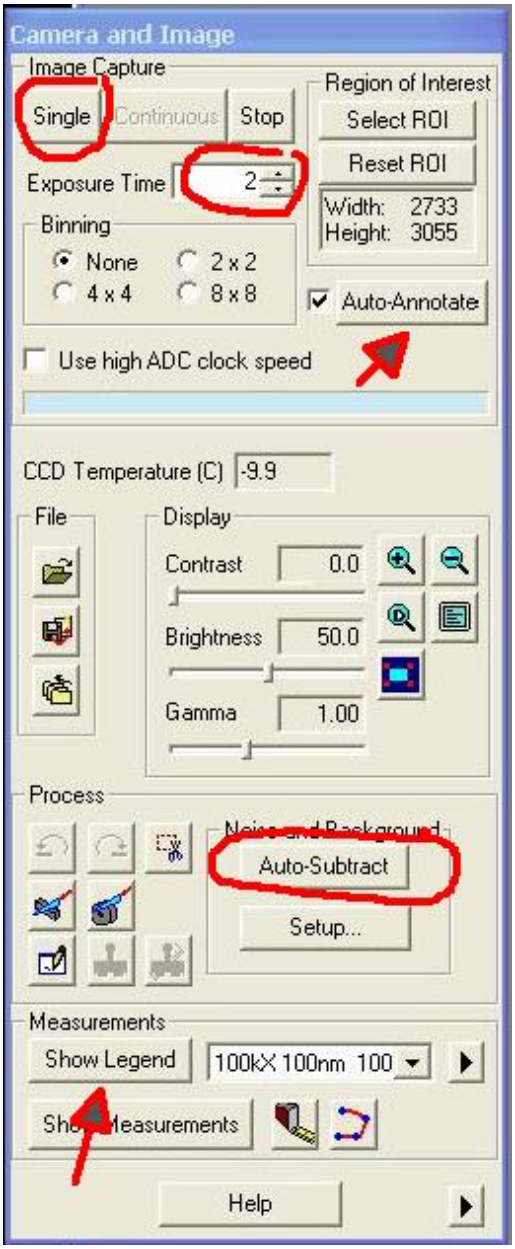
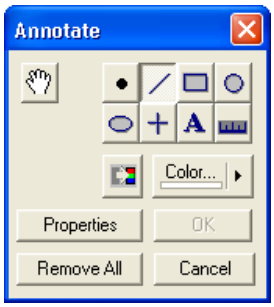
Image Acquisition on JEOL-1200Ex TEM

Image acquisition:

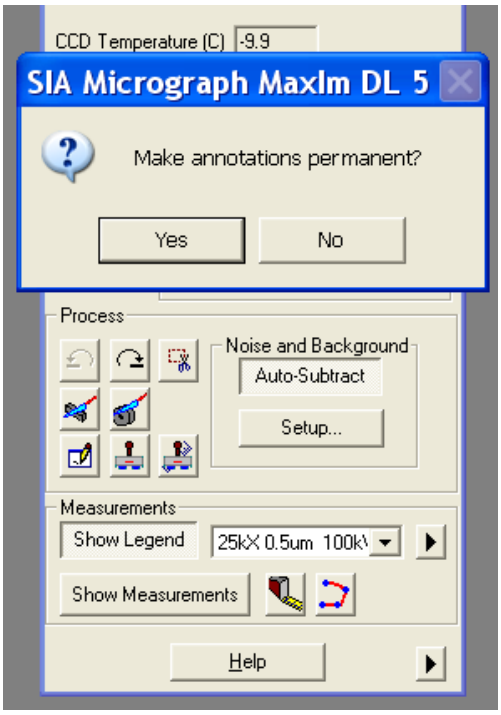


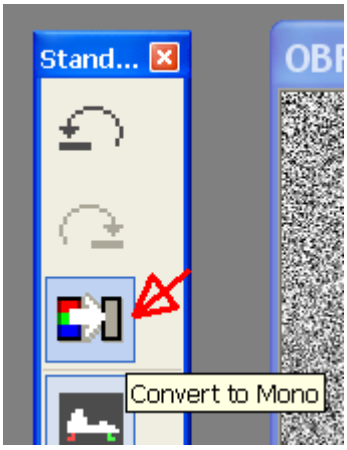
- Start the camera software (MaxImDL) at the beginning of the session. The camera should cool down to -10 °C before taking any images. Cooling reduces noise.
- Set exposure to 2 seconds for most samples. If the image is very bright (i.e., when using Spot Size 1), set exposure to 1 s. Ignore the suggested exposure time displayed on the microscope TV screen – this was for the old film camera.
- Make sure the "Use high ADC clock speed is NOT checked (high speed readout increases noise)
- Activate (click) the "Show Legend" button. Make sure the correct magnification is selected in the drop-down menu.
- Make sure "Auto-Annotate" is checked. Click the Auto-annotate button to set what annotation will be put on the image.
- After you take the first image, make sure to activate the "Auto-Subtract" button - in the "Noise and Background" section of the control panel. Initially, the button is greyed out and you will be able to activate the button only after the first image is acquired. See a section at the end of this document on the importance of this subtraction.
- Focus the image, spread the beam somewhat beyond the edge of the large phosphor screen
- Press the "L" button on the microscope console to raise the phosphor screen
- Click "Single" in the software to acquire the image. DO NOT TOUCH OR LEAN ON THE MICROSCOPE TABLE. VIBRATIONS WILL MAKE YOUR IMAGES blurry.
- After the image is displayed on the screen, push the "L" button again to lower the phosphor screen, so protect the phosphor screen used for imaging.

