

Constructing a Printable Grid with Photoshop

Introduction

The purpose of this procedure is to enable a printable grid to be overlaid on a print of a technical image. The primary application is to reduce the need of looking through a microscope to count objects in an eye-piece grid. Instead, using a micrograph, objects can be counted and identified outside the laboratory environment. The grid available in Photoshop will not print.

The calibration of the resulting grid is a function of:

1. A stage micrometer. This is a microscope slide that contains a series of vertical lines and is similar to a ruler. A typical ruling is 1mm with 10 major divisions and 100 least divisions. Each least division is 10 micron from center to center or like edge to like edge. Inch unit stage micrometers are also available.
2. Objective lens.
3. Camera to microscope adapter.
4. Capture resolution. This is the megapixel setting of the camera.

Saving Image of Stage Micrometer

This procedure is based upon a millimeter stage micrometer. **In order that the grid be calibrated, use the same objective lens, imaging system and capture resolution (megapixels) as that of the specimen.**

1. Capture a .jpg image of the stage micrometer and save it in a folder named *Grid*.
2. Use the following exemplary retrieval code format: 100X10mu5MP, where 100X stands for your objective lens, 10mu for the stage micrometer least division and 5MP stands for capture resolution of the camera.

Preparation for Making Vertical Grid Lines

1. Open the stage micrometer image in Photoshop.
2. Assure that the Tool bar is displayed.
3. Open the Layers palette (F7).
4. Double click on the name *Background*.
5. The *New Layer* dialog box appears. Click OK.
6. Click the *New Layer* icon which is just left of the trash barrel at the bottom of the palette.
7. *Layer 1* has been created and appears just above *Layer 0*. Click on the Set foreground color tool (third tool from the bottom of the *Tool bar*). Drag the slider to the color blue. Move the circle cursor to the upper right corner. L. click. Click OK.
8. Select the *Paint bucket* tool in the middle of the Tool bar. Its location is shared with the *Gradient tool*. Right click for menu.
9. Click in the image field to fill the layer with the color blue.
10. Click on the Layer 1 eye image. The Layer 0 image appears in the image field.
11. Drag Layer 1 below Layer 0.
12. Click on Layer 0 to make it active. We need to make the stage micrometer scale vertical:
13. Select the *Ruler tool*. It is 7 from the bottom and shared with the *Eyedropper tool*.
14. Click and drag a line up the center of one of the vertical scale lines.
15. Click *Image* at the top of the Menu bar and select Rotate canvas> Arbitrary. Click OK. The scale lines are now exactly vertical.

Making the Vertical Grid Lines

1. Assure that the Layers palette is open.

2. Click the *New Layer* icon located next to the trash barrel. *Layer 2* has been created.
3. Click the *Single Column Marquee* tool. Its location is shared with other tools. It is the second from the top.

NOTE

Determine a convenient number of horizontal divisions for your grid. Examples: 10 divisions, each of 10 micron for a 10X objective lens. Also, 10 of 5 micron for a 20X objective, 5 of 10 micron for a 40X and 10 of 2 micron for a 100X. The following steps are based upon a 10X objective lens and 10 micron wide divisions.

4. Position its cursor over the scale line farthest to the right and left click until a line of “marching ants” appears.
5. Select the *Set foreground color* tool (the two blocks, three up from the bottom of the Tool bar).
6. Select *red*. OK. This step selects the grid color.
7. Press the Shift and F5 keys simultaneously to set the grid color. Click OK in the *Contents* box.
8. Observe the Layer tab and to the far right a smaller tab. This tab is the *Options* tab. L. click on this tab to open a menu.
9. Click on the *Duplicate layer* option. Click OK in the *Duplicate Layer* dialog box to make *Layer 2 copy*.
10. Select the *Move tool* at the very top of the tool bar.
11. Press the left arrow key to move the marching ants line 10 least divisions (one decadal).
12. Click the *Option* tab and select *Duplicate layer*.
13. Click OK in the Duplicate layer dialog box.
14. Select the *Move tool* and position the marching ants over the next decadal using the left arrow key.
15. Repeat Steps 12 thru 14 until all of the vertical grid lines have been made. **Do not click the Options tab after moving the marching ants to the last scale line.**
16. Simultaneously press *Ctrl* and *d* to remove the last marching ants line.
17. Hold the *Shift* key down and click on all layers except Layer 0 and Layer 1. Start at the top.
18. Click the Layer 0 eye icon.
19. R. click any Layer 2 copy and L. click *Merge visible*.
20. Make Layer 1 visible by clicking the eye location.
21. Double click on Layer 2 copy and change the name to *Vertical Lines*. Click outside to set the name.

