



Morgagni manual online

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This is a quick manual for a users who are new to the Morgagni microscope and negative staining method.

A bottom mount model of an AMT CCD camera is installed on the Morgagni 268.

This is a 1k X 1k camera.

IMPORTANT: BEFORE USING ANY OF THESE INSTRUMENTS\METHODS THE FIRST TIME IT IS MANDATORY TO BE TRAINED.

Preparing grids

- a) Fill a round Pyrex dish to the top with clean water. Skim a Kimwipe across the surface of the water to remove any dust on the surface.
- b) Using a Pasteur pipette, dispense one drop of collodion (toxic) onto the water surface. The plastic will float on the surface of the water and spread to the size of the Pyrex dish.
- c) Examine the surface to find an area free of creases in the plastic.
- d) Using forceps place the grids, light-side up, on the smooth area of plastic. Arrange the grids in regular rows and columns.
- e) When all desired grids have been placed on the plastic, cut a piece of clean printer paper slightly larger than the grid arrangement and place it on top of the grids. Allow the paper to become saturated with water (overnight).
- f) Free the paper and grids from the surrounding plastic by slashing around the paper with forceps. Then carefully pick up the paper and place it with grids facing up on a filter paper in a glass Petri dish. Do not use a plastic Petri dish as static electricity can damage the grids. Allow the grids to dry for at least 24 hours.

Carbon coating the grids (carbon evaporator)

There are two carbon evaporators: one is in the EM suite and the other is in the Ohi lab. Refer to specific operation instructions for each carbon evaporator.

Uranyl formate preparation for negative staining (0.7% w/v)

- a) Weigh 37.5mg of uranyl formate in a beaker and cover it to prevent exposure to light.
- b) Bring 5ml H₂O to boil in small flask.
- c) Add the boiling H₂O to the uranyl formate in the beaker. Stir for 5 minutes.
- d) Add 5μl NaOH (5M). A color change to a darker yellow should occur. Stir for 5 minutes.
- e) Filter and store in container protected from light exposure.
- f) Mark the tube with your name and date.

Glow discharge grids

- a) Before applying the sample to the grid you should glow discharge the grid to recover the hydrophilic properties of the carbon.
- b) Put the grid on a glass slide wrapped in parafilm in the glow discharger.
- c) Close the lid and make sure it aligns well.
- d) Push the start button and wait until the process will finishes (you should see purple glow).
- e) Take your grids and close the lid.

Preparation of negative stain grid

- a) Before starting, prepare parafilm, filter paper, H₂O and stain.
- b) Grab a glow discharged grid on the edge with forceps with the dark side (carbon) up.
- c) Pipette two drops of H₂O and two drop of stain (30-40μl each) onto the parafilm.
- d) Apply the sample (~3-5μl) and wait 30-60s.
- e) Blot the excess sample on the filter paper.
- f) Wash the grid briefly (5-10s) in H₂O by touching the drop and blot it on the filter paper X2.
- g) Wash the grid once with the stain drop and blot on the filter paper.
- h) Put the grid on the second stain drop for 1-1.5 min then blot the stain and dry the grid with vacuum.

