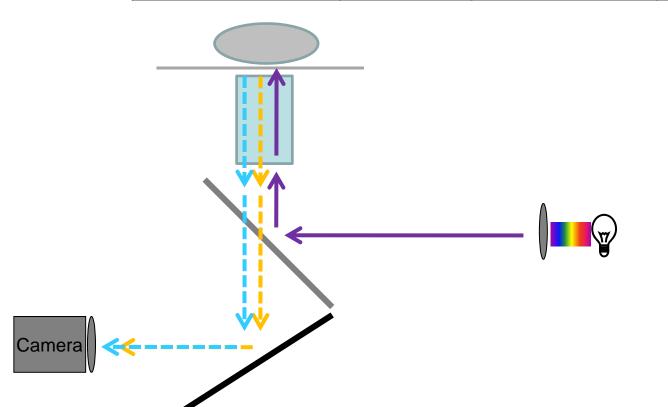
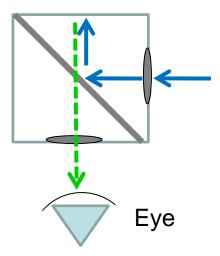


	Excitation Filter (nm)	Dichroic Mirror (long pass; nm)	Emission Filter (nm)
CFP	420/20	450	475/40
YFP (FRET)	420/20	450	535/25
mOrange2	548/20	560	572/20
mCherry (FRET)	040/20	560	630/20
mCherry	580/20	600	630/20

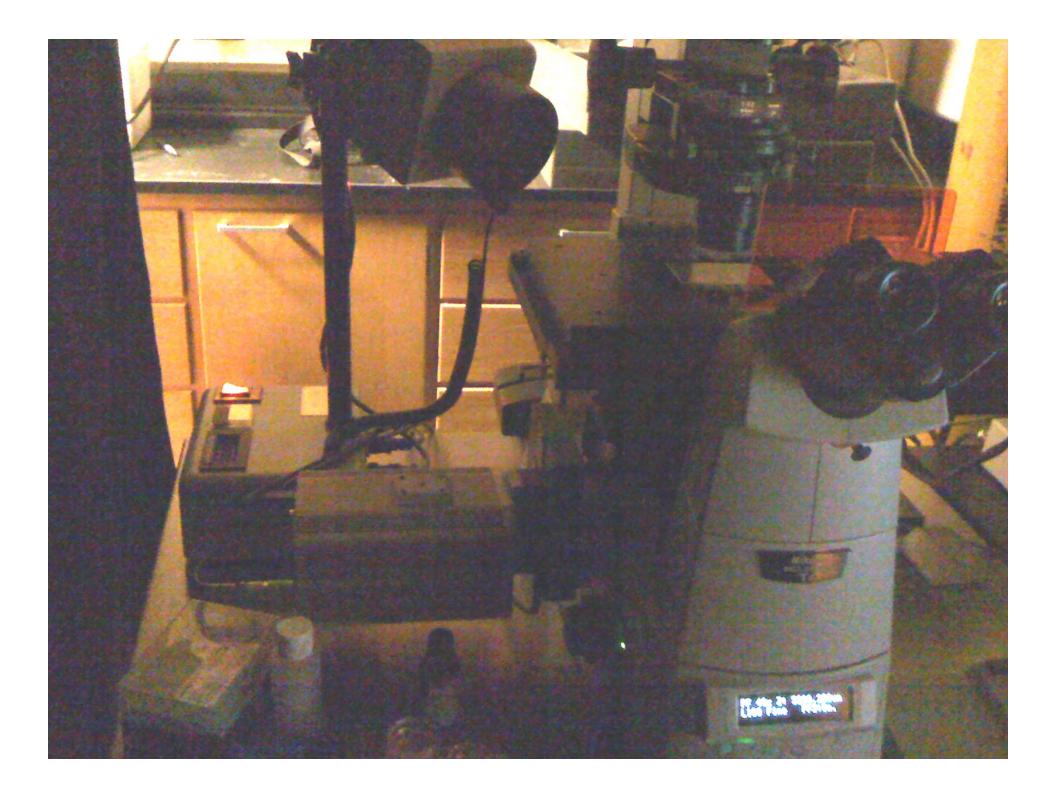




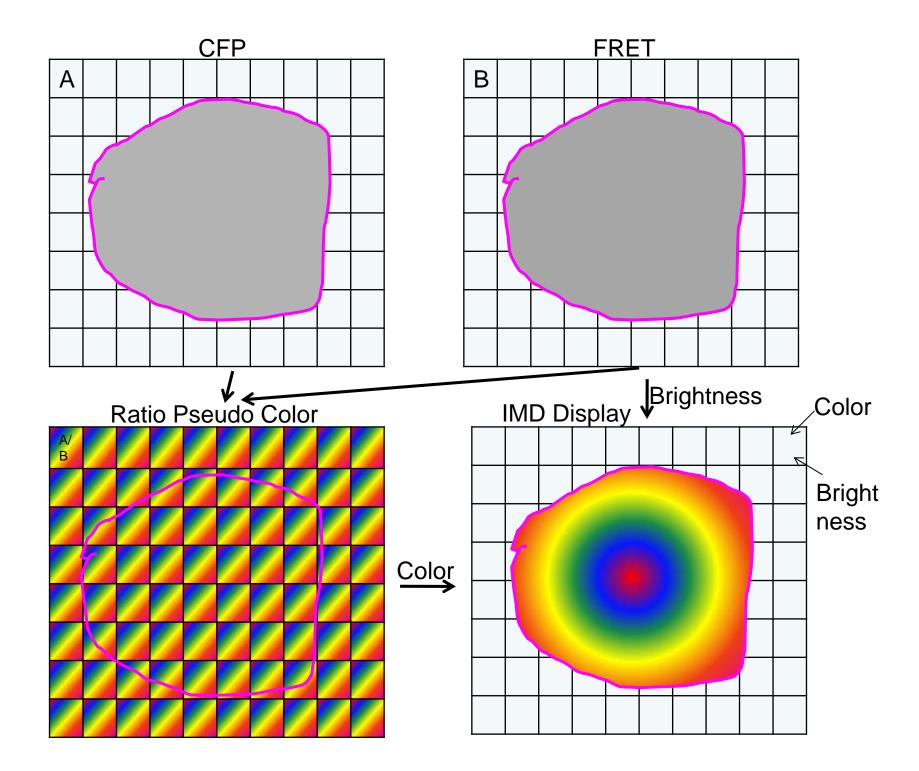


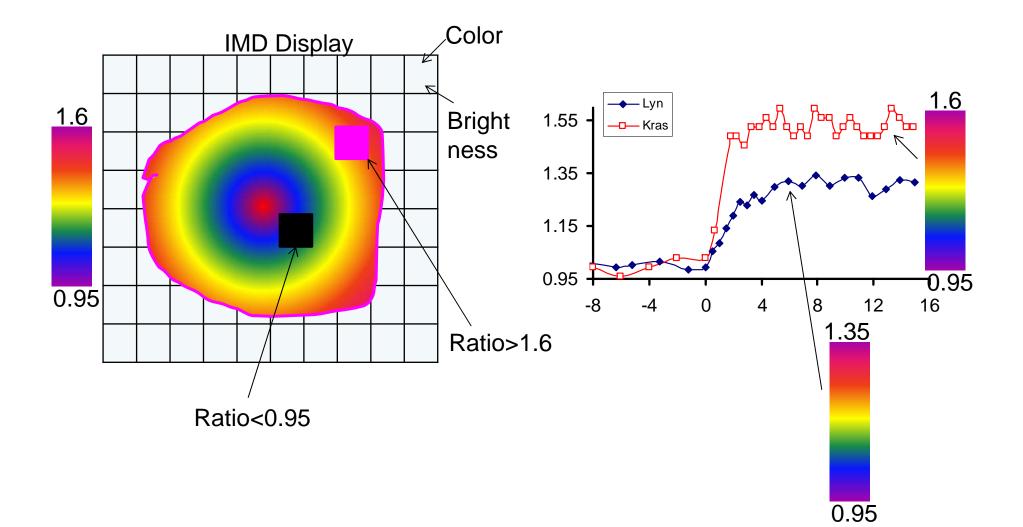












File Configure Run Experiment Graphs Utilities Calibration Journals Windows Help

New Open Protocol Movie Archive Extract Extract Ext

2.61 GB physical memory 3.99 GB virtual memory

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MAG Biosyster	ns Software						
File Configure	Run Experiment	Graphs	Utilities	Calibration	Journals	Windows	Help
New Open							
Protocol Movie				ſ	Load Proto	ocol	
Archive					Protocol Fi	les (*.FSF)	
Extract					CFP_FR	ET_YFP_DIC.	FSF
Exit							
More >>							