Making the Grid

1. Assure that the *Vertical Lines* layer is selected. L. click *Option tab>Duplicate layer*.
2. In the Duplicate layer dialog box, rename the layer *Horizontal Lines*. OK.
3. Click Edit>Transform>Rotate 90 CW.
4. Hold the Shift key down and click the *Vertical Lines* layer.
5. Press Ctrl and E simultaneously to merge the two layers into one.
6. Double click the single layer and name it, *Grid*. Click outside to set the name.

Grid Finalization

1. Select the Rectangular marquee tool. Use it to make a box next to the right edge of the grid. Make the box extend to the image field edges.
2. Zoom in as desired in order to be precise. **Assure that the Grid layer is selected.** Use the left arrow key to nudge the left edge of the box until it just kisses the vertical grid line. Press the delete key to remove the overhanging horizontal grid lines.
3. Click outside the box to close out the Rectangular marquee tool.
4. Use the Rectangular marquee tool to remove unwanted portions of the rest of the grid.
5. Click on Layer 1.
6. Select the Magic wand tool. Hold the shift key down and click on each of the four empty triangles in the

image field.

7. Set the Foreground color to blue. OK. Simultaneously press the Shift and F5 keys. OK.
8. Press *Ctrl* and *d* keys to deselect the marching ants.
9. Click on Layer 0 and drag it into the trash barrel.
10. Save the image using this format: objective magnification-grid size-capture resolution. For example,
10X50mu7.7MP.psd
11. Use *Save as* under the *File* menu. Be sure to save as a Photoshop document by using the Photohop file extnsion (.psd) and **with the Layers box checked**.

Using the Printable Grid

1. Open the specimen image. Assure that the image layer is selected
2. Open the Grid. Assure that the grid layer is selected.
3. L. click Window>Arrange>Tile Vertically in the Menu bar. Select the specimen image.
4. Use the Move tool to slide the grid from the grid image over the specimen image. L. click and move the grid to a favorable position. The grid image may be deleted and the specimen image enlarged.
5. Adjust the grid brightness by selecting *Opacity* in the layers palette and moving the slider.
6. If the image is saved at this point, then when it is retrieved the move tool can be used to move the grid. To freeze the grid position, right click on the active layer and left click on *Flatten Image*, then save.
7. The image with grid overlay may now be saved and printed using your usual means.

Printing an Enlarged Section of the Image/Grid

1. Select the Rectangular marquee tool.
2. Outline the desired area.
3. Click *Image>Crop*.
4. Click *Image>Size*.
5. In the *Document Size:* dialog box, enter the largest paper dimension in the width box for landscape printing.
6. Click *File>Save As* and follow instructions on the display.
7. Your enlarged image area may now be printed.

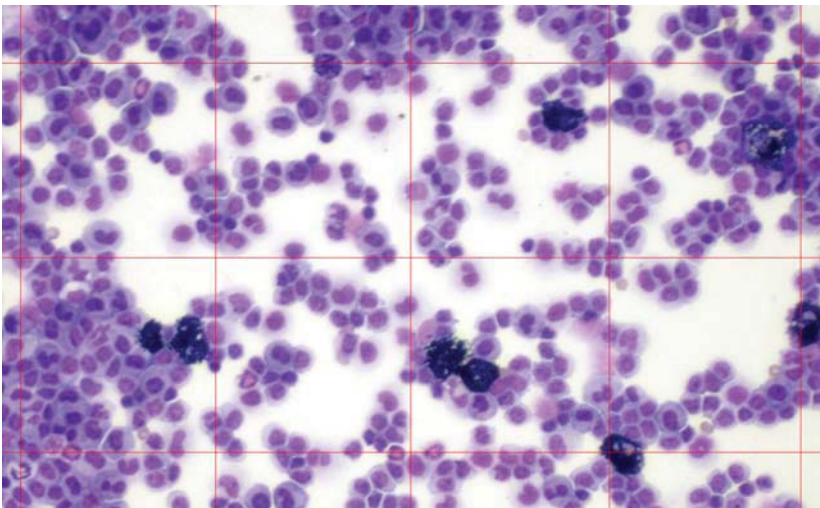


Image of cells with 10 X 10 micron grid overlay. Red grid lines chosen in order to have contrast over white and black areas.

Objective lens = 10X for both the specimen image and the grid overlay.

Capture resolution = 7.7MP for both images.

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