



Zeiss LSM 900

With Airyscan 2

Standard Operating Procedure

Interdisciplinary Life Sciences Building

Room 324

Text Dr. deCarvalho for time-sensitive issues: 301-529-5008



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	POWERING ON	
1.	Turn on the main power switch (left).	PSU 1
2.	Turn on components switch (right).	PSU 2 PSU
3 & 4.	Turn on the power strip. Wait a few seconds to turn on the PC.	
5.	Open ZEN 3.5 from the desktop.	Zen 3.5 (blue edition)
6.	A web browser will pop up with the Google Session Start Form that you must fill out. The acquisition software will not load until the form is submitted.	Zeiss Confocal Session Start (Unassisted) Log in to record the start of your imaging session. You will be pre- same way when you close the program. Sign in to Google to save your progress. Learn more * Required Enter your first and last name. * Your answer



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LOADING THE SLIDE			
1.	Select your desired objective in the Microscope menu on the right side of the program.	✓ Microscope 5x 10x 20x 40x 63x 0.16 0.45 0.8 1.2 1.4	
2.	This is an inverted scope, so place the coverslip facing downwards. Note: the 40x objective requires Immersol and the 63x objective requires immersion oil. Place immersion media directly onto the objective before slide placement.		
*Befc	ore changing objectives from oil to dry	<i>, clean your slide AND the objective</i> *	



	LOCATING THE SPECIMEN	
1.	Use the joystick for XY movement.	
2.	Use the mini display for focusing (Z movement).	
3.	Use the Locate tab to view your sample. Select transmitted light, DAPI, GFP or DsRed.	Coate Acquisition Processing An Transmitted Light Off On Reflect Switch To * Transmission Favorites Configure BF DIC II DIC III DsRed ALL OFF
4.	If you'd like to save a position to return to later, it can be specified with a 'Mark' under the Stage menu on the right-hand side of the screen. You can return to this exact position using the 'airplane' button.	X/Y Position Set Ze Calibr Marks # Name X (µm) Y (µm) +



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CONFOCAL ACQUISITION				
1.	Open the Acquisition tab.	C:\Users\bhaller\Pictures\temp\Snap-13.czi - ZEN 3.0 (ZEN system) File Edit View Acquisition Graphics Tools Window Image: C:\Users\bhaller\Pictures\temp\Snap-13.czi - ZEN 3.0 (ZEN system) Image: C:\Users\Bhaller\Pictures\Bhaller\Pictures\temp\Snap-13.czi - ZEN 3.0 (ZEN system) Image: C:\Use		
2.	Select your customized experiment that was set up during training or a KPIF DEFAULT program.	Acquisition Acquisition Processing Analysis Applications A - KPIF 2-Track Confocal DEFAULT with BF * Smart Setup * Smart Setup		
3.	Imaging Setup menu If you need to alter the experiment settings for a new sample type, please see the appendix (pg. 19) on how to configure tracks and dye settings. Save modifications as a new experiment using the 'gear' button.	 Imaging Setup Show All 488 661 Confocal Confocal Confocal Confocal Switch track every Frame Switch track every Frame DAPI: SP 470 Ch3: LP 575 Ch2: LP 575 Ch2: Sp 470 Ch3: LP 575 Ch2: Sp 470 Ch3: LP 575 Ch2: Sp 470 Ch3: Ch3: Ch3: Ch3: Ch3: Ch3: Ch3: Ch3:		



	Acquisition Mode menu	
	Crop area : Set your region of interest by either moving the slider to increase the crop area or adjusting the scan area.	Acquisition Mode Show All
	Frame size : Press the 'Confocal' button to have the frame size optimized for spatial resolution.	Crop Area Crop Area Crop Area Scan Area Image Size 638.9 μm × 638.9 μm Pixel Size 0.22 μm
4.	Scan speed: Can slow down for better signal-to-noise (instead of line averaging) or speed up for live samples (but reduced image quality).	Frame Size 2909 px 2909 px Presets ▼ Sampling 1.0 x Confocal Frame Time 28.79 s Pixel Time 0.73 μs Scan Speed 6 ♦ Max
	Direction : Bidirectional scanning reduces scan time. Click on 'Auto' to correct scanning resignation if you see artifacts. Use unidirectional scanning for publication quality images.	Direction Line Step 1 Averaging None 2x 4x 8 16
	Averaging: Select optimal line averaging to reduce noise.	
5.	View your specimen with the 'Live' button. Safety note: Do not look directly into the laser light while scanning.	AF



