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# TECHNICAL NOTE

## REDUCING BIAS IN HANDWRITING AND RELATED IMAGES USING BEST PRACTICES IN MICROMETRY FOR CASE WORK REQUIRING THE STEREOMICROSCOPE

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**Abstract.** *This paper provides techniques and procedures to calibrate the stereomicroscope in a forensic document examiner's laboratory and thus to reduce bias in producing microscopical images of handwriting, inks, writing and printing, or other evidence for more accurate presentations in forensic document examiners' reports and for trial exhibits. Instructions are provided on how to determine field sizes at any magnification, how to calibrate a stereomicroscope eyepiece reticle for taking precise measurements, and how to create labeled scale bars for inclusion into photomicrographs.*

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**Reference:** Barabe, J.G. (2015). Reducing Bias in Handwriting and Related Images Using Best Practices in Micrometry for Case Work Requiring the Stereomicroscope. *J. Forensic Document Examination*, Vol. 25, pp. 47-57

**Key Words:** Micrometry, Stereomicroscope, Calibration, Stage micrometer

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### I. Introduction

#### 1.1 What is micrometry?

*Micrometry* is the use of a calibrated microscope to make measurements of microscopic objects and phenomena. Stereo and other microscopes provide magnified images that allow the forensic document examiner (FDE) to see, study and photograph many subtle details in handwriting and other evidence that are difficult to discern or are invisible to the naked eye. Micrometry provides the added dimension of allowing numerical measurements to be made, and to place calibrated and labeled scale bars onto the images to indicate the actual size of the object in the image. Thus, the use of micrometry provides a more solid scientific foundation for collecting and presenting handwriting and related evidence.

#### 1.2 History of micrometry

With the invention of photography in the early 19th Century, scale was often indicated by the presence of an object of approximate known size, such as the human body or, for smaller objects, a coin (this method is still useful when appropriate). For more precise indications of measurement, a ruler of some sort was included in the photograph. Today, a large number of scales are used for a wide variety of purposes, and specific scale designs are used in the geology, art conservation, medicine and dentistry, and forensic science.

The ability to make precise measurements with microscopes has been of considerable value to scientists from the time the first microscope was invented in the late 16th Century. Micrometry, however, did not become an established part of microscopy until the invention of the stage micrometer and the eyepiece reticle in the early - mid 19th Century. The stereomicroscope was not invented until 1894 with Greenough's design, which came into wide use in the 20th Century, especially for dissections and

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examinations that did not need high magnifications. This design was supplanted by one using single interchangeable objectives, and then by zoom lens configurations, the most common configuration in today's stereomicroscopes.

In much of the early scientific literature using microscopical images, scale was indicated by stating the magnification alone, which is easily calculated by multiplying the eyepiece magnification by the objective magnification. In the pre-digital imaging era, there was no practical way to include an image of a scale in the photomicrograph, especially for transparencies. For publication, a scale was photographed and printed at the same magnification as the subject, and the scale was then drawn on to the photographic print by an artist. With the advent of digital photography, the insertion of a scale into a photomicrograph has become much more common, although still not universal. When present, it significantly raises the scientific value of the image.

### 1.3 Importance of measurements for FDEs

Measurements provide objective information in casework. For example, if measurements point to a specific pen type (say a 0.7 mm ballpoint rather than the suspect's usual 0.5 mm, or 11.5 versus 11 point type), then showing these differences could potentially play a critical role in the case if the pen type becomes at issue. Even when measurements play a less critical role, the fact that FDEs took measurements or incorporated bar scales into micrographs, the FDE is then in a stronger position to defend his/her work as being conducted using best scientific practices. If an opposing FDE did not include these best practices, then that examiner's work will be less credible. Knowing how to calibrate the microscope to a known standard also shows that the FDE is knowledgeable about the microscope as a scientific instrument.

The use of scale bars in micrographs, rather than just reporting the magnification or including rulers in the image, has been the standard in the scientific community for the past decades. Reporting magnification alone is ambiguous, for several reasons:

- Cameras come in widely different formats and this can affect the size of the field of view.

- The field of view may be affected by optical components of the imaging path.
- Reproduction sizes or projected images may also distort the *perception* of magnification.

One can photograph a standard ruler with the specimen, but this is often impractical. It might take up too much space, or, at the higher magnifications, the lines designating individual millimeters may be too imprecisely printed in the original ruler. The presence of a ruler in the photograph may also distract from the subject or simply be aesthetically unpleasant. Therefore, it is incumbent on FDEs to begin using long held, best practices of scientists and microscopists to use scale bars in photomicrographs.

### 1.4 The stereomicroscope

To implement micrometry, it is useful to review some parts of the microscope and how these parts affect measurements. For this paper, the author's Olympus SZH stereomicroscope will be used as the example, but the principles apply to all similar instruments. Like all standard microscopes, the stereomicroscope has two fundamental magnifying optical components, the objective, which provides the initial magnification, and the eyepiece, which magnifies the real image projected by the objective. Total magnification is the objective magnification times the eyepiece magnification. The objective is inscribed 1 X, but the additional zoom optics multiply that at factors ranging from 7.5 to 64. The eyepiece has a fixed magnification of 10 X. Some microscopes have click stops at each inscribed magnification; this is preferred for repeatability, but not essential.

