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SEM User Guide (Hubbs Hall)

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Greg Rouse[1]

[1]ucsd

Rouse Lab

Marina McCowin
UCSD- Scripps Institution of Oceanography

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We use this protocol and it's working

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Abstract

Basics on how to use the SEM machine at Hubbs Hall at SIO.

Guidelines

Contact Greg Rouse or Martin Tresguerres with any questions.

Power On/Set-up

1 The machine will be either shut down (red button) or standby (yellow button). If red press the yellow button and wait a few minutes. Then press green button (**ON**) - this turns on the computer, and activates the vacuum pump and powers the SEM.

2 From the PC desktop, double click on the **SmartSEM user interface** icon. You will then see a user dialogue/boot-up menu pop up (EM Server) and begin initializing on the right hand screen.

- *GREEN and BLACK/BLUE is good, RED is bad.*
- *The only red item that is okay, is the stage alignment. All other red items mean you may have to reboot the software.*

3 A login window will appear: (**USERNAME**: hubbssem ; **PASSWORD**: hubbssem). The SEM software will load.

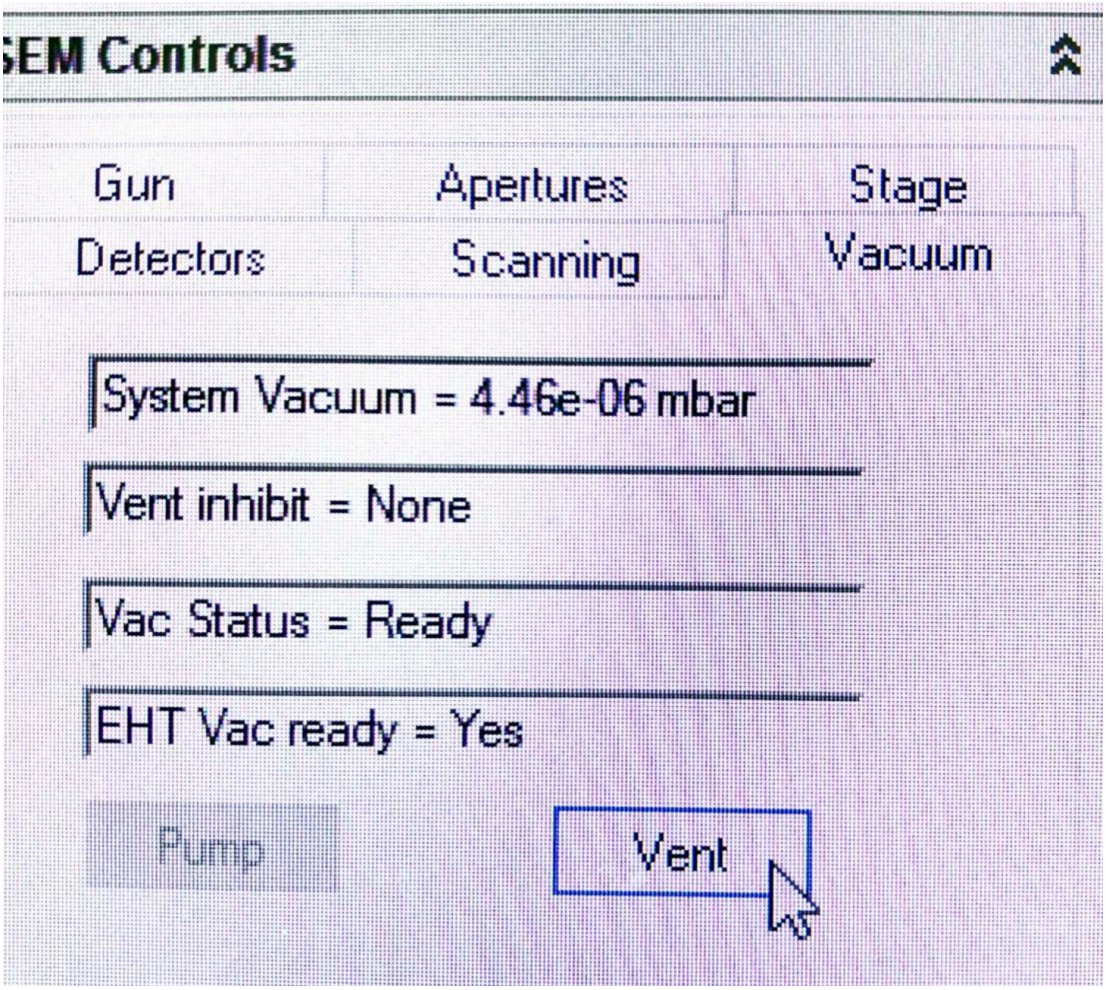
4 On the right side of the left hand screen, you will see an illustration of the stage. Check to make sure the stage is initialized (this should automatically happen as part of the boot. If it doesn't, you must manually initialize by clicking on "stage init.")