Morgagni introduction

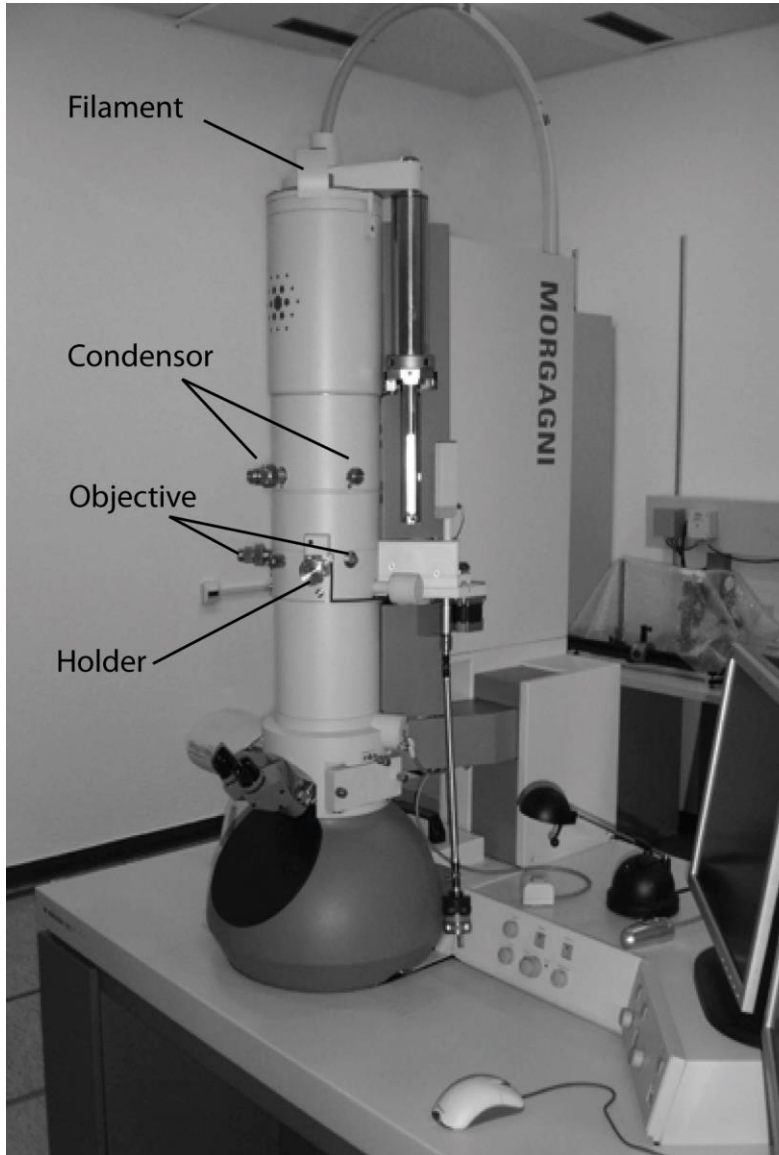


Fig. 1: Morgagni 268 equipped with AMT [1Kx1K] CCD

IMPORTANT: USUALLY THE SCOPE IS KEPT ON ALL THE TIME. HOWEVER, THERE ARE SITUATIONS WHEN THE SCOPE IS SHUTDOWN. IF YOU COME TO THE MORGAGNI ROOM AND FIND THE MORGAGNI OFF, PLEASE ASK BEFORE YOU TURN IT BACK ON!

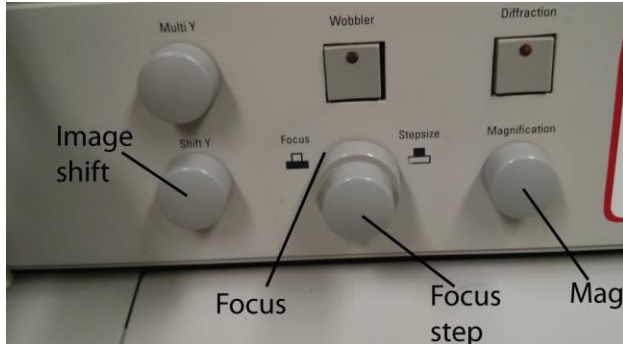
The Morgagni microscope (Fig. 1) operates at 100kV and uses an AMT CCD camera for imaging. This microscope is used for screening samples for quality before proceeding to data collection or cryo work.

The microscope is operated with one computer while the CCD camera operates with another computer.