		Channels	✓ Show All 🗹
		✓ 405 Confocal	DAPI Ref. 🗖 🔻
	Channels menu	✓ 488 Confocal	AF488
		561 Confocal	AF568
	If you want to mayimize image	✓ 640 Confocal	AF647
	signal to poise quality increase		us Ref. 🔅 🛪 🗸
	laser power and lower Master	🔲 High Intensity Laser F	Range
	Gain (< 800 V). If you want to	405	
6.	minimize photobleaching, increase Master Gain and lower laser	Lasers 🗹 40	5 🔲 488 🛄 561 🔛 640
	power.	405 nm —	0.7 %
		Pinhole ————————————————————————————————————	37 μm 🛟
	Ensure the pinhole size is optimal by selecting the '1 AU' button.	1.00 Airy Units ≙ 0.4 μm	section I AU Max
		DAPI	
		Master Gain ——	700 V 🛟
		Digital Offset	
		Digital Gain – – – – – – – – – – – – – – – – – – –	1.0 ‡
	Ontimiza imaga kuiahtu asa sia tha	Display	
	bistogram in the Disnlay tab	AF488-T2	Spline Mode
	histogram in the Display tab.		
7	Adjust laser power/master gain	Auto Min/Max Best Fit 2.00	↓ 0.01 ↓ Current ▼ Reset
7.	until the brightest pixels just	50 100	
	stretch to the right without	harman	
	clipping (see pg. 25).	- Mu	MMMMM abdaused



		Channels
8.	Note: Ensure that the 'Display Settings' is set to 'Default' in the Channels menu to visualize the full dynamic range.	\checkmark 405-561ConfocalDAPIRef.AF568 \checkmark \checkmark 488ConfocalAF488 \checkmark </td
9.	Use the 'Snap' button to capture an image. Save the file by selecting File > Save as.	AF C Continuous Snap
10.	Please review "Essential Checklist" on pg. 25 for obtaining optimal images.	



ACQUIRING Z-STACKS			
1.	Check the 'Z-stack' button.	✓ Z-Stack 18 Slices Tiles Time Series All Tracks per Slice ✓ Start Experiment	
3.	Z stack menu Focus to one end of the specimen and click 'Set First' and then to the other end of the specimen and click 'Set Last'. Click the button labeled 'Optimal', to automatically set the interval width (step size). Note: You can change the interval if you check 'Slice' under 'Keep' and vice versa.	 First / Last First / Last Center Set Last -124.69 µm Slices 18 Interval 0.10 µm Optimal 0.46 µm Keep Interval Slice Slice Slice Slice Slice Slice Slice Slice Auto Z Brightness Correction 	
	Note: If the signal intensity diminishes through a thick Z-stack, select 'Auto Z Brightness Correction'. Go to the 'Last' Position and set Master Gain. Click 'Add/Update'. Then go to 'First' position and set Master Gain. Click 'Add/Update'. Select 'Enable Test' and choose 'Continuous' to preview how the laser power changes at each position in the Z-stack.	970.4 Slice Center 1766.25 µm Position 970.36 µm Slice # 1 Offset 0.00 µm Slice # 1 Optimize Sectioning and Step ✓ Auto Z Brightness Correction Add / Update Z-Position Add / Update Z-Position Move to 970.358 µm Spline Interpolation Extrapolate ✓ Enable Test	
4.	Click 'Start Experiment' instead of 'Snap'.	✓ Z-Stack 18 Slices ☐ Tiles ☐ Time Series All Tracks per Slice ✓ ✓ Start Experiment	



from all z-slices.

5.

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After image acquisition, use the **Ortho Display** tab to view the Z-stack.

Check the 'Maximum Intensity Projection (MIP)' button to generate a Z-projection, which is a single image that depicts peak signal intensities

Image will be labeled as an 'Experiment' vs a 'S. Save as a .czi file to include metadata.