CFP_FRET_YFP_DIC.FSF	Select Protocol Directory	
	Protocol Files are Located in:	
	C:\MAG\app\mmfluor\Yijia	
Contents of Selected Proto	al Files	
	or rile.	
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CFP/FRET/YFP/DIC		1
Timelapse Interval: 60 min		-
Timelapse Interval: 60 min Load INF Path: C:\dat	.tes ∖he huang\41510\washout\MDA\ \yY\12_16_2011\FN2.5\2\ Event Marks:	
Timelapse Interval: 60 min Load INF Path: C:\dat Save INF Path: C:\dat	s\he huang\41510\washout\MDA\ a\Yi\12_16_2011\FN2.5\2\ Event Marks:	
Timelapse Interval: 60 min Load INF Path: C:\dat Save INF Path: C:\dat Wavelength Details:	a\he huang\41510\washout\MDA\ a\Yi\12_16_2011\FN2.5\2\	
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Timelapse Interval: 60 min Load INF Path: C:\dat Save INF Path: C:\dat Wavelength Details: Acquire Image: Yes Update Image: Yes Display Window: Yes Save Image: No	s\he huang\41510\washout\MDA\ a\Yi\12_16_2011\FN2.5\2\ Event Marks:	
Timelapse Interval: 60 min Load INF Path: C:\dat Save INF Path: C:\dat Wavelength Details: Acquire Image: Yes Display Window: Yes Save Image: No Low Threshold: 0	s/he huang/41510/washout/MDA\ \/Y/\12_16_2011\FN2.5\2\ Event Marks: GM6001 wash	
Timelapse Interval: 60 min Load INF Path: C:\dat Save INF Path: C:\dat Wavelength Details: Acquire Image: Yes Update Image: Yes Display Window: Yes Save Image: No Low Threshold: 00 High Threshold: 409	s/he huang/41510/washout/MDA\ \/Y/\12_16_2011\FN2.5\2\ Event Marks: GM6001 wash	
Timelapse Interval: 60 min Load INF Path: C:\dat Save INF Path: C:\dat Wavelength Details: Acquire Image: Yes Display Window: Yes Save Image: No Low Threshold: 0	s/he huang/41510/washout/MDA\ \/Y/\12_16_2011\FN2.5\2\ Event Marks: GM6001 wash	
Timelapse Interval: 60 min Load INF Path: C:\dat Save INF Path: C:\dat Wavelength Details: Acquire Image: Yes Update Image: Yes Display Window: Yes Save Image: No Low Threshold: 00 High Threshold: 00	s/he huang/41510/washout/MDA\ \/Y/\12_16_2011\FN2.5\2\ Event Marks: GM6001 wash	