Microscopes with a trinocular head have a third port for installing a camera. The trinocular head generally has a photo eyepiece in addition to the two viewing eyepieces. The function of the photo eyepiece is to project an image onto the camera sensor of the same field of view as seen in the eyepieces. If the stereomicroscope lacks a separate camera port, photographs may be taken through an eyepiece directly.

Microscope				
Configuration				
Example: $MSL/ERD = 1000 \mu\text{m} / 10 \text{ ERD} = 100 \mu\text{m} / \text{ESD}$ At 10 X magnification, each Eyepiece Reticle Division = 100 $\mu\text{m}$				
Magnification X	Field Size (mm)	Eyepiece Reticle Divisions	Micrometer Scale Length MSL ( $\mu\text{m}$ )	Eyepiece Reticle Division at X magnification MSL/ERD ( $\mu\text{m}$ )
			1000	=(D5/C5)
			1000	=(D6/C6)
			1000	=(D7/C7)
			1000	=(D8/C8)
			1000	=(D9/C9)
			1000	=(D10/C10)
			1000	=(D11/C11)
			1000	=(D12/C12)
			1000	=(D13/C13)
			1000	=(D14/C14)
			1000	=(D15/C15)

Table 1. Microscope Calibration Form

## 2. Taking measurements

### 2.1 Calibration of the stereomicroscope

Historically, many FDEs considered calibrating the stereomicroscope as superfluous, as it is an unchanging instrument. Today, however, in all branches of science, all data-generating instruments should be calibrated so that their data, including their error rates, can be objectively evaluated.

### 2.2 Starting with the microscope calibration form

While going through each of the necessary steps to calibrate your microscope, it is important to keep good notes and to place all of your resulting data in a convenient file for quick reference. Table 1 is a simple Excel spreadsheet, the Microscope Calibration Form, ideal for both collecting the measurements and for calculating calibrations. Print out a copy and enter data into the fields during your calibration measurements.

### 2.3 Measuring Field Sizes

A useful starting point is to determine the field size at every magnification. This is easily determined by placing a standard metric ruler on your microscope stage and measuring the field of view as the diameter of the image circle. Figure 1 is a photomicrograph of the field of view at 10 X magnification, using my Olympus SZH stereomicroscope with the eyepiece reticle in place. Note that the field of view is circular, with a diameter of 2.4 cm.

Several factors can affect the field of view and our other measurements and should be taken into consideration. Changing eyepiece magnifications will affect the field of view, but even eyepieces of the same magnification may have differing field numbers, which affects field size. The magnifications of the modular Olympus SZH can differ with the different modules, but adding the trinocular head does not change the field of view (nor any of the other measurements!). However, the epi-illumination module has a magnification factor of 1.25, thus requiring a separate calibration.

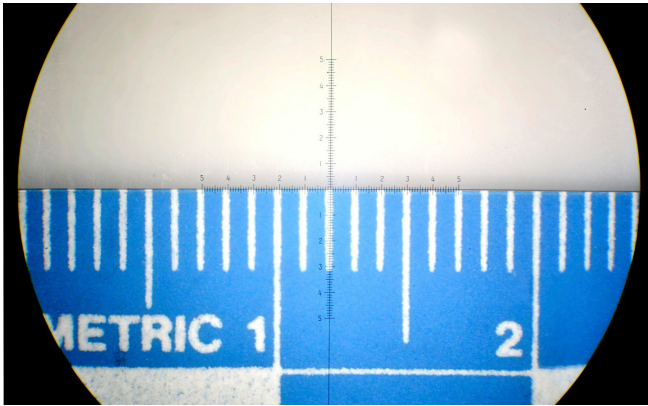


Figure 1. Photomicrograph of field of view of author's microscope at 10 X.

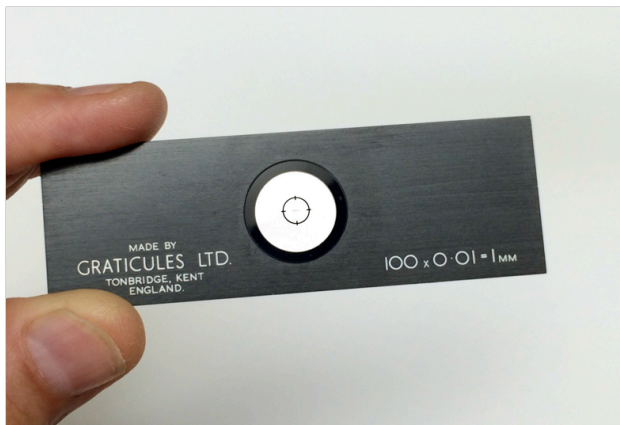


Figure 2. Stage micrometer. The circle surrounds a one-millimeter scale (see Figure 3).

