Digital Image Apodization

When film cameras were replaced with electronic cameras, the sensor size became smaller. As a consequence, a smaller field of view resulted. Large digital image sensors found in SLR cameras have become less expensive and adapting these cameras to microscopy has become attractive. However, with larger sensors and fields of view, brightness non-uniformities are becoming apparent.

These non-uniformities result from the very valuable feature of modern digital SLR cameras to be able to detect very small brightness differences. This feature when combined with a widefield objective lens and large sensor will result in camera images having edges darker than the centre. Fortunately, with digital image processing, the image can be made uniformly bright through a process referred to here as apodization.

Image Apodization

An apodization filter was historically placed in a pupil plane rather than the image plane. Use of the image plane was very difficult at the time, but less so by placing an optical element in the lens pupil plane. With digital image processing the image plane is now easier, especially for the removal of dark areas and hot spots. Also, with digital image processing, a digital library of apodization filters can be created for each applicable objective lens and applied electronically rather than with physical elements.

When looking through eyepieces using a wide field objective lens such as 2.5X, the field will look uniformly bright. However, a digital image will show dark corners for micrography systems that cover most of the objective lens field. The reason is that a digital SLR has much greater contrast sensitivity.

The human eye is limited to 32 shades of gray (a bit depth of 5 or 25 shades of gray). Whereas digital SLRs typically have a bit depth of 12. The resulting dark edges (vignetting) are in all imaging lenses and is a function of the \( \cos^4 \) column and as a percentage in the vignetting column. Other ancillary data includes, \( \cos \), \( \cos^0 \), and comments to help digital micrographers judge the significance of vignetting.

**Table 1.** Vignetting as a Function of Objective Lens Magnification.

<table>
<thead>
<tr>
<th>Mag.</th>
<th>Cos 0</th>
<th>Cos^0</th>
<th>Vignetting % of 1.00</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>6.756</td>
<td>9930</td>
<td>9723</td>
<td>2.77</td>
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<tr>
<td>2.0</td>
<td>3.572</td>
<td>9935</td>
<td>9813</td>
<td>1.87</td>
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<td>2.5</td>
<td>3.907</td>
<td>9973</td>
<td>9892</td>
<td>1.08</td>
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<tr>
<td>4.0</td>
<td>4.185</td>
<td>9973</td>
<td>9892</td>
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</tr>
<tr>
<td>10.0</td>
<td>3.849</td>
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<td>25.0</td>
<td>2.904</td>
<td>9986</td>
<td>9944</td>
<td>0.56</td>
</tr>
</tbody>
</table>

Table 1 presents the reduction in edge brightness for various microscope objective magnifications. The calculations apply to an eyepiece field number of 20 mm and 160 mm tube length DIN objectives and a bit depth of 12. The brightness is taken as 1.00 on axis and the reduction is expressed by the values in the \( \cos^0 \) column and as a percentage in the vignetting column. Other ancillary data includes, \( \cos \), \( \cos^0 \), and comments to help digital micrographers judge the significance of vignetting.

The Apodization Process

The work flow for apodizing an image is illustrated by the four images in Figure 1. A 2.5X lens was used in order to yield a large \( \cos^4 \). Fig. 1(a) is an image of the specimen. Fig. 1(b) shows the illumination field. It was captured immediately after removing the specimen used in Fig. 1(a). Fig. 1(c) is the apodization filter which is the result of processing Fig. 1(b) using Photoshop CS3 Extended. It was made by discarding the colour information of Fig. 1(b) and then inverting it. The effect of inversion is apparent by comparing Fig. 1(b) and 1(c). Fig. 1(d) is the result of apodizing Fig. 1(a) and also adjusting it by the application of Auto Colour.

Auto Colour or some other means of expanding the tonal range of the image to cover the brightness range of the display is essential and is part of the apodization process. When the apodization filter is applied to the specimen image, it loses brightness contrast since the image is only 50% opaque and the filter used was optimized at 45%. The filtered image is in the middle of the tonal range and has no highlights or shadows. When Auto Colour is applied both the colour saturation and brightness contrast are increased. This is observable by comparing Fig. 1(a) and 1(d). A procedure for apodizing images is presented in the final section.

Image Apodization Effects

The observed optical image was adjusted to closely resemble its electronic image, represented in Fig. 1(a). Except for the dark edges shown in the figure, the image is a good representation of that seen through the eyepieces. The apodized image, Fig. 1(d), is corrected for vignetting. Although elimination of illumination field nonuniformities may be the most important application for image apodization, there may be more. Note, that the apodized image has more brightness and colour contrast while still maintaining the integrity of the hue (colour).

Our experience indicates that at 20X and above, the benefits of apodization are so marginal that normal image processing can achieve nearly the same result. This is because the apodization filter at the higher magnifications is so uniform that it has almost no effect.

**Fig. 1. Apodization of a 2.5X Image. (a) Captured Image (b) Illumination Field (c) Apodization Filter (d) Apodized Image.**

The method presented in the above link does not require generating the apodization filter.

**Equipment Details**

All of the research for this article was through the use of a basic bright-field microscope and a stained slide. The images were captured with a 4.3 format Olympus E-510 SLR. The microscope was carefully adjusted to yield images with symmetrical vignetting and a central hot spot for the 2.5 X objective. Also, the illumination was made as uniform as possible by means of diffusers. As a consequence, the only non-uniformities are the result of vignetting resulting from natural off-axis light loss.

**Photoshop Procedure for Apodizing Images**

1. Open the filter which you generate per the method described under The Apodization Process.
2. Open the Layers palette.
3. Duplicate the filter by Rt. clicking in the Background, then Lt. click Duplicate layer. Click OK in the dialog box.
4. Adjust the Opacity to 50%.
5. Open the object image.
6. Duplicate the object image as per Step 3.
7. Adjust the Opacity to 50%.
8. Arrange the images side by side by clicking Window + Arrange + Tile vertically.
9. Reduce each image to a size of 25% or smaller so as not to clip the edges.
10. Select the Move tool.
11. Slide the filter image over the object image.
12. Align perfectly with the Move tool.
13. Select the Object Background Copy and simultaneously press Ctrl + Lt. click.
14. Rt. click on one of the selected layers and Lt. click Flatten Image at the bottom of the menu.
15. Adjust the image by pressing Image + Adjustments + Auto Colour or use whichever adjustment works best.

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