- Once the image has been captured, check the annotation at the bottom of the image. If the text is overlapped and not readable, you can move the text using the following procedure:
- Click on the Annotation icon  (bottom left icon in the "Process" area). This will bring up the "Annotate" tool box. Regardless what tool you select, when you move the cursor over the annotation on your image, the cursor will change to a hand symbol. You can drag the annotations to a desired position so that they are readable and do not overlap. When done, click OK in the Annotate toolbox. Next time you acquire an image, the annotations will be in their correct locations.

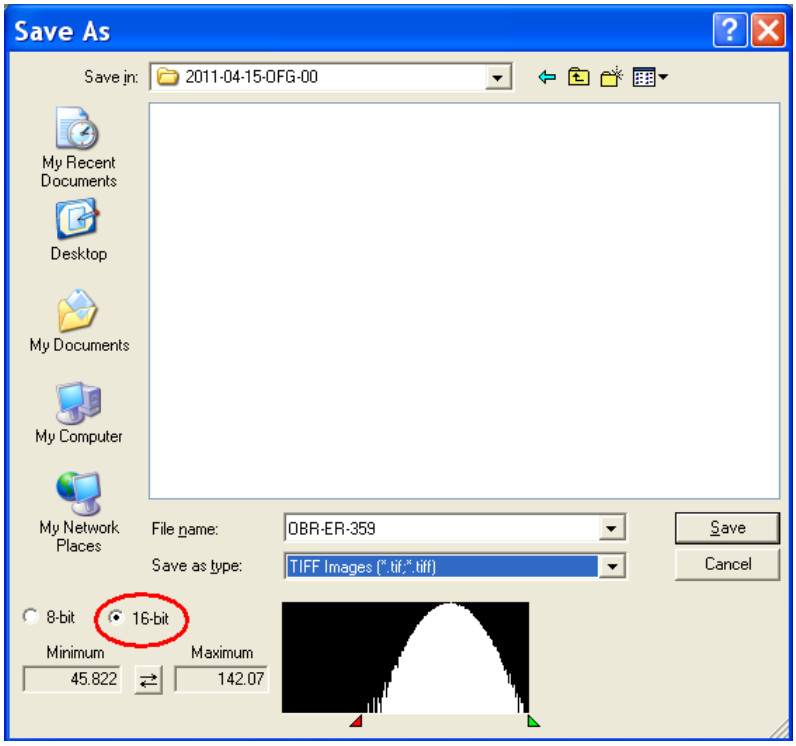


- Click on the Stamp tool  in the "Process" area to make the annotations permanent.
- Drag the scale bar to a desired location, and click the  "stamp scalebar" button in the "Process" area. If you do not see the scalebar anywhere, you forgot to turn on the "Show Legend" button.
- Stamping the scalebar caused the original grayscale image to become RGB color, which would take three times the disk space than the garayscale one (~51 MB vs. 17 MB). Convert the image to Grayscale by clicking the "convert to Mono button in the Standard toolbar on the left.





- Save your image to disk. The camera can capture high dynamic range (many shades of gray). Save your images as 16-bit TIFF, unless you have a very good reason to use only 8-bit TIFF format. The 16-bit image allows you to adjust the contrast and brightness without serious degradation in image quality. With 8-bit images, you only have 256 intensity levels, and contrast adjustments can easily lead to saturation and loss of information.



Opening the images in FIJI/ImageJ

When the saved 16-bit TIFF images are opened, they will have low contrast/seem too dark on the screen. This is because the software looks at the brightest pixel (the white background of the annotation area) and the darkest pixel (the black text in annotation) and sets the on-screen display to cover this range of pixel values. Use the Adjust – Brightness and Contrast (Ctrl-Shift-C keyboard shortcut) to set the minimum and maximum values to get a good image. Adjusting on-screen contrast is non-destructive (does not change the original data) so even if you save the image again as 16-bit image, you will have to do these adjustment again next time.

