THE UNIVERSITY OF BRITISH COLUMBIA

Centre for High-Throughput Phenogenomics

CHTP News

September 29, 2022

Chill-up with CHTP

To be more energy efficient, CHTP is participating in the Chill Up Challenge to reduce energy consumption. We're setting the temperature of our -80C freezers to -70C, reducing our freezer's energy consumption by as much as 40%. All our users are encouraged to join us in this challenge and help reduce UBC's carbon footprint.

Check out UBC's Green Labs Program for more information on how you can increase sustainability and reduce the environmental impact of your lab.

Fall Image Clean-Up

It's a new semester at UBC, which often means new projects and more data to organize. It also means many images are saved on CHTP microscope computers and our TeamShare server. To keep our microscope computers performing at optimum levels, we need to clear out data left on them by our users periodically. As a reminder, TeamShare and microscope computers are intended to be used for short-term storage only to facilitate the transfer of the images to your lab or desk computers. Please remember to transfer all your images off the microscopes and TeamShare. They are subject to removal without notice should they be stored on the server for longer than six months. Don't wait until it's too late – transfer your images today!

The Problem with Magnification

Anyone who has undergone Helios training with the resident SEM technician at CHTP will likely be subject to a rant about magnification. It is an important topic worth discussing with all researchers who may use a microscope at some point. Let's address why this measurement is less useful than you probably think. To begin, we must clarify the definition of magnification.

$$Magnification = \frac{Size\ of\ Scanning\ Area\ on\ Display\ Device}{Acutal\ Size\ of\ Specimen\ Scanning\ Area}$$

In the definition, the "Size of Scanning Area on Display Device" is the display size of the screen. So, simply put, if the screen area is 10cm wide, but the actual sample scanning area is 10µm wide, then this would equate to a magnification of 10000X. This all makes logical sense, so why then is this a terrible way to specify an image size? The answer becomes obvious when you consider using two different SEMs to view the same sample area or display the image on a different screen. What happens if you decide to acquire a micrograph of 10,000X on two different SEMs with different size screens? To achieve the same magnification, one would need to image different size areas on the specimen. Let's say the first SEM has a 10cm screen, and the second has a 20cm screen. Imaging a 10um wide area would yield a 10000X magnification, while imaging the same size region yield a 20000X magnification on the second SEM. Put another way, if you acquired a 10000X image on both microscopes, then the specimen area in each image would be different even though the magnification is technically the same due to the difference in screen sizes. This is why a 10000X image capture on the Hitachi microscope will not be of the same size area as a 10000X image on the FEI Helios microscope or any other microscope.

Some SEM software will allow users to specify a reference point for the magnification. In this case, you may specify either the screen size or size of a Polaroid film (as was used on the very first SEMs). Thus the magnification calculation is performed as if the image was displayed on Polaroid film rather than on the screen. This is a better reference for the calculation as it is a fixed size and does not vary, unlike SEM screens. Not all SEMs offer this feature, and many that do offer it do not indicate what reference was used on the databar of the image itself, so it is impossible to know which reference was used retroactively.

Also, the magnification displayed on the databar is only accurate when the image is displayed on the screen for the SEM from which it was acquired. If it is displayed on a different screen or in print, then the displayed size will be different from the size used to calculate the magnification. Consequently, the magnification listed on the databar is then incorrect.

The solution to the magnification problem is simply not using the magnification number. To specify image size, specify the actual scanning area size. This is an intrinsic measurement that does not depend upon a reference point. Most modern SEMs provide this value. It is often abbreviated as "HFW" an acronym for "horizontal full width." This number is the exact width of the scan area from one edge of the image to the other. So an image with an HFW value of 10µm is an image of a 10µm wide area. Acquiring images with the same HFW value on two different SEMs with different size screens will always yield the same size images. Thus, when acquiring images, it is best practice always to use the HFW value to specify image size and ignore the magnification number entirely.

Facilities & Equipment

Facilities

- Main Lab
- Cell Culture Facility
- Data Analysis Room

Scanning Electron Microscopy

- Helios FIB-SEM
- Hitachi SU3500

Light Microscopy

- Nikon Confocal
- Leica White Light Laser Confocal
- Axioplan II Fluorescent Microscope
- Zeiss AxioVision/PALM Laser Capture
- Optical Projection Tomography (OPT)
- Olympus LEXT Confocal

X-Ray Imaging

- Micro-CT Specimen Scanner
- Micro-CT *In Vivo* Scanner

Sample Preparation

- Leica Cryostat
- Leica EM MED020 Coating System
- Critical Point Dryer
- Microwave Preparation
- Grinding/Polishing Suite

New Users

To access the equipment housed within the Centre for High-Throughput Phenogenomics, principal investigators (normally faculty) need to fill in an Access Request Agreement Form (PDF).

Access Updates for Regular Users

Have new personnel in your laboratory who need access to the Centre? Or does existing personnel require additional equipment? Fill in an Access Amendment Form (PDF).

Has the billing information for your project changed? Fill in a Change Billing Information Form (PDF).

Submit Forms

Submit completed forms to Dr. Nancy Ford, Director of the Centre, at nlford@dentistry.ubc.ca

Pricing

Pricing for each item of equipment, as well as sample preparation, professional assistance and training is conveniently posted online.

