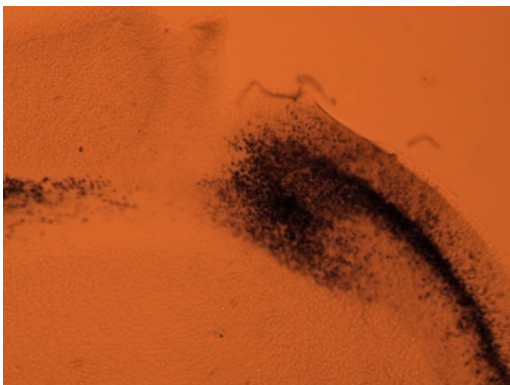


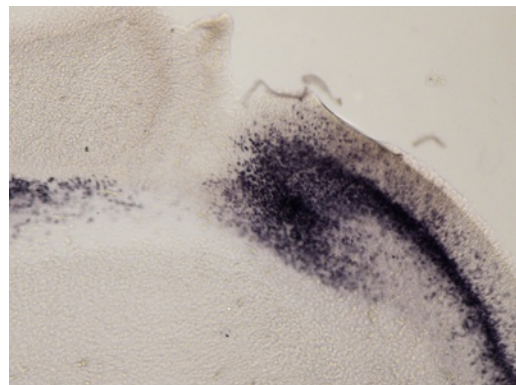
Guide to Brightfield Imaging

Getting Started

1. Switch on the microscope
 - For brightfield imaging only the power supply box needs to be switched on.
2. Put slide on stage and focus sample with 10x objective
3. Switch on the transmitted light. **(1)**
4. Press the **3200K button (2)**, this turns the light up bright enough to give the image a white background.
5. Adjust the **neutral density filters** to the lowest/low settings - on the right side of the microscope **(3)**
 - Usually **12** and **0.4**
 - Do not look down the eye pieces before this, as unfiltered light at 3200K is very bright



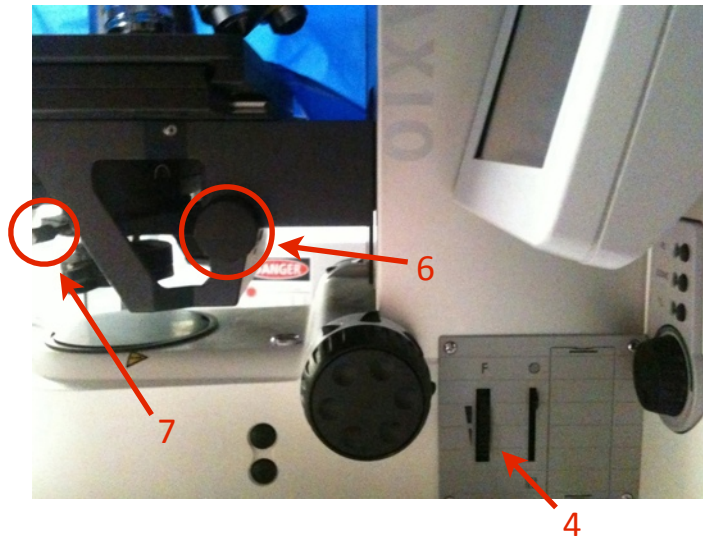
Sample with lamp turned down low
(no neutral density filters)



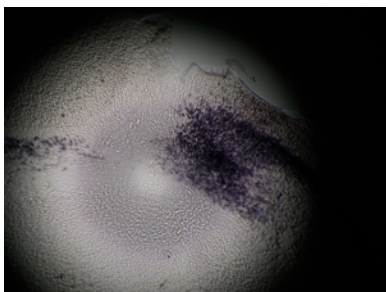
Sample with lamp at 3200K
(12 and 0.4 filters)

Setting up correct Koehler Illumination

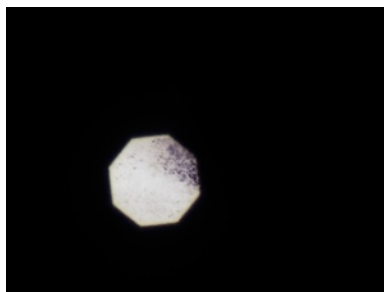
1. Close down the lamp field stop (diaphragm/iris) - on the right side of the microscope. (4)
2. Ensure the top condenser lens is in place, directly under the slide
 - You can swing this in and out of place using the centre button at the front of the condenser (5)



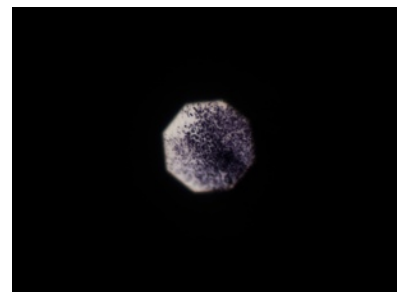
3. Bring the condenser all the way to the top - use the black focusing screws below the stage (6)
4. Adjust the condenser down so that the diaphragm image is in focus (octagon shape)
 - Ensure the sample is still in focus at the same time
5. Centre the octagon by adjusting the two silver screws at the front of the condenser (7)
6. Reopen the lamp field stop (diaphragm/iris), so that the full field of view is visible again (4)