2.48 GB physical memory 3.99 GB virtual memory

File Configure Run Experiment Graphs Utilities Calibration Journals Windows Help

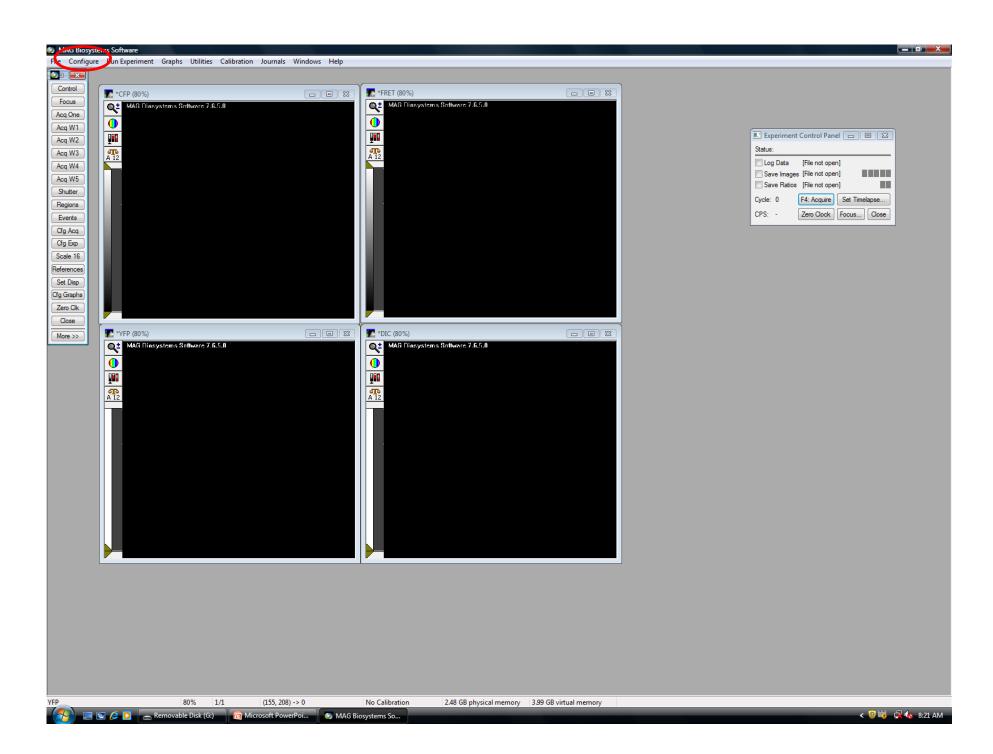
New Open Protocol

File Configure

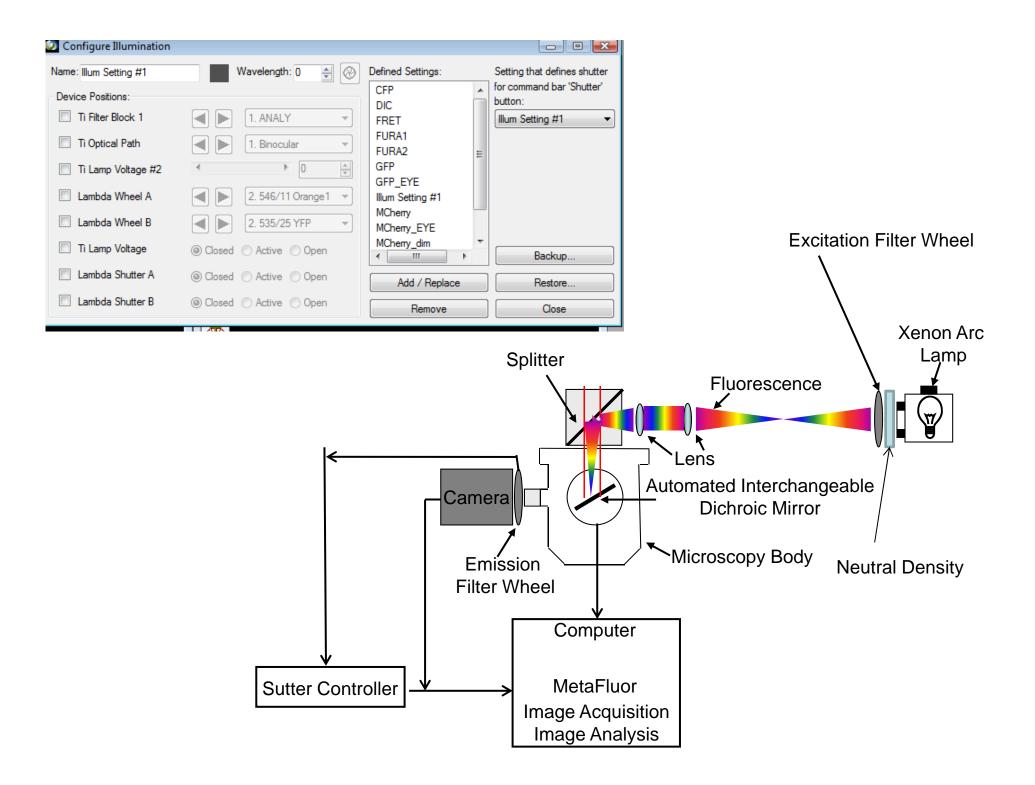
Protocol Movie Archive Extract Exit

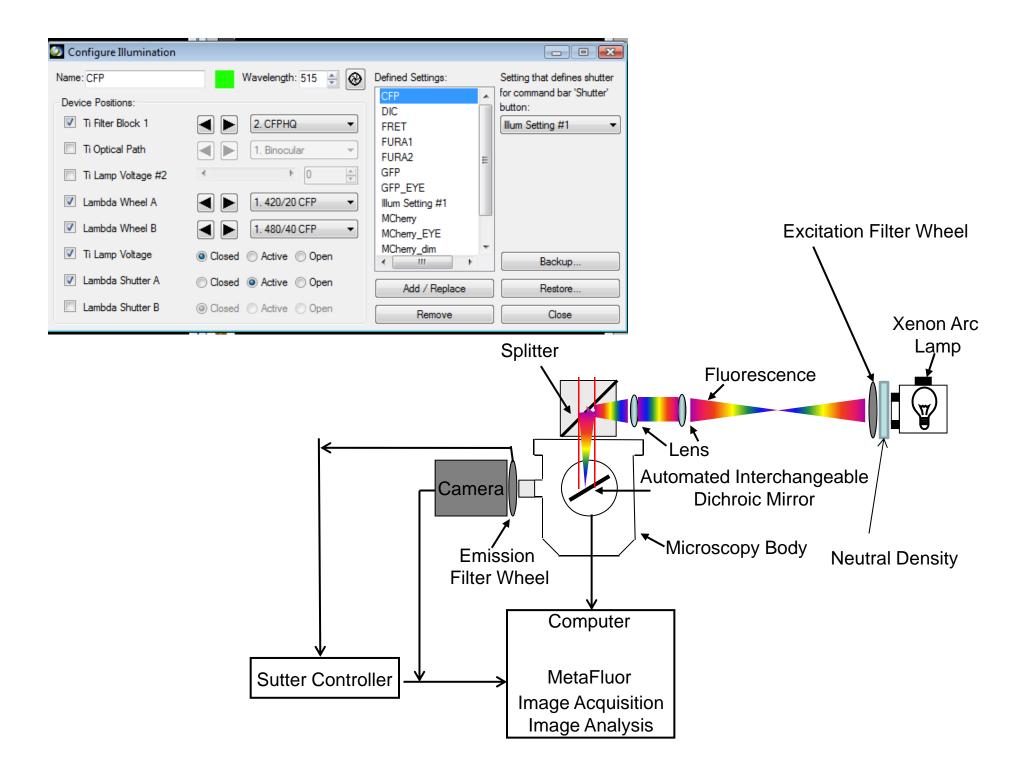
More >>

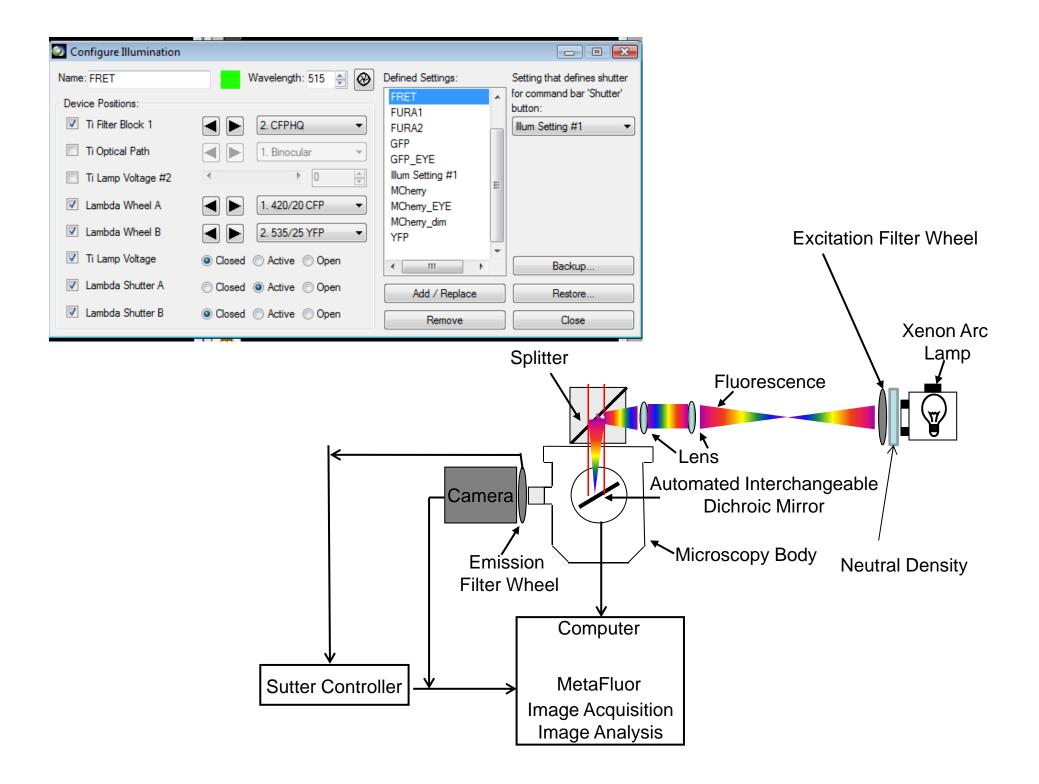
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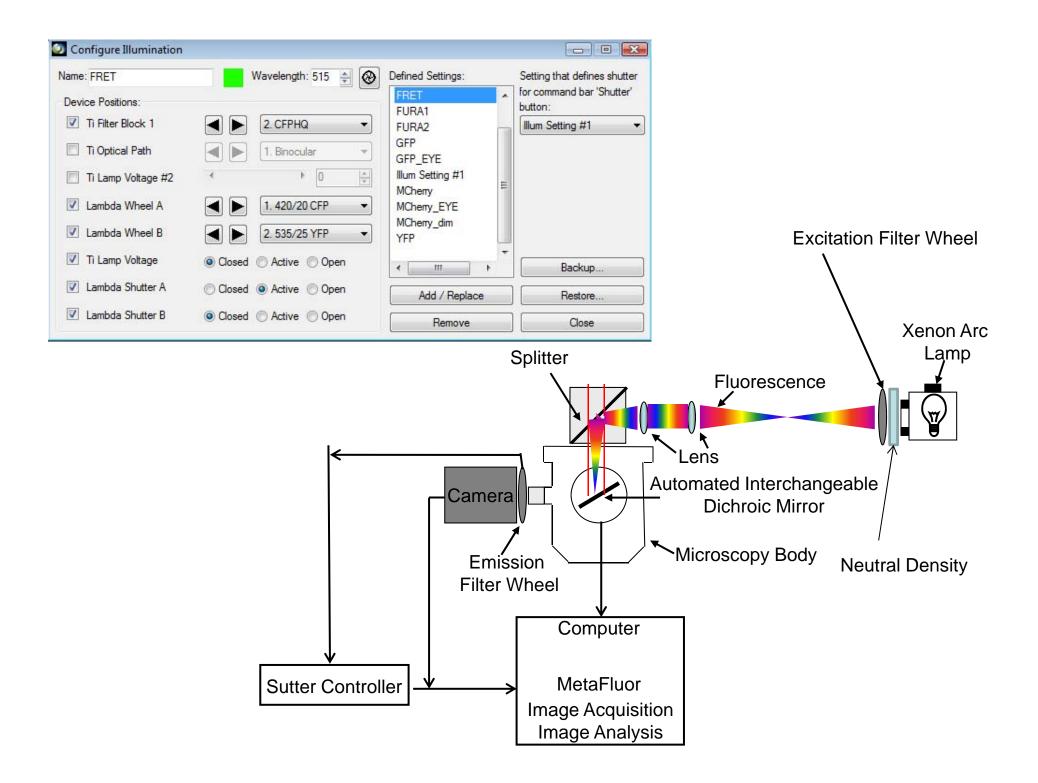


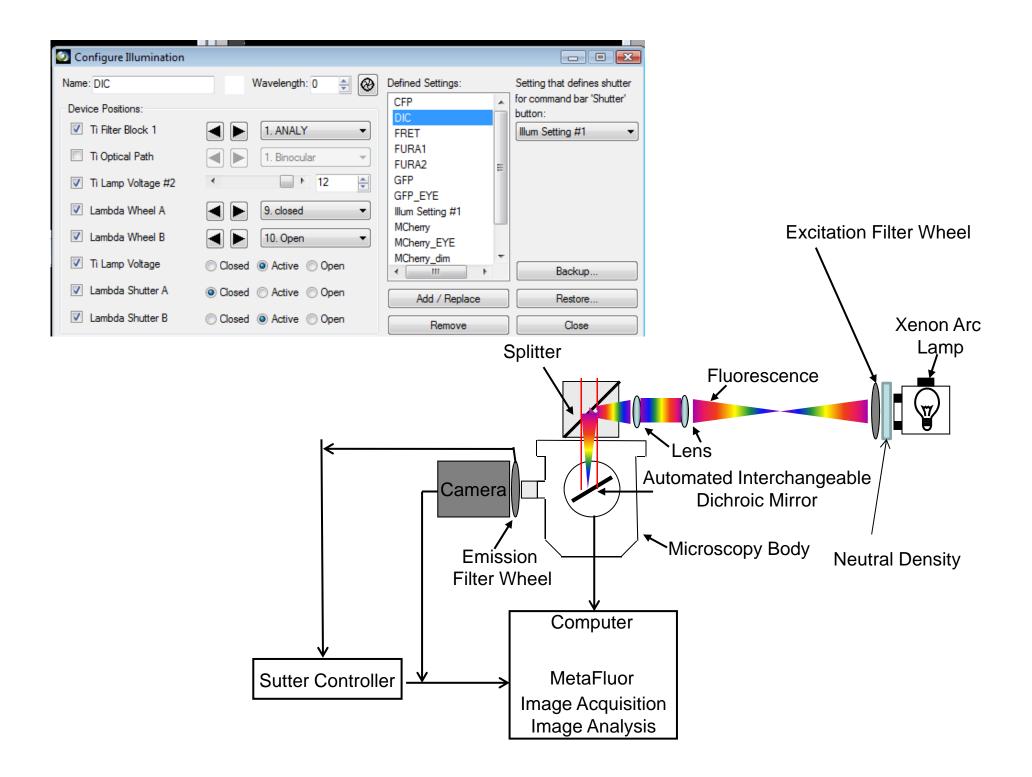
*CFP (80%)		FRET (80%)			
AG Dinsystems Software 7.6.5.0		Q± MAG Diosystems Software			
					Experiment Control Panel
4 12					Status:
A 12					Log Data [File not open]     Save Images [File not open]
					Save Ratios [File not open]
					Cycle: 0 F4: Acquire Set Timelapse
	2 Configure Illumination	>			CPS: - Zero Clock Focus Clo
	Name: Illum Setting #1	Wavelength: 0 🌲 ⊗		defines shutter d bar 'Shutter'	
	Device Positions:		DIC button:		
	Ti Optical Path		FRET Illum Settin	<u>g #1</u>	
	<ul> <li>Ti Lamp Voltage #2</li> </ul>	<ul> <li>▲ ■ 1. Binocular ▼</li> <li>▲ ■ 0 ♠</li> </ul>	FURA2 E		
			GFP_EYE		
	Lambda Wheel A		Illum Setting #1 MChemy		
▲*YFP (80%)			MCherry_EYE MCherry_dim		
C <sup>±</sup> MAG Diosystems Software 7.6.5.0	Ti Lamp Voltage	Closed Closed Active Open	≺ III ► Ba	ckup	
•	Lambda Shutter A	Closed Active Open	Add / Replace Re	store	
	Lambda Shutter B	Closed Active Open	Remove	Close	
4 12		A 12			