	AIRYSCAN ACQUISIT	ION
1.	<i>NOTE: You must use 63x objective for Airyscan</i> In the Acquisition tab, select your customized experiment. Use the 'KPIF Airy DEFAULT' template to set up a new experiment.	Acquisition Processing Analysis KPIF Airy DEFAULT * Smart Setup AF Image: Continuous Find Focus Set Exposure Live Continuous
2.	Imaging Setup menu If you need to alter the experiment settings for a new sample type, please see the appendix on pg. 19 on how to change tracks, filters and dye settings. Save modifications as a new experiment using the 'gear' button.	Switch track every Frame Under the second s
3.	Acquisition Mode menu Optimal parameters have already been set for Airyscan experiments. Please refer to this image to double check your experiment settings. Crop or scan area can be reduced to 1.0x, but not below.	✓ Acquisition Mode ✓ Show All LSM Crop Area 2.0 x Scan Area ↓ 0.00 µm ↓ 1436 px Y Presets Sampling 2.0 x SR Confocal Frame Time: 10.44 s Bits per Pixel 8 16 16
4.	View your specimen with "Live".	AF C C Continuous



		✓ ▲ Channels ✓ Show All	
5.	Under the Channels window, adjust each channel's laser power and Master Gain.	405 SR DAPI Ref. ✓ 488 SR AF488 C ✓ 561 SR AF568 C ✓ 640 SR AF647 C ✓ 640 SR AF647 C ✓ A + Tocus Ref. X High Intensity Laser Range 640 Lasers 405 488 561 ✓ 640 Lasers 405 488 561 ✓ 640 640 Master Gain 800 V Digital Gain 1.0 10 10 10 10 10	
6.	Adjust laser power/Master Gain until the pixels just stretch to the brightest pixels at the right without piling up (i.e., "clipping" see pg. 25).		ne Mode P Reset
7.	Open Airyscan adjustment (gray hexagon) at bottom of screen.	Frame Rate: Pixel Value: Position: - fps X:- Y:-	28 28
8.	Select each channel individually to allow adjustments in 'Live' mode, which will be finished when the hexagon turns green.	Airyscan Detector Adjustment Quality and Status OK Activation Adjust in live and continuous Adjust in time series with inter To adjust the Airyscan detector manually during at the automatic adjustment above. Fiber position X Position Y Position Store Current Pos	scans rval. c quisi
9.	Press 'Snap' to capture an image.	AF C C Continuous Snap	,
10.	To save the SR image, select the Airyscan tab. Click on 'Create Processed Image' which results in a new image labeled 'Snap-Airyscan Processing.czi'. For Z-stacks, choose 'Current image (3D)' on the drop-down menu.	Dimensions Player Graphics Custom Graphics Airyscan Display Mode Sum Channel SR Super Resolution 6.80 Current Image (2D) Create processed image	o Filter age(s)



	TILING	
1.	Stage menu The stage must be calibrated before proceeding with tiling. Click the 'Calibration' button under the Stage menu on the right side of the screen.	 Stage Show All Caution! Risk of Crushing Stop Stop X-Position µm 0µm 0µm 0µm 0µm × ×
	Click 'Continue' on the pop-up.	Caution! Risk of trapping fingers and damaging the instrument. Ensure that people stand clear of the instrument and that the full travel range is not obstructed by any objects (e.g. sample carrier, stage insert, TL condenser or other special device arrangements). Continue
2.	Select 'Tiles' from the experiment menu.	□ Z-Stack ✓ Tiles No Tiles □ Time Series 7.88
3.	A new tab will open. Select 'Show Viewer' to open the Advanced Setup Window.	Tiles Show All Show Advanced Tiles Viewer Show viewer Tile Persions
4.	Select 'Tile View' in the bottom right corner and then turn on 'Live'. There are several options to choose from:	Tiles Display Tiles Display Show All Show Live/Continuous in New Tab Separate Container Center to Stage Position



option 1:	'Set up new tile region from a predefined size'. This option will image a 3x3 tile centered around a specified position. Move the stage to a position of your choice and press 'F9' on the keyboard to specify it under 'Tile Regions'.	Tiles - Advanced Setup Keep Tool Draw Tile Rec Preview Tiles Tiles ++++++++++++++++++++++++++++++++++++
option 2:	'Set up new tile regions by drawing a contour'. This option will image a tile within a boxed region that is drawn. Choose the 'Rectangle' at the top left of the Advanced Setup Window and use your mouse to draw a rectangle directly onto the preview screen.	joisizi · I · · · · I joiojiži · I · · · · · joiojiži · I · · · · · I joiojiži · I · · · · · i joiojiži · I · · · · · i joiojiži · I · · · · · · i joiojiži · I · · · · · · i joiojiži · I · · · · · · i joiojiži · I · · · · · · · · · · · · · · · · ·
option 3:	'Set up new tile regions by specifying two or more marker positions. This option will image a tile that encompasses everything between two positions. Move the stage to your desired starting position and click '+' at the top of the Advanced Setup window. Repeat for the desired end position.	Preview Um 1
option 4:	'Single positions'. This option will image only specified positions and nothing in between. Move the stage to your desired position and press 'F10' on the keyboard to specify it under 'Positions'. Repeat for every position you want to image.	New Keep Tool Current X 7084 µm Y 30827 µm Preview µm 1 \$500 1 \$6000 Tiles 0000 0000 1 \$500 1 \$6000 Positions 0000 0000 0000 1 \$500 1 \$6000
5.	Click 'Start Experiment' to capture the Tiled Image. Save the experiment as a czi.	



