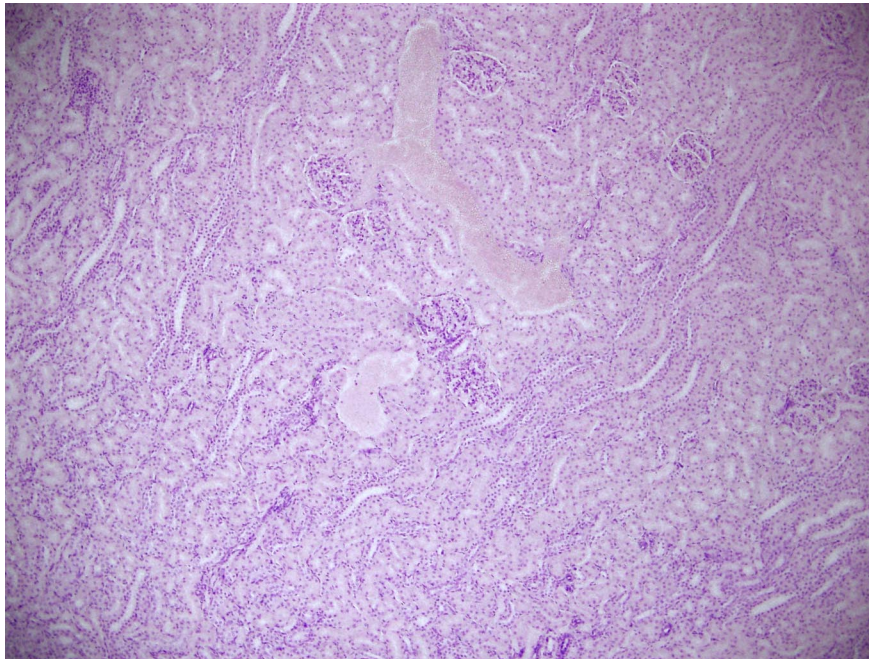


Microscopy QuickTip – Flatfielding and Photoshop

Consider the following image of kidney stained with H&E:

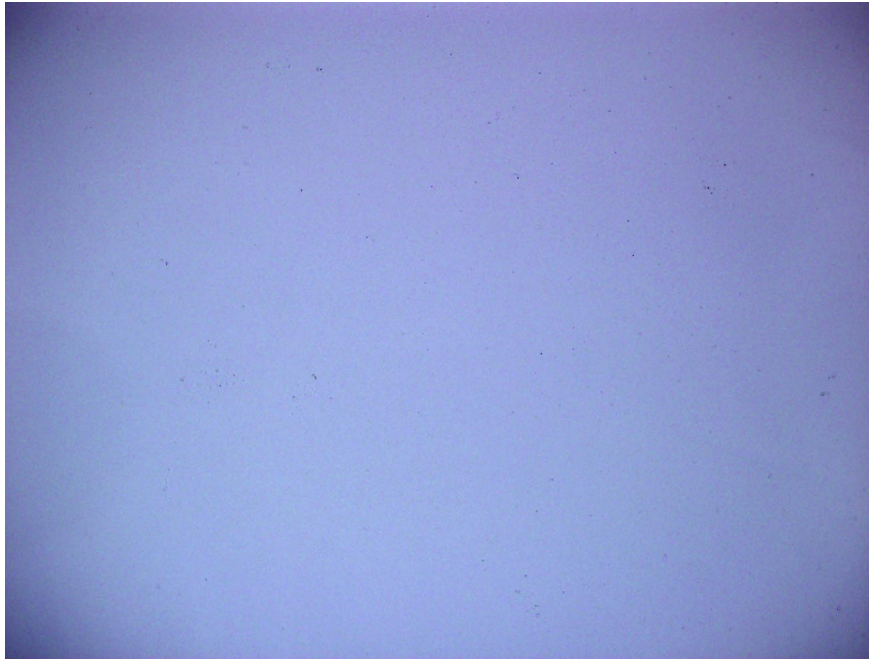


This image was collected on Fluoro1 with the microscope properly aligned. The centre of the image is brighter than the corners. To correct for this, we need to take an image of a blank region of the sample, a region with nothing in it like so:

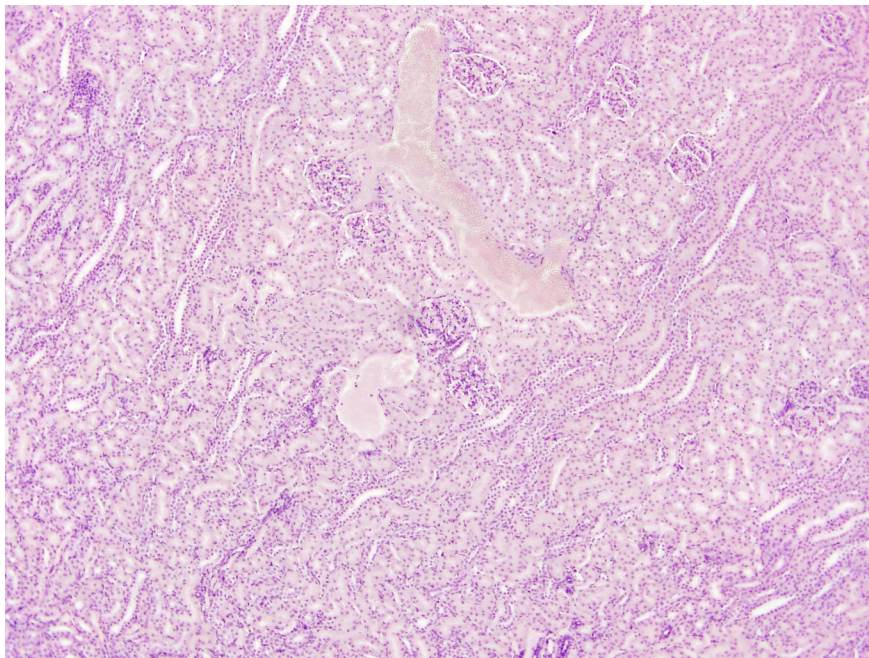


Here we can see that the recorded illumination is slightly brighter in the centre of the image than it is in the corners. If we enhance the contrast of the image, this becomes more evident.

Microscopy QuickTip – Flatfielding and Photoshop

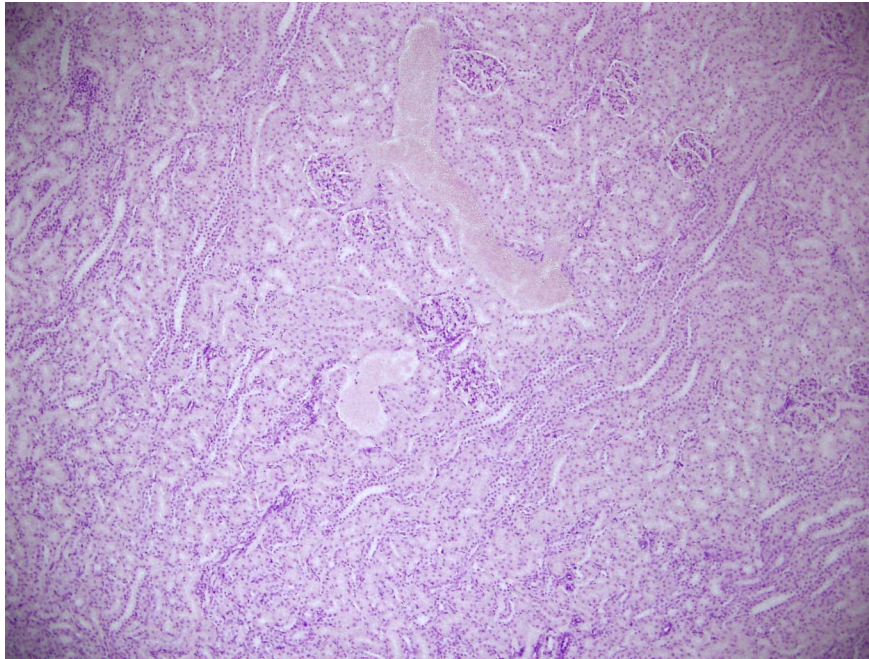


Correcting this in Photoshop is fairly straightforward. First, open the image of the stained kidney tissue. Then open the image of the empty field of view. Copy the image of the empty field of view and paste it on top of the kidney image. Set the *blending mode* of the new empty field of view image to *Divide*, select *Menu > Layer > Flatten Image*, and you're done. Bear in mind that both your original image and the image of the blank field of view should ideally be collected at the same settings. Here's the corrected image:



And the original for comparison:

Microscopy QuickTip – Flatfielding and Photoshop



Fluorescence images can be handled in the same way, but you'll need flat field images for each channel. To collect flatfield images for fluorescence imaging, you'll need to use a separate sample that is evenly fluorescent, as well. We have evenly fluorescent plastic slides at the microscopy facility and will be happy to assist you in collecting flatfield images.