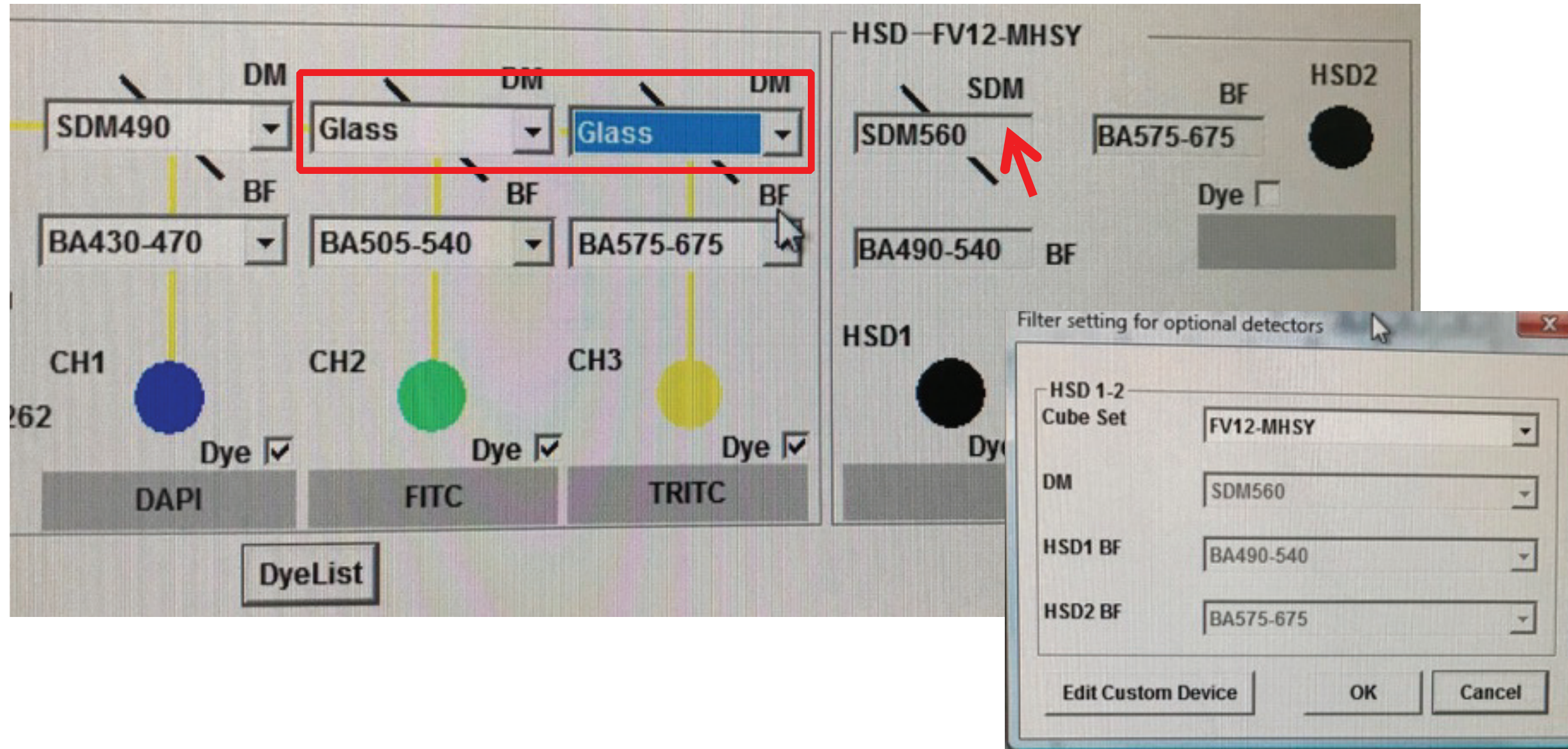


1. 一開始請照舊，選擇 Dye List，並選擇樣本的螢光種類。  
在此以 DAPI，FITC & TRITC 為例。



2. 選擇 Light Path & Dyes，進行光路設定

# HSD 光路設定



3. 在此我們將 DPAI 維持原本的 PMT，將 FITC & TRITC 設定以 HSD1 及 HSD2 擷取影像。因此將紅框處更改為 Glass，讓訊號可以通過進入 HSD1 & HSD2。