	LIVE IMAGING WITH INCUBATION		
1.	If using CO ₂ and/or nitrogen (for O ₂ levels), open the valve(s) of the gas tank(s). They are labeled on the regulators.		
2.	Tilt the neck of the microscope back and remove the slide holder.		
3.	The heated plate holder and lid are located within the microscope chamber on the bottom right.		
4.	Gently place the heated plate holder on the stage.		



5.	Add your sample and place the lid on top of the plate holder.	
6.	In Zen, open the Incubation menu and select H Insert P (if only heating stage) and H Unit XL (if heating the entire chamber). Set the desired temperature. If heating the chamber, close the side doors and top. If using CO ₂ , check CO2 Small V and set the desired percentage. If using nitrogen, check O2 Small V and set the desired percentage.	 Incubation Temperature H Unit XL 37.0 °C 24.4 °C 29.0 °C 28.5 °C H Dev Humid 37.0 °C 22.8 °C 37.0 °C 0.1 °C 0.1 °C 0.5 % 20.5 % 1 1 1 1
7.	When completed, uncheck the temperature and atmosphere settings.	
8.	Remove the plate holder apparatus.	
9.	Replace the slide holder by lining up the two red dots and pushing that corner down first. Close the CO ₂ /nitrogen gas tank valves.	



POWERING DOWN		
1.	Save all images or close the ones you did not need.	
2.	Clean immersion media from 40x or 63x objectives.	
3.	Close the ZEN program.	
4.	A web browser will popup with the Google Session End Form that you must fill out BEFORE shutting off the computer.	Zeiss LSM 900 Confocal session end (Unassisted) Leg out to record the end of your session time. Sign in to dioagle to save your progress. Learn more *Required Enter your first and last name * Your answer: Image: This is a request question Please note minor issues here. For serious issues, directly contact KPIF staff.
5.	Shut down the computer.	
6.	Turn off the microscope by turning off the power strip.	
7.	Turn off the components.	PSU
8.	Turn off the main power.	PSU



APPENDIX - For imaging setup			
	A. Setting Up Fluorescent Channels Without Transmitted Light		
	Important Consideration:		
	One Track: Fastest acquisition; recommended for ≤ 2 fluorophores without spectral bleed through.		
Two or more Tracks (pg 21): Slower acquisition; recommended for multiple fluorophores with emission spectra overlap/bleed through.			
One Track			
1.	Choose 'A - KPIF 1-Track Confocal DEFAULT' from the Experiments menu as a template to modify.	Locate Acquisition Processing Analysis Applications	
	Imaging Setup menu In this default program, the blue, green, and red emission channels are checked. Uncheck any channels you do not require.	 E Imaging Setup Show All ≥ Standard All chann Confocal Switch track every 	
2.	Channel 1 - Blue emission (400-480nm) Channel 2- Green emission (480-560nm) Channel 3 - Red emission (560-700nm)	400 500 600 700 DAPI: No Filter AF568: No Filter Use Dye Color Name Range	
	Channel 4 - Transmitted light	Ose Dyc Color Name Range V DAPI * * DAPI 400 nm - 492 nm V AF488 * * AF484 492 nm 567 nm V AF568 * * AF568 567 nm 700 nm Bright * * Bright 400 nm - 400 nm 100 nm	
Note:	If you are concerned about the potential for spectral bleed through, it is recommended to separate channels into multiple tracks (see Two or more Tracks sub-section, pg. 21)		



