These are simple notes taken by Charlie Sanabria while learning how to use the Focused Ion Beam (FIB) in the Scanning Electron Microscope (SEM) at the Applied Superconductivity Center (ASC). The instructions are intended for anyone interested in viewing the FIB operation procedure through an inexperienced eye—in order to familiarize themselves with the most common obstacles of this technique. These instructions were written based on information provided by Dr. Fumitake Kametani during a standard FIB training session, and they assume the user already has general SEM proficiency.

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Description of task

The Focused Ion Beam (FIB) [1] technique uses charged particles (Gallium Ions) to remove material from a metallographic sample in a very precise manner—allowing one to cut, carve, and sculpt the surface of a microscopic sample—acting essentially as a micro drill press.

The *modus operandi* of this technique is very similar to scanning electron microscopy (SEM), in the sense that *charged particles are accelerated towards a target*. In fact, the interaction of these Gallium ions with the target is similar enough to the electrons in the SEM, that a secondary electron image can be produced as well. The main difference between SEM and FIB is the size of the particles, and because the particles in the FIB are much heavier and have much more energy, not only they can produce secondary electrons, but they can also knock atoms out from the area under the beam—a task that is much more difficult to do with an electron beam[2].

Because of their similar *charged* nature, both techniques use a "gun" to accelerate these charged particles towards the target. However, the guns have different voltage ranges and are physically bulky, so they can't overlap on the same axis. In our microscope, they are located 54° with respect to each other, and because of this axis difference, the main challenge is to calibrate the two guns so that they both hit the same exact place.

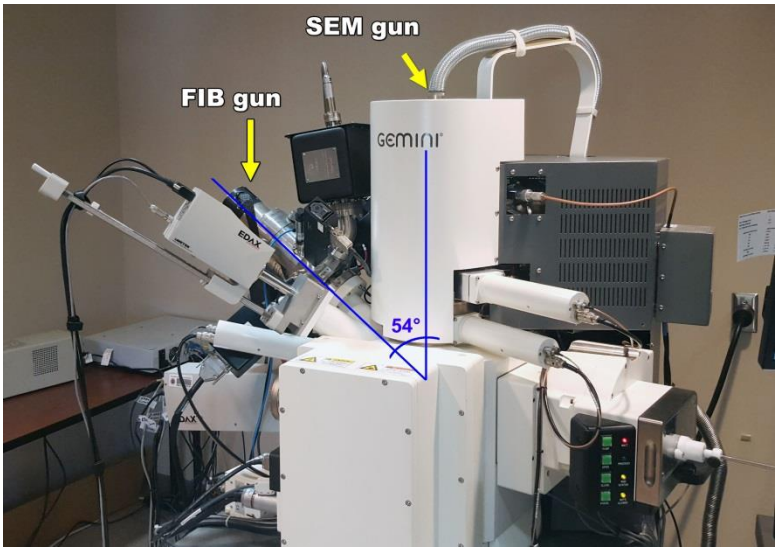

Figure 1 the SEM showing the two main guns

Sample preparation

The necessity to place the sample within the range of both guns at the same time, poses certain dimension restrictions on your sample. First of all the sample must be as flat as possible, meaning that its surface needs to be as parallel as possible to the surface of the SEM sample-holder. Additionally, this small window in which both guns can access the same spot requires the sample to be much thinner than your average metallographic puck—so your sample should be 5 mm or thinner.

Now, in order to minimize any drifting during milling[3], electrical conductance is extremely important. Make sure your sample is attached to an SEM stub using silver paint or other highly conductive media (avoid carbon tape).You may use any of the stub-holders in our SEM room, however we recommend that you use the holder with a Cu-raised center if you have a very thin sample, and that you use a regular stub-holder if your sample is reaching the upper limits of 3 or 4 mm in height.

[1] Commonly referred to by saying the word "fib", unlike SEM in which the letters are spelled out—S. E. M

[2] Gallium ions are almost two hundred thousand times more massive than electrons.

[3] This is how using the FIB is often referred to: milling.

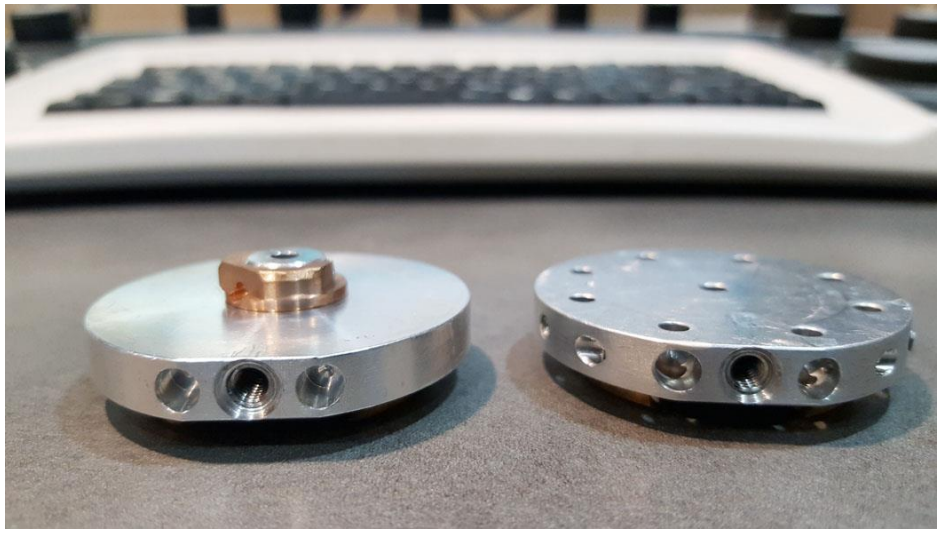

Figure 2 Two different stub holders. The one on the right is recommended if you have a sample thicker than 3 mm.

Figure 3 My first FIB samples, extracted from regular metallographic pucks and glued to SEM stubs using silver paint

Sample insertion and SEM settings

You may insert your sample and prepare the SEM for operation in the same way you would if you were just taking images with a comfortable working distance of 10 mm or larger.