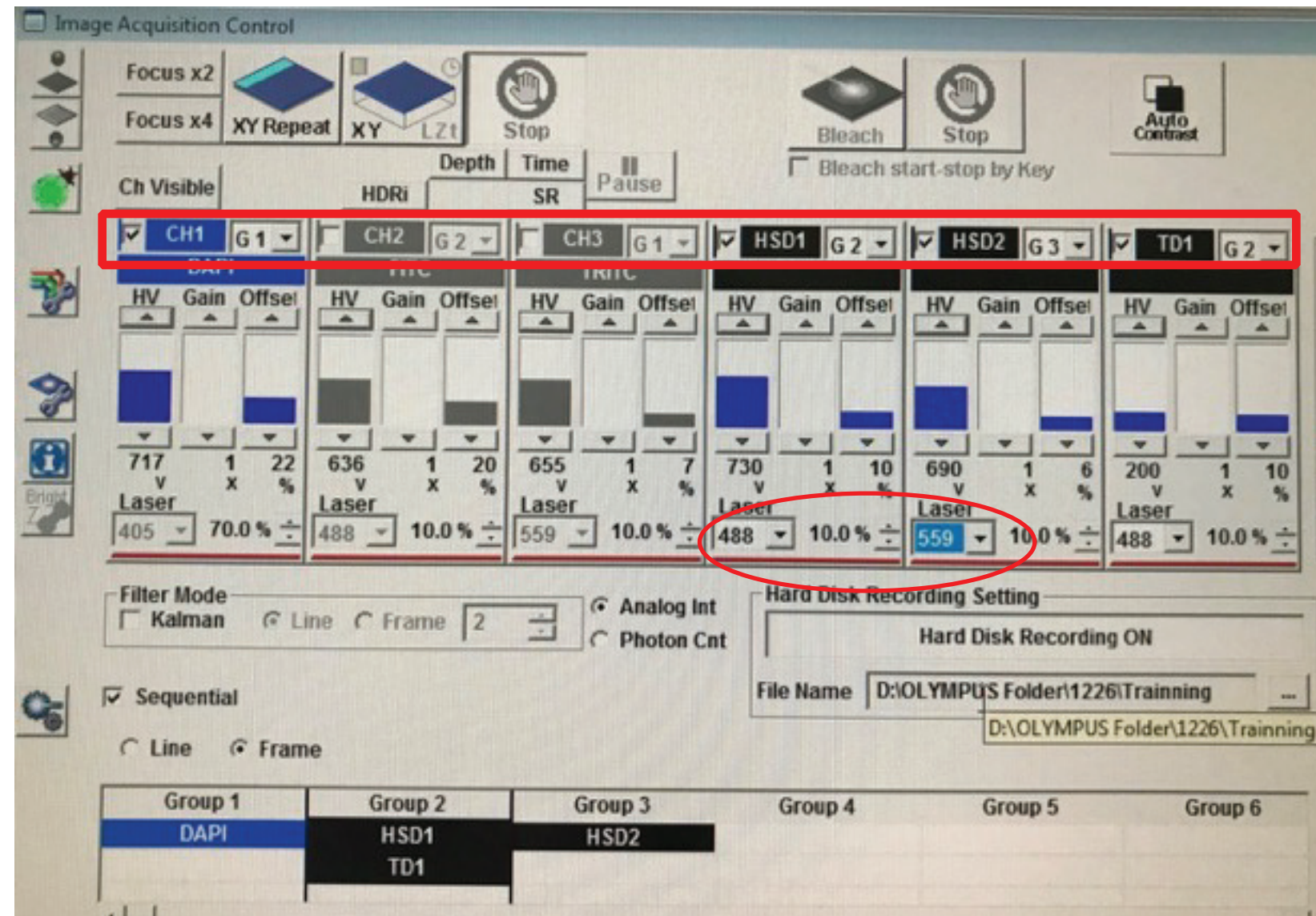
4. 於紅色箭頭處點一下滑鼠左鍵，選取我們所安裝的濾片匣。

# HSD 濾片匣選項

目前共有 4 組 HSD 的濾片匣，收光波長範圍請參閱下表

濾片名稱	HSD1 範圍	SDM	HSD2 範圍
<i>FV12-MHBVE</i>	<i>480-495</i>	<i>510</i>	<i>535-565</i>
<i>FV12-MHBY</i>	<i>505-540</i>	<i>559</i>	<i>575-675</i>
<i>FV12-MHSY</i>	<i>490-540</i>	<i>559</i>	<i>575-675</i>
<i>FV12-MHYR</i>	<i>575-620</i>	<i>635</i>	<i>655-755</i>