Condenser out of focus



Condenser in focus



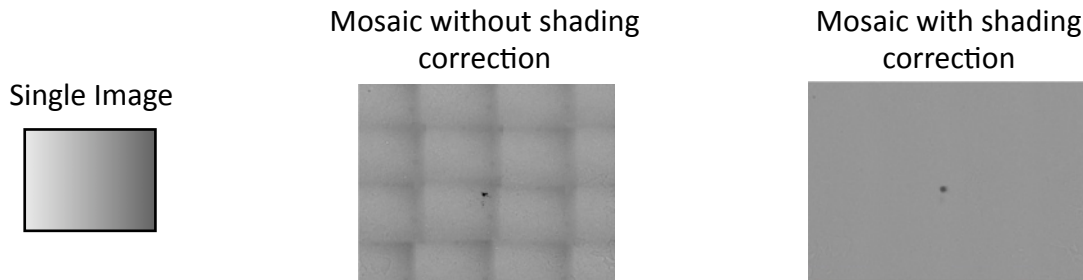
Condenser centered

Tips for brightfield MosaicX

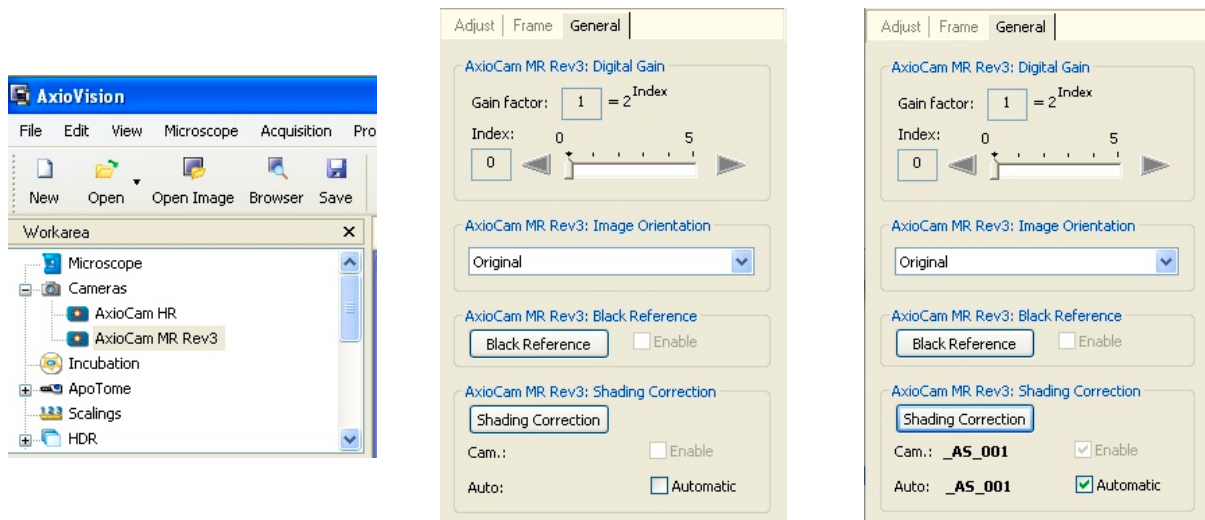
1. Ensure you have switched on and calibrated **shading correction** - see over.
2. Image with at least 10% overlap.
3. When stitching use a search depth of 12 and a minimum overlap of 2.
4. If there is a problem and the tiles don't stitch properly, in **stitching mode** choose **original positions** and rerun stitching - then go back to stitching, but this time choose a **minimum overlap of 1** (this will take longer but Axiovision will make less mistakes).
5. If you are stitching mosaic images which are larger than 3x3 Axiovision will shrink them when you run **convert tile images** - to stop this: in the top menu click on **Tools** then select **Options**. In the **acquisition** tab make the maximum image size much larger than 4096.
 - Always check and make sure the zoom is set to 1 when you are converting tile images.

Adjusting Shading Correction and White Balance

When taking images, especially transmitted light images, the field of view may not always have an even amount of illumination. This can result in a gradient of dark to light across every image you capture. Whilst not always noticeable in a single image it can cause problems when capturing a MosaicX/tiled image.



1. Set up correct light and Koehler illumination first (pages 1-2).
2. Focus on sample but then move the stage so that you are off the slide (nothing between objective and condenser lens).
3. In the workarea (left side of screen), expand "**cameras**" and click on the camera you are using.
 - The colour camera is called *Axiocam HR*.
 - The shading correction controls are under the **general** tab.
4. Tick the **Automatic** box, and then click **Shading Correction**.
 - If the Axiovision says the image is too bright, or too dark, then adjust the exposure and try again
 - If shading correction still comes up with an error, close the *Live Window* and try again.



5. An even shading will now occur automatically when you capture images with this objective **BUT** if the condenser focus is changed, or a different objective is used then the shading correction will need to be updated.
6. Move the stage so that you are back on the slide, but in a clear area without any sample.
7. In the same camera menu, under the **adjust** tab, click **white balance**.

Image **before** Koehler, shading correction and white balance

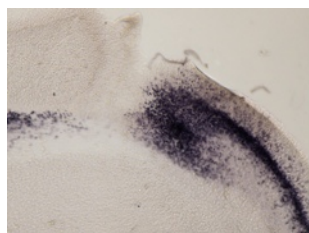


Image **after** Koehler, shading correction and white balance