After recording the name of the **Microscope** and any **Configuration Data**, list each of your microscope's magnifications in the **Magnification** column. Measure the field sizes at each magnification and write them into the cells.

### 2.4 Stage micrometer

To measure with the microscope, a reference is essential. A stage micrometer is a microscope slide with precisely ruled measurements at the center; see Figure 2. A typical design is a 1 mm bar subdivided into one hundred ten-micrometer sections (Figures 3a and 3b). One micrometer (abbreviated  $\mu\text{m}$ ) is 1/1000 of a millimeter, so 1 mm = 1000  $\mu\text{m}$ . There are 100 divisions, so each division is 0.01 mm, or 10  $\mu\text{m}$ . Stage micrometers can be obtained from many scientific and microscopy suppliers.

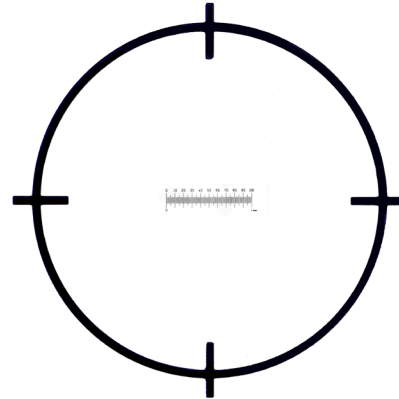


Figure 3a. The circle at the center of a stage  $\mu\text{m}$  surrounds a one-millimeter scale subdivided into one hundred 10 micrometer units.

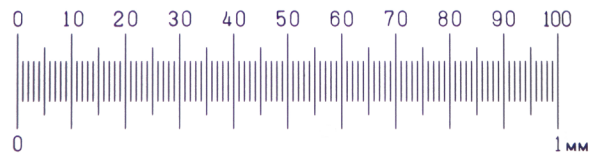


Figure 3b. Enlargement of center of the stage micrometer.

Having a 1 mm ruler handy is fine, but we need a way to use it. One way is to use it to calibrate our eyepiece reticle. Once calibrated, we can use our reticle to measure objects in our microscope's field of view.

### 2.5 The Eyepiece Reticle

The eyepiece reticle, also called a graticule, is a thin piece of glass that has been etched with a pattern that is visible at the same plane of focus as the specimen. Many patterns are available, for many different uses. For the purposes of this paper, the reticle needs to be the kind that will allow making measurements with the stereomicroscope. The ideal pattern is a simple scale such as illustrated in Figure 4.

The reticle for my Olympus SZH can be seen by unscrewing the bottom retaining portion (Figure 5). Notice that the reticle includes lines exactly one centimeter long, subdivided in ten one millimeter units, which are then subdivided into ten 100 micrometer units. The reticle, itself, is *without* units of measurement. Without calibration, these lines are meaningless.



Figure 4. A reticle with this pattern is uncluttered and ideal for use. The author's reticle also has a vertical scale 90 degrees to the horizontal scale (see Figure 5).

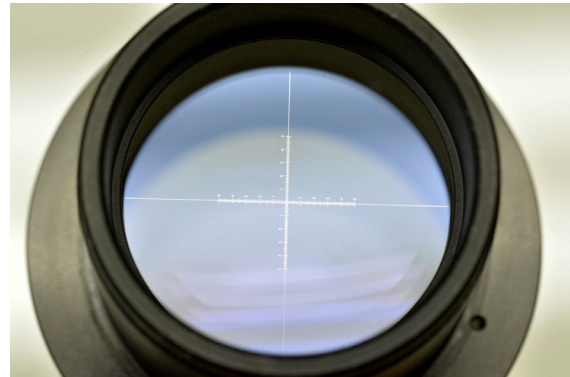


Figure 5. A view of the eyepiece reticle in place

## 2.6 Calibrating the eyepiece reticle

Before you begin, have the Microscope Calibration Form in Table 1 ready.

The first step in setting up your stereomicroscope is to bring the reticle into good focus by turning the eyepiece helix. This should be done without a specimen in place, so as to avoid interference. This step can also be performed with the eyepiece removed from the microscope. Next, put the stage micrometer on the microscope and bring the scale in the center into good focus at high magnification, viewing through the right eye piece, preferably with your left eye covered. Then, to complete the process, focus the scale using your left eye piece only with your right eye covered. At this point, both eyes will precisely focus on the specimen. Adjust the inter-eyepiece distance to make a perfect circle in the binocular view for maximum sharpness and comfortable viewing with both eyes.

Next, set your microscope to 10 X magnification. While you can start at any magnification (and will have to use all magnifications to complete the calibration), 10 X magnification is a good starting place.

