UCL Institute of Child Health

User guide
Olympus 1X71

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Table of contents

Ownership
Prof. Jane Sowden, Developmental Biology Unit (Purchased in 2011)

Access Rules

- No access without prior training by the Light Microscopy Facility Staff
- Free of hourly charge for Sowden and Ferretti groups, £1 hourly charge for all other users towards the cost of the consumables is expected
- Prof. Sowden team has priority over other users.
- Users must always record their activity in the Log book
- Problem(s) with the microscope should be reported as soon as they are noticed

Olympus customer support service: http://www.olympus.co.uk/microscopy

The system is not covered by a maintenance contract
Olympus requires a PO number before sending an engineer

General specifications

Microscopy techniques available

- ***Brightfield***
- ***Phase contrast***
- ***Epitluorescence***

Objectives

- Olympus UPlanFLN 10x Ph1 NA 0.3 WD 10.0 mm
- Olympus LUCPlanFLN 20x Ph1 NA 0.45 WD 6.6-7.8 with correction collar
- Olympus LUCPlanFLN 40x Ph2 NA 0.6 WD 3.0-4.2 with correction collar
- Zeiss objectives can also be used

Filter cubes (see p11-15)

Position	Filter set name	Exciter	Beamsplitter	Emitter
1	DAPI	350/50x	400LP	ET460/50m
2	GFP	ET470/40x	495LP	ET 525/50m
3	DsRed (TRITC/Cy3)	ET545/30x	T570LPXR	ET620/60m
4	Cy5	ET620/60x	T660LPXR	ET700/75m
5	Cy7	ET710/75x	T760LPXR	ET810/90m
6	empty (brightfield)			

Camera (see p16)
Hamamatsu ORCA R^2 CCD Camera with HClmage Capture Software

Fluorescence illumination (see p16)
Prior Lumen 200 Metal Halide Light Source (2000 hours/bulb)

Pixels to Microns calibration

5x objective binning 1x1	1 pixel = 1.88 um
10x objective binning 1x1	1 pixel = 1.03 um
20x objective binning 1x1	1 pixel = 0.514 um
40x objective binning 1x1	1 pixel = 0.256 um

Calibration with a micrometer under transmitted white light

Consumables list

Price correct as of November 2013

- Prior Lumen 200 bulb LM375 (£550, Prior Scientific Instruments Ltd)
- Prior Lumen 200 light guide LM587 (£400, Prior Scientific Instruments Ltd)
- Halogen bulb 12V/100W (£1.8, Technical Lamp Supplies UK)

Quick user guides

Users must always record their activity in the Log book

Transmitted light

1. Halogen Lamp Power Supply Unit TH4 "ON"
2. Light Path selector on "Ocular"
3. Kohler illumination adjusted
4. Correct phase ring in position (10x & 20x Ph1, 40x Ph2)
5. Filter cube on position #6

Epifluorescence

Warnings:
Do not shut the unit down within 30 minutes of powering up the unit.

- ***After shutting down the unit allow 30 minutes before re-powering up***
- ***After shutting down the unit allow 30 minutes before changing the bulb.***

Failure to do so is likely to result in damage to the bulb.

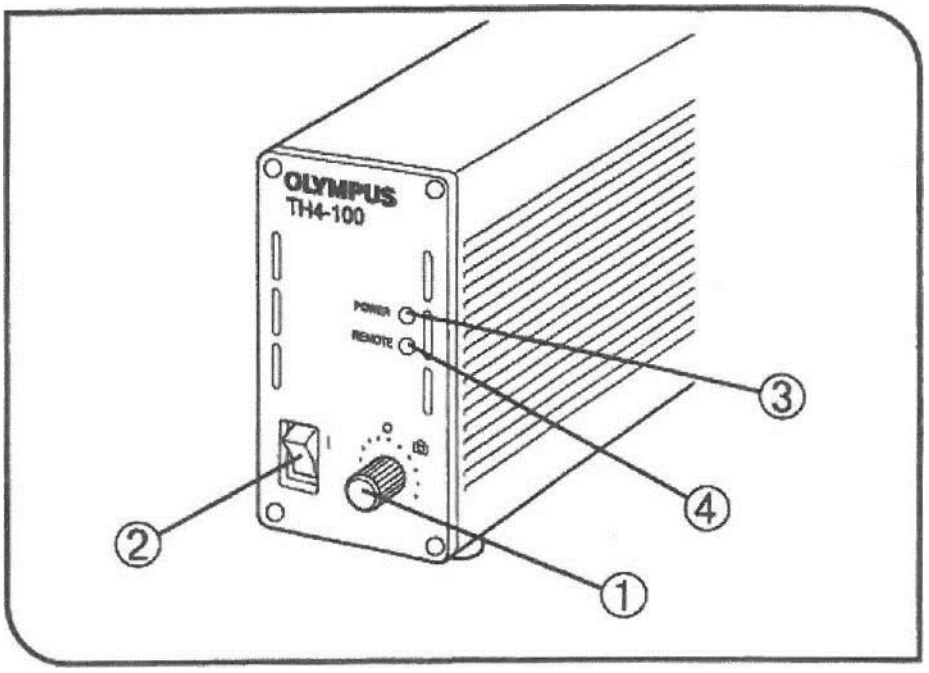
1. Prior Lumen 200 module on
2. Prior Lumen 200 intensity knob >0%
3. Light Path selector lever on "Ocular"
4. Correct filter cube in position
5. Fluorescence shutter open