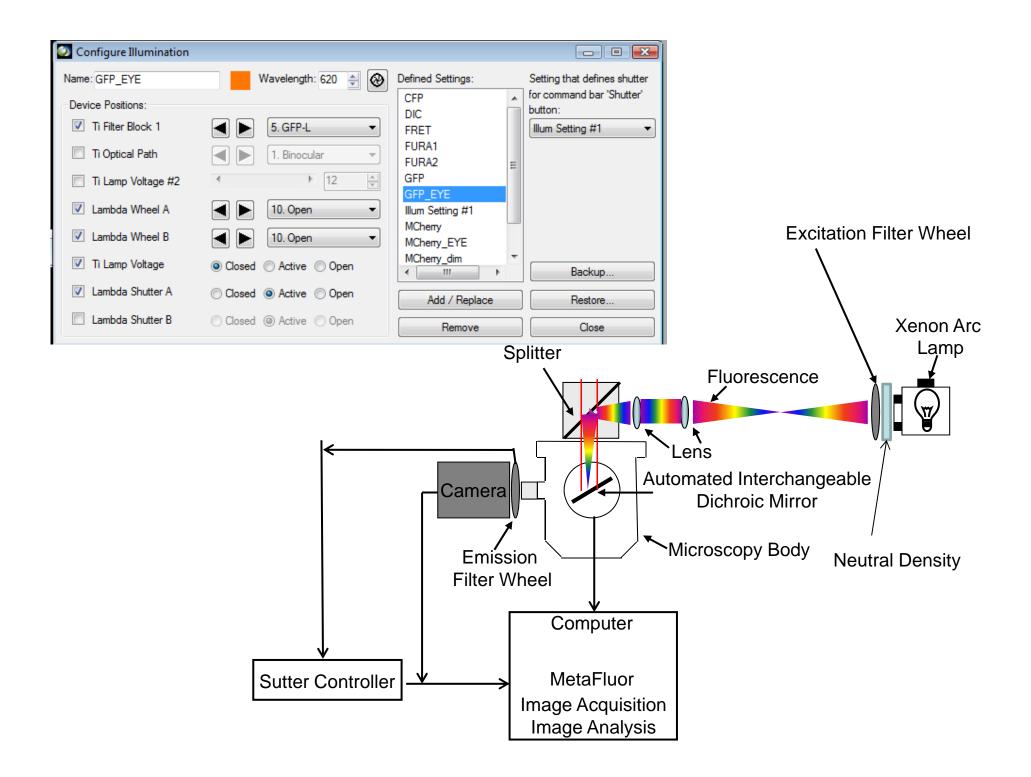












MAG Biosyst	ems Software					
ile Configure	Run Experiment Graphs Utilities Calibration Jou	irnals Windows Help				
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Focus	*CFP (80%)					
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Acq W2					💷 Experiment Control Panel 👝 🔳 🖾	ו
Acq W3					Status:	
	A 12	210 216 A 12			Log Data [File not open]	
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Shutter			Wavalasath 1	relength Name:	Cycle: 0 F4: Acquire Set Timelapse	
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Events			Wavelength 3			
Cfg Acq			Wavelength 4 T			
Cfg Exp			Way	relength Changing Hardware:		
Scale 16			Wavelength Use:	ination Device: DIC -		
References			Wavelength 4 is the			
Set Disp			numerator for Ratio 2:			
Cfg Graphs			Ratio 2 = W4 / W5			
Zero Clk			7 P	usition:		
Close				Set Z Position for this Wavelength to: 0		
More >>	*YFP (80%)	— — 🖾 🎽 🚾 *DIC (	(8)			
More >>	Q± MAG Diosystems Software 7.6.5.0	Q± M		Digital Camera Acquisition Parameters:		
			Left: 0 🜩 Width: 512 🌩	Exposure Time: 400 🚔 Milliseconds 🔻		
			Top: 0 🚔 Height: 480 🚔	Gain: Gain 3 (3x)		
			Binning: 1 🚔	Bit Depth: n/a		
	A 12	A 12	Image Size: 512 x 480	Transfer Speed: 1.25 MHz (Standard)		
			Select Region	Camera Shutter: Open for Expose		
			Use Entire Image	EM Gain: 100 🛬 < 🕞 🕨		
				Allow items to differ for each wavelength		
				Close		
		1				

MAG Biosyste	ems Software					_ 0 ×
File Configure	Run Experiment Graphs Utilities Calibration Jo	urnals Windows Help				
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Focus	*CFP (80%)	🗆 😐 🐹 💽 *FRET				
Acq One	Q± MAG Dissystems Software 7.6.5.0		AG Diosystems Software 7.6.5.0			
Acq W1						
Acq W2					🔳 Experiment Control Panel 👝 🔳 🔀	
					Status:	
Acq W3	412				Log Data [File not open]	
Acq W4			Configure Acquisition		Save Images [File not open]	
Acq W5			Wavelength to Configure:		Save Ratios [File not open]	
Shutter			Way	elength Name:	Cycle: 0 F4: Acquire Set Timelapse	
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Events			Wavelength 3	CFP		
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Scale 16			Wavelength Use:	ination Device: CFP -		
References			Wavelength 1 is the			
Set Disp			numerator for Ratio 1:			
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Zero Clk			ZPo	isition:		
Close			-	et Z Position for this Wavelength to: 0		
More >>	T *YFP (80%)	— — 🖾 🌌 *DIC (	(8)			
	AG Dissystems Software 7.6.5.0	Q± M	Camera Binning and Sub-Region:	Digital Camera Acquisition Parameters:		
			Left: 0 vvidth: 512	Exposure Time: 800 🖨 Milliseconds 🔻		
			Top: 0 🚔 Height: 480 🚔	Gain: Gain 3 (3x) 👻		
			Binning: 1 🌲	Bit Depth: n/a 👻		
	A 12	A 12	Image Size: 512 x 480	Transfer Speed: 10 MHz (EM Gain) 🔻		
			Select Region	Camera Shutter: Open for Expose 🔹		
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				Allow items to differ for each wavelength		
				Close		

MAG Biosyste	ems Software								
File Configure	Run Experiment Graphs Utilities Calibration Journals Window	/s Help							
Control	*CFP (80%)		*FRET (	80%)				x	
Focus	Q± MAG Dissystems 8nttware 7.6.5.0			G Dinsystems	s Software	7.6.5.0			
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Acq W1									
Acq W2									
Acq W3	A 12		A 12						
Acq W4									
Acq W5									
Shutter									
Regions									
Events									
Cfg Acq									
Cfg Exp									
Scale 16 References									
Set Disp		Configure	Experiment						
Cfg Graphs					Acquire		Save		Update
Zero Clk			Image:	Acquire?	Interval:	Data Logging:	Interval: Show?	Update?	Interval:
Close		On / Off	CFP	Acquire	1	🗌 Avg 🔲 Sum 🔲 Area	1 🚔 🔽 Show	🔽 Update	1
More >>	*YFP (80%)	On / Off	FRET	Acquire	1	🔲 Avg 🔲 Sum 🔲 Area	1 🚖 🔽 Show	Update	1 🛓
	Q± MAG Diosystems Software 7.6.5.0	On / Off	MCHERRY	Acquire	1	Avg Sum Area	0 🚖 🗌 Show	Update	1
		On / Off	DIC	Acquire	1	🗖 Avg 📄 Sum 🔲 Area	1 🚖 🔽 Show	🔽 Update	1 🚔
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	A 12	On / Off		n/a	n/a	Ratio (W1 / W2)			1
		On / Off	Ratio 2	n/a	n/a	Ratio (W4 / W5)	0 🚖 🔳 Show	Update	1
		On / Off	Calcium	n/a	n/a	Calibrated Ratio 1	Use Acquire Calibratio	on Standards	
							to select which image	to calibrate	
		ОК							Cancel
					_				