One thing that we have noticed over the years is that a gun voltage of 7 kV and an aperture of 120 mm in *high current* mode are the most convenient settings for aligning the FIB and SEM guns. Also, be aware that changing the voltage or the aperture in the middle of your session may shift the beam and cause gun misalignments. Therefore, once the voltage and aperture are set, make sure you stick with them. Also make sure your magnification mode is “Polaroid545”. You can check this by going to the ‘User Preferences’ as shown in Figure 4.

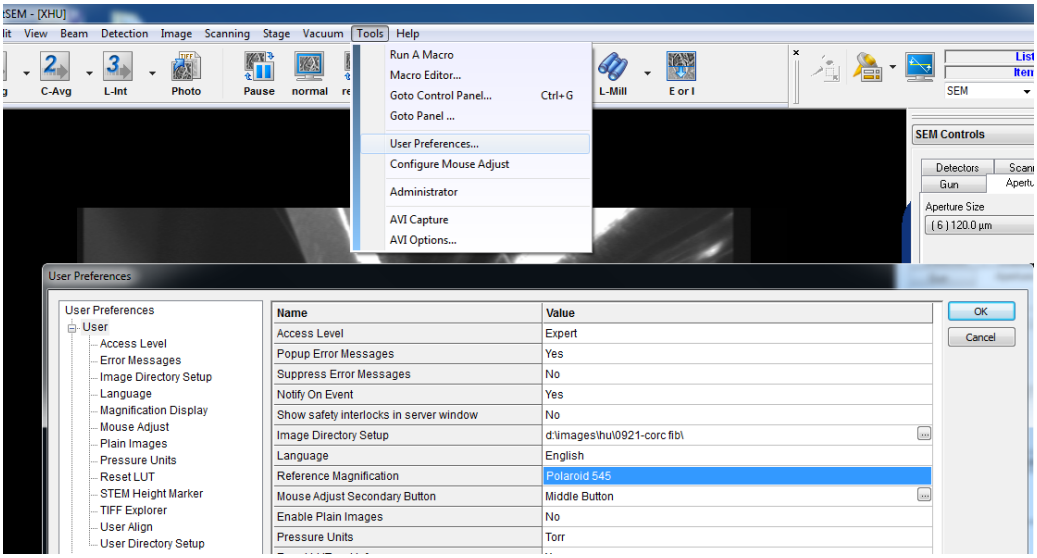

Figure 4 User preferences…
Image obtained using ZEISS SmartSEM software
www.zeiss.com/smartSEM

Eucentricity

In order to align the guns we need the sample surface to reach eucentricity, meaning there will be a point (or line) on the surface that remains unmoved when rotating the stage. Once you know where this point is, you probably want to stay close to it for the rest of the fibbing[4] operation. Let's familiarize ourselves with the stage in order to understand eucentricity.

You may have never noticed the M axis before.

Figure 5 Stage controls highlighting the M axis buttons

The M axis, just like the x and the y axis, is dependent of the stage Tilt. This means that when you tilt the stage, these axes will *tilt with it*. The z axis on the other hand, will always move the stage up regardless of the stage Tilt, as seen in Figure 6.

[4] Yes, saying the verb 'fibbing' is acceptable.

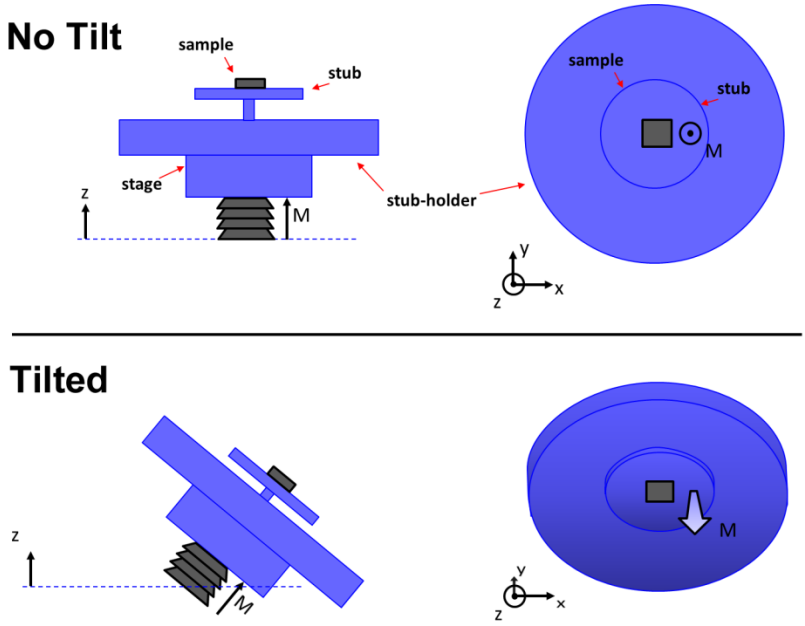

Figure 6 Side and top views of the sample holder array, and its axes at two different Tilt values.

M is always in the direction *normal* to the sample surface, and it allows you to move the eucentricity point (or line) up or down. The goal is to move it closer to the sample surface. This is done at a *safe* working distance first, and fine-tuned later closer to the electron gun.

