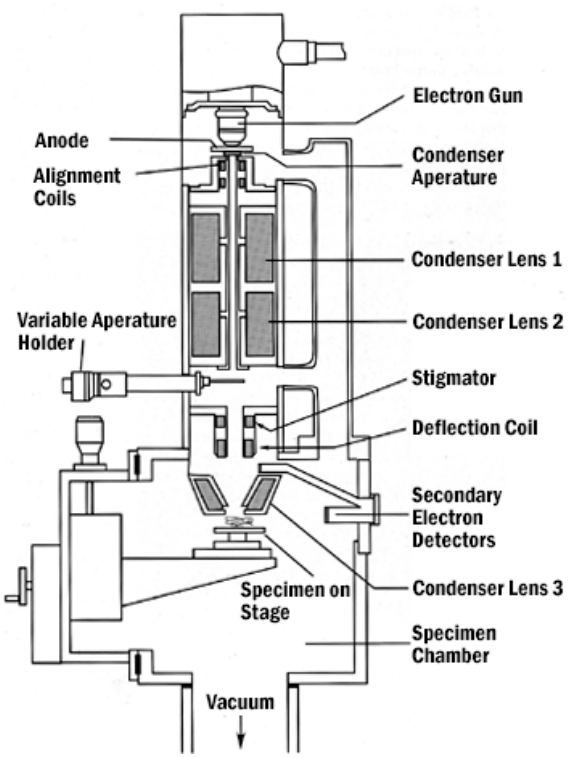
Hitachi TM 1000 Standard Operating Procedure

Background Information on Scanning Electron Microscopy (SEM)



- Scanning electron microscopes produce images of a sample by scanning the surface with a focused beam of electrons (instead of using light).
- The beam of electrons is produced at the top of the microscope by heating a metallic filament.
- The electron beam follows a vertical path through a series of electromagnetic lenses which focus and direct the beam down towards the sample.
- The electrons interact with the atoms in the sample and eject secondary electrons from the sample. These electrons are converted using a detector to produce signals that then display the surface topography and composition of the sample.
- Specimens are observed within a vacuumed chamber.

- The SEM is not a camera → the detector is not continuously image-forming
    - Each pixel in the micrograph is synchronized with the position of the beam of electrons that are targeted at the specimen
    - Therefore, the resulting image is a distribution map of the intensity of the signal being emitted from the scanned area of the specimen
    - The speed of the scan determines the resolution of the produced image
        - Faster scan = lower resolution
        - Slower scan = higher resolution
    - When navigating/viewing your specimen, you should use a fast scan
    - Images will be saved using a slow scan, providing a better resolution than what you viewed when navigating around the specimen stage

- Samples with a width less than 70 mm can fit on the specimen stage with the TM-1000

- Conventional imaging in SEM requires that specimens are electrically conductive on the surface
    - Non-conductive specimen will collect charge when scanned by the electron beam (visually, this will appear as a bright area)
    - To prevent charging, non-conductive samples should first have a thin coating applied to their outer surface of an electrically conducting material (such as gold) using a sputter coater.
    - The detectors in the TM-1000 operate under low-vacuum conditions, which allows specimen to be imaged without being coated
    - If your specimen does still emit a charge (bright white areas blocking your view of the specimen) then you should try sputter-coating it with a conductive material. This can be done at the Electron Microscope Lab on campus (21 Barker Hall) for $18 per run (3 to 4 samples).

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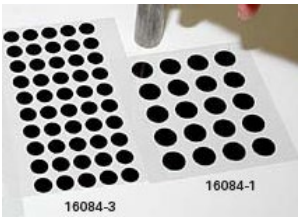
Sample Preparation

- Always wear gloves while handling the specimen mounts
- Specimen mounts for the Hitachi TM1000 can be purchased at tedpella.com
    - Some specimen mounts are available in the imaging lab as well
    - If processing a single sample at a time or a larger sample
        - Hitachi M4 Aluminum Specimen Mount (Prod No. 16324 or #16327)

    - If processing multiple samples at a time
        - Standard SEM Pin Stub Mount, Ø12.7mm x 8mm pin height (Prod No. 16111)
        - Multi-Pin Specimen Mount, 4 x 3.2mm (Prod No. 15310)
            - Allows 4 samples at a time in the ESEM

- Attach sample to specimen mount using either:
    - PELCO Carbon Conductive Tabs, 12mm OD (Prod No. 16084-1 or 16083)
        - This carbon tape is more temporary, a less secure hold
        - The surface dries quickly, so only place tab on the specimen mount if you are ready to place sample on the mount

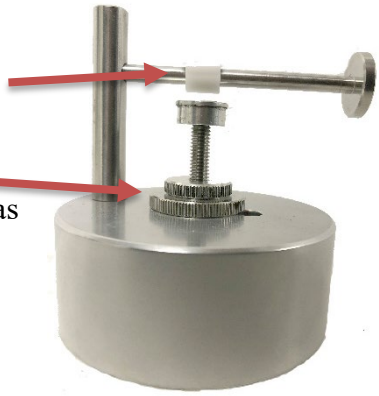


    - PELCO Colloidal Graphite (Prod. No. 16053) Isopropanol Based Graphite Paint
        - If using colloidal graphite, allow to dry completely before processing
    - Be gentle when fixing your sample to the adhesive, only touch the sample with clean and flat surfaces (no sharp objects that would alter/damage the surface)
    - The electron beam has a limited area that can be scanned/imaged
        - If using larger samples, situate the area you would like to image towards the center of the specimen mount

