Fluorescence image capture:

1. Switch on: Xcite (Hg burner)
   Power supply 231
   Microscope (should be on all the time)
2. Login (normal CBDM lab login)
3. Launch Slidebook 5.0

Focus Window – you can control all focus/filter controls from here.

4. Click on Focus [F] window icon to launch focus window.
5. Open brightfield (BF). Click Open.
6. Focus on sample.
7. Close BF.
8. Select fluorochrome filter of choice.
9. Open fluorescence.
10. Click on objective buttons to move objectives (x20, x40, x60 etc)
11. Click on camera icon to launch Image Capture Window.

Image Capture Window:

12. Select fluorochromes/filters required
13. Click 'Test' to check exposure times e.g. 100/200 ms
14. Or can click 'Find best'.
15. Can set different exposure times for different fluorochromes.
16. Click Start to capture image. Image will be generated in a new slide window.
17. Add scale bar: Annotations > Scale Bars. A 10 μm scale bar will appear in the bottom left hand of your captured image.
    Should you wish to change the size, position or color of the scale bar:
    Annotations > Settings > Scale Bars.

Slide Window – you can clean up the image here:

18. Click histogram icon to contrast enhance image.
19. Make changes using the slider bars to the left and right of the spectra as follows: Move left line to middle of peak and right line to right edge of tail.
20. Click apply -> ok.
21. **Click thumb icon to <u>save</u> changes**.
22. Image -> deconvolve -> no neighbours -> select channels -> ok.
23. Save files
24. Export files: View -> Export -> Tiff

25. Ensure burner has been on for 30 min before switching off.

Extra notes:

i. Pancreas autofluorescent in green channel, but not in Cy3 (KR) channel.

ii. Slidebook has two export functions*. The Image>Export>Tiff menu will export the raw unscaled image data into a tiff file, i.e. if the internal data format is a 12-bit or 16-bit image, the tiff file will contain the original image data in a 16-bit format. Most standard image processing programs handle only 8-bit grayscale or 24-bit RGB tiff files, but not the 16-bit format and hence won't display them correctly.*

Use this one:

The View>Export>Tiff or View>Export>BMP menus write 8-bit or 24-bit image files of the current view, based on the renormalization selected with the histogram. Therefore, use the export function in the View menu to get image files that look like the views on the screen. Use the Image menu export function to preserve the quantitatively correct image data.

Brightfield image capture

1. Switch on: Xcite (Hg burner)
   Power supply 231
   Microscope (should be on all the time)
2. Login (normal CBDM lab login)
3. Launch Slidebook 5.0

Focus Window – you can control all focus/filter controls from here.

4. Click on Focus window icon to launch focus window.
5. Open brightfield (BF). Click Open.
6. Focus on sample through eyepiece then push shutter in and adjust focus for camera view if necessary.

Image capture window:

7. Check box next to Virtual RGB.
8. Check box next to White Balance and draw a box on white (negative) part of your image and click Update -> ok.
9. Click Start.
10. In new window that is generated there will be 4 boxes marked 'None'. Select vRed from top box, vGreen from 2nd box and vBlue from 3rd box.
11. You should now see a merged image of your RGB planes.
12. To save this image as a TIFF file ready for opening in other software programs, click View -> Export -> TIFF and save wherever you desire.

Troubleshooting:

If white parts of image look orange then increase the brightness (using the brightness knob situated at the back right part of the scope).