In order to make the adjustments permanent, you will have to change the image type from 16 bit to 8-bit (Image-Type-8bit). Save the 8-bit image as a new TIFF file (do not overwrite your original file). Saving as JPG is OK for sharing or a presentation, but repeated opening and saving in JPG format will degrade image quality and resolution.

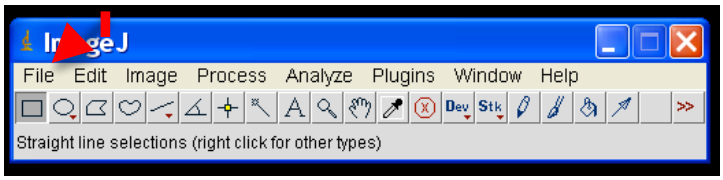
Measurements on digital micrographs using Image J

Spatial Calibration, Measurements

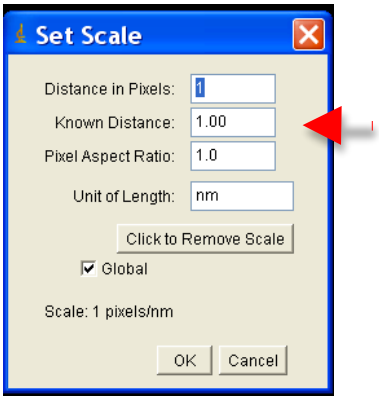
To measure a distance or area on your micrograph you need to know the pixel size. On the JEOL 1200EX using the SIA-15C camera the pixel sizes for each magnification are shown in Table 1. For example, at 25,000X each pixel corresponds to 1 nm. So if a distance on the image is 100 pixels the distance is 100 nm. We are of course assuming that the image has not been re-sampled (scaled-up or reduced from its original number of pixels), otherwise the pixel values in Table 1 are not valid.

To measure a distance, follow this method.

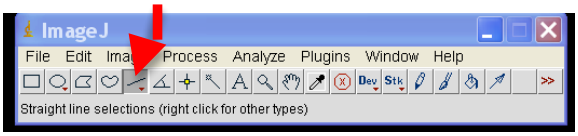
1. Open the image in ImageJ (File-Open; or drag the image on the ImageJ window).

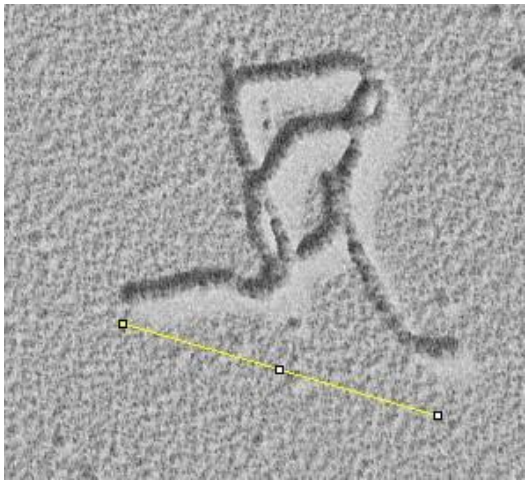


2. Under the "Analyze" menu select "Set Scale"
3. Type "1.0" under Distance in Pixels
4. Find the appropriate magnification in Table 1 (at the end of this document) and enter the pixel value in nanometers under "Known Distance". Leave "Pixel Aspect Ratio" at 1.0.
5. Enter "nm" under unit of length. Make sure "Global" is checked. Click "OK" .

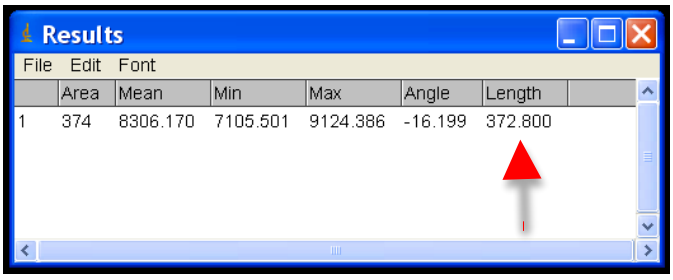


6. Now that we have set the scale we can draw a line between 2 points of interest. Select the line toolbar and draw a line between 2 points. Or you can select the freehand ROI tool and draw around an object whose area you wish to measure.





7. In the "Analyze" menu select "Set Measurements" to select what parameters will be measured. Then click on "Measure" (Ctrl-M keyboard shortcut). This will pop out a new "Results" window.



If you open more images taken at the same magnification you can use the same scale settings. You will have to uncheck the "Disable Global Magnification" box if Image J pops up the following window. Also check "Disable these messages" box. If you open images taken at a different magnification you will have to set the scale again (Step 2).