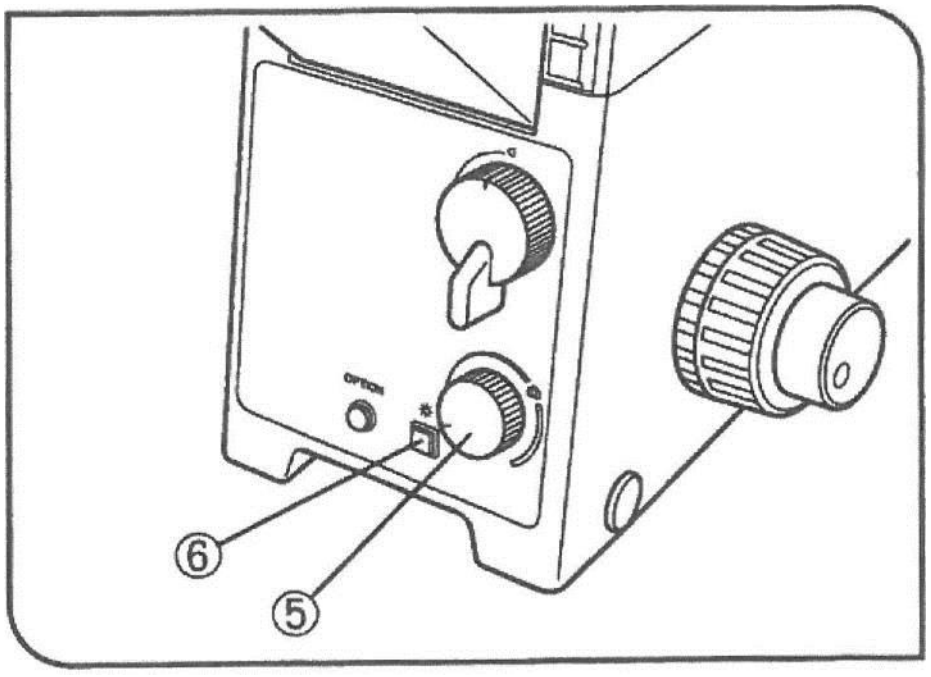
Image Capture

1. Start Camera controller (press until LED turns green)
2. Computer on (Login: Jane/ Password: Ja*e)
3. HClmage software open
4. Light Path selector lever on Camera
5. Correct transmitted light/epifluorescence set-up
6. "Live" mode
7. Adjust exposure time accordingly. Make use of the Histogram and the Saturation options
8. "Abort"
9. "Capturel"
10. Save as in My Documents>UserName_Unit>FileName.tif
11. Shut-down: exit HClmage, log out windows session, camera on stand-by (press until LED turns orange)

Halogen lamp operation:
Turning on the lamp

1. Make sure the light intensity control knob **(5)** is in the MIN (minimum intensity) position on the microscope frame.
2. Make sure the light intensity control knob **(1)** is in the MIN (minimum intensity) position on the TH4 module.
3. Set the main switch **(2)** to **"I"** (ON) on the TH4 module.
4. On the microscope front, press the transmitted light ON-OFF button **(6)** so that the button is illuminated.
5. Adjust the brightness with the light intensity control knob **(5).**
6. To turn OFF, set the transmitted light ON-OFF button **(6)** to OFF





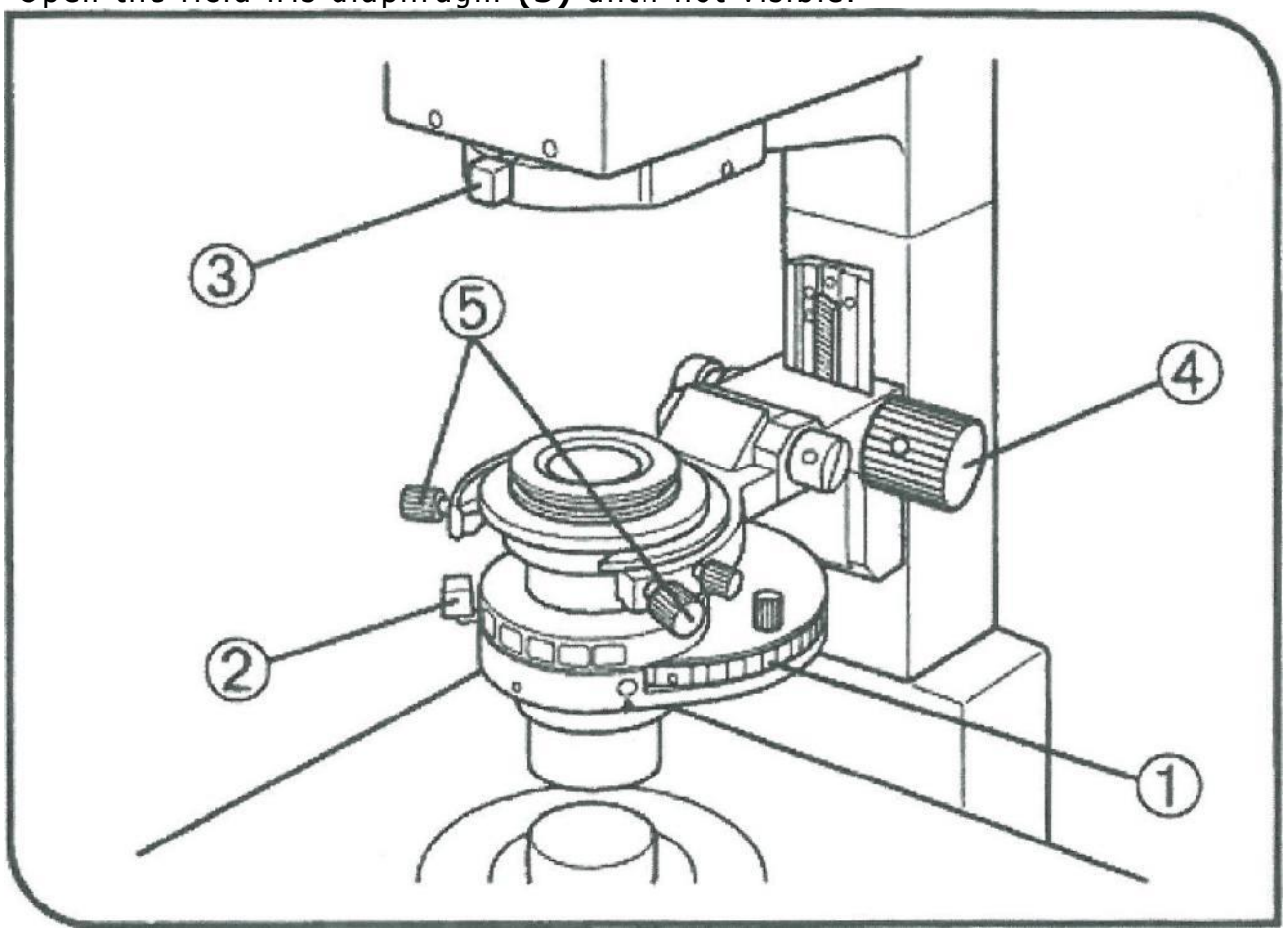
Halogen lamp operation:

