

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).

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