

Using Olympus dotSlide for polarising microscopy

Background

Because the dotSlide system is built using a conventional microscope frame it lends itself to acquiring scans in different imaging modes whose components can be added to the frame. Polarising microscopy can be performed by adding polarising filters below and above the sample.



However, because the polarising image has a dark background, there can be are problems with camera exposure, with using automatic tissue content recognition and with generation of the focus map, especially if the crystalline features are rare events within the sample.

The following is a generic approach which **should** work for most samples. It relies on setting a manual exposure time, acquiring the overview in bright field and then generating the focus map and scan in polarising mode

Method:

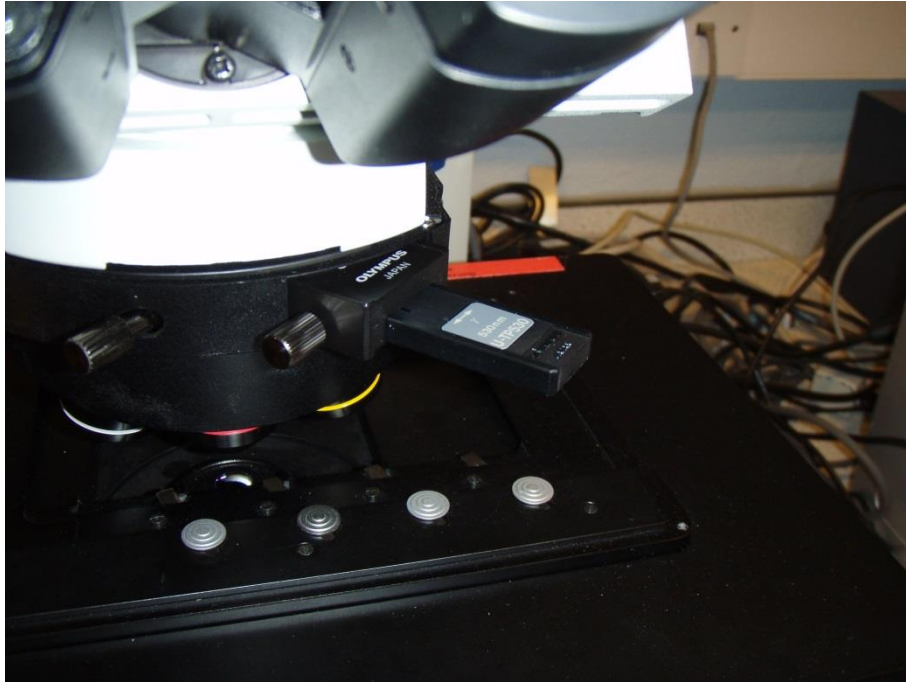
Startup and initial setup

- Start system
- Start dotSlide software
- Perform the usual calibration of stage limits (XY and Z) and overview area

Using Olympus dotSlide in Polarising Mode

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- Remove filter holder from nosepiece and replace with the analyser (with tint plate retracted (out))



- Place polariser (U-POT) on field diaphragm with lettering and notch to front



- Retract ND6 filter from stand (button out – only button in should be LBD (colour correction))



- Place sample on stage and focus using eyepieces
- Rotate polariser clockwise to extinction point (crossed polars) or whatever degree of polarisation you want – I suggest that the polariser isn't set to full extinction so that the non tissue background is a bluey grey and non refractile tissue can still be seen.



Calculate exposure

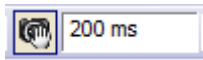
- Start live view



- Change to objective required for higher power scan



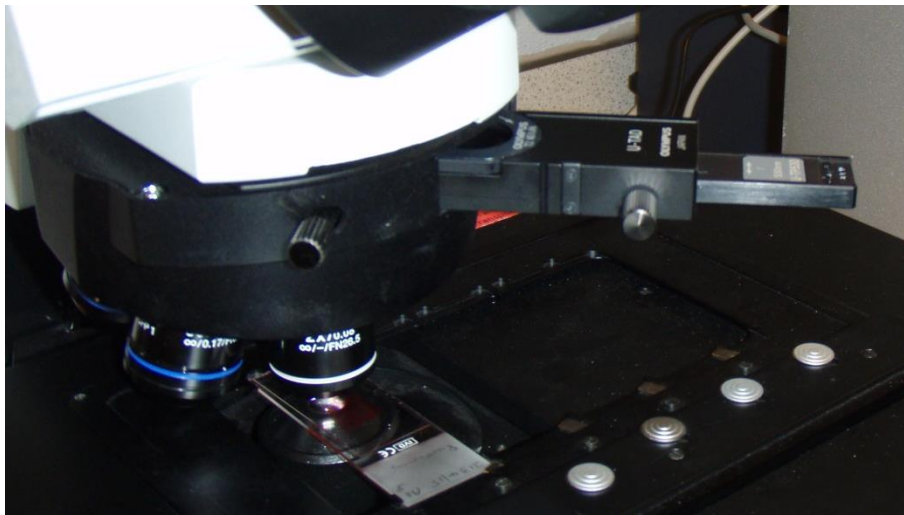
- Focus live view using wheel on joystick box
- Toggle camera exposure to manual exposure mode and adjust exposure to desired image intensity