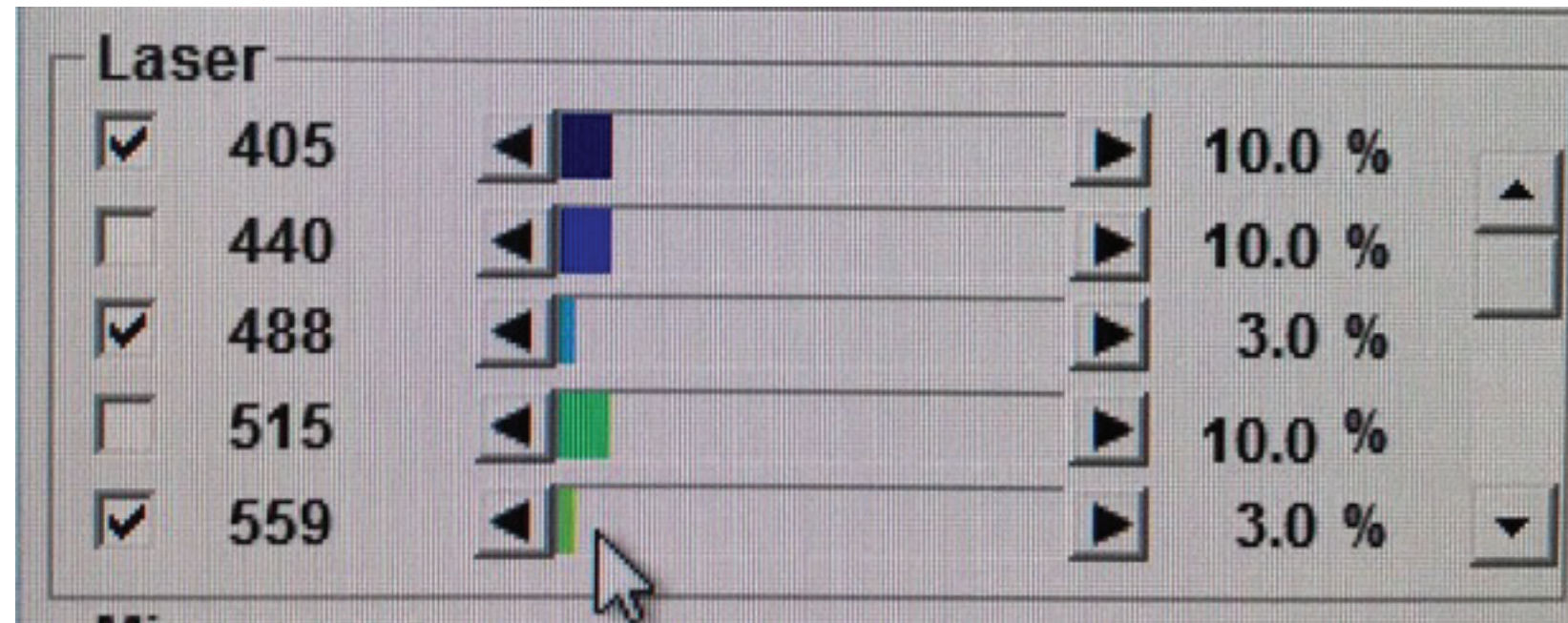
# HSD 光路設定



5. 回到原視窗，確認要使用的偵測器是否被勾選，以及 HSD1 & HSD2 的雷射設定是否正確。

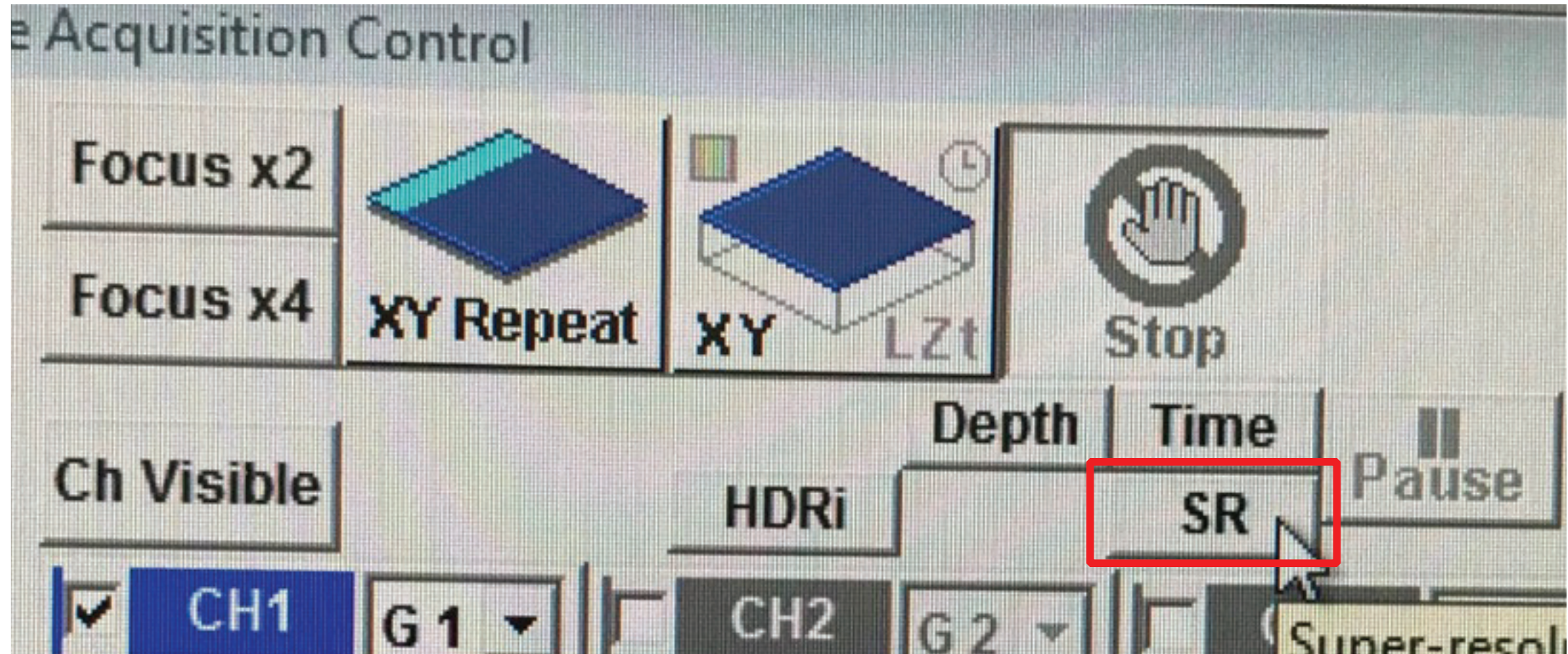
6. 其餘的設定以及影像調整均與之前操作方式相同。可開始進行掃描。

# HSD 使用注意事項

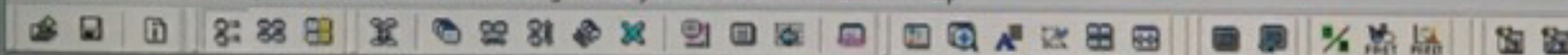


由於 HSD 的敏感度相當高，所以雷射盡量由低輸出開始掃圖，再慢慢地提高雷射輸出，避免影響 HSD 的使用壽命。

# OLYMPUS Super Resolution (OSR) 使用方式



若要使用 OSR 功能，請先選取 SR 選項，就會跳出 SR 影像的控制視窗，如下頁



### Acquisition Setting

**Mode**

<< Fast 2.0us/Pixel Slow >> AutoHV

P:2.0us L:84.640ms F:44.351s S:88.863s

**Size**

Aspect Ratio 1:1 4:3 arbitrary

X 512 by 512

**Area**

Rotation 0.0 0 Zoom 9.6 1

PanX 0 um 0 PanY 0 um 0

**Laser**

<input checked="" type="checkbox"/> 405	10.0 %
<input type="checkbox"/> 440	10.0 %
<input checked="" type="checkbox"/> 488	3.0 %
<input type="checkbox"/> 515	10.0 %