Now, place the stage micrometer on the microscope stage or the area under your microscope where you place your documents for viewing. As most stage micrometers are transparent, a lighted stage is ideal, but the micrometer can simply be placed on a white sheet of paper. Bring the ruled portion in the center into sharp focus. Note that the entire stage

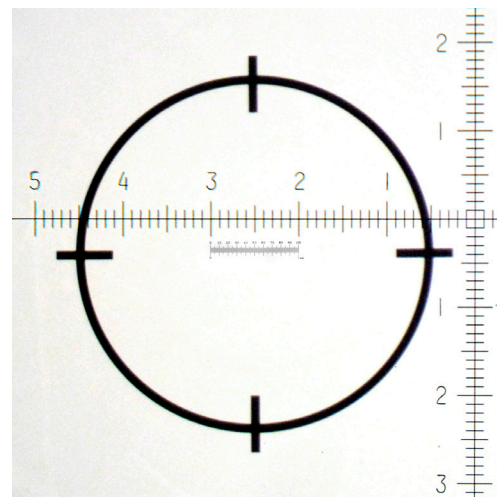


Figure 6. View of the one millimeter stage micrometer scale aligned with the eyepiece reticle scale. The millimeter scale subtends 10 the eyepiece reticle units at 10 X magnification.

micrometer scale is in view; in your Micrometer Scale Length (MSL) column, write in 1000. Next, position the stage micrometer scale to align as precisely as possible with the reticle scale, close but not touching, and preferably at a zero point. See Figure 6. Count the number of Eyepiece Reticle Divisions (ERDs) for the full one millimeter scale. Enter that number in the ERD field coinciding with 10 X magnification.

Since one millimeter is equal to 1000 micrometers,

**1000 micrometers / 10 Eyepiece Reticle Divisions equals 100 micrometers per Eyepiece Reticle division (ERD).**

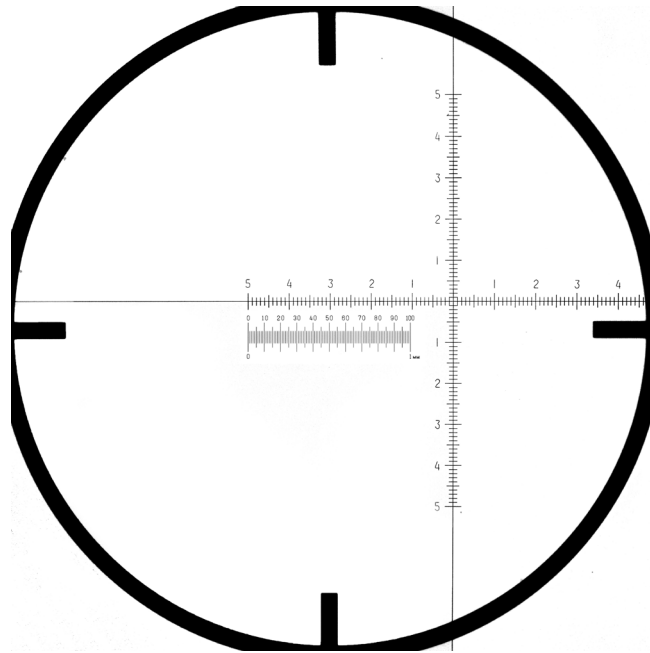


Figure 7. View of the one millimeter stage micrometer scale aligned with the eyepiece reticle scale. The 1 millimeter scale subtends 40 units in the eyepiece reticle at 40 X magnification.

Microscope	Olympus SZH # 892775			
Configuration	1X objective, trinocular head			
Example: $MSL/ERD = 1000 \mu m / 10 ERD = 100 \mu m / ERD$				At 10 X
magnification, each Eyepiece Reticle Division = 100 $\mu m$				
Magnification X	Field Size (mm)	Eyepiece Reticle Divisions ERD	Micrometer Scale Length MSL ( $\mu m$ )	Eyepiece Reticle Division at X magnification MSL/ERD ( $\mu m$ )
7.5	32	7.8	1000	128.2
10	24	10.0	1000	100.0
15	16	15.2	1000	65.8
20	12	20.5	1000	48.8
25	9.5	25.0	1000	40.0
30	8	30.1	1000	33.2
40	7	40.0	1000	25.0
50	5	50.0	1000	20.0
60	4	59.8	1000	16.7
64	3.5	64.0	1000	15.6

Table 2. Calibration of the entire series of magnifications.

Expressed more generally,

Each ERD = length of the stage micrometer units in micrometers / number of ERDs.

Since the length of the stage micrometer is 1 mm, and 1 mm = 1000  $\mu\text{m}$ , then, at 10 X magnification, with this particular microscope and reticle,

Each ERD = 1000  $\mu\text{m}$  / 10 ERDs, or 100  $\mu\text{m}$  per ERD.

When I increase my magnification to 40 X, I find that the stage micrometer image now covers about four times as much of my eyepiece reticle (Figure 7).

Measuring carefully, I see that the 1000  $\mu\text{m}$  scale now covers close to 40 ERDs. Doing the math, at 40 X magnification, 1000  $\mu\text{m}$  / 20 ERDs = 25  $\mu\text{m}$  per ERD. Continuing, I calibrate the entire series of magnifications (Table 2).