- "Initialize" allows the stage to move and calibrate the scale.

MAKE SURE TO CLICK ON THE SAFE NAVIGATION BOX*, so the stage doesn't hit the beam.* Box must be checked.

5 Turn ON nitrogen tank (**turn knob to left**).

6 Flood chamber by clicking on **vacuum**, then clicking on **vent** (upper left of screen).



7 When the vac status says, **at air**, and the chamber door pops open and you hear gas streaming, turn OFF the nitrogen tank (**turn knob to right**).

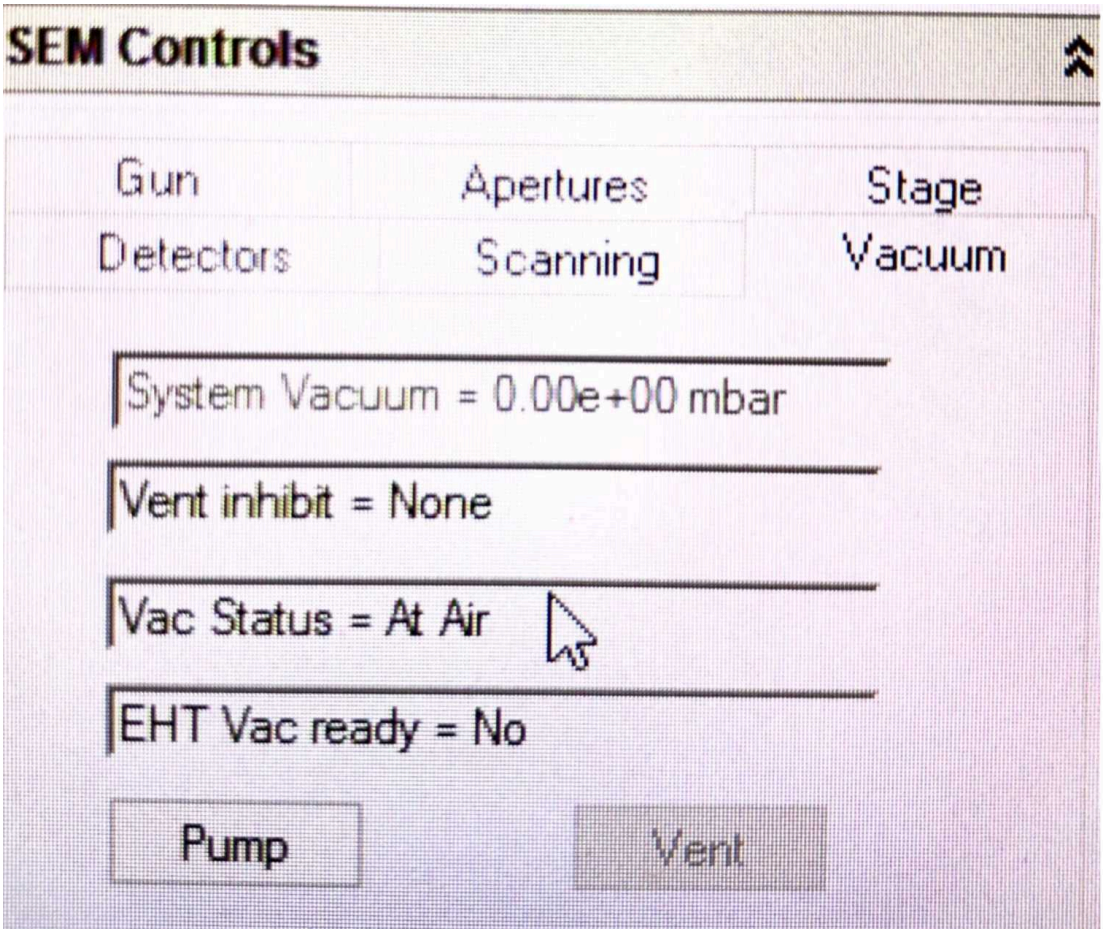


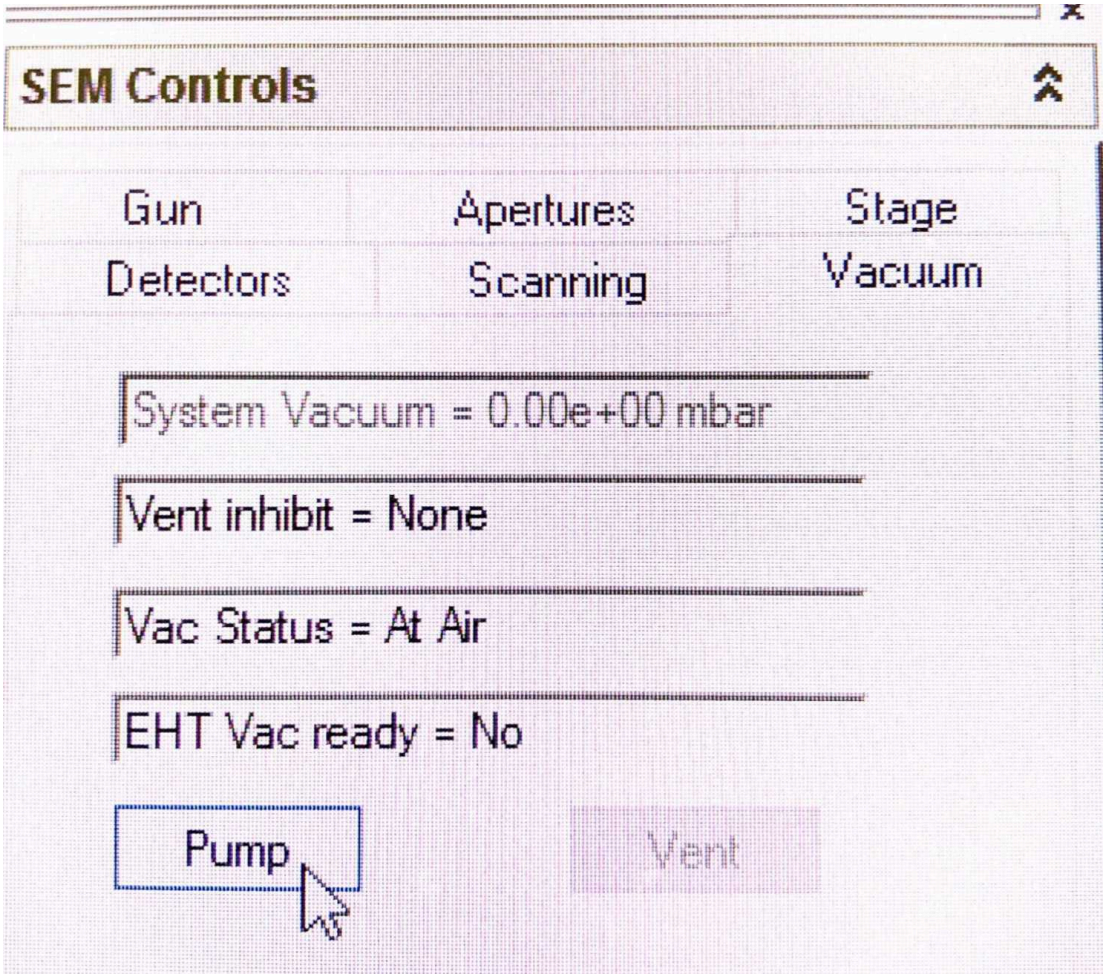
Image Specimens

8 Open the chamber, gently slide it open. Dont pull any cables..

9 Put in specimens. There are a maximum of 9 slots. They are numbered on the side of the specimen stage.