Review the pricing sheet (PDF)

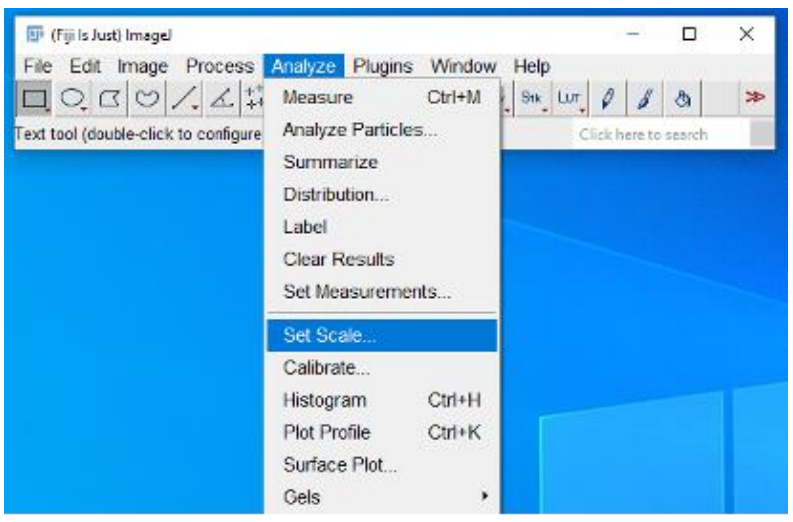
Booking

Registered users may book equipment online. Click the **booking link** on the CHTP homepage to find booking calendars for each item of equipment.

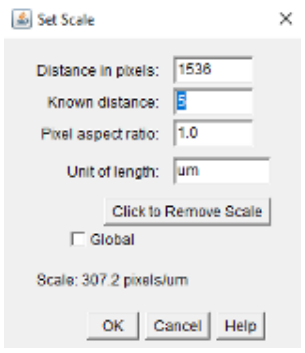
Making Ex-Situ Measurement on Images

Have you ever needed to make measurements on a feature in one of your images well after your imaging session ended, or have you simply forgotten to annotate the image while working on the microscope? Some easy options exist for making ex-situ measurements on images you've already acquired. Free image editing/analysis software such as ImageJ is the most cost-effective choice. ImageJ is an open-source program that all microscopists should be familiar with. It allows basic measurements, filters, annotations, and more advanced image editing features. The only information needed to begin measurements would be the scale information for the image, which should be provided on the micrograph as a scale bar and/or a width value (often abbreviated as "HFW") and the dimension of the image in pixels. The image dimensions, if unknown, may be determined in Windows by simply right-clicking the mouse pointer on the file, selecting "Properties" from the contextual menu that appears, and clicking on the "Details" tab.

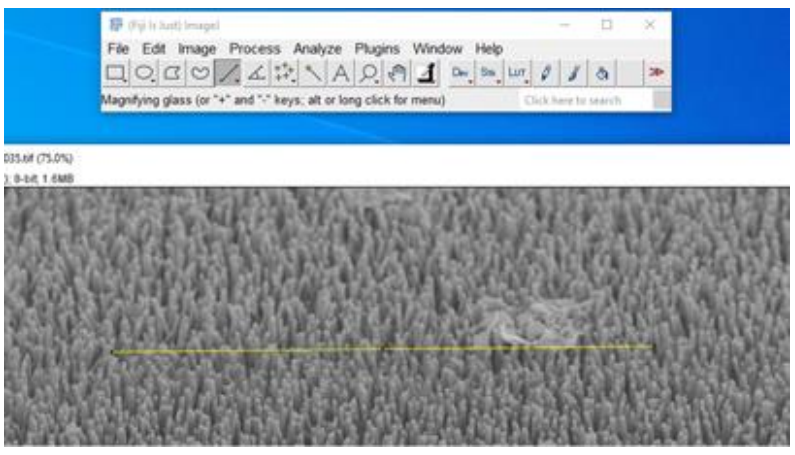
The first step in performing measurements is to set the scale for the image. In ImageJ this can be done by clicking on the "Set Scale" option in the Analyze menu:



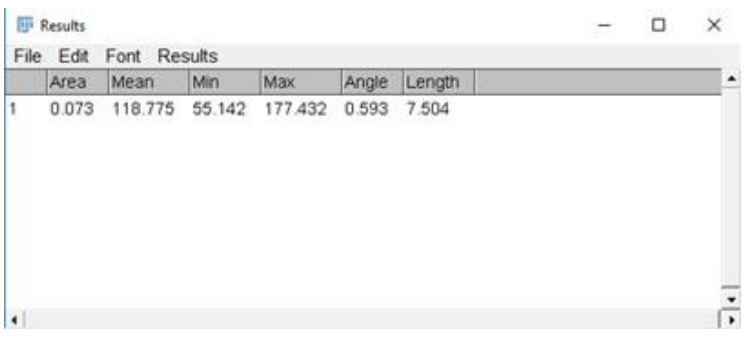
Then specify a known width in both pixels and physical distance and a measurement unit (i.e. µm). This is where knowing the HFW comes in. You may specify the HFW as the known distance, and the pixel distance is just the number of pixels wide the image is (i.e. 1536 is the default for the photo preset in the Helios microscope):



Next, use the line drawing tool to draw a line over the area or feature you wish to measure.



Click the Measure option in the Analyse menu, and the results should appear in a new window.



The Length field will provide the length of the drawn line. Subsequent measurements will populate new lines in the table. The results may then be exported to a text file. This method may also be used to measure other properties, such as areas and angles. With this simple method, you needn't fret over a forgotten measurement.

CHTP Corporate Sponsors

Significant in-kind CFI support provided by the following corporate sponsors:

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