At this point, we're capable of precise measurements, but not yet able to communicate these measurements in our photomicrographs. That's next.

### 3. Creating Scale Bars and Placing Them in Photomicrographs

Indicating scale in non-microscopic situations is rarely problematic; a ruler can be photographed or scanned with the case document, or photographed separately but at the same magnification, and the ruler copied and pasted into the document after the fact. In a photomicrograph, however, including a ruler of any sort is impractical. One solution is to photograph a high precision ruler (the stage micrometer scale) at the same magnification as our micrograph and then paste the ruler, or a cropped portion of it, somewhere in the image. This works, but a more elegant solution is to create a set of scale bars, one for each magnification, and, when appropriate, insert the scale bar into the micrograph. The scale bars can be created in image processing software such as Adobe Photoshop™, or other similar software. Even older versions of Photoshop Elements™ have this capability. While the following discussion will occasionally involve computer jargon, each step will be explained. The following steps are specific to Photoshop™ but should be easily adaptable to other image processing software.

1. Create a folder in your pictures folder entitled something like Photomic Scales Olympus SZH.
2. Photograph the stage micrometer scale at each magnification that your microscope provides and place each image file in the folder. Save each image as a TIFF file. Make sure you name the file with the microscope and magnification name, such as Olyp\_SZH\_10X.TIF as an example.
3. Open an image file of one of the photomicrographs in a graphics program such as Photoshop. With the Magnifying Glass, enlarge the image so that you can work with it easily (Figure 8).
4. If necessary, rotate the image so that it's straight horizontally. This is important, as it affects the length of the bar we're about to create.
5. With the Line Tool, draw a line from one end of the 1 mm scale to the other. Select a width of about 5 – 8 pixels, and the color black (a matter of professional taste; any color can be used). It should be a single straight line, not a series of dots or dashes. Holding down the shift key while dragging the mouse keeps the line straight and parallel to the bottom of the image. The line should be as precise as possible. You will notice that this operation created a separate layer in Photoshop (Figure 9).
6. Click on the eye icon for the Background Layer that includes your original micrograph of the stage micrometer; it will disappear, leaving only the bar floating in space (Figure 10).
7. Next, select your Text Tool and click somewhere near the bar. When the cursor appears, type in the length of the bar you just created, for example 1 mm. Chose a typeface and size aesthetically suitable, generally something conservative, non-decorative.
8. Click on the arrow cursor to leave Text Mode.
9. With the Arrow Tool or the arrow keys, move the text to center it close to the bar but not overlapping (Figure 11).