<input checked="" type="checkbox"/> 559	3.0 %

**Microscope**

UPLSAPO 60X O NA:1.35

IX Start Set -8.16 um Go

1.00 -17.18um Go

End Set -26.21 um Go

-5744.56um Set 0

Clear Start/End StepSize 3.61 um Op. Slices 6

Focus Handle On Escape Fine

X:0.182um Y:0.182um Z:0.683um

ZDC ZDC Setting Enable ZDC AF during Time Series Scan

**Time Scan**

Interval FreeRun Num 37 Trig

### Image Acquisition Control

Focus x2 Focus x4 XY Repeat XY LZ1 Stop Bleach Stop Auto Contrast

Bleach start-stop by Key

Ch Visible HDR SR

CH1 G1	CH2 G2	CH3 G1	HSD1 G2	HSD2 G3	TD1 G2
DAPI	FITC	TRITC			
HV Gain Offset	HV Gain Offset	HV Gain Offset	HV Gain Offset	HV Gain Offset	HV Gain Offset
717 1 22	636 1 20	655 1 7	500 1 10	500 1 6	222 1 10
Laser 405 10.0 %	Laser 488 3.0 %	Laser 559 3.0 %	Laser 488 3.0 %	Laser 559 3.0 %	Laser 488 3.0 %