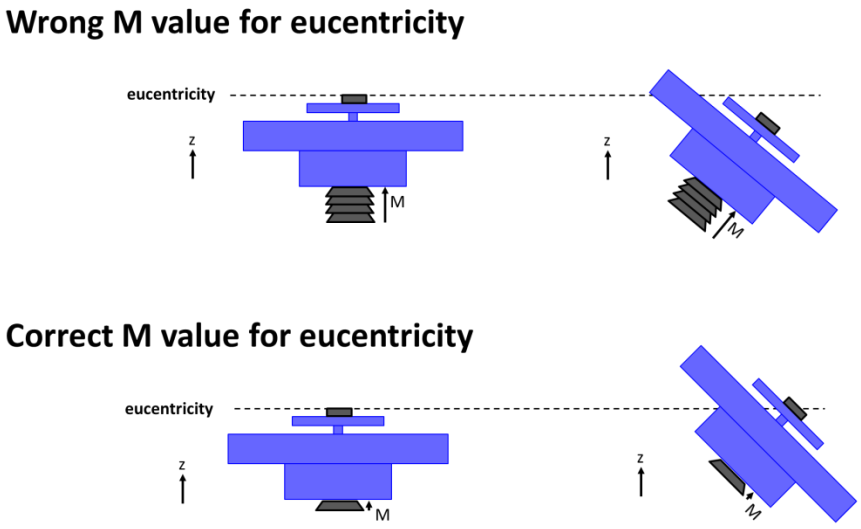

Figure 7 Side and top views of an example where eucentricity is not at the sample's surface and another one where it is

Finding the correct M for eucentricity

Eucentricity is found by tilting the sample (you may go as far as 54°) at a working distance higher than 10 mm (for safety), and at a relatively low magnification between 100x and 500x. The first step to find eucentricity would be to focus your sample, and find to a spot that is easy to see and keep it as reference (the crosshairs are essential for this[5]. Tilt the

[5] You may activate the crosshairs by going to View → Crosshairs

sample to 54° using the Stage Navigation window. Notice that your point of reference has moved away, **the idea here is to use M to find that spot again**[6].

It is very likely that the spot doesn't match exactly in the x-axis (of your screen), but the idea is to tweak M until this spot remains unmoved (in the x-axis) when switching from zero Tilt to 54° Tilt. In Figure 8, I'm showing a yellow line very close to a y-axis value of 0 on screen (if we think of the center of the screen as the origin); the goal is to get the reference spot (pointed at by the purple arrow) to remain on that line at both Zero Tilt and 54° Tilt, notice that in this image there still needs to be a little more M tweaking in order to get that spot to match horizontally in both view frames.

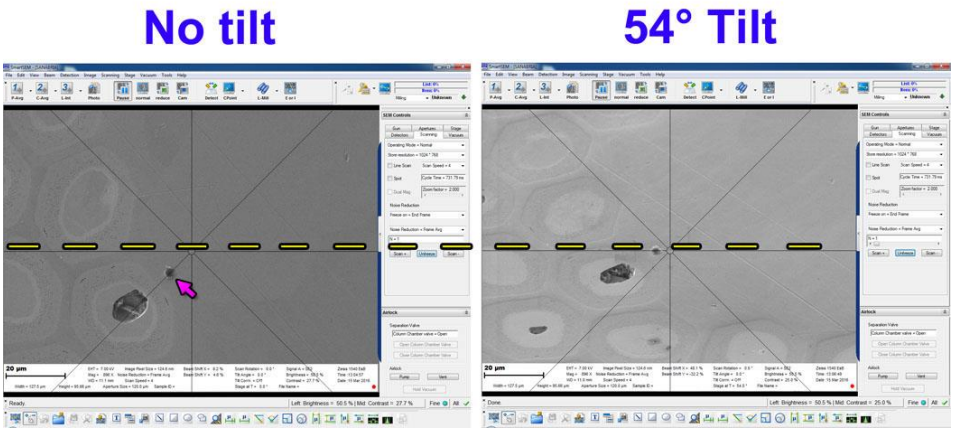

Figure 8 The two views (no tilt and tilt at 54°) showing eucentricity that could still be improved
Image obtained using ZEISS SmartSEM software
www.zeiss.com/smartSEM

You may have to go back to a lower magnification or go to a lower Tilt value if the reference spot is hard to find. Keep in mind that the effects of changing M are smaller as the Tilt approaches zero. You may also have to repeat these steps at least once more, because very often you will find that going back to Zero Tilt has moved the reference point. Notice that x, y and z still remain unchanged, you have only altered M.

Once the eucentricity is found at this low magnification you may set the stage closer to the electron gun. The easiest way to do this is to go back to Zero Tilt and set the working distance (on the screen) to 5.11 mm. Then, move the stage in the z direction until the image is focused. Make sure you uncheck track-z, and that you are aware of how close the sample is to the gun!

After focusing (using z), the sample surface should be 5.11 mm from the gun.

Fine-tuning eucentricity

Now that you are at a working distance of 5.11 mm[7] you may repeat the same steps but at a higher magnification (1500X should do). The steps are:

1. Find a reference spot at zero Tilt.
2. Tilt the stage to 54°.
3. Tweak M to align the reference point to the screen's y-axis.
4. Repeat.

Figure 9 shows examples of good eucentricity.

[6] At this point the z should not be adjusted.

[7] You may have to check your stigmation and apertures again

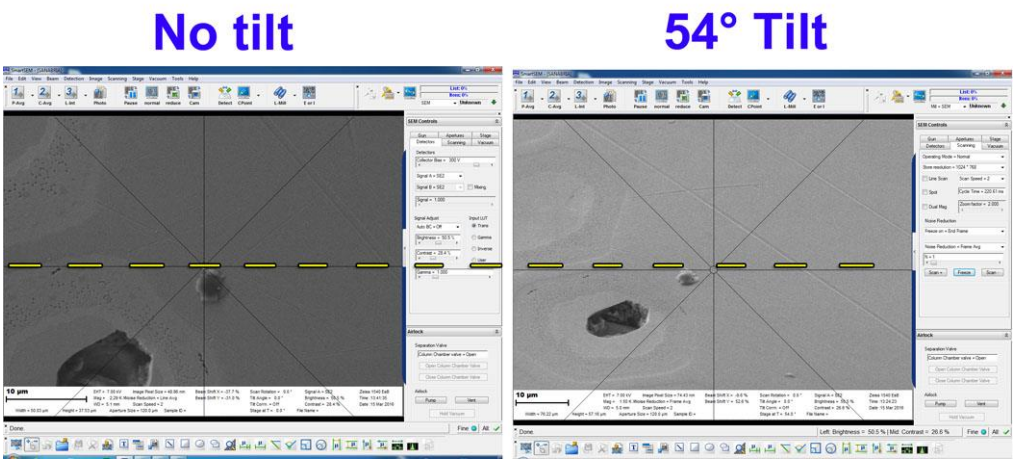

Figure 9 Good eucentricity
Image obtained using ZEISS SmartSEM software
www.zeiss.com/smartSEM

If at the end of this, the working distance is not 5.11 mm you may have to adjust z and re-adjust M again. Once M is aligned it shouldn't be messed with anymore.