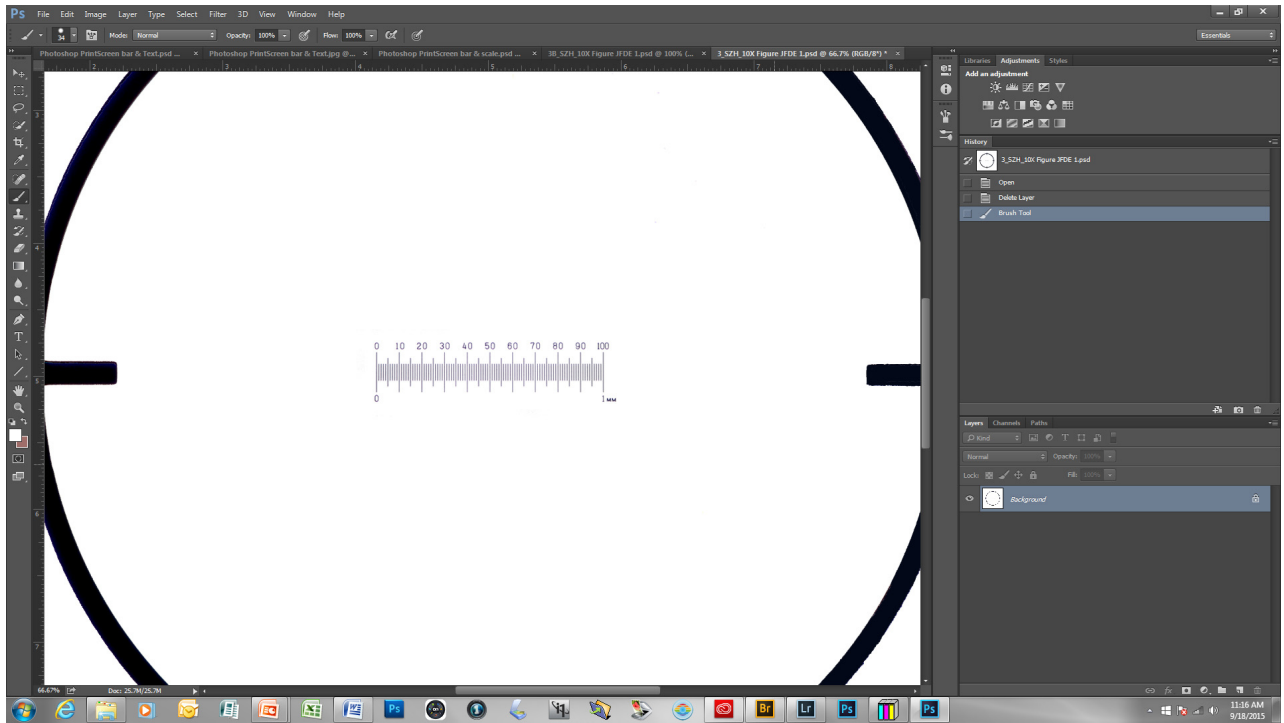


Figure 8. Photoshop screen view of the one millimeter stage micrometer at 10 X magnification.

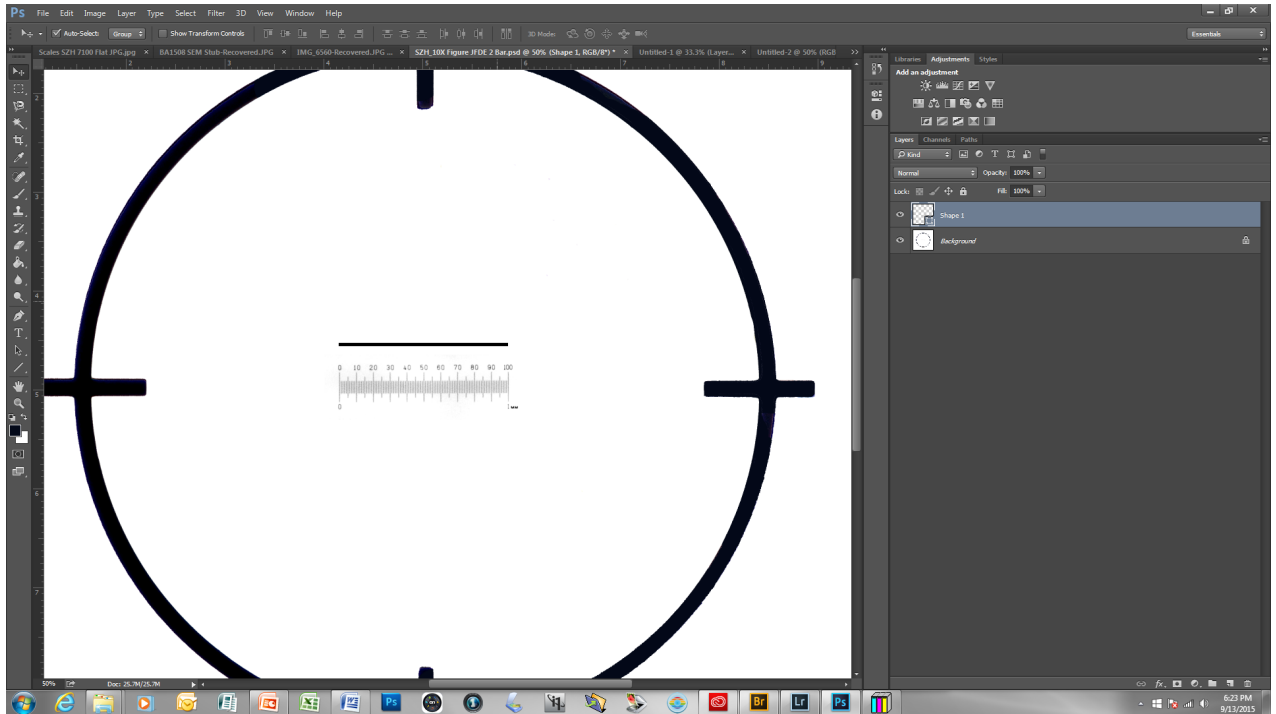


Figure 9. Photoshop screen view of the one millimeter stage micrometer at 10 X magnification. An 8 pixel-wide bar was drawn to exactly match the 1 mm scale.