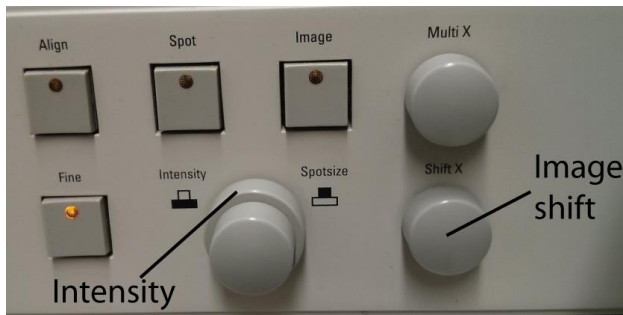
The microscope has 3 physical operating panels (Fig. 2). The main panel (A) controls OI of the microscope and should not be touched. The right panel (B) controls image shift, focus, and magnification. The left panel (C) controls image shift and intensity.



A



B



C

Fig. 2: Control panels. A) main control. B) right panel. C) left panel.

Getting in the room

The Morgagni user interface is usually ON and so is the HT. This should look like Fig 3. Before starting use, check whether these are ON. If one of them is off, **ask for help**.

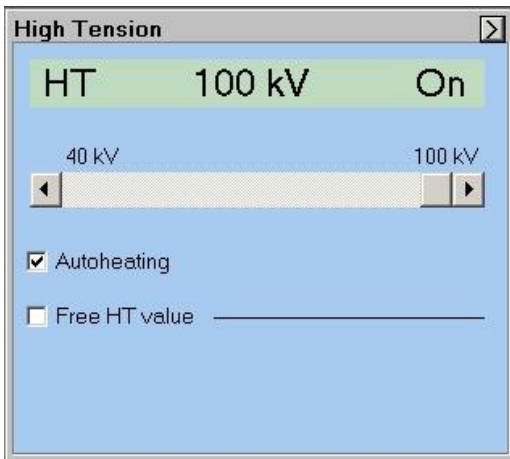
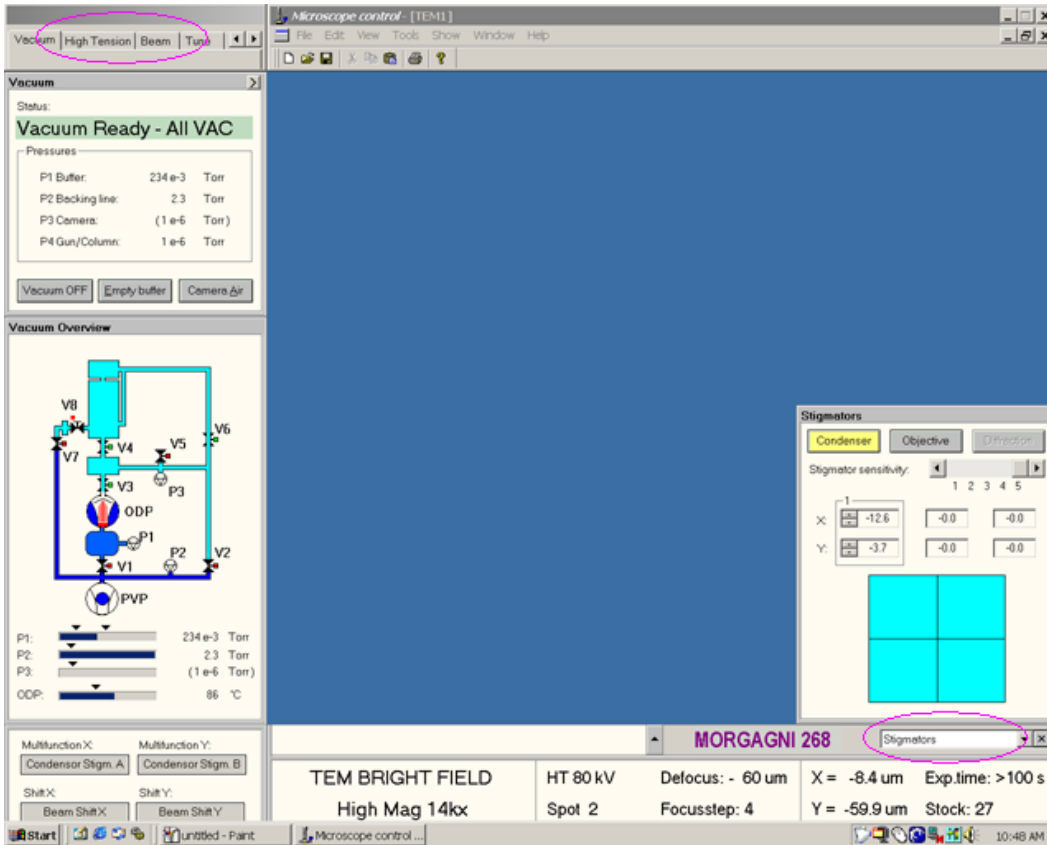