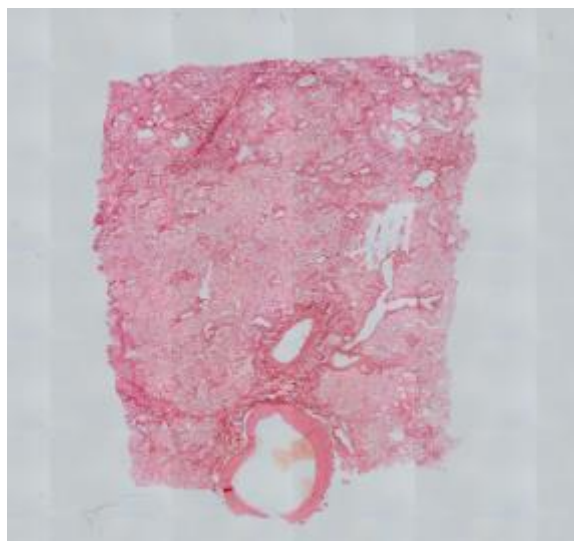
- Rough guide for exposure (exact exposure will depend on how crossed the polars are):
 - x4 = 30-50 ms
 - x10 = 30-50 ms
 - x20 = 70-100 ms
 - x40 = 250-400 ms
- Stop live view – leave exposure set on manual with desired exposure time

Overview scan

- Start expert mode
- Remove analyser by retracting it to click stop but leave polariser in place

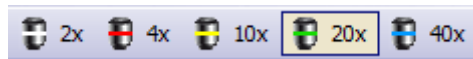


- Do overview (in BF mode) – this won't look perfect as the polariser gives a slight unevenness to the illumination

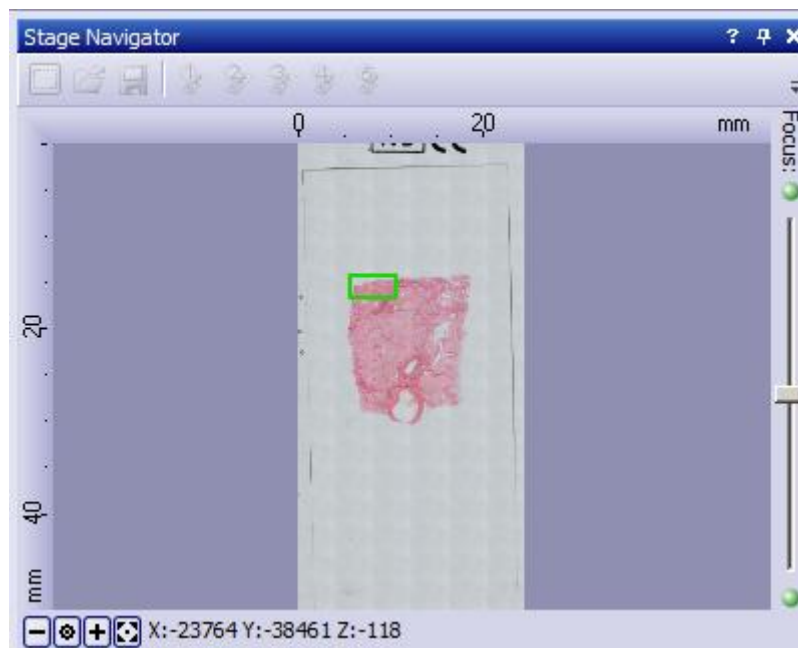


High power scan

- Select magnification
- Check “More Options” settings
- Select scan area – hopefully the tissue content algorithm will be able to see the stain and identify the tissue. Modify scan area options as required
- Check and edit focus map as required*
- Reinsert analyser (with tint plate out)
- Change to desired objective for high power scan



- In Stage Navigator window, click on an area of the sample within and near the bottom of your defined scan area