🗸 🖪 Imaging

3.	Choose a dye from the dropdown menu for each channel. If you do not find your specific dye, select one with the closest spectral properties (so you can visualize the emission curve).	Search Hoech Standard Dye Database Hoechst 33258 Hoechst 33258 Hoechst 33258 Hoechst 33258 Hoechst 33242 Switch track every Frame DAP: No Filter DAP: No Filter DAP: DAP AF488 Bright AF568 Bright Bright Bright
4.	Adjust the emission capture window for each channel to a desired range by moving the sliders. Make sure the green emission window doesn't overlap with the 488 laser and/or the red emission window doesn't overlap	Switch track every Frame
	with the 594 laser (This does not apply to the 405 or 640 laser line).	
Note:	Changing the capture window for each channel will influence the capture window for other channels	
5.	Select your track and lasers from the Channels menu under the Acquisition tab. If you deleted any emission channels (step 2) uncheck any lasers that you don't require.	All channels Confoca DAPI AF488 Ref. AF568 AF568 High Intensity Laser Range All channels Lasers ✓ 405 ✓ 488 ✓ 561 640 405 nm 0.8 %
6.	Save your modifications as a new experiment using the 'gear' button and choose 'Save As'. DO NOT CLICK 'SAVE' as this will overwrite the default template.	Column Locate Acquisition Processing Analysis Applications A - KPIF 2-Track Confocal DEFAULT with BF Analysis Processing # Acquisition Para * Smart Setup ************************************



is Applications
Show All 2 Confocal Confocal Show All 2 Confocal Show All Confocal Confocal Show All Confocal C
✓ Show All +LSM Confocal



3.	Choose a dye from the dropdown menu for the appropriate channel. If you do not find your specific dye, select one with the closest spectral properties (so you can visualize the emission curve).	Climaging Setup Standard Standard Gol5-561 488.86 Contocal Switch track every Frame Switch track every Frame Switch track every Frame South track every South track every Golf track every South track every South track every South track every South track every South track every South track every South track every Color South track every South track every South track every
4.	Adjust the emission capture window for each channel to a desired range by moving the sliders. Make sure the green emission window doesn't overlap with the 488 laser and/or the red emission window doesn't overlap with the 594 laser (This does not apply to the 405 or 640 laser line).	Switch track every Frame
Note:	You can select multiple dyes per track with confocal imaging, as long as their emission curves do not overlap.	
5.	Remember to select your tracks and lasers from the Channels menu under the Acquisition tab. If you would like to exclude tracks, uncheck the track. If you would like to exclude individual lasers from the track, uncheck the laser from the track.	 ✓ Channels ✓ Show All ✓ Track1 Confocal DAPI Ref. tdTom Track2 Confocal EGFP Bright Focus Ref. W W High Intensity Laser Range Track1 Lasers 405 488 561 640
6.	If you would like to save your current settings for future use, save your modifications as a new experiment using the 'gear' button and choose 'Save As'. DO NOT CLICK 'SAVE' as this will overwrite the default template.	Locate Acquisition Processing Analysis Applications A - KPIF 2-Track Confocal DEFAULT with BF A - KPIF 2-Track Confocal DEFAULT A - KPIF 2-TFAULT A - KPIF 2-TFAULT A - KPIF 2-TFAULT A - KPIF 2-TFAU





	B. Fluorescence with Transmitte	ed Light Channels
1.	For Brightfield (BF) images choose 'A - KPIF 2-Track Confocal DEFAULT with BF' from the Experiments menu as a template to modify.	Column 2 Acquisition Processing Analysis Applications A - KPIF 2-Track Confocal DEFAULT with BF Image: Column 2 Image: Column 2 Image: Column 2 * Smart Setup Image: Column 2 Image: Column 2 Image: Column 2 Image: Column 2
	For Differential Interference Contrast (DIC) images choose 'A - KPIF 2-Track Confocal Default with DIC' from the Experiments menu as a template to modify.	Locate Acquisition Processing Analysis Applications A - KPIF 2-Track Confocal DEFAULT with DIC *
2.	In the Imaging Setup menu: For BF, ensure the transmitted light channel labeled "Bright" is checked in the 488-BF track.	405-561 488-BF +LSM Confocal Confocal m * Switch track every ■ Frame • 400 500 600 70 Ch1: No Filter Ch3: No Filter Ch3: No Filter
		Use Dye Color Name Range ✓ ✓ Ch1 400 nm - 490 nm ✓ AF488 ✓ ✓ AF488 490 nm - 569 nm ✓ ✓ ✓ ✓ Ch3 569 nm - 700 nm ✓ Øright ✓ ✓ Bright 400 nm - 400 nm 405-561 488 DIC +LSM Confocal *
	For DIC, ensure the transmitted light channel labeled "DIC" is checked on the separate DIC track.	Switch track every Frame
	Note: DO NOT modify the DIC track. Only DIC should be specified in this track.	400 500 600 700 Ch1: No Filter Ch3: No Filte Use Dye Color Name Range Use Dye Color Name Range Ch1 400 nm - 490 nm Ch2 490 nm - 569 nm DIC ▼ Ch3 569 nm - 700 nm DIC ▼ DIC 400 nm - 400 nm