- Make a specimen map (on a piece of paper) of which number on the SEM stage holds which specimen.
- Gently close the chamber after making sure no dust is on the seal/O ring (run gloved finger around seal).

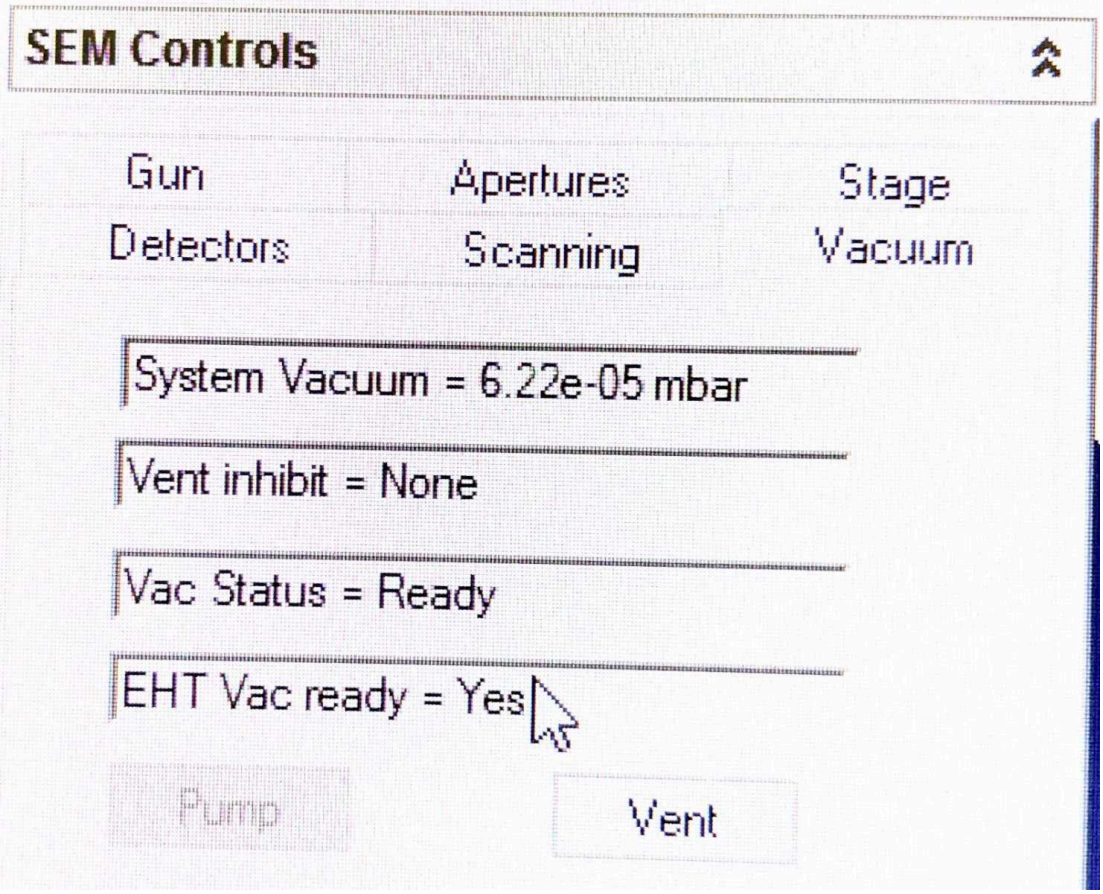
10 While holding the chamber door closed, select **pump** from the vacuum controls. Hold the chamber closed for ~10s while vacuum is created.