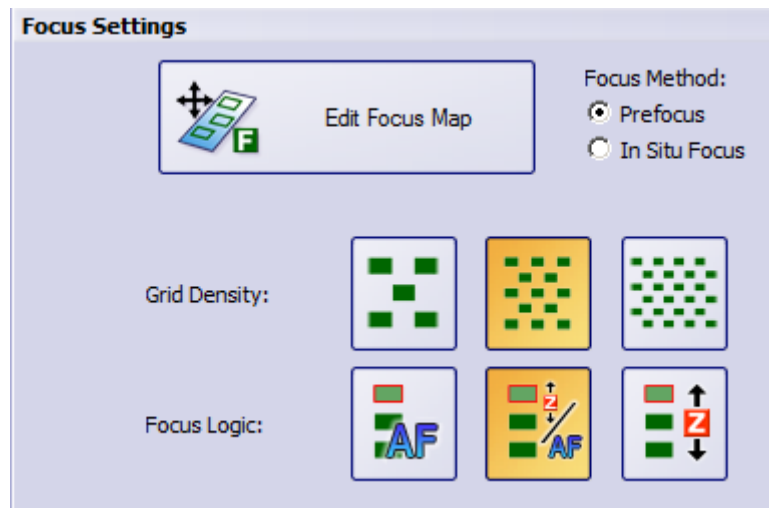
- Focus on the sample down the eyepieces using wheel on joystick box
- Autofocus



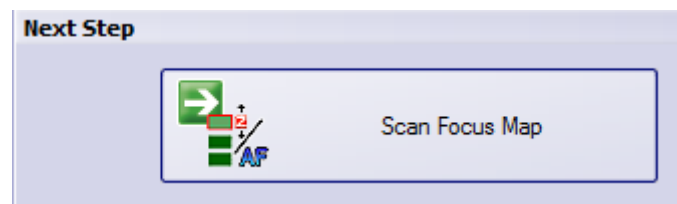
- Scan now – the scan will then be performed using the preset, manual exposure time

* Generation of the focus map will only work if the camera can see sample content within each field of view being sampled - i.e. when there is a significant amount of refractile material within each field and /or when the polariser isn't set to full extinction so that the non tissue background is a bluey grey and non refractile material can still be seen. If this isn't the case then you will need to adopt the following strategy at the focus map stage:

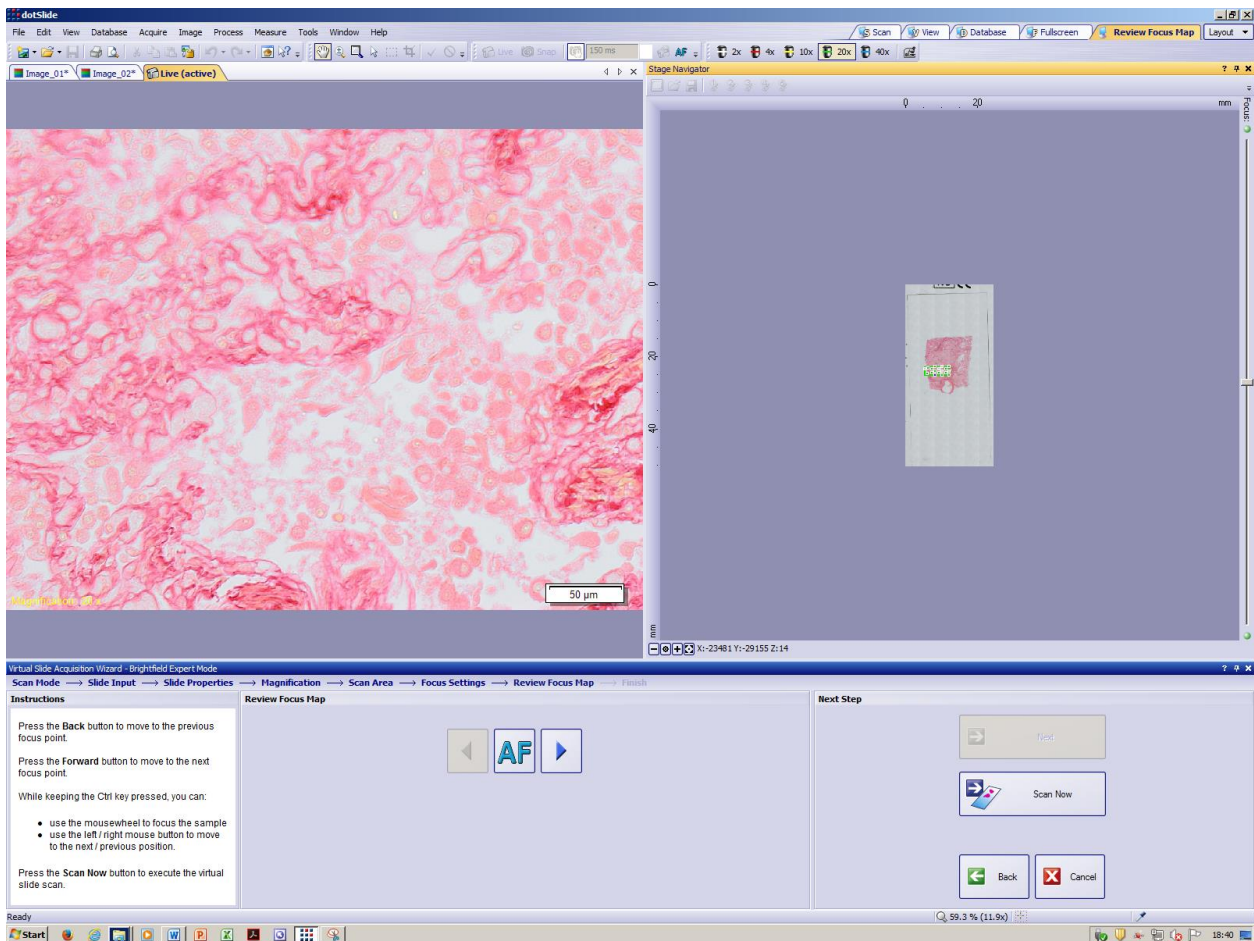
- Keep the analyser out
- Set focus map to semi-automatic focussing