Fig. 3: Morgagni user interface and the HT panel.

Check vacuum

Check the vacuum status from "vacuum" and "vacuum overview" modules. In the vacuum module, the status should show "Vacuum Ready - All VAC" on a green background and the "vacuum overview" should be a light-blue color.

Turn on the beam

Make sure the screen is down and click on "Heating" button and wait until it gets to the heating limit. You should see the beam on the screen. Center the beam using the beam shift x,y knobs (Fig. 2).

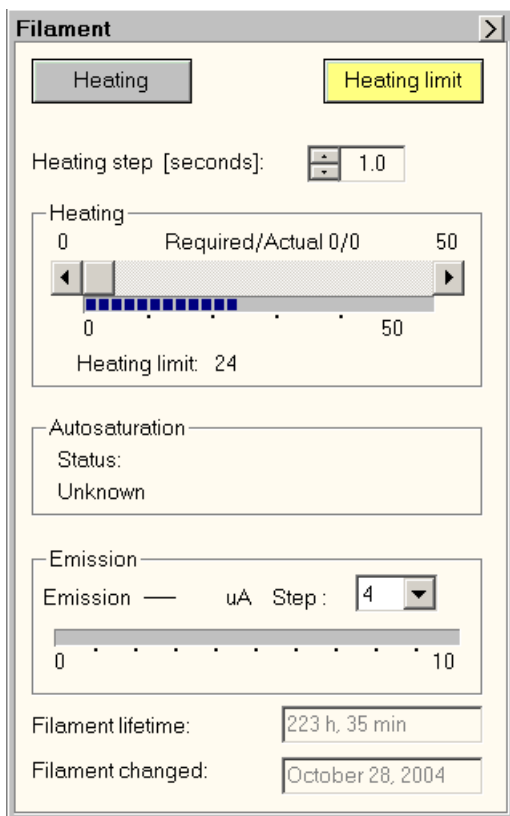


Fig. 4: Filamen tab and heating button

Check specimen holder & load grid

This is **important**. If you see any problem with the holder, report it immediately. Otherwise, you could be the one held accountable for the problem. Several things about the holder need to be checked before use.

- Overall shape, not bent, not obvious damage.
- The O-ring. Make sure there are no cracks or any other damage. If you do see any damage like a cut etc., ask to replace it. Check to see if there is any dirt or fibers on the O-ring. You might want to gently clean it with air.

Gently mount the specimen grid in its position. Use the tool pin to open and close the clamping device (Fig. 5).



Fig. 5: Grid holder

Insert specimen holder into column

IMPORTANT: BE CAREFUL! ONLY AT THIS STEP YOU MIGHT DAMAGE THE SCOPE OR SPECIMEN HOLDER MECHANICALLY. BE SURE THAT YOU UNDERSTAND WHAT YOU ARE DOING. SHOULD YOU FEEL ANY UNCOMFORTABLE OR CONFUSION ABOUT THE ACTION, PLEASE STOP AND SEEK HELP.

