Measuring from SEM Micrographs

By

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• A Micrograph is essentially an accumulation of data, for example:
  • Cell diameter
  • Insect Head Width
  • Sensory Hair Length

• To Measure Structures Off Photographs we need to use:
  • Scale Bar Information or
  • HFW Information

• There are Two Basic Ways to Measure off Photographs:
  • The **Old Way by Hand** (but still very useful for micrographs that are not digitized).

• The **New Way by Computer Measuring Programs** such as **Image J**
• The Previous Slide Shows an Environmental SEM (ESEM) Micrograph of C2C12 Mouse Myoblasts Cells grown on a coverslip.

• In the data bar of the picture we see:
  • HFW: The width of the picture. Here it says that the **actual distance on the specimen** is 0.17 mm or 170 um.
  • The Scale Bar indicates that the actual distance on the specimen (for that bar) is 50 um.

• Let’s now use the above information to measure off the micrograph: first we will use the **Old Way** (see next slide)
The HFW says that on the specimen this distance is really 170 um (0.17 mm), but if we measure it using a ruler on the print of the Micrograph it measures 226.6 mm.

The Scale Bar here indicates that on the specimen this distance is really 50 um (0.050 mm) but on the print it measures 66.8 mm.
Using **Magnification** to Measure off Micrographs

- You can **not** use the Magnification printed in the databar unless your print of the SEM screen is the same size as the SEM screen!!!!
- Therefore you need to calculate the magnification of YOUR PRINT
- You will do this using either the **Scale Bar** or the **HFW** since they represent **REAL DISTANCES ON YOUR SPECIMEN** (if your microscope is calibrated (see **Microscope Measuring Calibration Training Module**))
• **Scale Bar**: On the print it measures (using a ruler) 66.8 mm, but really represents 50 um or 0.050 mm.
  • Calculating the Print Magnification:
  • \( \frac{66.8 \text{ mm}}{0.050 \text{ mm}} = 1336 \text{ X} \). This means you are seeing an image of the scale bar 1336 times larger than it really represents. Also anything on the print really appears 1336 times larger than life!

• **HFW**: The width of the print measures on the paper 226.6 mm but in real life that distance on the specimen is really 0.17 mm.
  • Calculating the Print Magnification:
  • \( \frac{226.6 \text{ mm}}{0.17 \text{ mm}} = 1333 \text{ X} \)
• $\frac{1333}{1336} = 0.998$

• Thus the two calculated magnifications are really close

• $\frac{1333 + 1336}{2} = 1334.5$ times for an “Average” Magnification. We will use that to measure the Cell Diameter of the Cell indicated in the Next Slide.
On the print the diameter of this cell (indicated by the white arrows) is 15 mm measuring with a ruler. Really in life this cell’s diameter is $15 \text{ mm} / 1334.5 = 0.01124 \text{ mm} = 11.24 \text{ um}$
Measuring Structures off Micrographs using Computer Measuring Programs

For Example:

• Image J
• Scion Image for Windows
• NIH Image (for Macintosh, see next 2 slides)
• Sigma Scan Pro
• Many Others
About NIH Image

NIH Image is a public domain image processing and analysis program for the Macintosh. It was developed at the Research Services Branch (RSB) of the National Institute of Mental Health (NIMH), part of the National Institutes of Health (NIH). A free PC version of Image, called Scion Image for Windows, is available from Scion Corporation. There is also Image/J, a Java program inspired by Image that "runs anywhere".

Image can acquire, display, edit, enhance, analyze and animate images. It reads and writes TIFF, PICT, PICS and MacPaint files, providing compatibility with many other applications, including programs for scanning, processing, editing, publishing and analyzing images. It supports many standard image processing functions, including contrast enhancement, density profiling, smoothing, sharpening, edge detection, median filtering, and spatial convolution with user defined kernels.
**Image** can be used to measure area, mean, centroid, perimeter, etc. of user defined regions of interest. It also performs automated particle analysis and provides tools for measuring path lengths and angles. Spatial calibration is supported to provide real world area and length measurements. Density calibration can be done against radiation or optical density standards using user specified units. Results can be printed, exported to text files, or copied to the Clipboard.