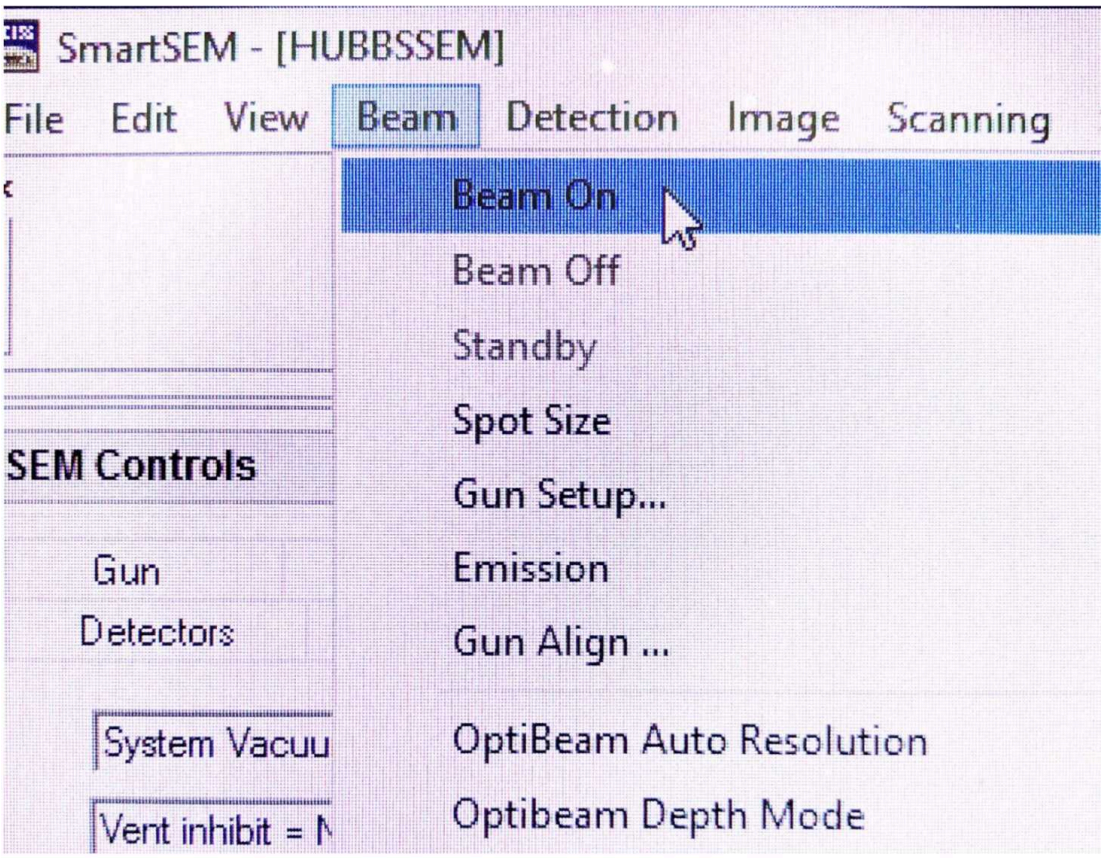
Note

You will see the pressure start to drop (unit= Torr).

11 Watch for chamber vacuum pressure to be reached (*you know the chamber pressure is reached when the **EHT Vac ready** switches from NO, to **YES**.*)



12 Turn the **BEAM ON** (equivalent to GUN ON). *You'll see the voltage increasing. It will stop at 10kV and then proceed. You normally want it to be at EHT= 20kV .*



13 Under the **GUN** tab, select the desired probe amp (*should automatically be at 100pAmp*). *Here, you can also change the kV. You don't normally need to do this.*

14 Under the **DETECTORS** tab, you can choose between the **SE detector** and **backscatter**. SE detector is the usual choice you should use for the best images.