The FIB Control panel

You can bring up the FIB Control panel by clicking on the button highlighted in Figure 10.

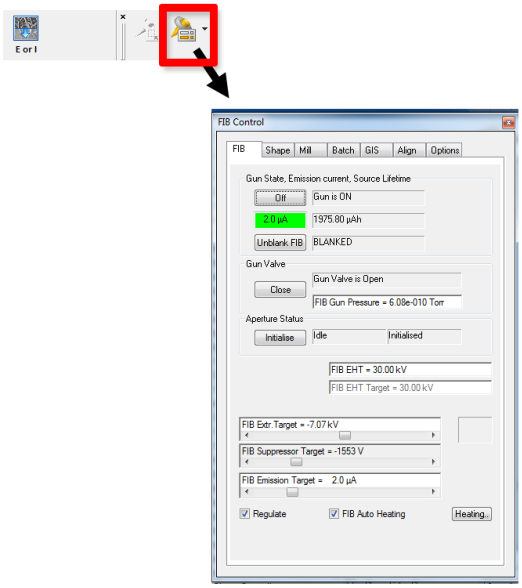

Figure 10 FIB button and Panel
Image obtained using ZEISS SmartSEM software
www.zeiss.com/smartSEM

Notice that on the FIB tab of this panel, you can turn the FIB on and off. Use this button to turn it ON, and wait for it to initialize (this may take up to a minute). The current indicator will change from red to green (currently green in Figure 10). Once green, it is ready to be used[8].

[8] The aperture should initialize automatically, but there was one time where I had to do it manually by clicking the Initialize button under Aperture Status (this might not always be the case).

A button that you will use often is the E or I button (also shown in Figure 10), this activates either the image produced by the SEM, or the one produced by the FIB (*i.e.* the image from the electron gun, "*E*", and the image from the ion gun, "*I*").

If you don't have these buttons in your toolbar you can pull them up if you go to View → Toolbar Views and check the box that says FIB Toolbar shown in Figure 11.

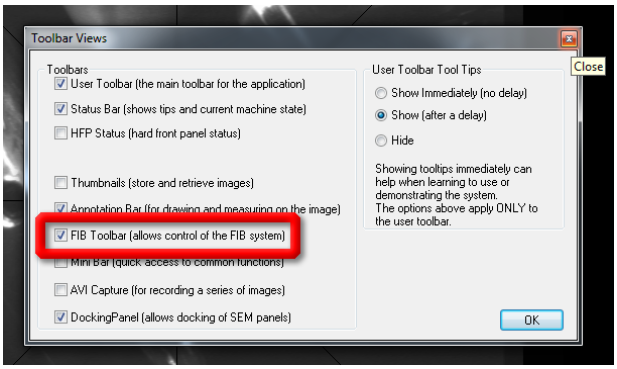

Figure 11 Toolbar views
Image obtained using ZEISS SmartSEM software
www.zeiss.com/smartSEM

Now we have to set up the current we want to use as the default FIB image or *"I"* image. Go to the Mill tab (Figure 12), and make sure you have an imaging current of 50 pA. This is a low current, which is good for imaging, but it will still eat through your sample if you leave it running for too long or stay at high magnification (more than 2000x). Therefore try pausing the "*I*" whenever you are not actively using it. The pause button is seen on the toolbar of Figure 9. No need to pause anything yet, we still have not activated the FIB, we have only turned it on and have set the default imaging current.

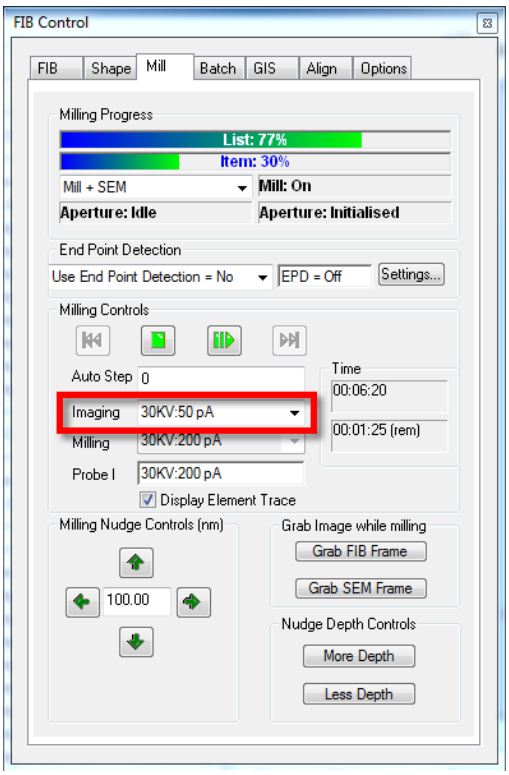

Figure 12 Mill tab
Image obtained using ZEISS SmartSEM software
www.zeiss.com/smartSEM

Finding the coincident point

We talked about eucentricity above, which is a stage-related property. But even when you have a perfect eucentricity, there is still a high chance that the guns are not pointing at the same place—and we need to adjust for this. This is called the coincident point, and it is very important in order to have a match between our "*E"* and your "*I*" images.

With the stage still tilted at 54°, activate the "*I*" image by pressing the E or I button[9], set the magnification to match the one you currently have in the SEM image, and roughly focus the image[10]. Notice that the focus, the magnification and the stigmation knobs used for the SEM work also for the FIB[11]. The brightness and contrast knobs work here as well, which could be the reason why your "*I*" image is black[12]. **Always use a scan speed of 5 when doing any kind of FIB operation**.