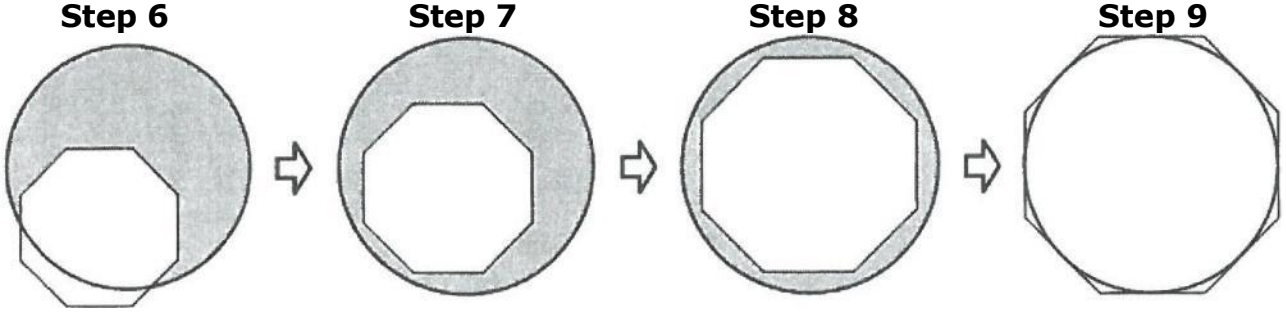
Turning off the lamp

1. Set the light intensity control knob **(5)** to the MIN (minimum intensity) position on the microscope frame.
2. Set the light intensity control knob **(1)** to the MIN (minimum intensity) position on the TH4 module.
3. Set the main switch **(2)** to **"0"** (OFF) on the TH4 module.

Kohler illumination:

1. Rotate the turret **(1)** to the "BF" position. (Any of positions 3,4 or 5, position 1=Ph1, 2 = Ph2)
2. Slide the aperture iris diaphragm lever **(2)** to fully open the diaphragm.
3. Slide the field iris diaphragm lever **(3)** to the fully open position.
4. Engage the 10x objective and bring the specimen into focus.
5. Using the field iris diaphragm lever **(3)**, completly close the field iris diaphragm.
6. Rotate the condensor height adjustment knob **(4)** to bring the field iris diaphragm image into focus.
7. Center the field iris diaphragm **(3)** using the condenser centering knobs **(5).**
8. Open the field iris diaphragm **(3)** until its image reach the limits of the field of view, adjust the centering if necessary.
9. Open the field iris diaphragm **(3)** until not visible.





Adjusting the objective correction collar

Correction is possible according to the vessel bottom thickness.

1. When the thickness of the vessel bottom is known, match the scale reading of the correction collar to the thickness of the vessel in use.

or

2. If the thickness of the vessel is unknown or diverge from the manufacturer specifications, the optimum position for the correction collar can be obtained by judging the image resolution and contrast. When a satisfactory image is not obtain after focusing:
    1. Rotate the correction collar to the left and right, refocus each time and compare the images.
    2 Then rotate the collar in the direction yielding a better image, rotate the correction collar to the left and right, refocus each time and compare the images.
    3 Repeat this cycle until the position with the optimum image is found.

20x Correction Collar

40x Correction Collar

Correction Collar Scale

0 mm
0.17 mm (glass coverslip #1.5)
0.5 mm
1 mm (most tissue culture plates)
1.5 mm
2 mm

Prior Lumen200 Metal Halide Lamp Operation:

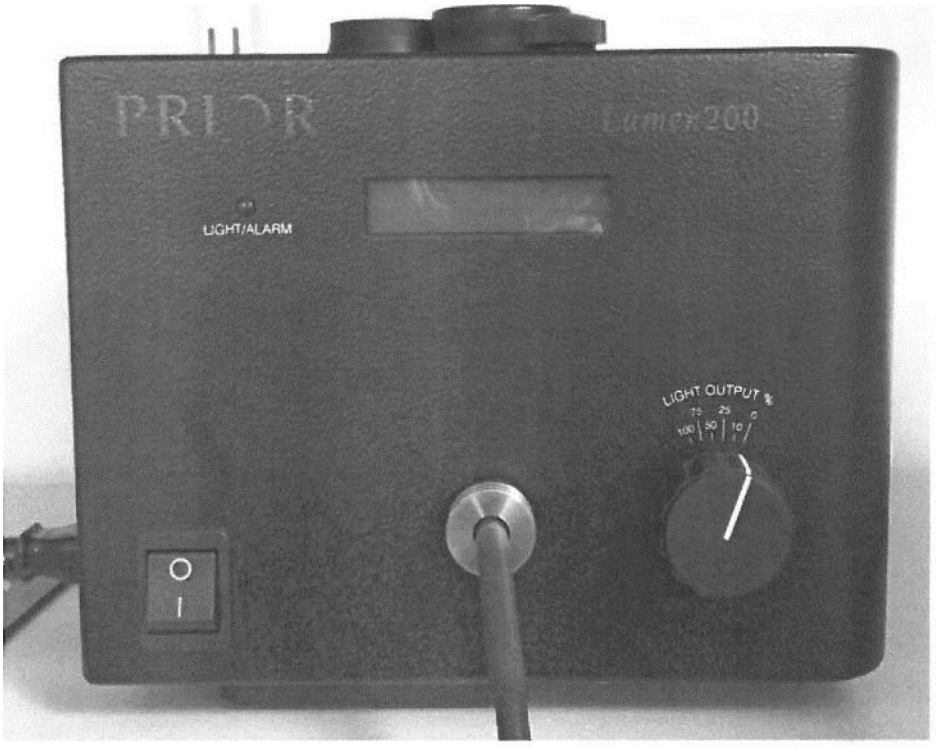
Starting Up the Lumen

1. Switch the Lumen power switch on.
2. Make sure the ventilation vent on the left hand side is unobstructed or the lamp will overheat resulting in automatic shutdown and damage to the module.
3. Allow 1-5 minutes for light to reach 70% of output.
4. Allow 30 minutes for the Lumen to reach operational temperature.
5. **Warning:** Do not power down the unit within 30 mins of power up. This may reduce the effective lifetime of the bulb.

Shutting down the Lumen

The following warnings apply as damage to the bulb may result if instructions not followed:

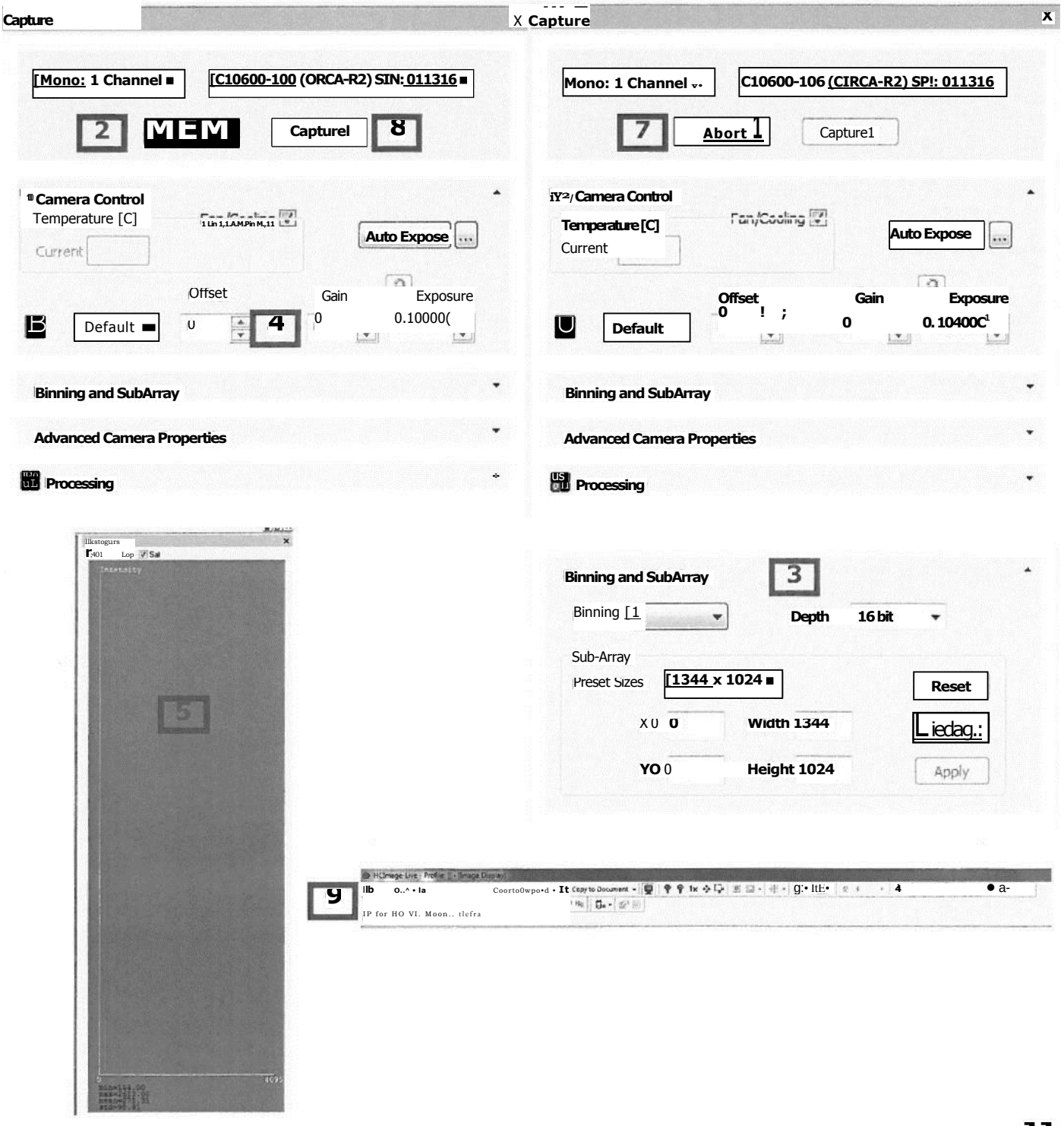
1. Warning: Do not shut the unit down within 30 minutes of powering up the unit.
2. Warning: After shutting down the unit allow 30 minutes before re-powering up or changing the bulb. Failure to do so is likely to result in damage to the bulb.