Filter Mode Kalman Line Frame 2 Analog Int Photon Crt

Hard Disk Recording Setting

Hard Disk Recording ON

File Name D:\OLYMPUS Folder\1226\Training

Sequential Line Frame

Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
	HSD1	HSD2			
	TD1				

100% Completed all initialize

### Super-resolution Setting

**Acquisition**

Scan counts (10-20 is recommended) 1 63 40

High Contrast Mode ON OFF

**Processing**

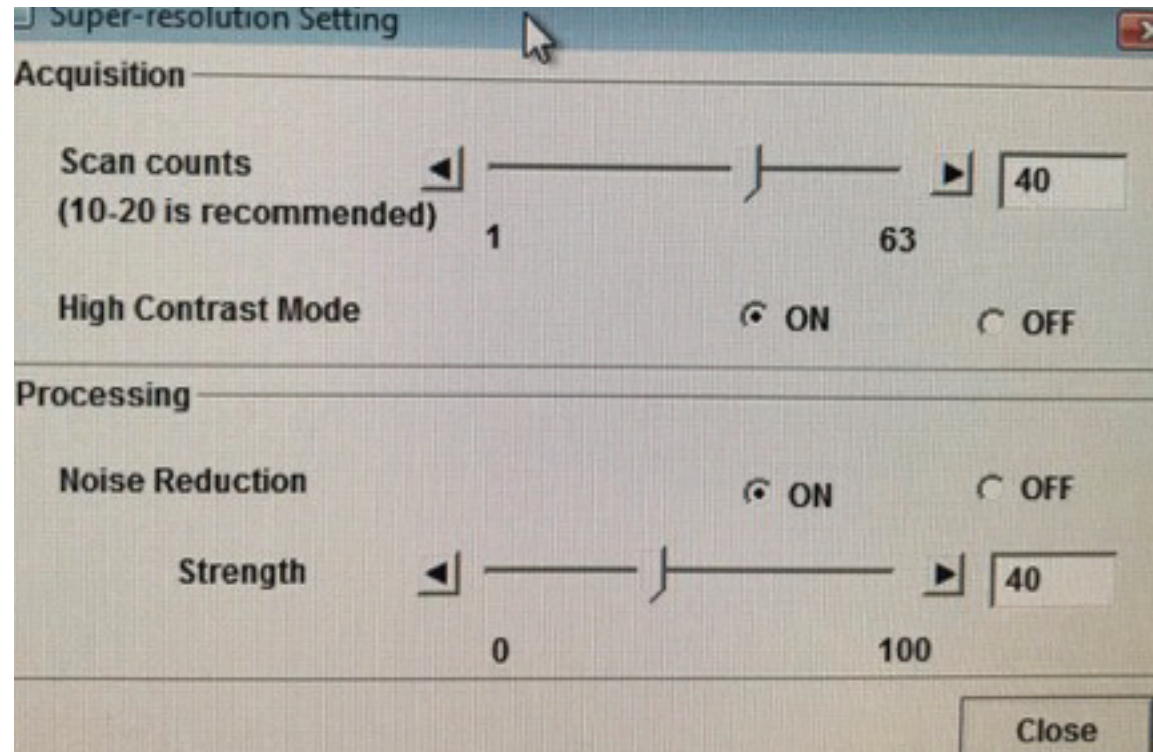
Noise Reduction ON OFF

Strength 0 100 40

Close

SR 影像設定視窗

# SR 影像注意事項



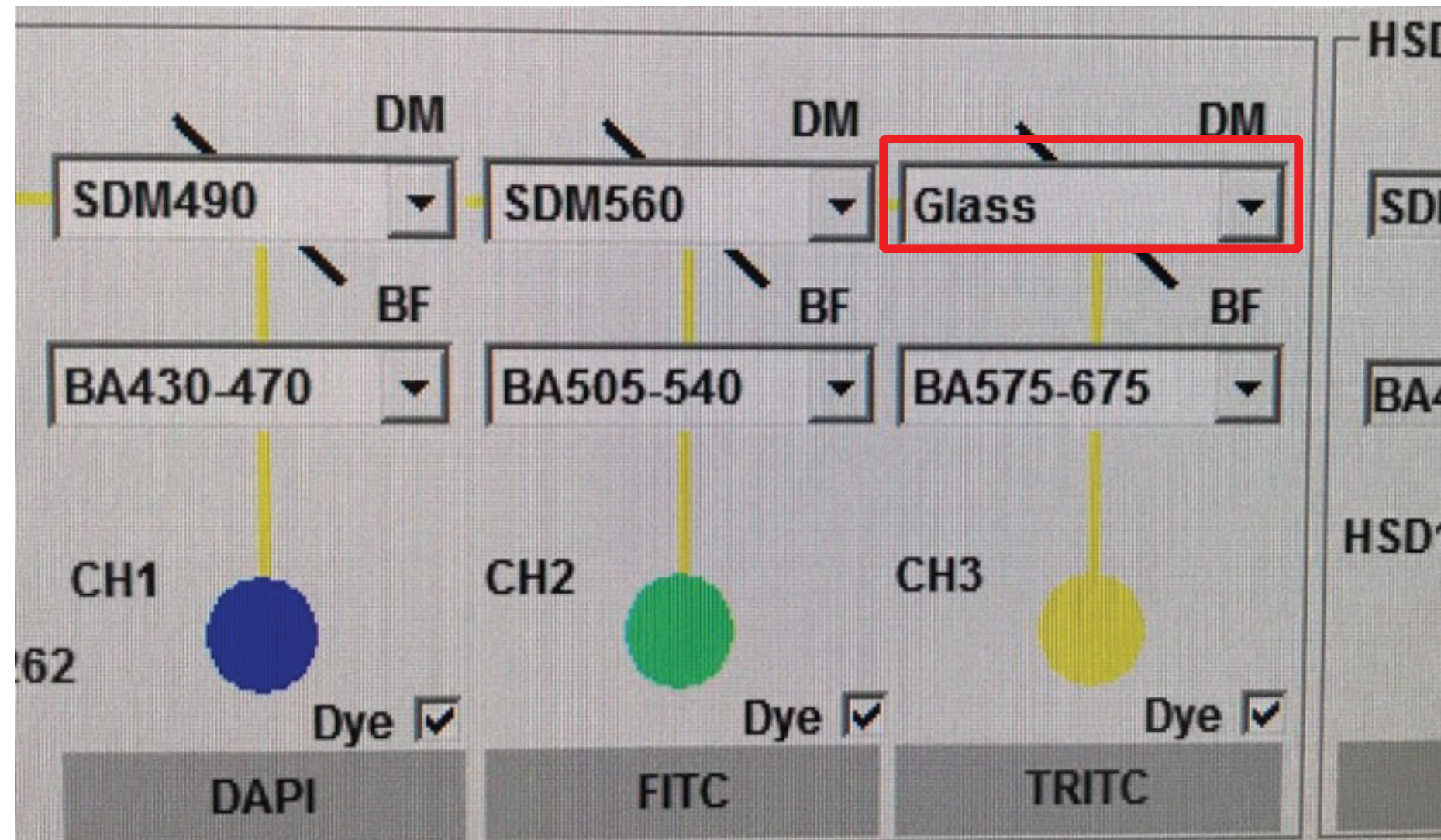
在此可以設定 SR 影像的擷取張數及影像處理參數，建議先嘗試預設參數， Scan counts: 20, Strength: 40。