- Insert the holder carrier to stage with the pin into 1'clock slot position. This will trigger the vacuum pump and opening valve of specimen stage.
- Turn the holder counterclockwise to let the pin fall in 12'clock slot.
- Wait about 7 seconds until the red LED and the vacuum pump goes off.
- Turn the holder carrier fully counter-clockwise to 6'clock position until a firm stop is encountered (half turn).
- Push the carrier gently into the microscope until it stops.
- Fully depress the push-button at the end of the carrier.
- Keep this push-button depressed and pull the carrier out as far as it will go.
- Release the push-button.
- Turn the carrier clockwise until a firm stop is encountered (half turn) - the pin back to 12'o-clock position and remove.

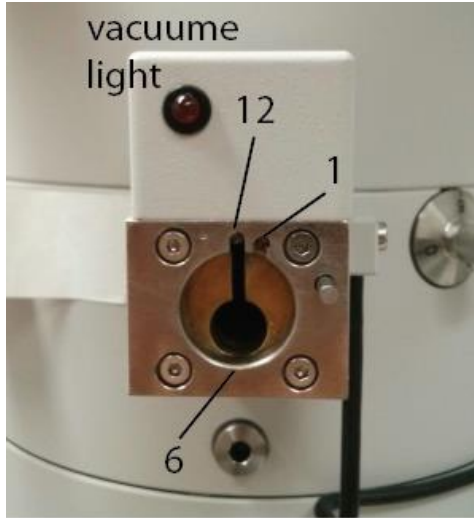


Fig. 6: stage entry

Turn on the AMT software

Launch to the AMT software from “Start” → “amtV542” or by click on the “amtV542” icon on the desktop.

Set up a "Case" for saving images

After a "case" (Fig. 7) is set up, the images saved will have the filename reflecting the case name and of incrementing if multiple images are taken.

- To start a new case, select *File - Case Study - Open New Case*.
- To use an existing case, select *File - Case Study - Continue Previous Case*.
- To exit a case, select *File - Case Study - Close Current Case*.
- The Button "CASE" on the left side of the main window has various meanings based on whether a case is open:
 1. if no case is open, it starts one.
 2. if a case is open and Final Image has been selected, it saves it.
 3. if a case is open and image is Alive or Aborted Image, then click on the button will creates a Final Image and initiates a save.

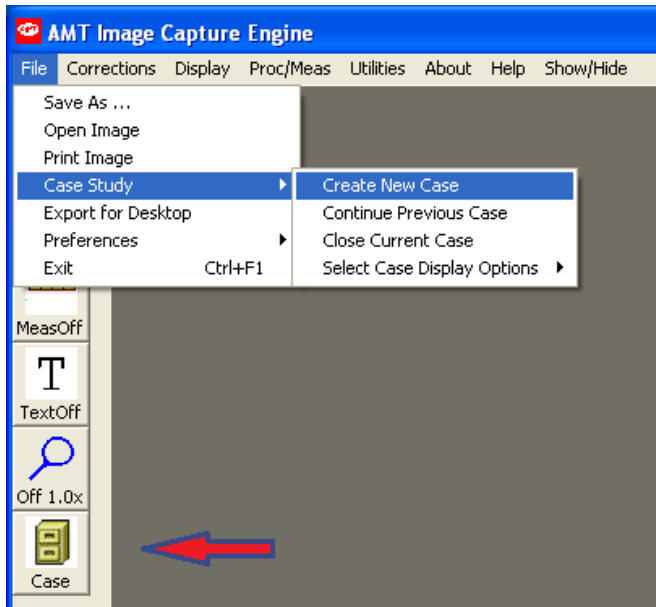


Fig. 7: the "case" button

View specimen "Click for Live Image"

- Click on command button "Click for Live Image".
- Adjust the brightness of the beam using the intensity knob to position the histogram approximately centered in the box.
- The Speed/Quality button switches between frame-averaging. This button has no effect on the final image.