Hopefully your reference point is nearby and you can see it (you may have to lower the magnification if you don't). If you want to use a different point of reference, you may go back to "*E*" and use x and y stage translate (or the usual Ctrl+Tab followed by a click on the screen to find a new spot), but don't move too far from the eucentric line or your alignment is going to be affected. Let me repeat that (because I had a hard time realizing this): It is OK to adjust the stage x and y at this point if you go back to the "*E*" mode.

Ok, once you find a proper reference point using the x and y, go back to "*I*" mode, and the goal is to use the z **axis** (not the x and y anymore) to move the view frame so that the reference spot matches its place in the "*E*" view frame. You will most likely need very small increments of z, in order for your eucentricity and focus to not be affected as much, but if you see that your working distance is diverging too much from 5.11 mm you may want to re-adjust your eucentricity.

In the same way as before, it is very likely that these two points don't match perfectly in the x-axis of the screen—similar to the situation in Figure 9—except that this time with "*E*" vs "*I*" images instead of Zero Tilt vs 54° Tilt in "*E*". You can see an example of the reference point not matching the x-axis of the screen in Figure 13.

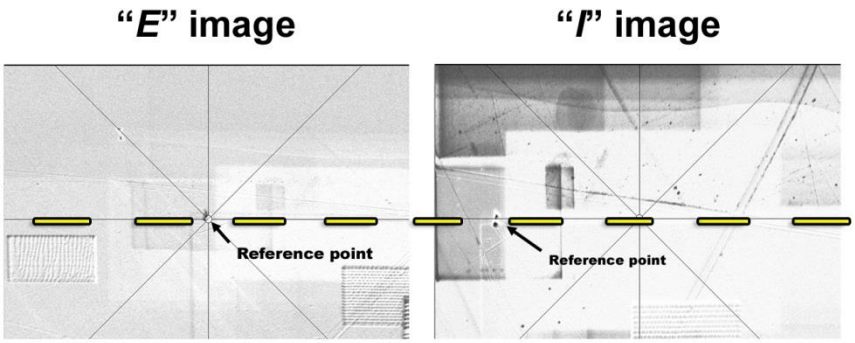

Figure 13 The "*E*" and "*I*" images showing the reference points aligning properly in the y-axis of the screen.

Now, to match the spots perfectly (in both the x and the y of the screen) you will have to use the Beam Shift knobs (seen in Figure 14) while on "*E*". The effects of this adjustment can be seen in Figure 15.

[9] Pressing this will activate the FIB. Here is where you want to be aware of situations in which you are not actively using it and press the pause button, or else your sample will be eaten away.

[10] Start with something low, between 100X and 500X.

[11] The Aperture X, Aperture Y and the Wobble don't work with the FIB gun. You could perform wobbling using the Align tab **Error! Reference source ot found.**. But even then, wobbling is not recommended for FIB apertures.

[12] There was one time when I couldn't focus for the life of me. All I could see was blurry blobs, and the stigmation and focus were not fixing the problem. It had to do with the probe not going all the way in. I had to switch probes for a second and it was fine (more on changing probes later).

Figure 14 The Beam Shift knobs work for both "*E*" and "*I*" images, but adjusting the *"E"* only is safer. Use these to fine tune your match between the two.

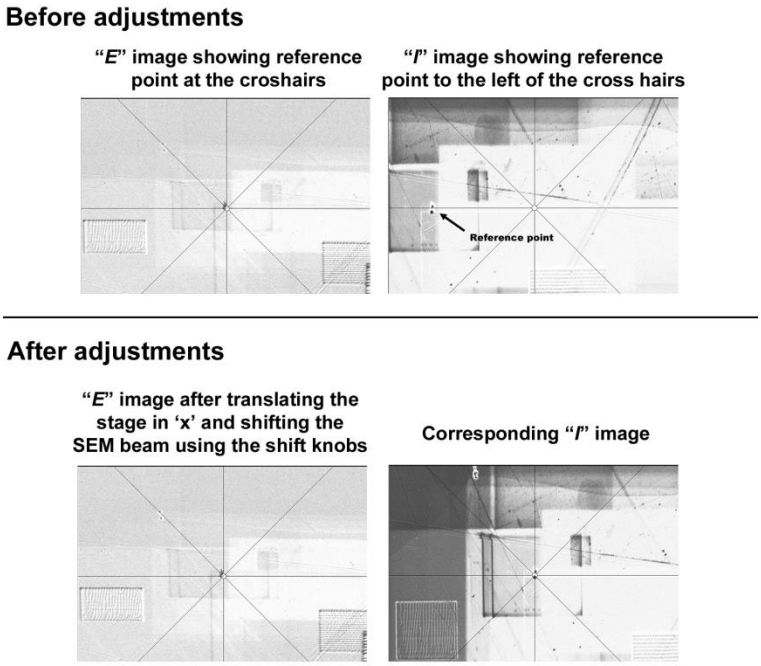

Figure 15 Images before and after using the Beam Shift knobs while in *"E"*.

After all of this alignment, you may freely navigate around your sample using x and y or pressing Ctrl+Tab and clicking on a specific point on the screen. Keep in mind that the "*E*" gun may go out of focus if you move in the y direction, but just focus it—there is no issue with this as long as you don't move too far. Don't forget: if you move too far from your reference point the guns may diverge. Stay close.

Adjusting the FIB

Most of the settings in the FIB tab should be left as they are, with a voltage of 30kV. Figure 16 below shows the settings as they were the first time I was using it.

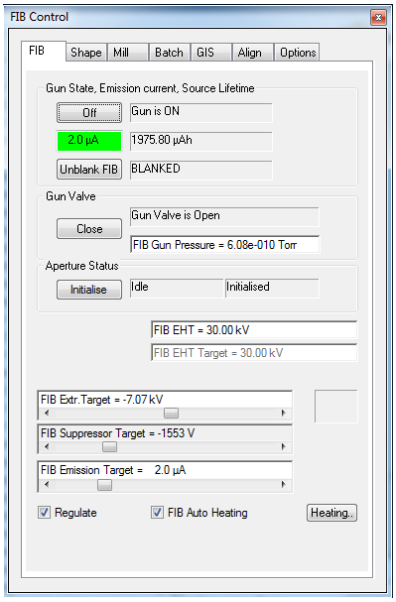

Figure 16 FIB tab
Image obtained using ZEISS SmartSEM software
www.zeiss.com/smartSEM

Oh, let me mention one thing that seems to happen sometimes and that could affect the system vacuum significantly. Go to the GIS tab[13] and **make sure all the valves are closed** (*i.e.* all boxes unchecked). Sometimes a mysterious macro opens them. Keep checking them once in a while (more on the GIS later).