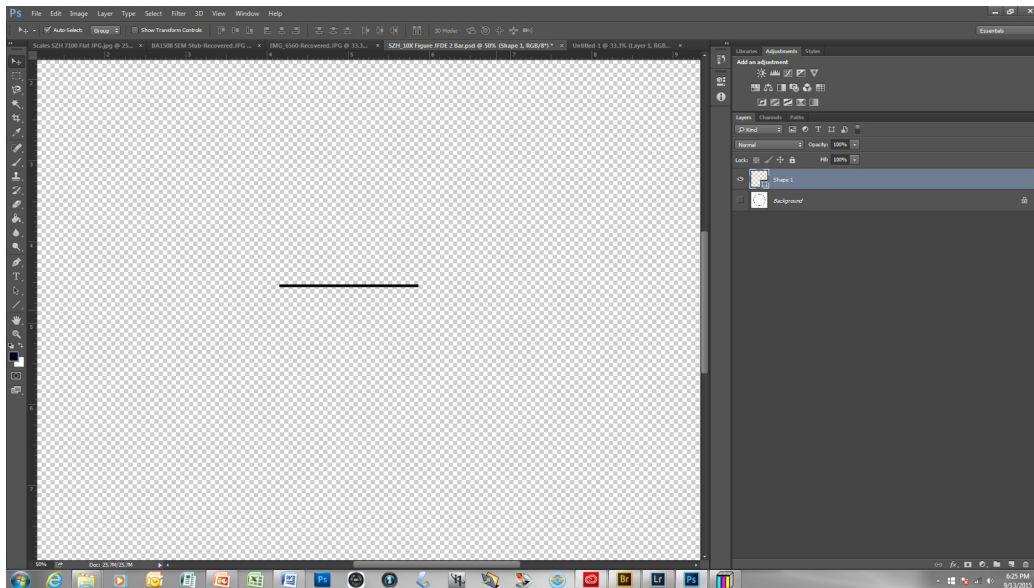


Figure 10. Photoshop screen view of the one millimeter bar at 10 X magnification. The background, including the scale, is now hidden: note that the eyeball icon of the background is grayed.

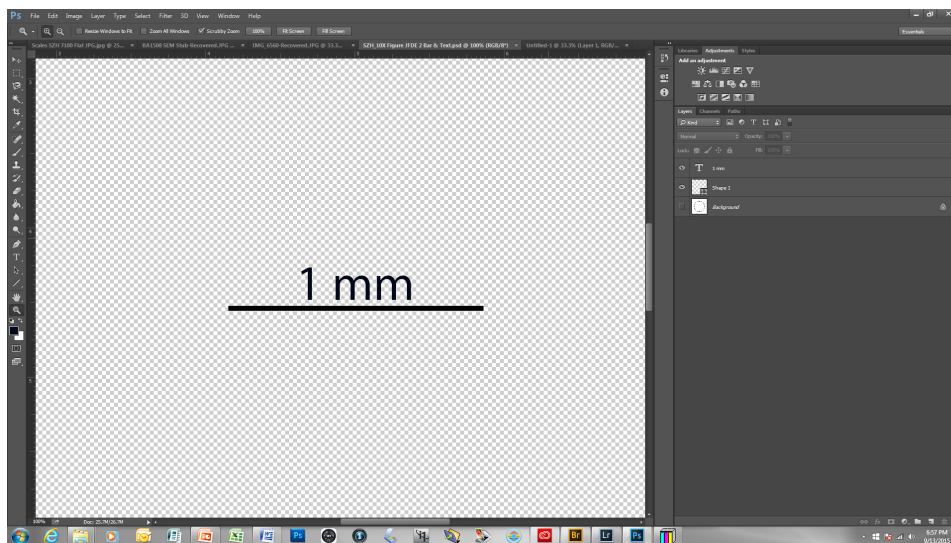


Figure 11. Photoshop screen view of the one millimeter bar at 10 X magnification; the text indicating units has been added and aligned. Note that there are 3 layers, but the background, which contains the stage micrometer scale, remains hidden.

**10.** Once satisfied with your bar, click on the Layer button at the top. From the drop-down menu, click on Merge Visible. Now, your text and scale bar have been converted from two vector files into a single raster file. Your original photo of the stage micrometer scale can be made visible by again clicking on the eye icon in the Layers palette.

**11.** Save this file as a TIFF file of the same name.

**12.** Repeat this for each of your selected magnifications.

At this point, you could be finished. To place a scale bar into a photomicrograph, simply open your file, and with the merged scale bar layer selected, select

