QBI Slide Scanners : Fluorescence Quick Guide

Name: .

Group: .

Tissue Staining / Labelling:

.

Pre-scan Classifier:

.prescan (5x) for 20x

Integration times (camera gain 16):

DAPI: .

Scan Classifier:

20x

Integration times (camera gain 5):

DAPI:	-
488:	-
568:	-
647:	-

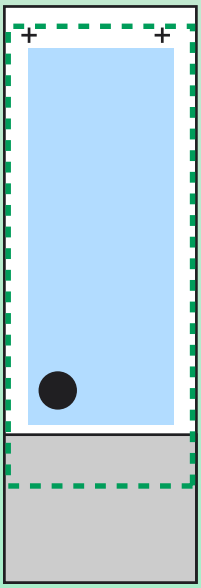
Scan Times:

Prescan:	~5 minutes
Focus Map:	~5 minutes
Final Scan (20x):	~25 minutes 1 colour 2-4 hours for 3 colour

Notes / Recommendations:

-

For Best Results



1. Use SuperFrost Plus slides
2. Use 24 x 60mm coverslips
3. Ensure some tissue / cells are positioned in the lower left corner (black dot)
4. Keep tissue away from the edges of the slide and below the + + (in blue area)
5. If possible, use DAPI as a counter stain

Instructions

1. Switch on the microscope and computer
2. Switch on the fluorescence Colibri LEDs
3. Load all of your slides - make sure they are ***CLEAN, flat and pushed all the way to the top left in the slide frame***
4. Start Vslide - START online mode
5. Start the scanning software
6. Click **Adv. Mode**
7. Load an example slide and check its exposure settings:
    i. Open the lamp shutter (foot pedal) and leave open
    ii. Open the RL-shutter
    iii. Select a fluorescent filter on the touch screen
    iv. Right click on image window
    v. Adjusting integration time with gain on 5 to get a good image without saturation
    vi. Close the RL-shutter
8. Copy the integration times for each fluorescent channel into your **Scan classifier**
    i. In the top menu: Metacyte>Classifier Setup
    ii. Choose ***your*** Scan Classifier
    iii. Select the **Capture** tab
    iv. Change the integration times to those you observed or add channels if necessary
9. Click **Setup** and setup your slides:
    i. Activate and Name each slide to be scanned (individually or in groups of 5)
    ii. Choose your recommended **pre-scan classifier** under **Classifier**
    iii. Under search window choose **Predefined area**
    iv. Under size choose **Adv. Mode ++**
    v. Ensure each activated slide has been named, and has the correct classifier selected as described above
    vi. Click **OK**
10. Activate the robotic arm (right click **SF+** in the top right corner of the main screen and choose initialization)
11. Click **Scan**

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