- Clean each specimen with compressed air (after adhesive dries) to remove any contaminants that may block your view of any features you would like to image

- Label the sample number on the underside of each mount with permanent marker, or its position within your storage container (for example 1 through 12)
    - Universal Specimen Holder and Tall Storage Box (Prod No. 16160)
    - Immediately label to top cover of the storage box with your sample number associated with each mount, if you are labeling them separately

- Test the height of your sample using the specimen preparation stage
    - The height of your sample should not be above the white section of the metal swinging rod. This ensures that your sample won't be damaged as you close the chamber door when you insert the sample into the machine
    - Adjust the height by rotating the bottom two disks along the central axis
    - If you are analyzing multiple samples simultaneously, use the tallest sample as your gauge
        - Ideally, your sample's height should be as close as possible to the white bar (within a few millimeters) in order to get the best resolution when imaging



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Operating the Hitachi TM 1000

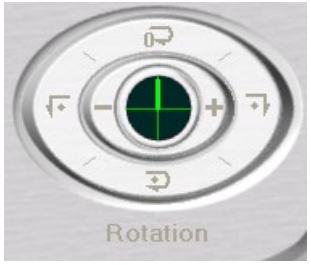
- Place disc or specimen mount in the chamber stage
- Close chamber door
- While holding the chamber door closed (apply pressure), press the green "exchange" button to begin pumping air out of the chamber
    - Keep applying pressure to the chamber door for ~10 seconds to ensure that the chamber door seals correctly (you should hear a "click" when it is okay to let go of the chamber door).
- Wait for the green "READY" light to appear on the machine
    - May take 3 to 5 minutes
    - The more porous the sample, the longer this process will take

- You can now open the program called "TM-1000"
    - The application will not open unless you have the machine on and the chamber has been pumped
- Click the "Start" button in the leftmost corner to begin (this turns on the electron beam and you will enter the "fast" scanning mode)

Navigation

- Use the silver dials on the left side of the machine to navigate around your specimen mount
- The left dial operates the x axis (left to right)
- The right dial operates the y axis (up and down)

You can also rotate the orientation of your specimen using the application's control panel



- The + and – will rotate the stage in small increments
- The outer options will rotate the stage by 90 degrees at a time
- If you rotate the specimen, the navigation dials are rotated as well!

Focus Once you have located your specimen, you should immediately adjust the focus of the beam

- To do this increase your magnification to a level that is higher than any images you will likely capture (at least 1000x recommended)
- This ensures that any images captured at lower magnifications will be in focus (repeat process if you begin to save images at higher magnifications)
- You can focus manually or use the Auto Focus button (Auto Focus recommended)

Brightness and Contrast

- It is more efficient to adjust the brightness and contrast at lower magnifications that are closer to where you will be saving an image (rather than while adjusting the focus at a high magnification)
    - Again, this can be done manually or automatically

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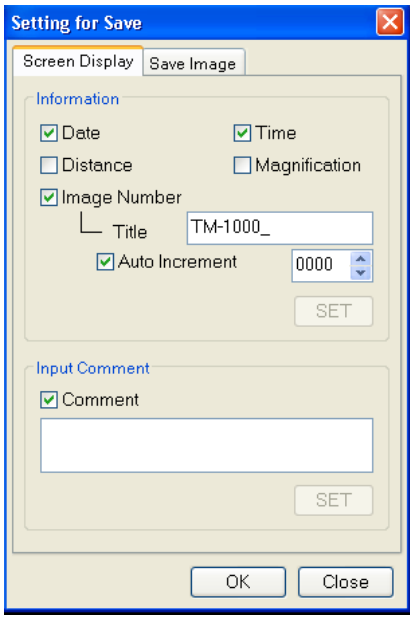
Image Capture (images are referred to as *micrographs*)

- Once you have positioned your view of the specimen to an ideal location you can save images/micrographs
    - Generally, it is best to record multiple views of your specimen at varying magnifications
        - For example, 50x, 100x, and 200x
    - Save as many images as it takes to record all of the diagnostic features of your sample
        - If there is a range or variation in those features, save images of each type
    - Always position image so that the most useful information is in view (try to not get broken/useless edges if possible)

    - If you are interested in using the images in a publication or report:
        - Try to have at least one "clean" image
            - i.e. a view that doesn't have broken/damaged/irrelevant features
            - increase your magnification if necessary
        - Rotate the specimen to an attractive angle if necessary

Edit the Caption



- Edit the text that will be included in your saved micrograph by selecting the icon in the bottom right corner of the screen
- Here you can choose what information will be included as text at the bottom of your micrograph (date, time, magnification, sample name)
- A scale bar will always save with your image
    - Suggested title: Site Name - Sample # - View - magnification
        - ex) Cerén 60001-001 transverse 100x

- How to save an image
    - First create a folder on the computer where you will save all of your images
    - Then select the "Save" button on the top right of the application screen and find your designated folder
        - Never select the "Quick Save" button (this saves a very low resolution version of your image)

How To Close

- Click "Stop" to shut off the beam (top left corner)
- Wait at least 20 seconds before venting the chamber (this helps to extend the life of the filament)
- Vent the chamber by pressing the green "exchange" button on the machine
    - this may take 2-3 minutes (there will be a green light when ready)
- Remove specimen disc/sample and return it to your storage container

If finished for the day, close the chamber and press the green button again to pump the chamber full of air (as if you were starting a new sample).
Once this is finished it is safe to turn off the machine using the "power" switch.