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# Confocal imaging on Nikon AXR confocal microscope

Andrew Hunter[1]

[1]Northwestern university



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divya.darwinarulseeli

ABSTRACT

This protocol provides a step by step protocol to acquire images on Nikon AXR confocal set-up.

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MATERIALS TEXT

***Equipment Setup***

- Nikon AXR Confocal microscope
- Plan Apo (lambda) 2x, NA=0.10 WD=8500um
- Plan Apo lambda 10x, NA=0.45 WD=4000um
- Plan APO VC 20x DIC N2, NA 0.75 WD=1000um
- Apo TIRF 60x Oil DIC N2, NA 1.49 WD=120umx
- Computer with monitor and software
- NIS-Elements AR 5.41.02 64-bit software

## Confocal set-up

1 In this order: Turn on power to microscope stand (15seconds to power up), power on the controller by pushing the button and waiting for the standby lights to go green, then provide power to the lasers by turning the activation key

2 If using CAM, begin reservation to receive access to the computer and software

3 Ensure connection to R drive for data storage

4 Select and load the NIS-Elements AR 5.41.02 64-bit software

5 Set lightpath to Eyepiece – EPI in order to locate brain slice

6 Turn on epifluorescent light to 50-75% intensity, look through eyepiece in order to see brain slices

7 Once slice is observable, change lightpath to AX

8 Check for lasers of interest and make sure that they are highlighted and in use (488: 499-551) (561: 571-625) (640: 663-738)



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9 Check the pinhole size (aperture) make sure it is set to 1.0 AU

10 Scanner set to Galvano with scattered sampling Channel Series for fixed tissue imaging

11 For scanning set resolution to lowest/fastest value at 256x256 with maximum field of view to make identifying slices and finding target regions as fast as possible

12 Adjust Z using knob to find slices and find depth of greatest intensity

13 Open up “Look Up Table” to view saturation histograms for each channel

14 Locate the highest intensity field in your region of interest

15 Use AutoSignal.ai (set to Fixed/Robust for mounted slides)

16 Use LUTs to determine saturation of image, adjust the power and the gain of the lasers to adjust saturation; try to make the laser power as low as possible while preserving image, do not let gain exceed 50-60

17 Once the histograms for all lasers are within acceptable ranges, begin to design scan

18 “Acquire” -> “Scan Large Image” to pull up the large image overview user interface (UI)

19 Using the controls, set the far left right, top, and bottom borders of the slice in order to identify the size of the image, ensure that the proper objective lens is selected in “objective” in the top



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left corner

20 Note the size of the image (will be ___ x ___ fields) as well as the expected size of the image, close “Scan Large Image”

21 Use the rendered image of the scan size and place the microscope in the center of the generated grid (if 8x8, move cursor to the point between square 4 and square 5 on both the X and Y axes, double click to move camera to center)

22 When ready to capture image: sample at Nyquist value and 1024x1024 or 512x512 pixel resolution

23 Go back into “Scan Large Image” the number of fields will have updated to match the new resolution

24 Scan an additional time to ensure that the camera shifted to the center of your ROI

25 On the side tab, select “ND acquisition – Define/Run Experiment”

26 Select XY channel -> “Custom” will pull up the custom image size pull-out

27 Select “Large Image” and enter scan area __ x __ fields, select the objective, and insert preferred overlap (10-15%), press “Finish”

28 Once the ND acquisition screen has populated, set “redefine Reference Z after Auto Focus, and select “Use PFS”

29 If Z-scanning, also check off the Z tab



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30 Use the Z adjustment to locate the top and bottom of the slice and set these boundaries using the “Top” and “bottom” buttons – WRITE DOWN THESE Z-VALUES FOR STEROLOGY PURPOSES

31 Set the Z step value, ideally use the step suggested by the software (0.122um [or 0.2um])

32 Press “Run Now” to begin scanning

33 Once image is produced, it will generate a deck of images that are not scanned, save the file as “XXXXXX_RAW”

34 Saving criteria:
mouseID_genotype_hemisphere_series#_Primary_Objective#_SamplingResolution_Slice (ex m8535_Gabrr3Cre_RightHem_series3_NeuN_Nyq1024_Slice7)

35 To construct stitched image go to “Image” -> ND Processing -> “Stitch Multipoint to Large Image”

36 Save image with same name as the RAW file but delete “_RAW”



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