📃 Experiment Control Panel 👝 🗉 🔀 Status: Log Data [File not open] Save Images [File not open] Save Ratios [File not open] Cycle: 0 F4: Acquire Set Timelapse... CPS: - Zero Clock Focus... Close

No Calibration 2.49 GB physical memory 3.99 GB virtual memory

File Configure Run Experiment Graphs Utilities Calibration Journals Windows Help

Focus	
Acquisition Region:	Camera Settings:
Binning: 1 Image Size: 512 x 512	Exposure Time:
Left: 0 📉	200 🖨 ms 🔻
Top: 0	Gain: Gain 2 (2x) 🔻
Width: 512 🚔	
Height: 512	Rate: 1.25 MHz (S 💌
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Store as Acq Rgn << CTR >> Full Chip	Store for this Wave
☑ Update Histogram    Depth: 16-bit	Illumination:
100 -	Wavelength:
	CFP 🔺
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E co	MCHERRY
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e	
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Histogram of Image	Close After Acquire
Auto Scaling:	
Lo % 0 🗢 👽 Autoscale Hi % 0 🜩	Start Focusing
	F2: Pause Focus
Use Wave Settings Store for this Wave	Close
Z Position:	
▲ 1 ▼ 1 Goto 0 Use Wave Z	Store for this Wave

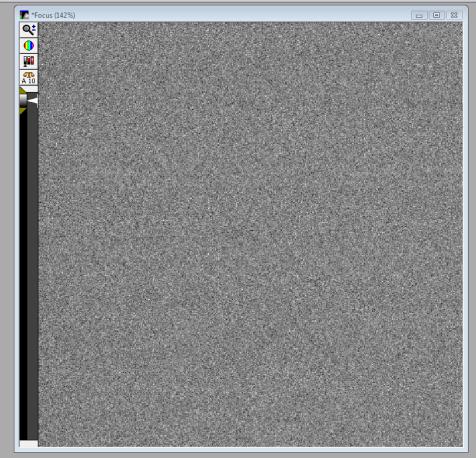
2.49 GB physical memory 3.99 GB virtual memory

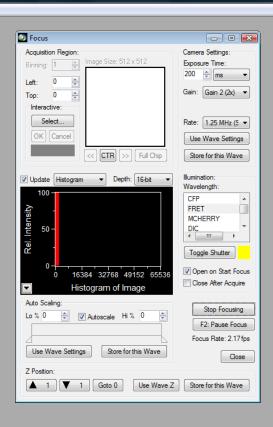
🚱 📃 🔄 🖉 🚬 Removable Disk (G:) 🛛 @ Microsoft PowerPoi... 🖉 MAG Biosystems So...

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Focus

File Configure Run Experiment Graphs Utilities Calibration Journals Windows Help



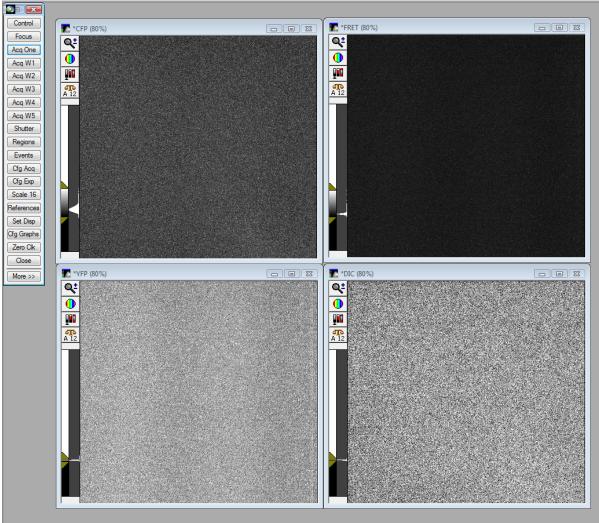


142% 1/1 (485, 438) -> 0 No Calibration 2.49 GB physical memory 3.99 GB virtual memory



YFP

File Configure Run Experiment Graphs Utilities Calibration Journals Windows Help



Experiment Control Panel

 Status: Paused

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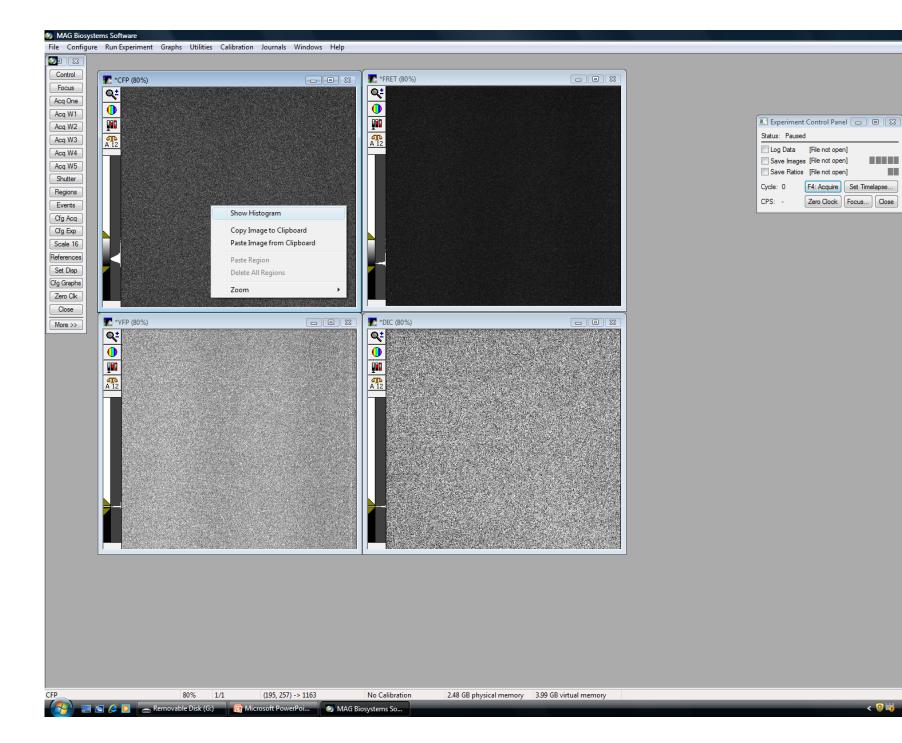
 Save Ratios
 [File not open]

 Cycle: 0
 F4: Acquire
 Set Timelapse...

 CPS: Zero Clock
 Focus...