15 Use the **Z toggle** to move the stage up towards the beam so WD is 10mm or below (under the blue line). *Again, make sure the z-limit **Safe Navigation** box is checked.*

16 Double click on the **stub number** to position the beam, then use the **X/Y toggle** to precisely position the specimen. You may need to use the **Magnification** and **Focus** toggles to locate the sample. Use **speed 1**, under the **Speeds** button.

17 Parameters to adjust for optimal imaging: (Set scan speed to **speed 4** for better adjusting).

- Adjust the **Brightness** and **Contrast** (keep brightness ~ 50%, and adjust contrast to preference).
- Adjust **Magnification**

- Adjust **Focus**. To fine tune, click the **Reduce** button, move the green box to the area of the specimen that should be in the most focus. Zoom in (using **Magnification** toggle) and re-focus.
- If things seem out of focus you can try and adjust **Aperture X&Y, especially at the beginning of a seesion. Adjust X and refocus and then Y and keep adjusting until the focus is optimised. Check again as you contine to work.**.
- Click off the Reduce button and lower the magnification and frame the image.
- If needed, press the **Scan rotate** button down and use this to position the image.

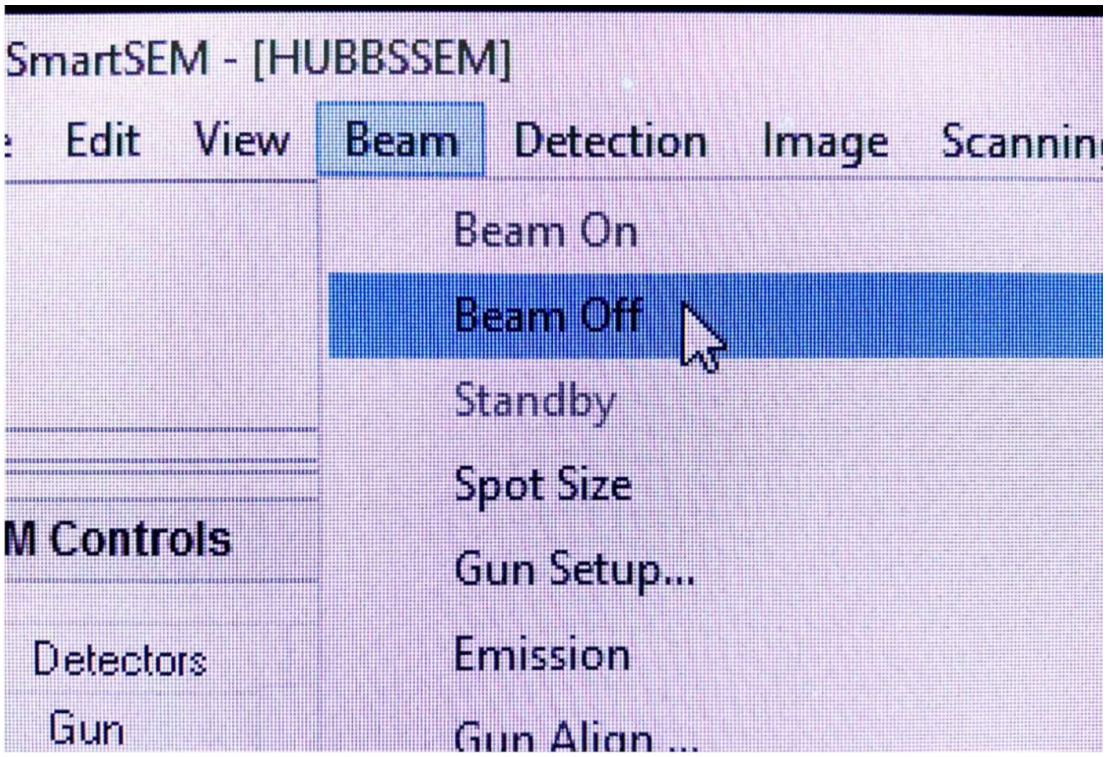
18 To take a picture:

- Set the scan speed to **speed 8**
- **Check the resolution is set to what you need.** Publication quality is 3072×2304 pixels
- Click **Freeze** button. Wait until the image is fully loaded/scanned.
- Click **File** > **Save Image**. Type in specimen information.