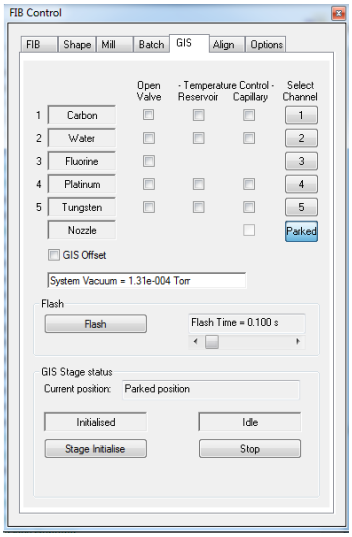

Figure 17 GIS tab with all boxes unchecked
Image obtained using ZEISS SmartSEM software
www.zeiss.com/smartSEM

Also, a word of advice when using the FIB: **Be patient** when changing probes and when ending or starting a milling process. You must allow the macros to run completely before you start a new one—you could end up with non-responsive commands[14].

[13] This stands for Gas Injection System. I would advise referring to this by spelling out the three letters G.I.S, instead of saying the word "gis" (especially if you tend to mispronounce the G in GIF).

[14] The E or I button can suddenly stop working if a macro is not terminated properly. I had this problem happen several times because of my impatience. I had to log out, close the EM server and log back in for it to work again.

Focusing the current probes

Ok, at this point you should be in "*I*" mode, seeing an image produced with the 50 pA probe. There are many probes (see Figure 18 for all the probes available), however, you will most likely only need 50, 200, 500, and 2 nA[15].

So, with the image you have in front of you (50 pA) try getting a sharp and well-focused "*I"* image using the same techniques as SEM, namely focus, stigmation adjust, etc[16]. But make sure you are **always using a scan speed of 5** because changing the scan speed can cause the image to shift. Also, **use InLens mode**, it might be easier.

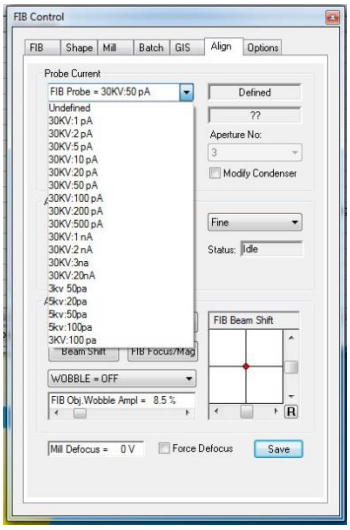

Figure 18 Different current modes available under the Align tab.
Image obtained using ZEISS SmartSEM software
www.zeiss.com/smartSEM

Once focused at 50 pA, go to Align tab (Figure 18), and click Save. This will save the focusing settings for the 50 pA current. Our goal is to align all the probes, therefore the steps of focusing and saving, will be repeated for 2 nA, 500 pA and 200 pA[17]. To change the probes just click on any of the available probes in the pulldown menu of Figure 18. Be patient when switching probes, they take a few seconds to swap. The steps are:

1. Select a probe
2. Focus and fix stigmation
3. Pause (to prevent your sample from eating away)
4. Click Save in the Align tab
5. Go to next probe and repeat

The higher the current, the coarser the milling will be, for example, the 2 nA probe is often used for digging trenches when you are preparing a lamellae. The 500 pA (which is still fairly coarse), perhaps can be used for making sharper trench boundaries, while 200 pA is often used for finer cuts. The 50 pA is used for very fine milling and/or imaging. And, as you will see later the 10 pA probe is used for the GIS deposition.

Aligning probes

Now that all probes are focused, the next step is to align then all to **match the imaging probe, so they all produce the same shape in the same position**. This is done using the Beam Shift Cor button in the Align tab. To perform the

[15] Don't use 1 nA it seems to be slightly off.

[16] As I mentioned before do not center the aperture by wobbling.

[17] The pause button comes in handy here while you are not focusing

alignment, you will be milling your first shape. To make a shape you can use the button next to the button that brings the FIB Control panel (see Figure 19). Select a shape and draw it on the screen (click and drag).

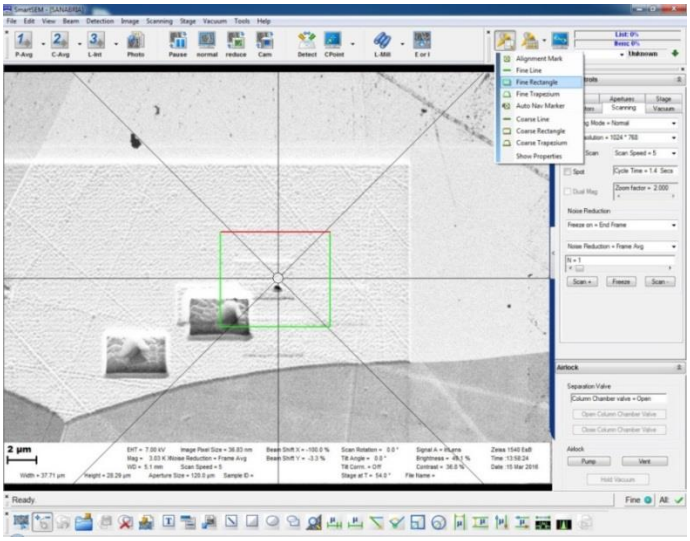

Figure 19 Drawing a shape
Image obtained using ZEISS SmartSEM software
www.zeiss.com/smartSEM

Try drawing a "Fine Rectangle" (I'm not sure what the "coarse" shapes do). Then go to the Shape tab (see Figure 20) and adjust its dimensions to make it 5 by 5 with an origin at 0[18]. For some reason it often can't go to whole numbers, don't worry about it.