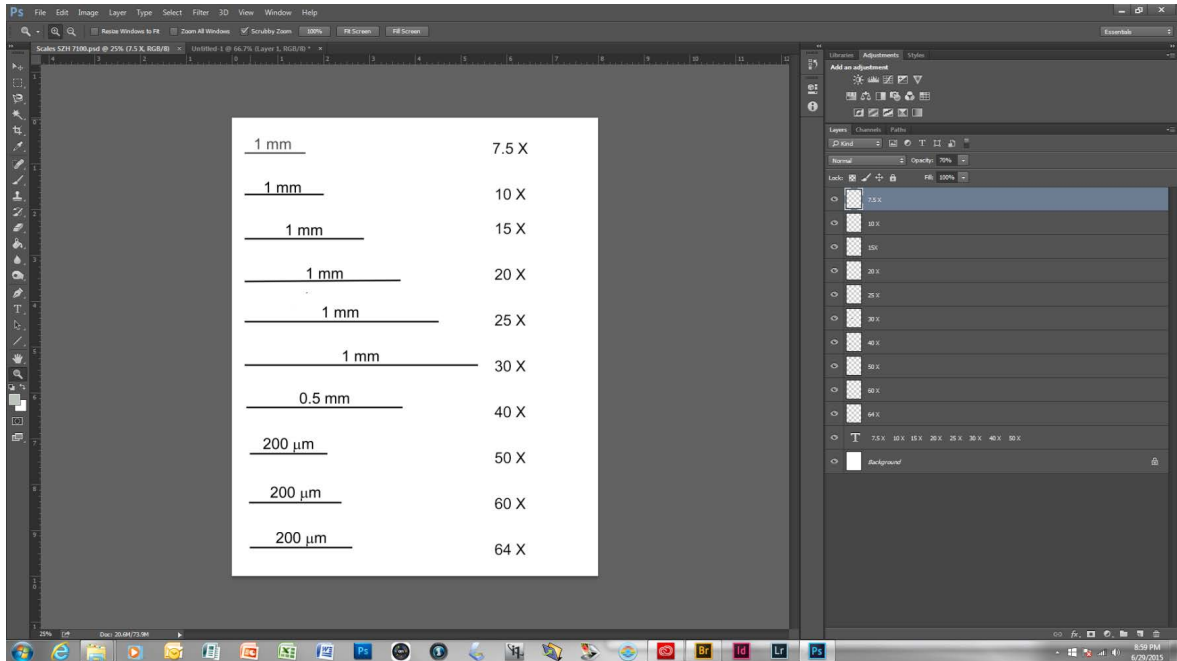


Figure 12. Photoshop screen view of the scale bars at 10 magnifications, with appropriate labels. In this view, each bar is in a separate layer.

your graphic, copy it into your clipboard, and paste it into your photomicrograph which was made at the same magnification. Using your arrow cursor or the arrow keys, position it appropriately; the author usually places it unobtrusively in the lower right corner.

Once satisfied with the placement, the scale layer can be merged with the photomicrograph to create a single-layered file.

### 3.1 Create a File Containing All Your Scale Bars

Once you've created labeled scale bars for each magnification, they can be collected into a single convenient file for use at any time. Figure 12 is a screen shot of my Photoshop file when opened. I store the file on my Desktop, for easy convenience. All the scale bars are on the left, and the identifying captions are on the right. Note that each calibrated scale bar occupies its own individual layer on the right.

#### 3.1.1 These are the steps:

1. Start by opening the lowest magnification scale bar file, in my case **SZH\_Scale\_7X.TIF**.
  2. Select the layer that includes the scale bar-text combination.
  3. Copy the layer (CMD-C or CTL-C).
  4. In Photoshop, create a new file, something like **Scalebars SZH.TIF**.
  5. Paste (CMD-V or CTL-V) the scale bar into the new file.
  6. Your layer palette will automatically label the layer Layer 1. Double click the label and rename the layer **7.5 X**.
- At this point, you have your new file started. Now, you need to create labels for each magnification.
1. Select the Text Tool, the big T on the tool bar.
  2. Put your cursor in the image box opposite your first scale bar, click once, and type the corresponding magnification, in this case **7.5 X**.
  3. Still in Text mode, hit ENTER at least twice to give yourself space.
  4. Type in your next magnification; for me it's **10 X**.
  5. Repeat this for each of your magnifications.
  6. Open the next scalebar file, in my case **SZH\_Scale\_10X.TIF**.
  7. Again, select the layer with the labeled scale bar and copy the layer. Close the file.

8. Returning to our **Scalebars SZH.TIF** file,
9. Paste in the 10 X scale bar file.
10. Rename the layer 10 X.
11. Move the scale bar file opposite the text **10 X**.
12. Repeat this procedure until all the scale bars are neatly in place opposite their labels.
13. Tweak the positioning, and you're done! Your final file should resemble Figure 12.

### **3.1.2 Now, how do you use them?**

When creating photomicrographs, record your magnifications, and, when necessary, any special characteristics such as name of microscope, etc. Process your image as appropriate to your use, adjusting tonality (brightness and contrast) as appropriate.

1. Open the Scalebars SZH.TIF file.
2. Select the layer that your magnification rests on.
3. Select and copy the appropriate calibrated scale bar.
4. Returning to your photomicrograph, paste the scale bar into the photomicrograph.
5. With your arrow tool or arrow keys, move the scale bar to an appropriate place, such as the bottom right corner.
6. Merge the scale bar layer with the background layer. Now the photomicrograph and the scale bar constitute a single rasterized unit.

## **4. Conclusion**

Calibrating the eye piece reticle and producing calibrated scale bars for inclusion into photomicrographs are indications of scientific and microscopical professionalism. The inclusion of objective numerical information may not in itself eliminate bias, but it does take a significant step in that direction.

It should also be remembered that a scientific photograph should exhibit the following characteristics:

1. Proper exposure.
2. Proper white balance (no color cast due to improper color temperature settings).

3. The subject matter should be centered in the image and cropped to include all relevant image data and exclude the irrelevant.
4. All lines should be straight and parallel.
5. In focus, with sufficient camera resolution.

Measuring field sizes, calibrating the eyepiece reticle and creating and using scale bars are one-time exercises that can add significant professional value to your reports and presentations.