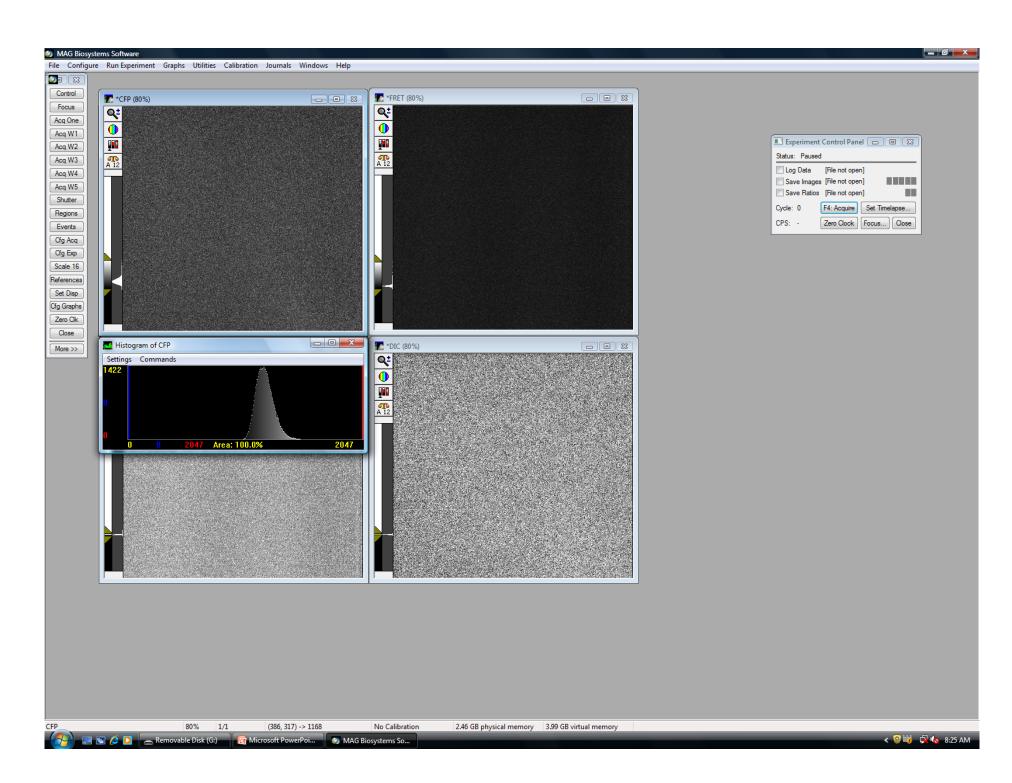
 Cose

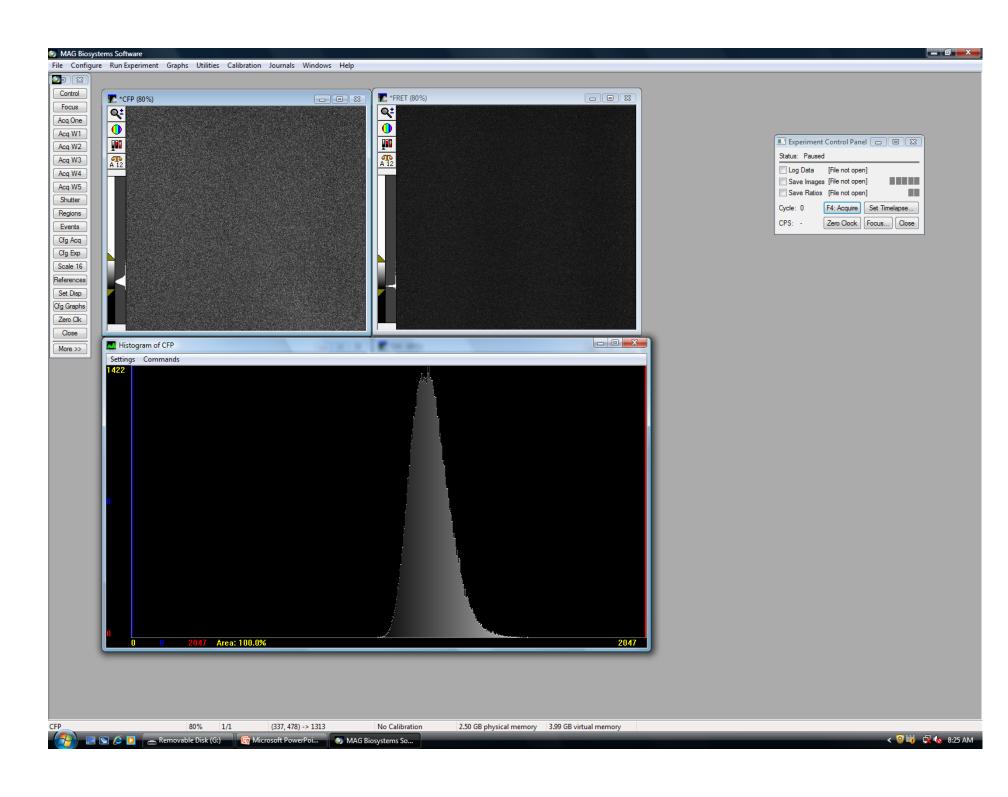
80% 1/1 (455, 438) -> 1001 No Calibration 2.48 GB physical memory 3.99 GB virtual memory

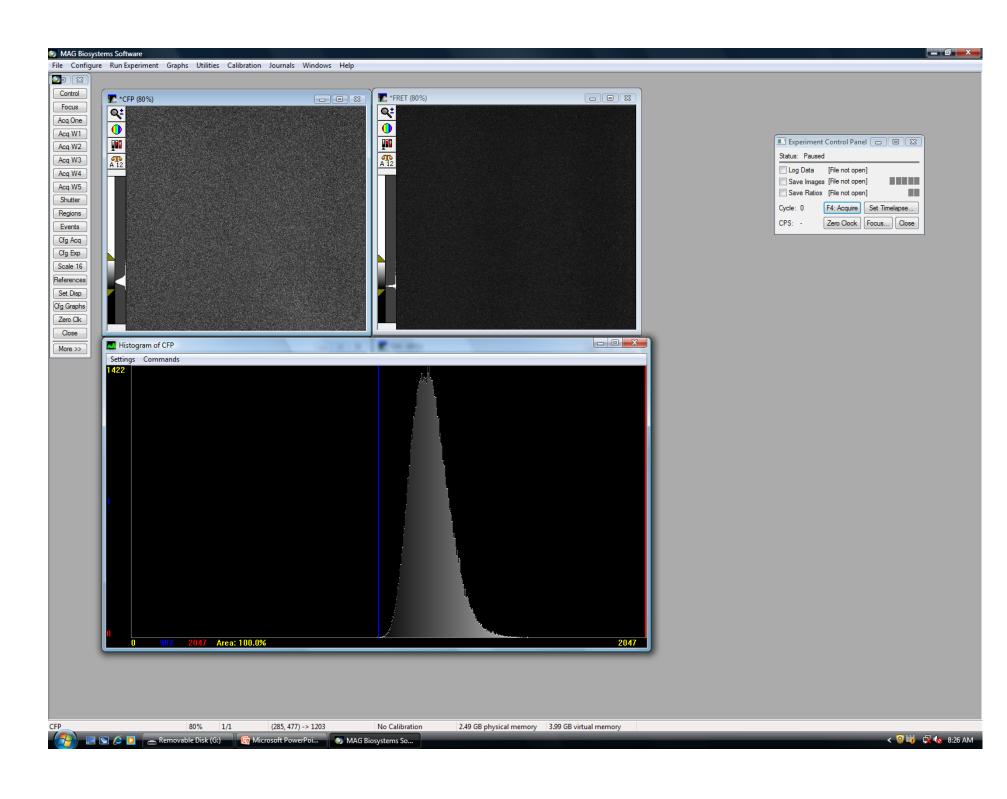


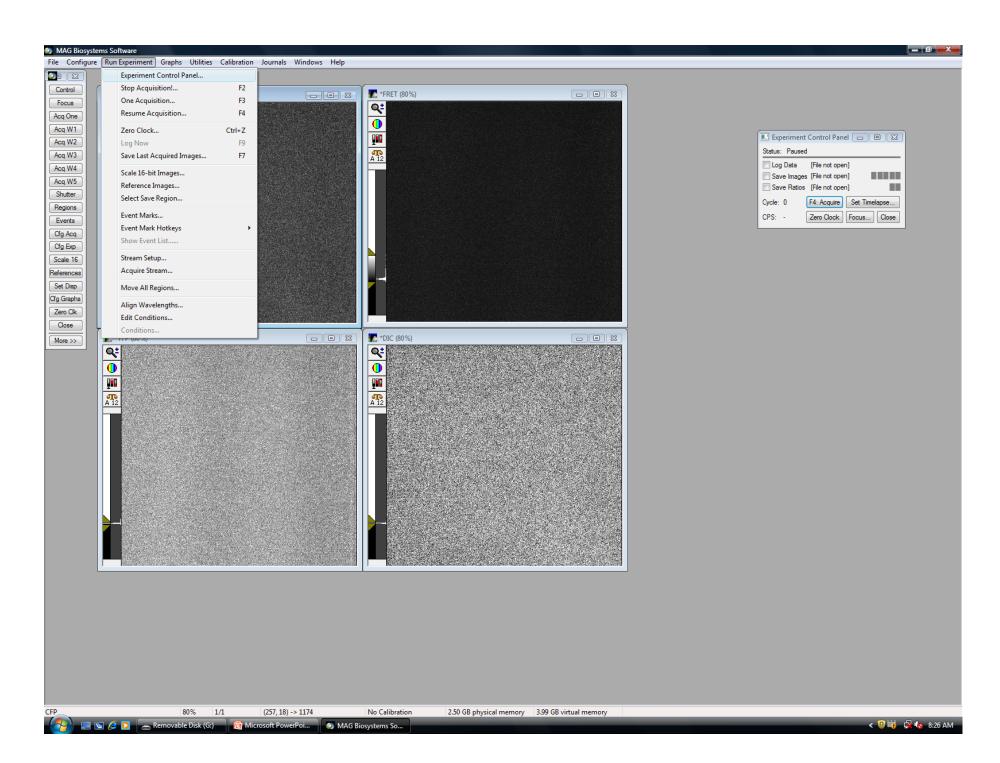
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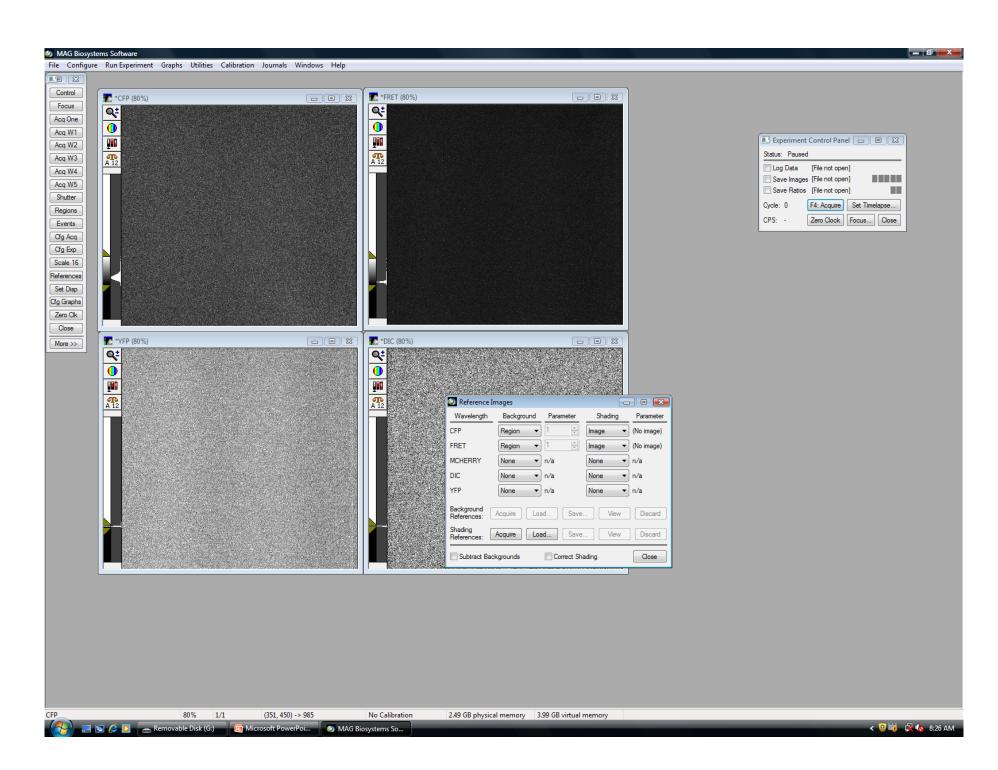
\_ 0 X