1. 在 SR 影像模式時，只能用 HSD 擷取影像。
2. 在 SR 影像模式時，掃描速度，pinhole，HSD 的 HV 值等參數會固定無法調整。
3. 在 SR 影像模式時，掃描範圍 (Zoom in) 會隨這著影像大小設定而變動。

Image Size	128	256	320	512	640	800	1024	1600	2048	4096
Zoom factor	38.5	19.3	15.4	9.6	7.7	6.2	4.8	3.1	2.4	1.2
Speed per Frame	7.507	17.173	22.985	44.351	61.767	87.221	129.870	276.319	427.162	1524.147



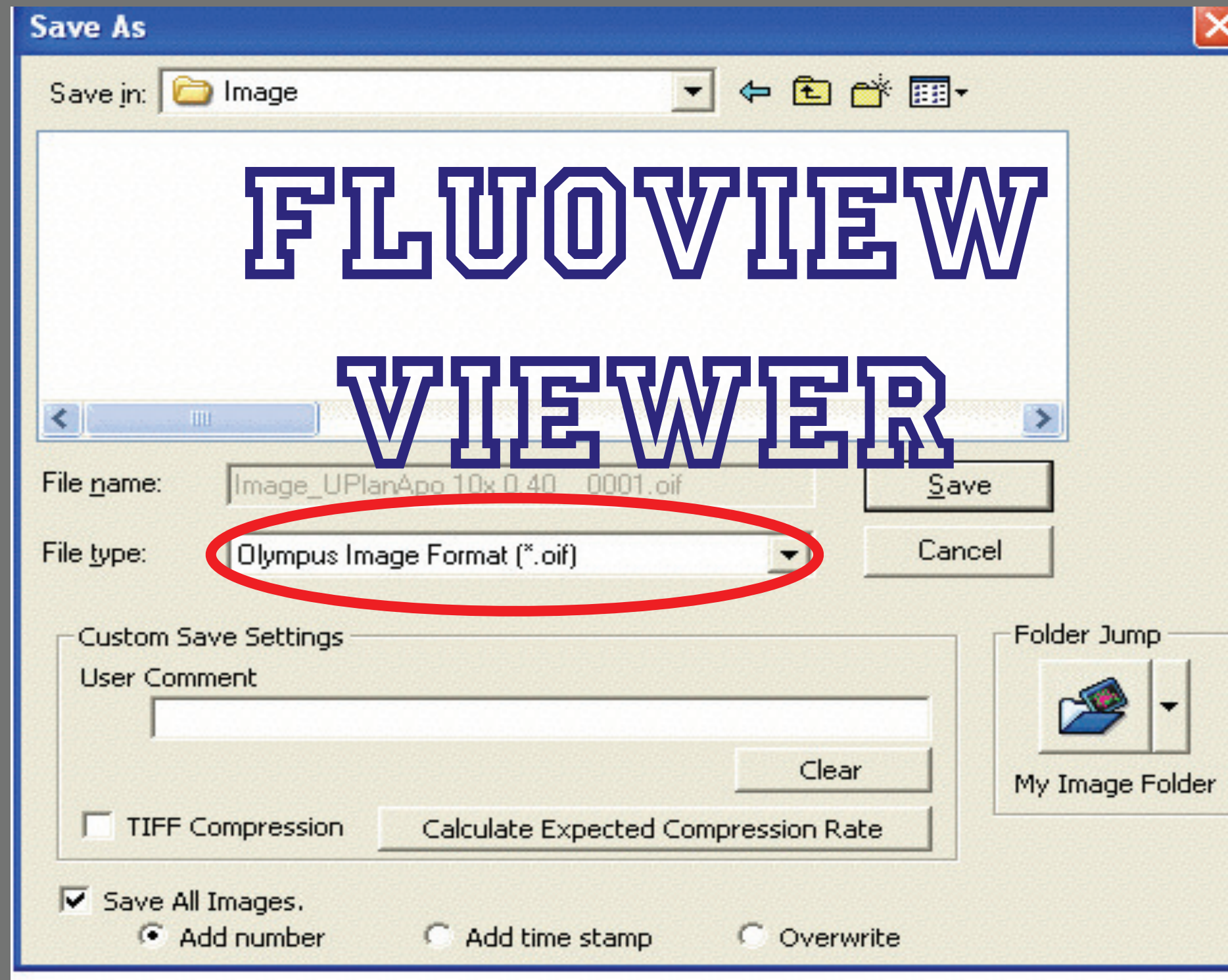
使用完畢後.....



使用 HSD 或 SR 之後，請回歸預設參數，請選擇 Dye List ，在按一次 Apply ，讓光路設定回歸預設參數即可。

不過，紅色框位置，請各位手動變更為 **Mirror** ，謝謝大家。

# File Save and Export



# File Save and Export

Save in: Image

Tiff, Jpg, Bmp.....

File name: Sample\_XYZT.jpg Save

File type: JPEG (\*.jpg;\*.jpeg) Cancel

Custom Export Settings

Information

Bit Depth 8 [ Bits/Pixel ]

Save the image files of each frame in the following new folder.  
"Sample\_XYZT.jpg.frames#"

Folder Jump

My Image Folder

ROI Overlay

No Overlay  All ROIs  Selected ROI on 2D

Selected ROI Shape (Select one or more in the following)

Output Format Merge Channel (using assigned LUT)

Merge Method Amount

Gray Scale (after merging)

Channel Selection

1 2 3 4 5 6 7 8

9 10 11 12 13 14 15 16

JPEG

Quality 70

1(lowest) - 100(highest) , other=default(70)

Range Selection

Single by 2D Range by 2D Reset

	Start	End	Step	
L	1	1	1	1 / 2
Z	1	3	1	3 / 3
T	1	5	1	5 / 5

Save Properties As ASCII Text.

Save All Images.

Add number  Add time stamp  Overwrite

OLYMPUS

Your Vision, Our Future

# OLYMPUS<sup>®</sup>

Your Vision, Our Future

**OLYMPUS**

Your Vision, Our Future