Fig. 8: "click for live image"

Acquire image "Click for Final Image"

- When the "live" image is focused and illuminated properly then "Click on Final Image" for an image that can be saved (Final Image).

- It takes about 8-9 seconds.
- This image is ready for digital storage.

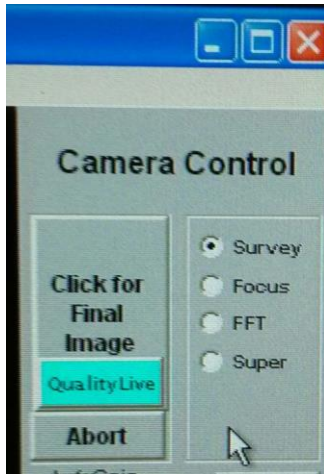


Fig 9: "click for final image"

Saving images

With AMT software, there are four image format options: 8-bit TIFF, 16-bit TIFF, lossless JPEG, (100% compress) and normal JPEG (50% compress).

There are two ways to save an image:

- Directly save a recorded image from File -> Save As. You have to give filename each time. You can define one of the four images formats in "File Type".
- Alternatively, one could utilize the button Case. As long as a Case is opened, each new file will be saved in "Case" folder with incremental index in the filename. One has an option to choose 8-bit or 16-bit TIFF as image file format. The default is 8-bit, which is sufficient for most screening purposes.

In both of the case you can enter information about the spaceman and the image (Fig. 10).

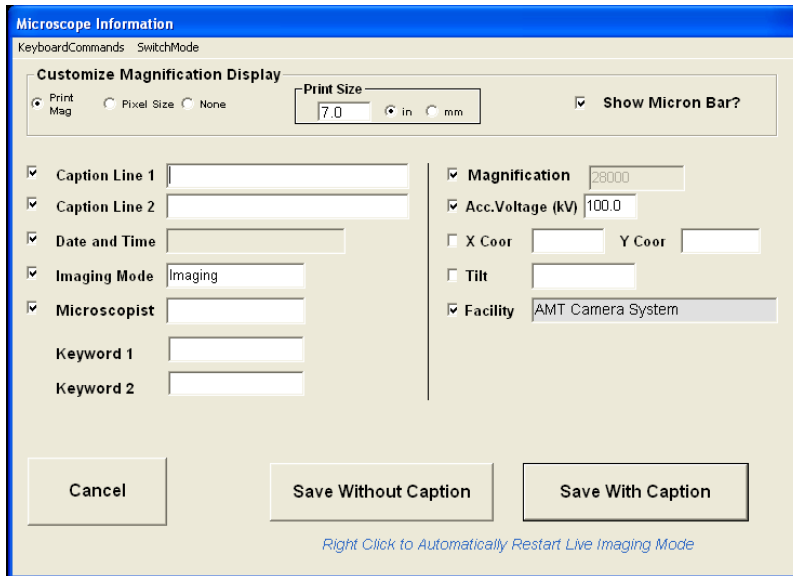


Fig10: image info.

Retract specimen holder

- Insert the holder carrier to stage with the pin into 1'clock slot position. This will trig the pumping and opening valve of specimen stage.
- Turn the holder counterclockwise to let the pin fall in 12'clock slot.
- Wait about 7 seconds until the red LED and the vacuum pump goes off.
- Turn the holder carrier fully counter-clockwise to 6'clock position until a firm stop is encountered (half turn).
- Push the carrier gently into the microscope **while holding** the push-button until it stops.
- Release the push-button and pull the carrier out as far as it will go.
- Turn the carrier clockwise until a firm stop is encountered (half turn) - the pin back to 12 o'clock position.
- Retract the holder from the stage.

Closing up

- Turn-off the heating button
- Close AMT software by selecting File - exit.
- Make sure the screen is down.
- Sign-up in the log book (time blocks)