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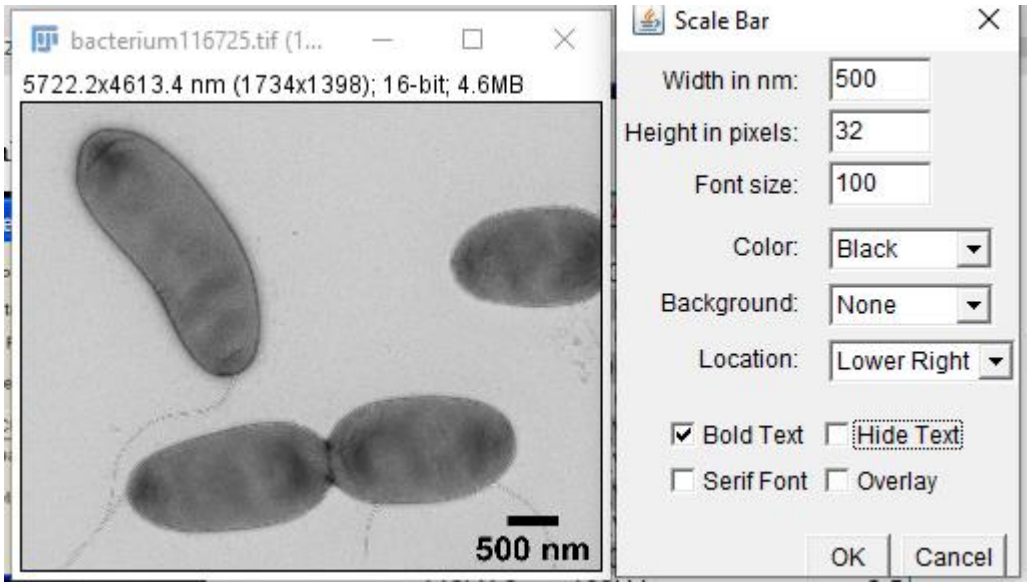
Adding a scale bar

- "Analyze – Tools – Scale Bar". Set the size and color of the bar, position and font size. If your image has a busy background in the bar area, you can fill the area around the bar ("Background" option in the dialog window).
- To add the scale bar permanently (burn into the image), make sure the "Overlay" is not selected.



The importance of Bias, Dark noise and Flat field (BDF) correction

Each sensor has a different bias level (zero point), dark current (sensitivity to temperature), and sensitivity to light. These effects vary from pixel to pixel in the same sensor. Each of these effects corrupts the intensity represented in every pixel of the image in a specific way. The majority of the problems caused by these variations can be removed by calibration. Performing basic calibrations on your images can result in a huge improvement in the signal-to-noise ratio, resulting in much greater sensitivity.

The BDF calibration has been pre-set by the MIC staff and should allow good quality images to be acquired under normal conditions. The flat-field calibration corrects for uneven illumination intensity across the field of view and for pixel-to-pixel differences in sensitivity. For best results, spread the beam beyond the size of the large phosphor screen in the microscope, but not much further. This is how the Flat Field calibration image was captured. If you spread the beam less or more than this, e.g.., to increase brightness in thick specimens, or to minimize damage unstable specimens, your image may show uneven intensity - the center will be brighter or darker than the corners.

If that becomes a problem, contact MIC staff. For general purpose imaging it is possible to correct for uneven illumination in the captured images using ImageJ software and applying a FFT bandpass filter with a very large radius (e.g., filter out features larger than 300 pixels).

In the “Camera and Image” pane3l of the MaximDL acquisition software, the BSD calibration is enabled by the “Auto Subtract” button in the Noise and Background” section of the panel (circled in the image on next page). This becomes available once you captured the first image (at least one image has to be open in the software).

Table 1: Calibrated pixel size for each magnification on JEOL1200Ex with SIA 15C CCD camera.

Magnification	Pixel size (nm)
1kX 20um 100kV	24.4
1.2kX 20um 100kV	20.4
1.5kX 20um 100kV	16.2
2kX 10um 100kV	12.5
2.5kX 10um 100kV	9.6
3kX 5um 100kV	8.1
4kX 5um 100kV	6.2
5kX 5um 100kV	5.1
6kX 5um 100kV	4.1
7.5kX 2um 100kV	3.3
10kX 2um 100kV	2.5
12kX 2um 100kV	2.1
15kX 2um 100kV	1.7
20kX 1um 100kV	1.3
25kX 0.5um 100kV	1
30kX 0.5um 100kV	0.84
40kX 0.2um 100kV	0.62
50kX 0.2um 100kV	0.51
60kX 200nm 100kV	0.42
75kX 200nm 100kV	0.33
100kX 100nm 100kV	0.25
120kX 100nm 100kV	0.2
150kX 100nm 100kV	0.16
200kX 50nm 100kV	0.12
250kX 50nm 100kV	0.11
300kX 50nm 100kV	0.08
400kX 20nm 100kV	0.06
500kX 20nm 100kV	0.05