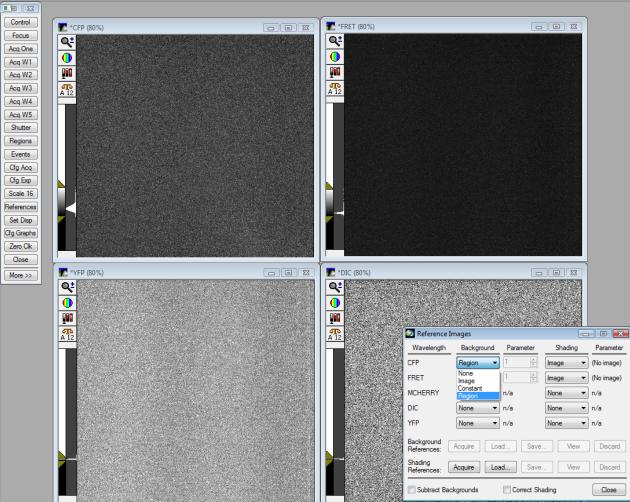






CFP

File Configure Run Experiment Graphs Utilities Calibration Journals Windows Help



 80%
 1/1
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 No Calibration
 2.50 GB physical memory
 3.99 GB virtual memory

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🔳 Experiment Control Panel 🗖 🗉 🔀

F4: Acquire Set Timelapse...

Zero Clock Focus... Close

Status: Paused

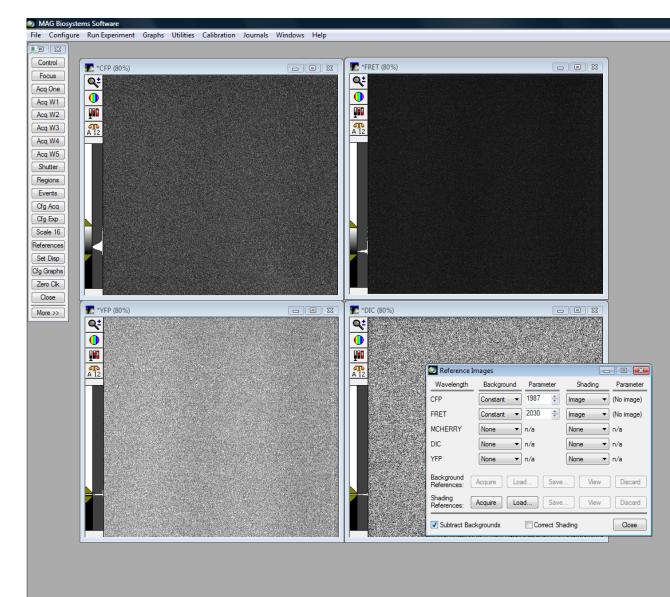
Cycle: 0

CPS: -

Log Data [File not open]

Save Images [File not open]

Save Ratios [File not open]



Exp	eriment	Control I	Panel		
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80% 1/1 (122, 258) -> 1007 No Calibration 2.50 GB physical memory 3.99 GB virtual memory

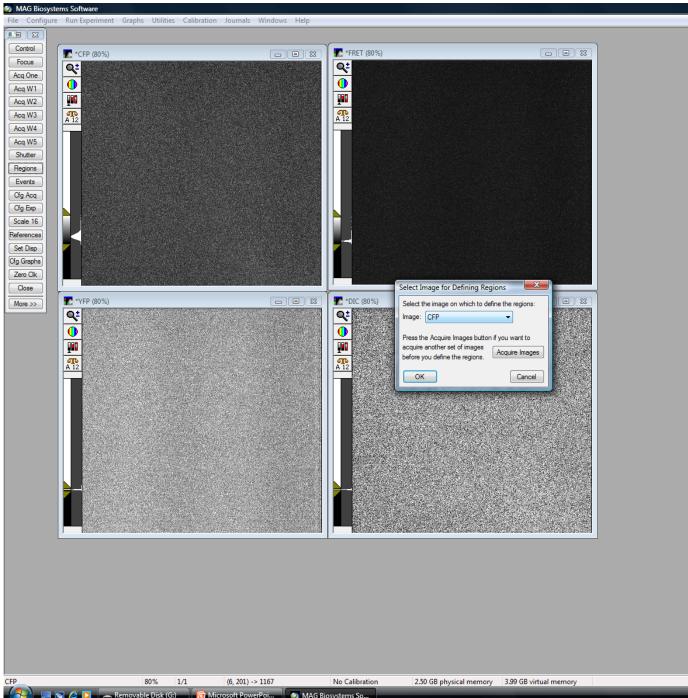
Removable Disk (G:) 👩 Microsoft PowerPoi... 🧑 MAG Biosystems So...

CFP

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— 0 — X

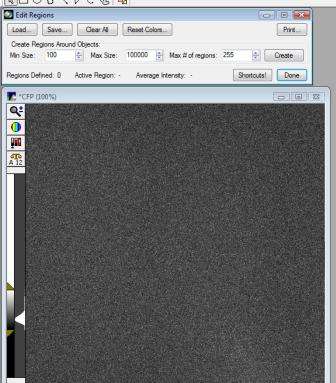


### 💷 Experiment Control Panel 👝 😐 🕱 Status: Paused Log Data [File not open] Save Images [File not open] Save Ratios [File not open] F4: Acquire Set Timelapse... Cycle: 0 CPS: -Zero Clock Focus... Close

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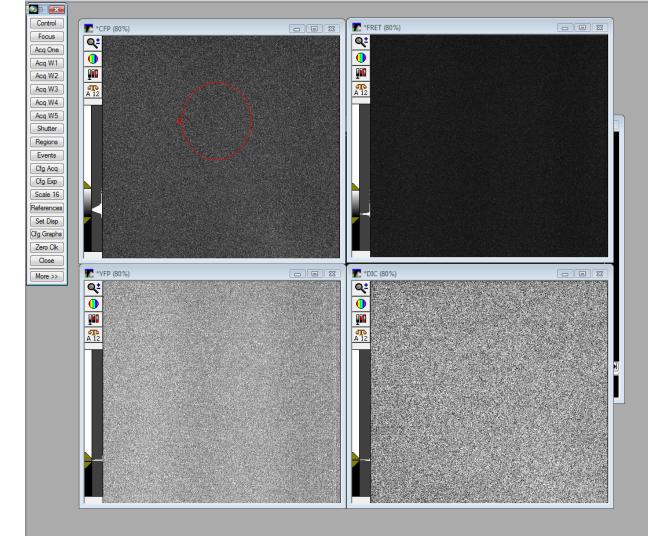
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No Calibration 2.50 GB physical memory 3.99 GB virtual memory