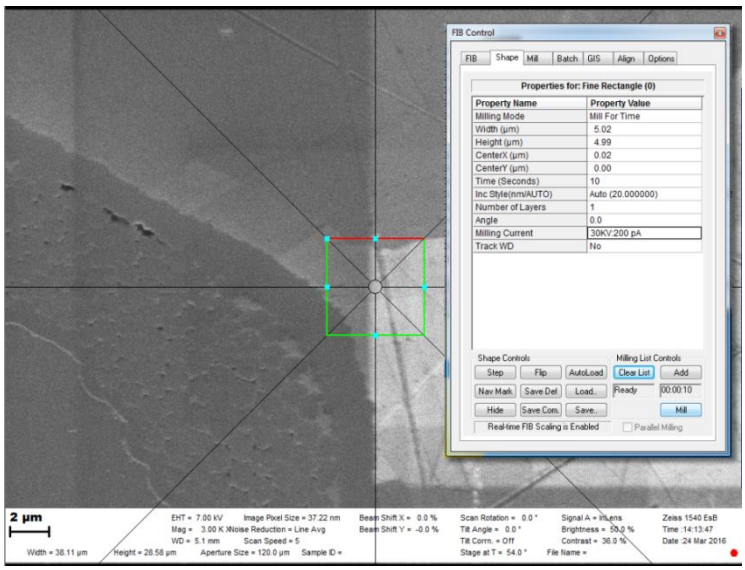

Figure 20 Shape parameters of the electronic shape drawn on screen
Image obtained using ZEISS SmartSEM software
www.zeiss.com/smartSEM

Make sure you select Mill For Time instead of Mill For Depth and mill for about 10 seconds. Let's start with the medium-low milling current of 200 pA (Figure 20). Push Clear List, Add and Mill[19]. While the milling happens, the

[18] You may often find that the table in the Shape tab is grayed out, it may be because you are not actively selecting a shape. Click on the shape and it should activate.

[19] Do this every time you are about to mill a shape

microscope will momentarily go to "*E"* so you can observe the milling with the SEM gun, but this time around you may not see it since it is such a short milling time. When done, it should go back to "*I"* but for some reason it doesn't work for mine—I'm sure that could be changed but I'm ok with it.[20].

Once you return to "*I"*, what you are seeing is the image produced by the 50 pA probe (not the probe you just milled with). Puse the image where it is.

Notice how the milled square is slightly off (*i.e.* the milled square is not in the same position as the square you drew on the screen, Figure 21).

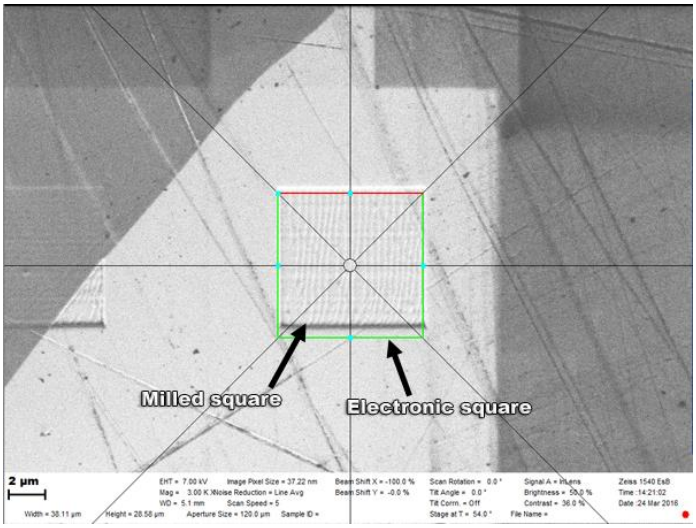

Figure 21 A square milled by the 200 pA probe showing a slight misalignment with the imaging probe (50 pA).

So, what we need to do here is move the electronic square (shape on the screen) by clicking and dragging it, so that it matches the milled square, as it was done in Figure 22—this is still at 50 pA, and can be done when the image is paused.

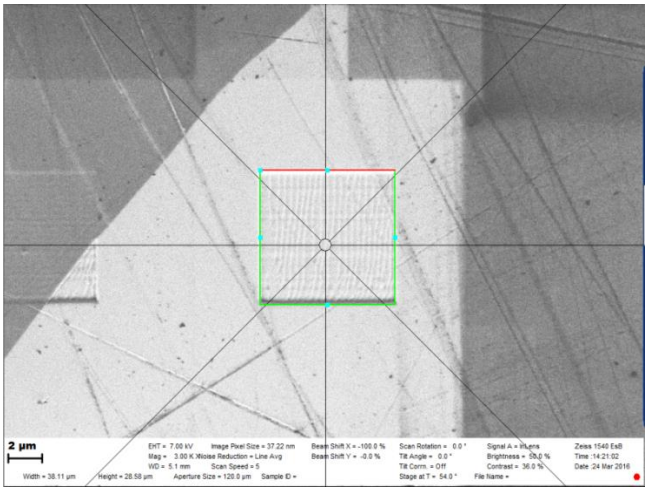

Figure 22 Matching electronic square with milled square as seen by the 50 pA probe

Then, switch to the current you just used (200 pA in this first case), and once the image is formed with this milling current, you will notice that the milled square and the drawn square are off again (naturally, since you just moved the electronic one), as seen in Figure 23.

[20] I actually like that it doesn't go back. I wouldn't want to be distracted and then have it go back to "*I*" and start eating away my sample.