Shut Down

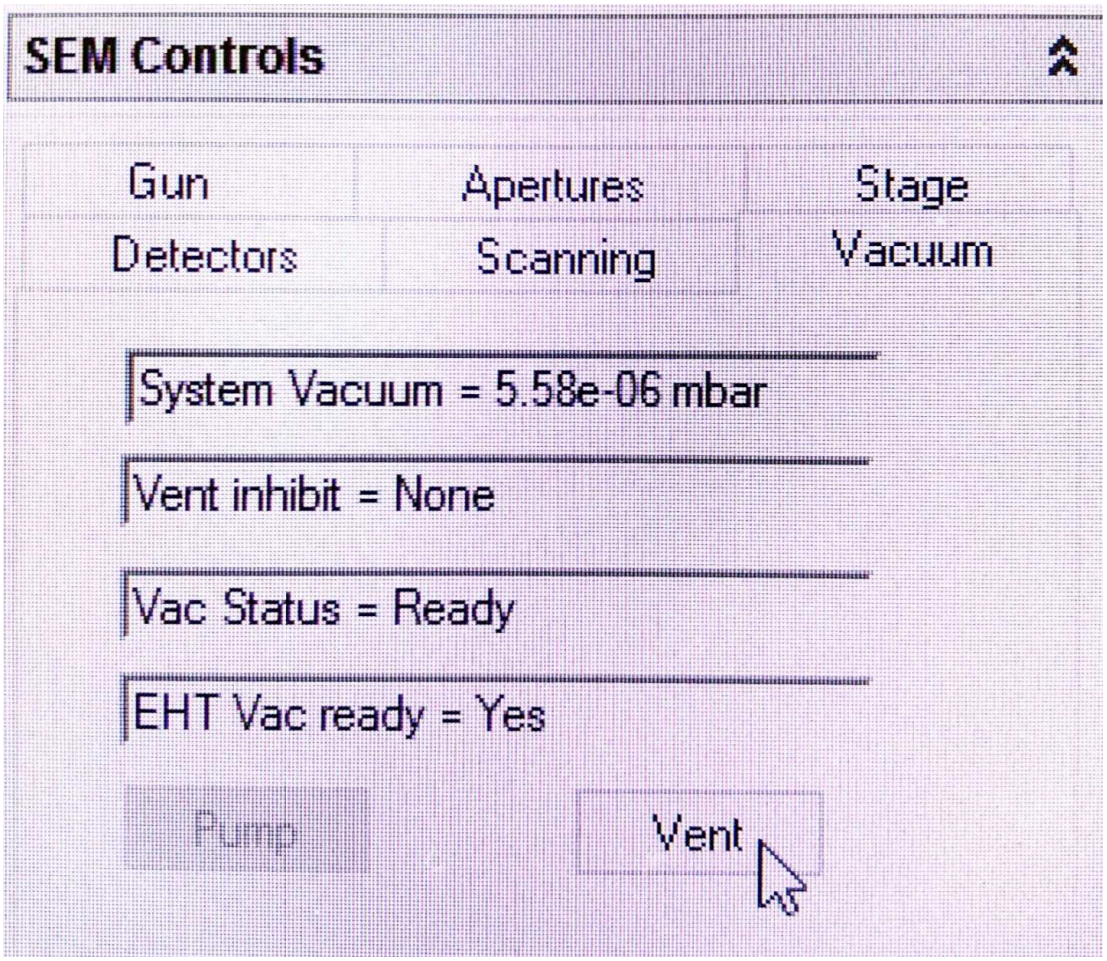
19 Lower stage completely and center it.