- Edit the focus map as desired
- Change to desired objective for high power scan
- In Stage Navigator window, click on an area of the sample within and near the bottom of your defined scan area
- Focus down the eyepieces
- Autofocus – if you get an “Images are too bright” message appear at bottom RHS of screen, reinsert one or both neutral density filters and try again.
- Then scan focus map with the ND filters in



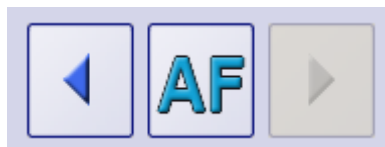
- Once the focus map has been generated, the Review Focus Map window will appear and the interface will look like this:



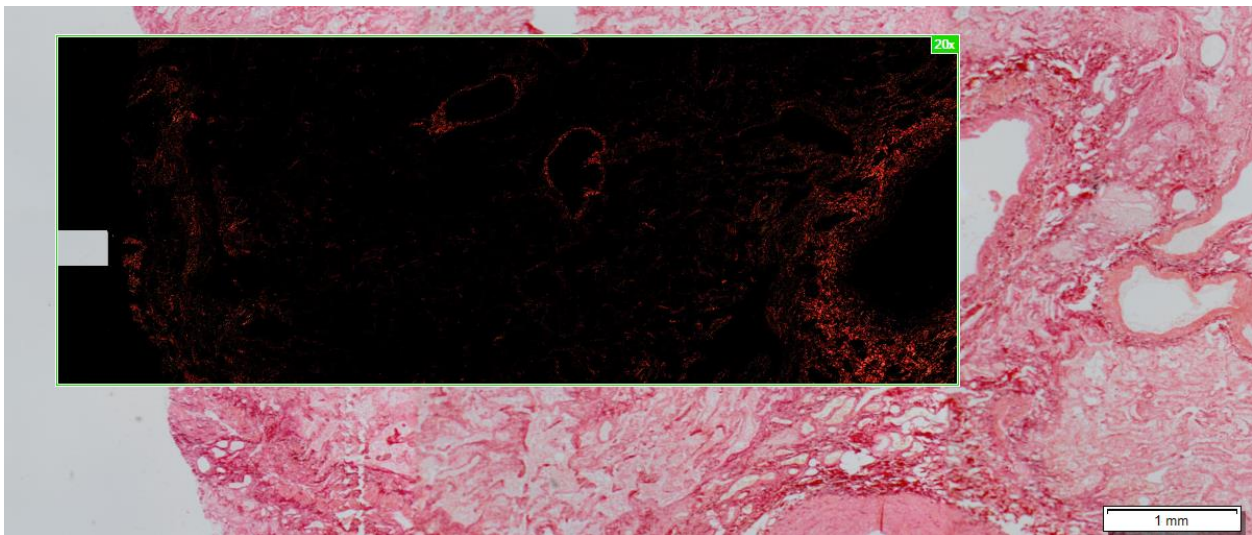
- The top LH window is a live view. Check the proposed focus of this field of view. If you are happy with the focus, simply click the rightward blue arrow to accept the proposed focus and review the next position



- If unhappy with the focus, refine it using the focus wheel on the RHS of the joystick box, then click the rightward blue arrow to accept the refined focus and review the next position
- Once all of the focus positions have been reviewed, the rightward arrow is greyed out



- VERY IMPORTANT – (1) remove any inserted ND filters (2) Reinsert the analyser with the tint plate out
- Scan Now – the scan will proceed immediately



At end of session

Please return system to its normal, brightfield setup so as not to confuse the next user:

- reinsert ND8 filter
- remove polariser and analyser and return them to storage
- replace standard filter holder above the nosepiece