Warning: the air outlet for heat ventilation must not but obstructed

Image Capture with HClmage

1. Click the **Capture** pane.
2. Click **Live** for a live image from the camera
3. Camera binning or image sub-array can be set in the **Binning SubArray** panel.
4. In the **Camera Control panel,** adjust exposure/gain manually or automatically by clicking on Auto Expose; view the intensity distribution in the histogram.
5. Check Sat. (saturation) in the histogram of the Image Display to guard against image saturation. Saturated pixel are indicated in Red. Yellow indicates pixels approaching saturation.
6. Adjust camera exposure and gain settings as necessary
7. Click Abort.
8. Click Capture1 to acquire an image.
9. Click the Save icon to save the image in My Documents>UserName_Unit>file name.tif



Selecting the right fluorochrome/filter set

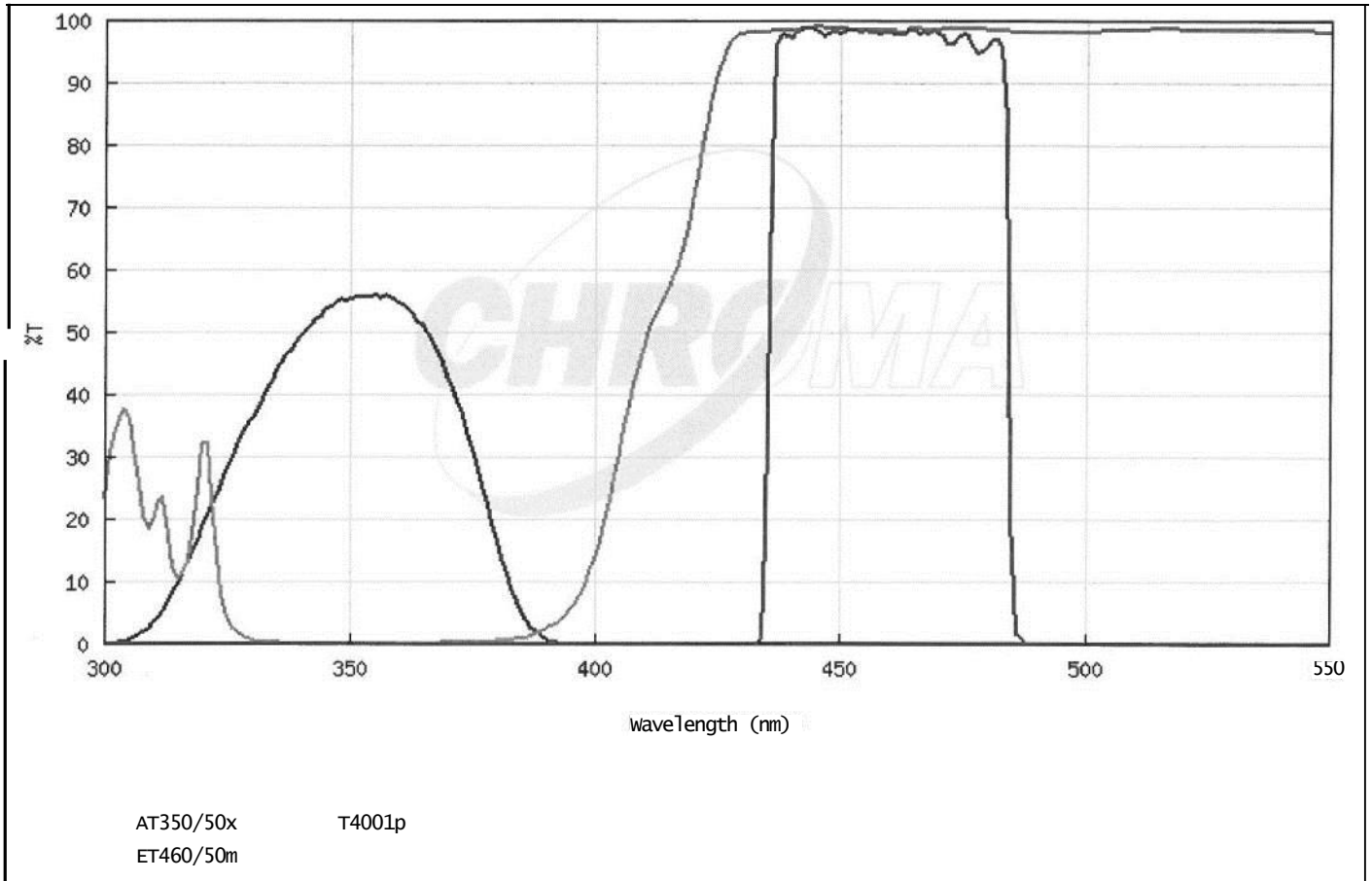
Position #1

49000 - ET - DAPI

Exciter 350/50x

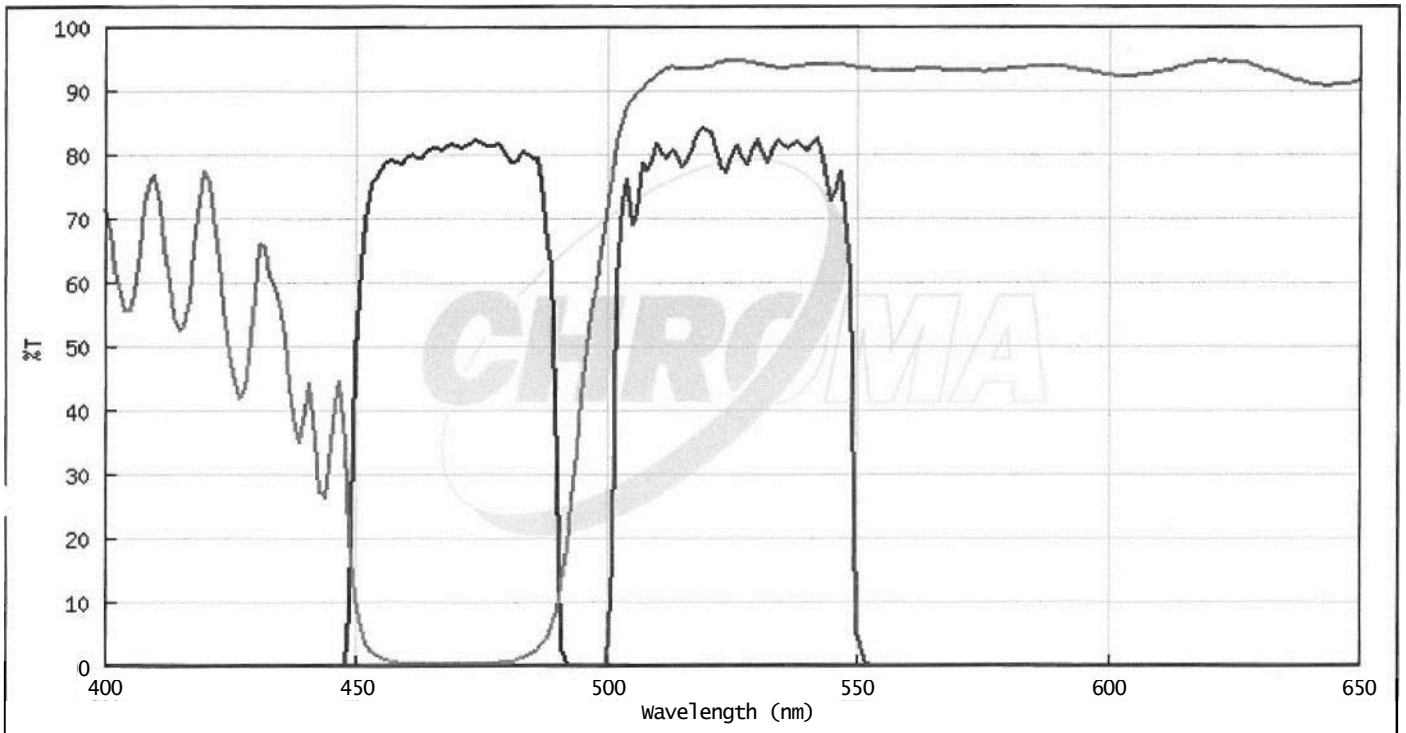
beamsplitter 40011[3]

Emitter ET460/50m



Fluorochrome	EX	EM	Use
Alexa Fluor 350TM	346	442	Recommended
Coumarin	384	470	Recommended
DAPI	359	461	Recommended
DyLight 350	352	435	Recommended
Hoechst 33258	352	461	Recommended
LysoTracker Blue/Me0H	373	425	Recommended

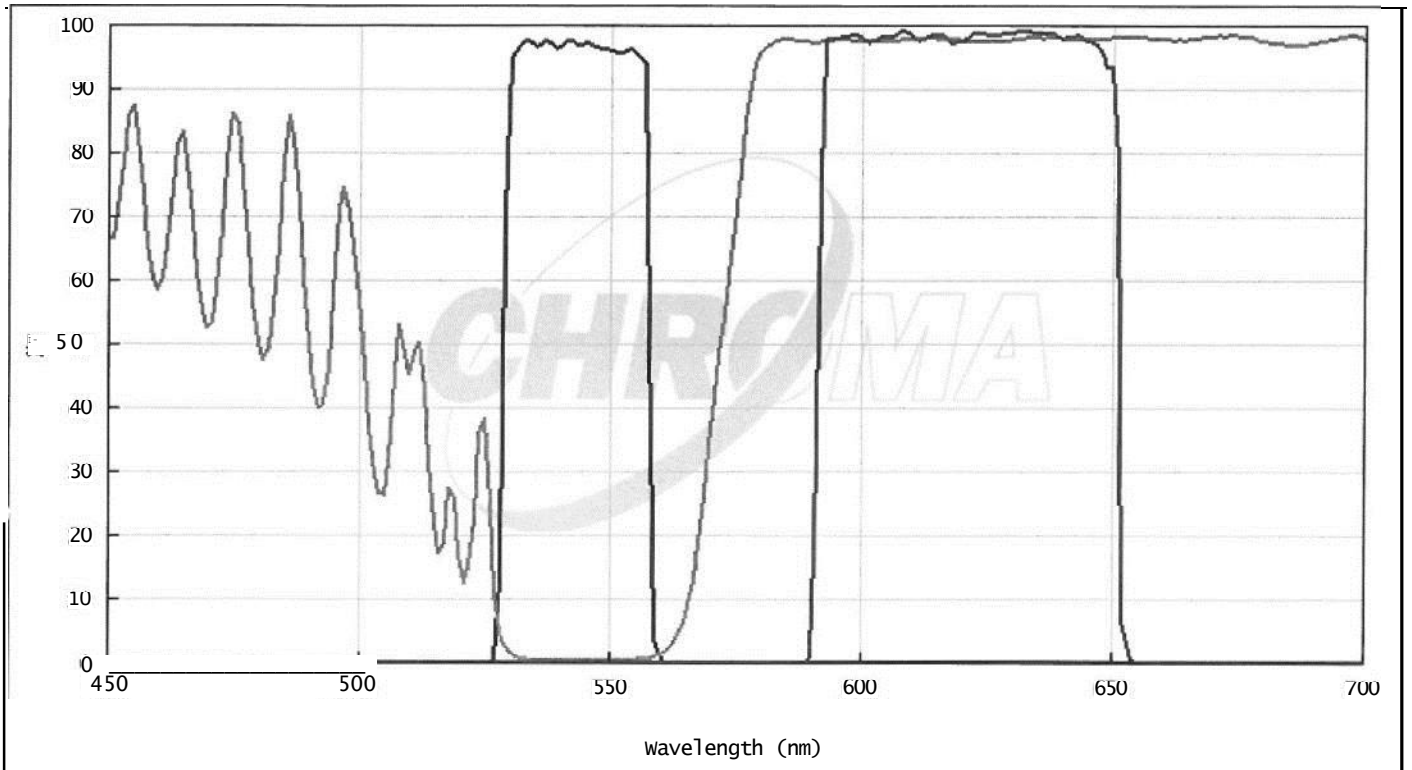
Position #2
41017 EndowGFP/EGFP Bandpass
Emitter ET470/40x
Beamsplitter 495LP
Emitter ET 525/50m



