



Image acquisition

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Image acquisition:

- Operating instructions for the arrayWoRx[e] autoloader CCD scanner ([jump](#))
- Operating instructions for the Genepix 4000B dual laser scanner ([jump](#))

Operating instructions for the arrayWoRx[e] autoloader scanner

Preparation for scanning:

1. Load the slides upside down in the carousel, with the calibration slide in position one. Take note of the slide orientation so that you can be certain that the slide hasn't also been rotated. The slide ID should be in the bottom left-hand corner.
2. Start the arrayWoRx^e program by clicking on the "arrayWoRx" icon, then press "Scan". The scanner will then automatically 'warm-up'. This will take about 10 minutes.

Focusing the scanner:

3. Go to the "Scan Table" and load the first experimental slide from the carousel.
4. Press "Preview" to obtain a low resolution image, then adjust the scan area such that the entire microarray is included.
5. Select the correct channel number (1, 2, 3 or 4), and change the exposure time to 0.06 seconds.
6. Go to "Panel View" and select a small field (3 rows by 3 columns of spots) with the yellow square (within the image)
7. Go to "Utilities" - "Focus Scanner", then press "Start" and manipulate the "Focus Knob" on the right-hand side of the scanner until you obtain a sharp spot image. This will only require gentle movements of the "Focus Knob", so be careful.
8. Press "Done" once you are happy with the focus setting. This will automatically save this setting.

Calibrating the scanner:

9. Go to the "Scan Table" and load the calibration slide from the carousel (should be in position one).
10. Go to "Utilities" - "Calibrate Scanner" - select "Cy3" - "Calibrate Now", then press "Done" once completed.
11. Repeat step 10 for all of the channels you wish to use (choice of "Cy5", "A350", "A488").

Setting the correct exposure time:

12. Go to the "Scan Table" and load the first experimental slide (should be in position two).
13. Press "Preview" to obtain a low resolution image and then go to "Panel View" and select a small field with the yellow square (within the preview scan image) that covers about 9 spots (3 rows x 3 columns).
14. Select channel (e.g. "Cy3", "Cy5", "A350", or "A488") and change the exposure time, then press the "Camera Icon" to obtain a single panel image.
15. Repeat step 14 for each channel using dark and bright areas (e.g. two to three of each). You're aiming to achieve the same saturation level for each channel. However this is almost impossible and so a saturation range of between 80% and 100% is acceptable.
16. Go to "Image" - "High SNR" and select "Resolution 6.504 μm per pixel".
17. Go to "Advance" and select "Enable stitch panel flattening", "Enable stitch panel connection", "Generate images in TIFF format" and "Rotate"; "Use barcodes for file names" should not be selected.

Scanning the slides:

18. Go to "Scan Settings", then "Create", type the project number, and then press "Save". This will save the scan settings using the project number as a file name.
19. Go to "Scan Table" and deselect slide position 1; this will prevent the calibration slide from being scanned along with the experimental slides.
20. Change the "Preset" field to the correct scan setting (the file you created in step 18) for each carousel position with an experimental slide. Then type the slide ID (e.g. S101025) within the "Data Name" field.
21. Press "Scan" and select the "Data File" location on the FlyChip file server (e.g. /production/project/) and then add the "Job name (e.g. project number).

Operating instructions for the Axon GenePix scanner

Preparation for scanning:

1. After power-on, give the scanner 15 minutes to warm up before acquiring any images
2. Load the slides upside down in the scanner
3. Start GenePixPro by clicking on the 'GenePixPro' icon

Preview scanning and defining the scan area:

4. Press 'Preview Scan' to start a preview scan
5. You can then press the 'Scan Area' button:
 - ◆ Move the mouse cursor to the top left of the features on the image
 - ◆ Hold down the mouse cursor and drag a rectangle around the region containing the features
6. Confirm that all features have been included within the scan area

Optimising the scanner settings:

Maximizing the dynamic range:

7. Open the 'Hardware Settings' dialog box
8. Start a 'Preview Scan' and change the 'PMT Gain' for each wavelength whilst scanning
9. You should aim to use the highest scan setting that does not lead to a significant level of saturation
10. Ensure that the image resolution has been set to 5 μm per pixel

Balancing the PMTs:

11. Start a 'Preview Scan' and 'Zoom' in on the features
12. Switch to the 'Histogram' tab and set the 'Min Intensity' and 'Max Intensity' fields to 500 and 65530
13. While scanning, adjust the PMT Gain in each channel until the ratio 'Count Ratio' is about 1.0
14. Save the 'Hardware Settings' by clicking the 'File' button and selecting 'Save Settings As'
15. Type in an appropriate file name and then press 'Save'

Performing a Data Scan and Saving an Image:

15. Press 'Data Scan' to start a full scan
16. To save the image, click on 'File' and select 'Save Images'
17. Navigate to the correct file location and select 'Single Image TIFF Files'
18. Type in an appropriate file name and then press 'Save'

Calibrate System (doesn't needed to be performed very often!):

19. Click 'Hardware Diagnostic' and select 'Calibrate System'
20. Check both the laser check boxes to calibrate both wavelengths
21. Click 'Start' and the wizard will then guide you through the procedure

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