		Channels	Show All
		✓ 405-561 Confocal DAPI	
		AF568	Ref. 🗖
		✓ 488-BF Confocal AF488	
	Under the Channels menu:	Bright	
			· * ·
2	For BF, ensure that the 488 laser is checked	High Intensity Laser Range	
3.	for the 488-BF track.	488-BF	
		Lasers 405 488 561	640
		488 nm	1.0 %
		Pinhole -0	25 μm
		1.00 Airy Units \triangleq 1.1 μ m section	1 AU May
		TL Brightfield	
		Channels	🗸 Show All 📝
		✓ 405-561 Confocal DAPI	
		AF568	-
		✓ 488 Confocal AF488	Ref. 🗖 🗸
	For DIC Linder the Channels monute onsure	DIC Confocal DIC	•
	the DIC togets and the 400 leases and the short	Focus Ref.	* ▼
	the DIC track and the 488 laser are checked.	☐ High Intensity Laser Range	
		DIC	
		Lasers 🔲 405 🗹 488 🔲 561	640
		488 nm	1.0 %
	To change brightness, adjust master gain		
		TL Brightfield	
4.	For BF, adjust the master gain under 'TL	Master Gain 0	236 V
	Brightfield'.	Digital Offset	0- <u>o</u> ;
	For DIC, adjust the master gain under 'TL		
	DIC'.	Master Gain	— 217 V
		Digital Offset	0-0



ESSENTIAL CHECKLIST - for optimal images		
Is 'Confocal' Checked in the Acquisition Mode menu? If not, optimal spatial resolution will not be not attained, However, you can choose lower resolution from the presets for faster image acquisition if optimal spatial resolution isn't required.	 Acquisition Mode ✓ Show All LSM Frame Line Crop Area Scan Area Image Size 638.9 µm × 638.9 µm Pixel Size 0.22 µm Frame Size 2909 px × 2909 px × Presets Sampling 1.0 x 	
Is 1 AU checked for each channel in the Channels menu? If unchecked, more out-of-focus light is collected than optimal and is not considered 'confocal'. However, you may want to collect more light if your sample is extremely dim.	 ▲ Channels ✓ Show All ✓ 405-561 Confocal DAPI Ref. ✓ 488 Confocal AF568 ✓ ✓ 488 Confocal AF488 ✓ <l< td=""></l<>	
Is your image properly exposed? Adjust laser power/gain until dynamic range fits below the end of the histogram and there is no clipping (red arrow on top image).	Auto Min/Max Best Fit 2.00 ; 0.01 ; Current S0 OVEROX DOSED 100 Auto Min/Max Best Fit 2.00 ; 0.01 ? Current Auto Min/Max Best Fit 2.00 ; 0.01 ? Current Reset Optimal Exposure 4 Auto Min/Max Auto Auto Auto Auto Auto Auto Auto Auto	
Lots of background noise? <i>Try adding Line Averaging from the Acquisition</i> <i>Mode menu.</i> Note: This increases acquisition time.	Direction ine Step 1 veraging None 2x 4x 8x 16x	



TROUBLESHOOTING	
Is the whole screen a single color with no visible	Channels
image during acquisition?	✓ 405-561 Confocal DAPI Ref
	AF568
<i>Double check that the Display Setting is set to</i>	✓ 488 Confocal AF488 ▼ ✓ ∧ + m Focus Ref. ✿ ▼
	High Intensity Laser Range
	400 Lasers 405 🗹 488 561 640
	488 nm 0.2 % :
	Pinhole -0
	1.00 Airy Units ≜ 4.0 μm section 1 AU Max
	Alexa Fluor 488
	Master Gain 700 V
	Digital Offset 0
	Digital Gain -1 1.0 :
	Display Setting Default
Is Zen and/or the computer unresponsive?	HELLO IT
Try restarting the computer and opening Zen	
again. If problems persist - contact Dr.	
deCarvalho.	RTIN
	HAVE YOU TRIED TURNING IT OFF
	AND ON AGAIN?