– H0470/40x Q4951p
– HQ525/50m

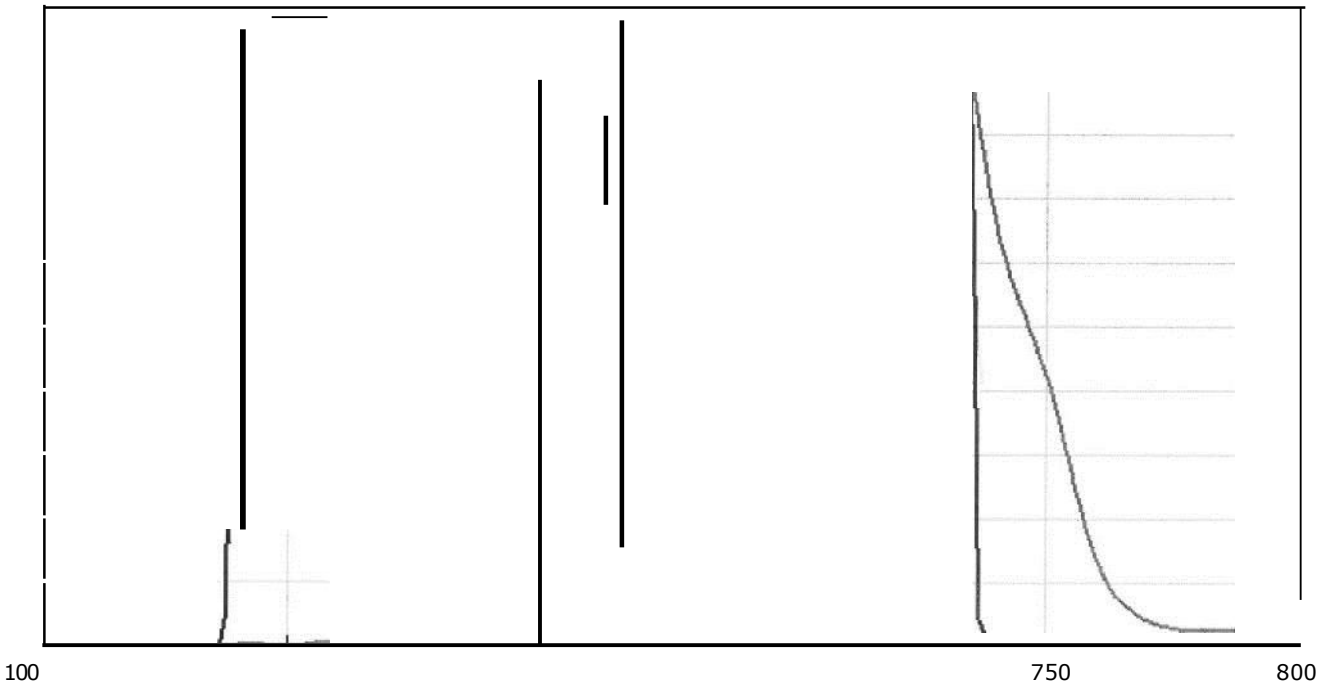
Fluorochrome	EX	EM	Use
Acridine Orange + DNA	500	526	Alternative
Alexa Fluor 488T"	498	520	Alternative
Azami Green	492	505	Alternative
BODIPY FL/pH7.2	505	512	Alternative
Calcein	494	517	Alternative
Calcium GreenTM-1	506	531	Alternative
Cy2TM	489	506	Alternative
DiO	484	502	Alternative
DyLight 488	492	517	Alternative
EGFP	488	507	Alternative
Emerald GFP	489	510	Alternative
FAM	492	518	Alternative
FITC	490	525	Alternative
Fluo-4	494	516	Alternative
GFP	488	507	Alternative
MitoTracker Green FM/MeOH	490	516	Alternative
mWasabi	493	509	Alternative
Oregon GreenTM 488	490	514	Alternative
ZsGreenl	493	505	Alternative

Position #3
49005 - ET - DSRed (TRITC/Cy3)
Exciter ET545/30x
Beamsplitter T570LPXR
Emitter ET620/60m