20 Click, **Beam**, then **Beam Off**. (*Synonymous with "Gun Off"*).



21 Turn Nitrogen on.

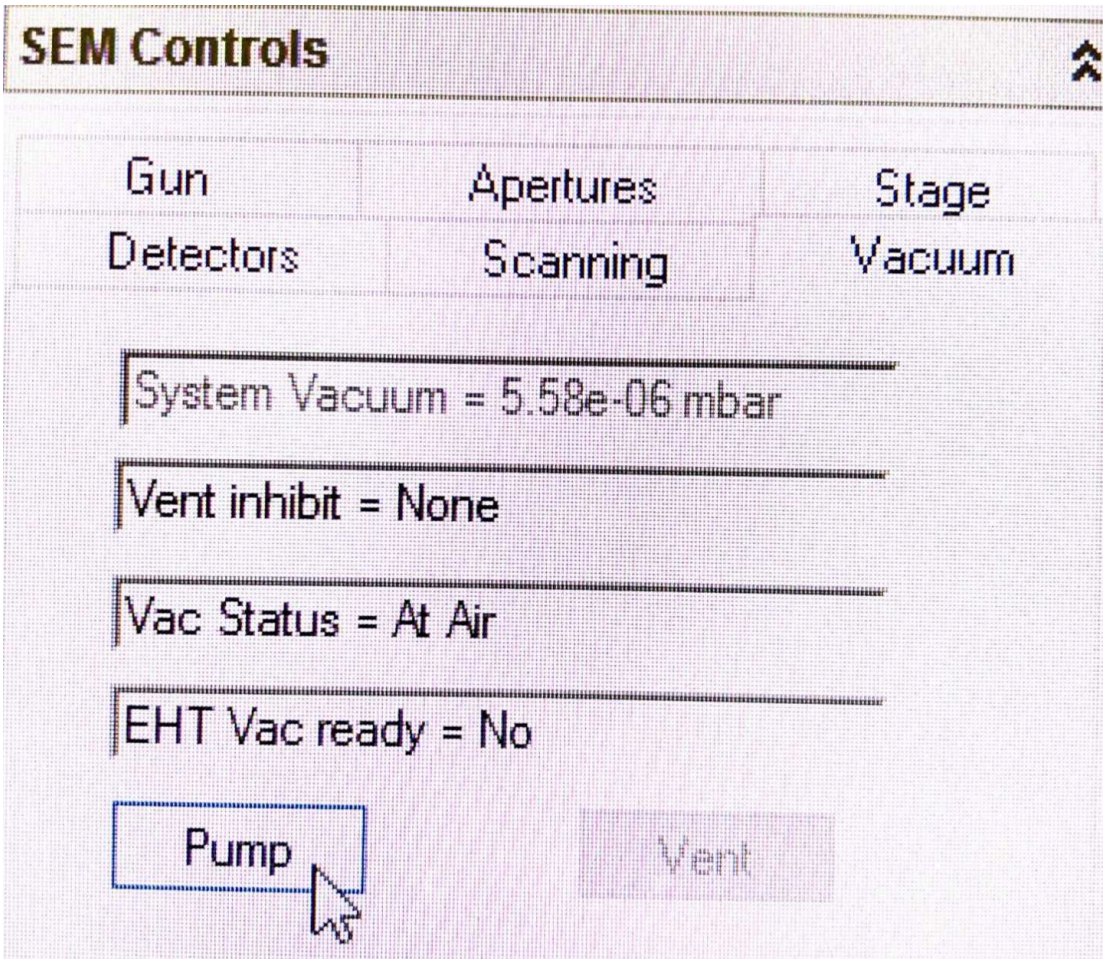
22 Click on **Vacuum**, then **Vent**. (*It may take a while until the gun is completely off for **Vent** to be clickable*).



23 Turn Nitrogen off.

24 Take specimens out (wearing gloves).

25 Gently close the chamber, click on **Pump** while continuing to hold it closed until a vacuum is created. *Wait until numbers stop moving.*



26 Click on **File in Smart SEM and** then **Exit**. **Close the EM Server** on the right screen by clicking the x upper right corner.

27 Shutdown the computer.

28 Push the yellow **standby** button. If no-one will be using the microscope for a few days or more then press the red button to turn the microscope completely off and shut down the pump

29 Sign out on the log sheet.