File Configure Run Experiment Graphs Utilities Calibration Journals Windows Help

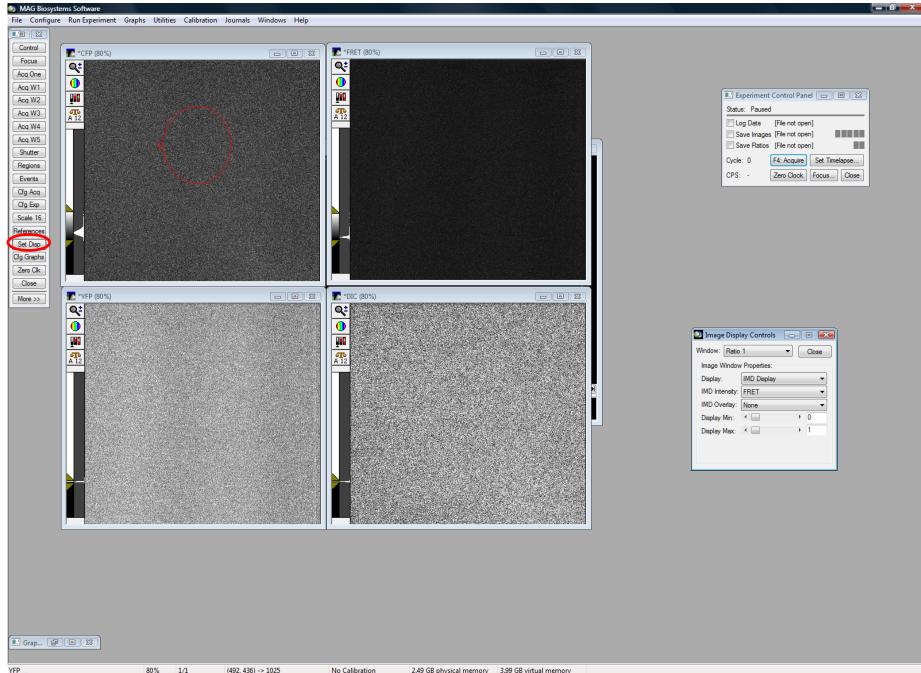


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💵 Grap... 🗗 😐 🕱

YFP

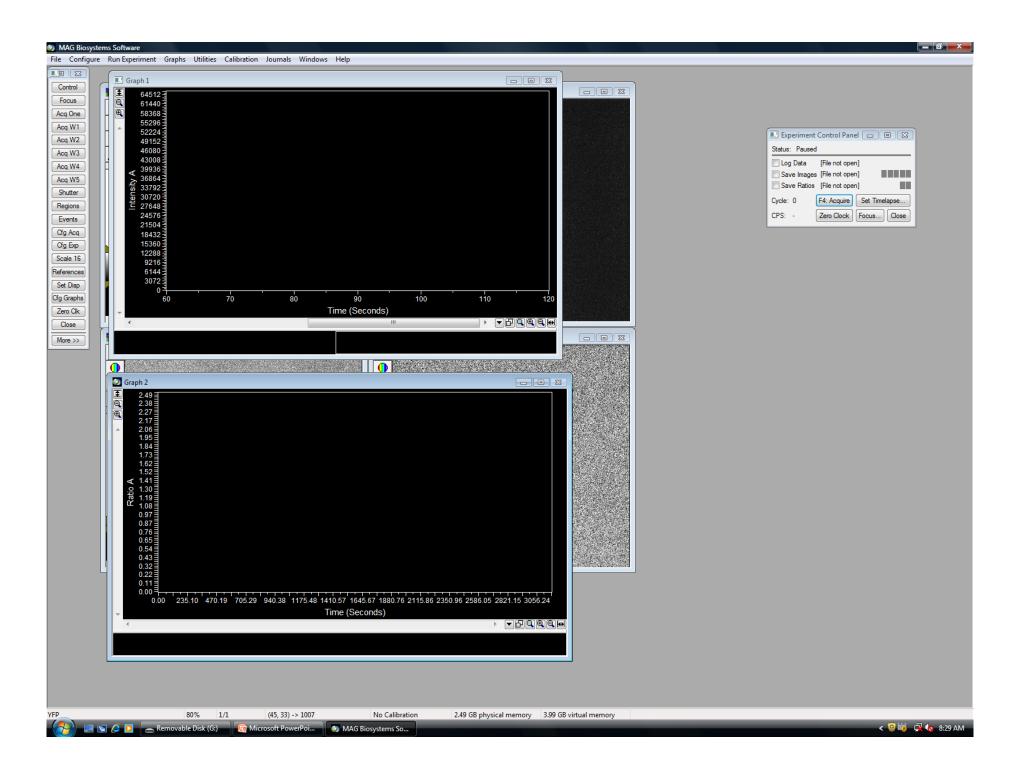
80% 1/1 (306, 471) -> 1008 No Calibration 2.49 GB physical memory 3.99 GB virtual memory

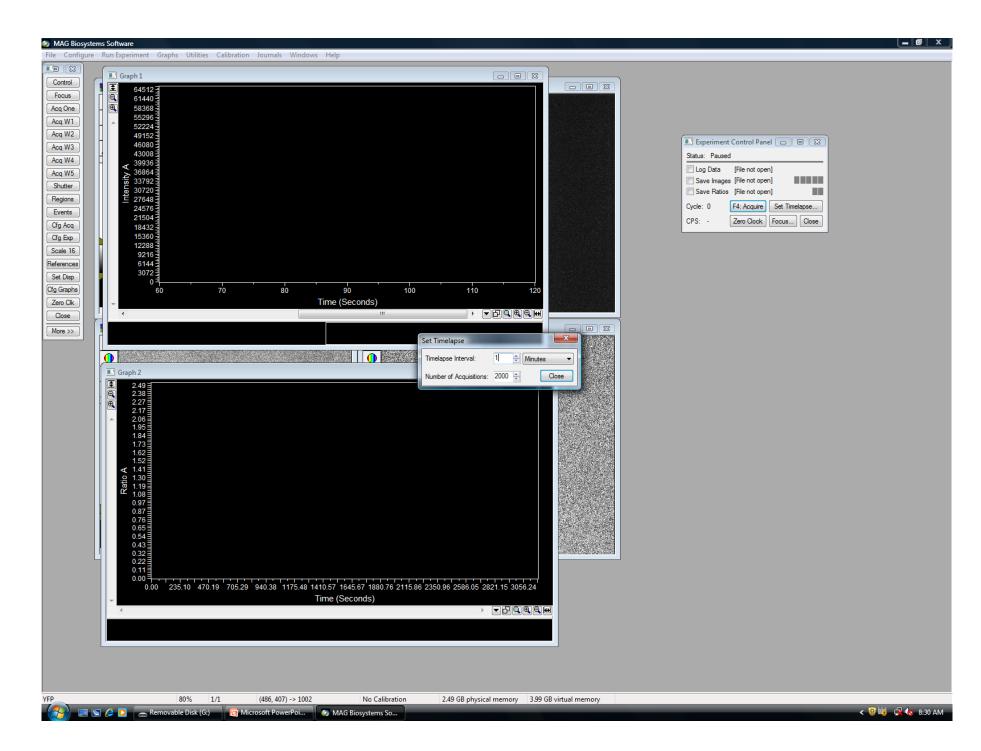


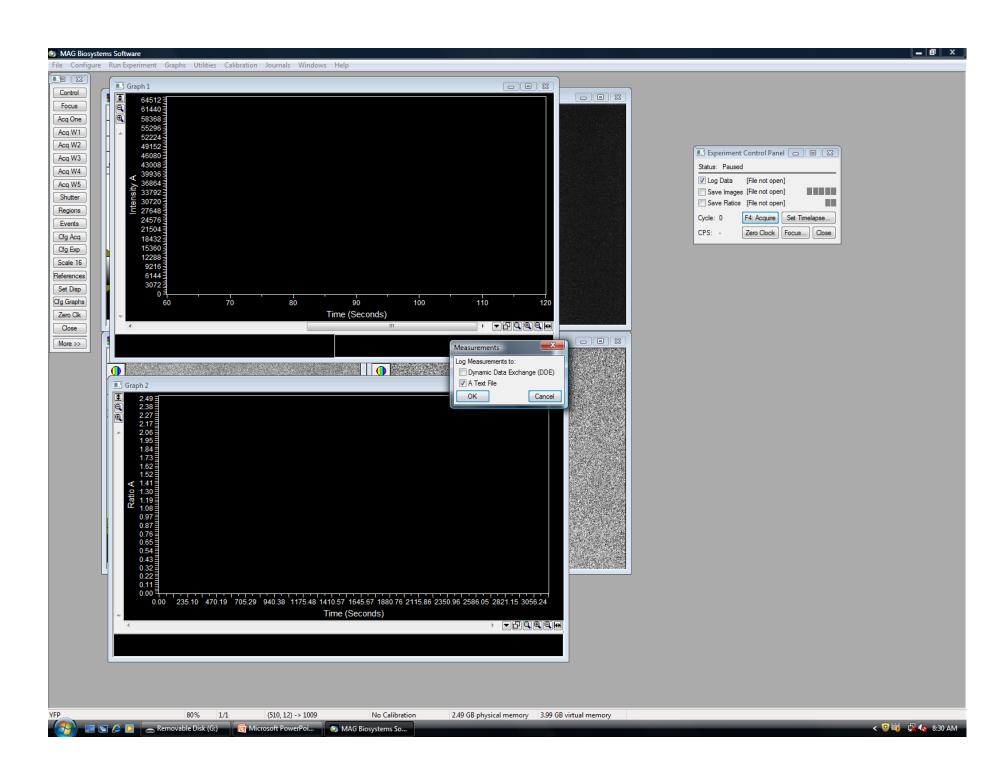
80% 1/1 (492, 436) -> 1025 No Calibration 2.49 GB physical memory 3.99 GB virtual memory

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Ø MAG Biosyste	ems Software								L D X
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YFP		👩 Microsoft PowerPoi					_		< 🧐 🙀 🙀 🚯 8:31 AM

Save Protocol		
Description: CFP/FRET/YFP/D	IC	*
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Wavelength Detai	ls:	Event Marks:
Acquire Image: Update Image: Display Window: Save Image:		GM6001 wash
Exposure Time: Camera Gain:	800 milliseconds 3	
Illum. Device: Wavelength: Intensity: Use Shutter:	Yes Yes Yes	
Wavelength: CF	P	Save Export Cancel

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