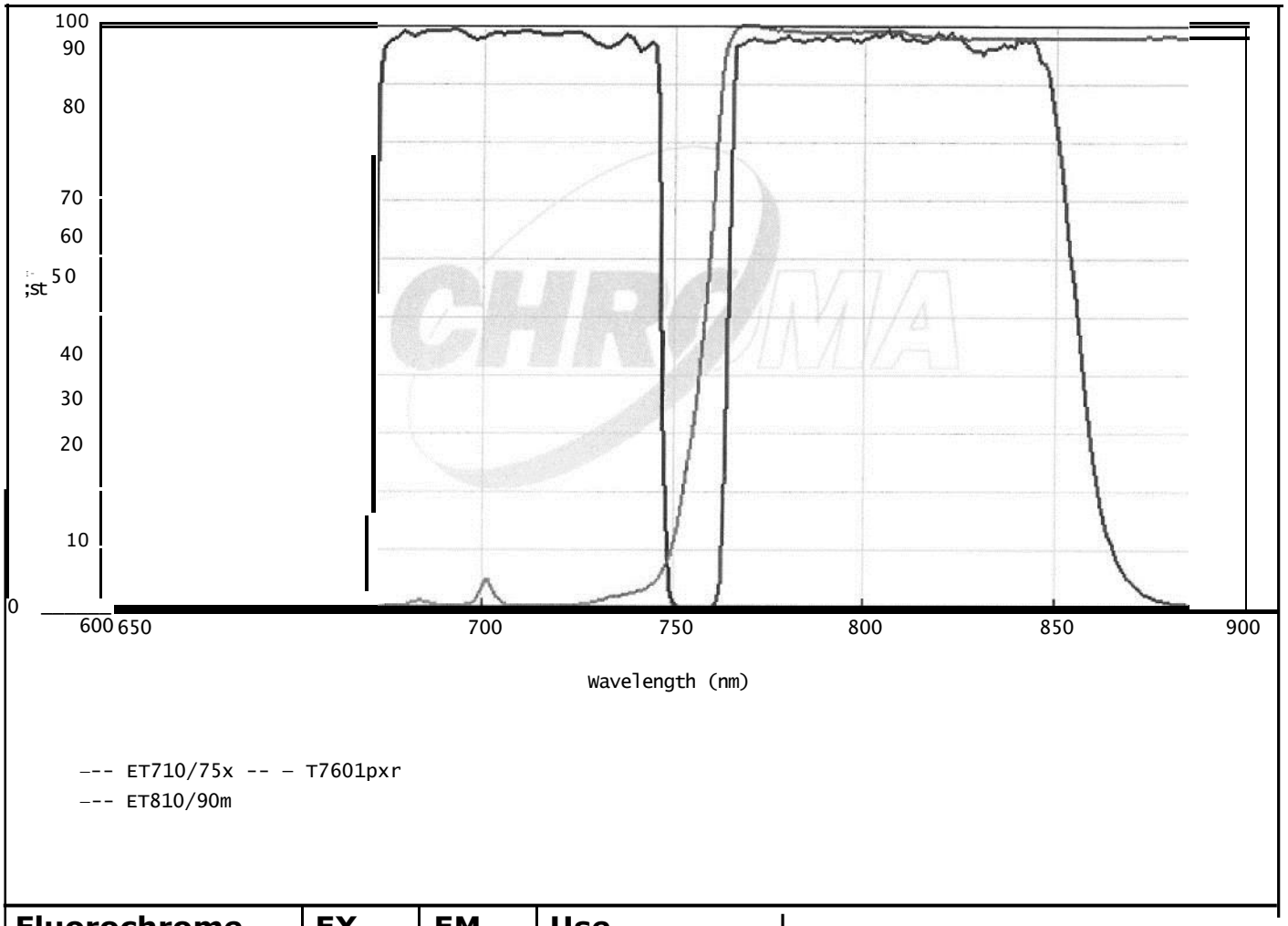
Fluorochrome	EX	EM	Use
AsRed2	576	592	Recommended
Ethidium Bromide	520	603	Recommended
Ethidium homidimer-1/DNA	527	617	Recommended
Propidium Iodide	536	617	Recommended
Resorufin	571	585	Recommended
Alexa Fluor 568TM	78	603	Alternative
Cy3TM	552	570	Alternative
Rhod-2	540	576	Alternative
TAM RA	555	580	Alternative
Tetramethylrhodamine isothio-cyanate	555	580	Alternative
TRITC	555	580	Alternative

Position #4 49006 - ET - Cy5
emitter ET620/60x
Beamsplitter T660LPXR
Emitter ET700/75n,



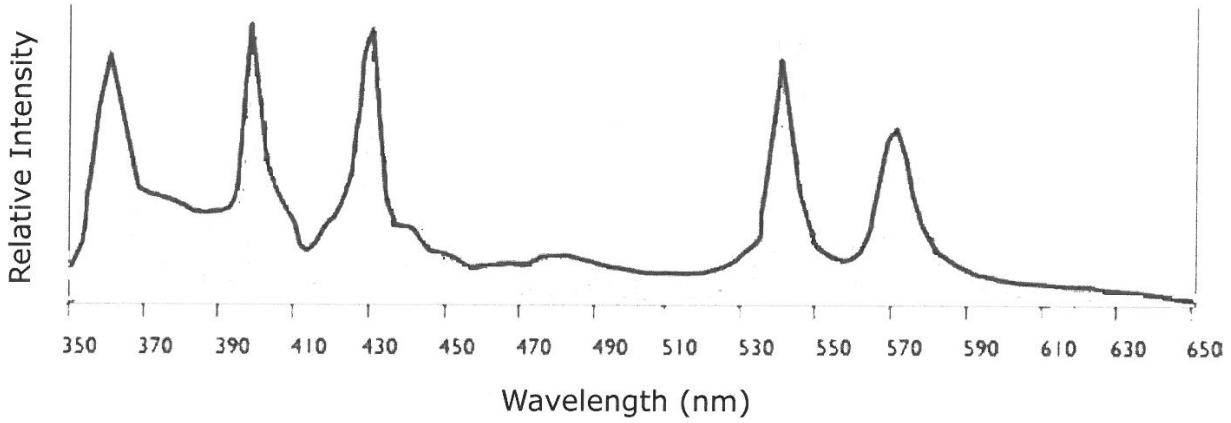
Fluorochrome	**EX**	**EM**	**Use**
Alexa Fluor 647TM	649	666	Recommended
Allophycocyanin (APC)	630	660	Recommended
Atto 647N	644	669	Recommended
Cy5TM	649	670	Recommended
DiD	644	665	Recommended
Draq5	647	683	Recommended
DyLight 649	652	667	Recommended
MitoTracker Deep Red 633/MeOH	644	665	Recommended
Nile Blue	631	660	Recommended
SYTO® 60	652	678	Recommended
TO-PRO ™3	642	661	Recommended

Position #5
49007 - ET - Cy7
Exciter ET710/75x
Beamsplitter T760LPXR
Emitter ET810/90m



Fluorochrome	EX	EM	Use
Alexa Fluor 750TM	752	779	Recommended
Cy7TM	743	767	Recommended
DiR	748	780	Recommended
DyLight 750	751	772	Recommended

Prior Lumen 200 Spectral Output



Hamamatsu ORCA-R^2 Spectral Response