A tool palette supports editing of color and gray scale images, including the ability to draw lines, rectangles and text. It can flip, rotate, invert and scale selections. It supports multiple windows and 8 levels of magnification. All editing, filtering, and measurement functions operate at any level of magnification and are undoable.

**Image** directly supports Data Translation and Scion frame grabber cards for capturing images or movie sequences using a TV camera. Acquired images can be shading corrected and frame averaged. Other frame grabbers are supported via plug-in modules.

**Image** can be customized in three ways: via a built-in Pascal-like macro language, via externally compiled plug-in modules and on the Pascal source code level. Example macros, plug-ins and complete source code can be downloaded from the [Download page](#). More information about NIH **Image** can be found in the [Overview](#) section of the manual.
All Image Analysis Programs:

• Correlate the Number of Pixels in a line, i.e. the Scale Bar, with the actual distance in or on the real specimen.

• Basically you click with the left mouse button on the left extremity of the scale bar, then click on the rightmost portion of the scale bar and type into the computer what the real distance is in mm or um. Thus the computer then knows that X number of pixels = Y mm on your specimen.
Many Image Analysis Programs:

• Can measure
  – Length, Area, Perimeter

• Do Particle Counting

• Do Densitometry (can analyze electrophoretic gels for example, as well as film)

• Can perform Basic to Advanced Image Enhancement (but Photoshop probably does it better so use Photoshop for image enhancement).
We Will Use Image J because:

- It is good.

- It is a free download from the NIH website: http://rsb.info.nih.gov/ij/

- It works on all computers
The Next Group of Slides are Screen Captures from Image J for Windows Version 1.41 where we will also measure the diameter of the cell in the same picture as earlier seen in this presentation.
When you first start Image J you see the screen on top

*Elliptical* or brush selections
Set Scale...
Use this dialog to define the spatial scale of the active image so measurement results can be presented in calibrated units, such as millimeters. Before using this command, use the straight line selection tool to make a line selection that corresponds to known distance. Then, bring up the *Set Scale* dialog, enter the known distance and unit of measurement, then click *OK*. ImageJ will have automatically filled in the *Distance in Pixels* field based on the length of the line selection.
1. Click

2. Click and then draw line to end of scale bar. You will end up with the yellow line.
Measure

Based on the selection type, calculates and displays either area statistics, line lengths and angles, or point coordinates. Area statistics are calculated if there is no selection or if a subregion of the image has been selected using one of the first four tools in the tool bar. Calculates line length and angle if a line selection has been created using one of the three line selection tools. Records coordinates if one or more points have been defined using the point selection tool. Use the *Analyze>*Set Measurements* command to specify what area statistics are recorded.

![Results Table](image)
With line selections, the following parameters can be recorded: *length, angle* (straight lines only), *mean, standard deviation, mode, min, max and bounding rectangle* (v1.34l or later). The mean, standard deviation, etc. are calculated from the values of the pixels along the line.
To export the measurements as a tab-delimited text file, select File > Save As > Measurements from the ImageJ menu bar or File > Save As from the "Results" window menu bar. Copy the measurements to the clipboard by selecting Edit > Copy All from the "Results" window menu bar. You can also save measurements by right-clicking in the Results window and selecting Save As or Copy All from the popup menu.

The width of the columns in the "Results" window can be adjusted by clicking on and dragging the vertical lines that separate the column headings.
I saved this as the File **Results.xls** in the File Folder: **Tissue Culture Cells Portbury**
This is the file just saved in the **Tissue Culture Cells Portbury** folder.
Click Yes
Our Cell Diameter Value (note same value as we Calculated by “Hand”)

Our Data Brought into an Excel Spreadsheet

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