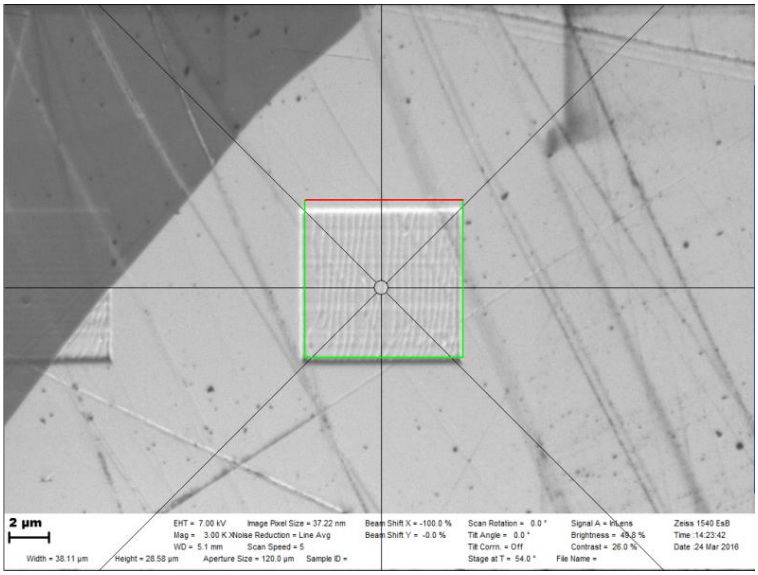

Figure 23 200 pA probe image showing a slight misalignment with the electronic square.

This time around you have to move the *milled square*, instead of the electronic one. To do this, use the arrows in the FIB Abs Beam Shift sub-window in the Align tab. Once they are aligned press the Beam Shift Corr button. And DON'T FORGET to click Save again (Figure 24)…You have aligned it!

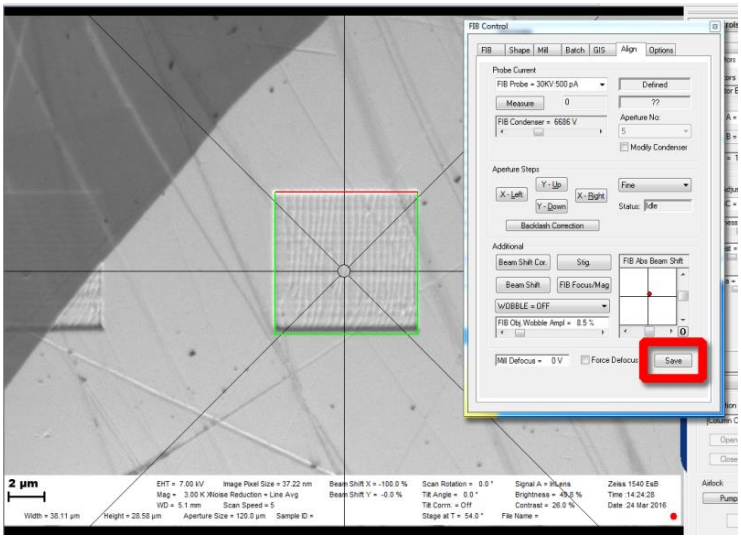

Figure 24 matching squares. Notice that the circle in the FIB Abs Beam Shift sub-window is no longer cantered. Don't forget to save!
Image obtained using ZEISS SmartSEM software
www.zeiss.com/smartSEM

Repeat these steps for all currents, using a new square every time[21]. Also, you better be quick, or your sample surface will whiter away! The steps are the following:

1. Draw a square, center, and adjust size using Shape tab.
2. Select all milling parameters (but with the next current value and perhaps a bigger square)
3. Press Clear List, Add and Mill.
4. Go back to 50 pA.

[21] At higher currents you may want a larger square since the edges tend to round off

5. Pause once the image forms and is stable.
6. Check the location of the electronic square with respect to the milled square.
7. Move the electronic square (click and drag) so that it matches the milled square.
8. Switch the probe to the one you just milled with (under Align tab)… be patient when switching probes.
9. Check the location of the milled square with respect to the electronic square, you shouldn't pause this time because you are about to move the beam.
10. Use the FIB Abs Beam Shift sub-window in the Align tab to match the milled square to the new position of the electronic square.
11. Press the Beam Shift Corr button.
12. Press Save.
13. Go to next probe and repeat.

Once this alignment is done for all the probes, you can remove the rectangle by selecting it and pressing Delete on the keyboard. Alignment is done and you are ready to perform FIB operations!

Making a lamella

FIB can be used for many things. Among these we have sample sectioning, nanofabrication, micro-lithography, Transmission Electron Microscopy (TEM) sample preparation, and much [much!] more. I am particularly interested in the TEM sample preparation since I want to identify the phases present in my samples.

A little background on my sample

Figure 25 shows that between the Nb filaments of these partially reacted Nb_3Sn wires, there are certain phases that we are not sure what they are. The problem is that they are too thin to be identified with the Energy Dispersive Spectroscopy (EDS) of our SEM. Therefore I need to prepare a thin lamella to analyze it using the EDS of the TEM at the MagLab (which is able to identify such small traces).

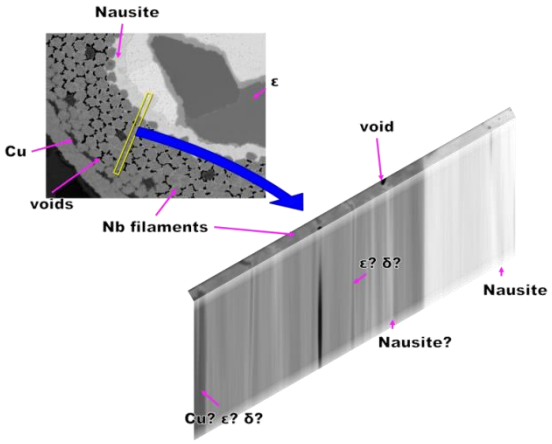

Figure 25 A transverse cross section of the area of interest with fake lamella made by manipulating the image.

Summarizing what we are about to do

Let me summarize the steps so we know *what* we are doing, before we jump into *how* we do it. What I want to do is use the FIB to dig two trapezoidal trenches with a thin piece of material between them. This piece then can be pulled out and welded to a TEM sample holder (or grid). Once on the grid, this thin lamella has to be shaven down even more until you get something that is electron transparent (less than 100 nm thick). Figure 26 shows the steps in a visual manner.

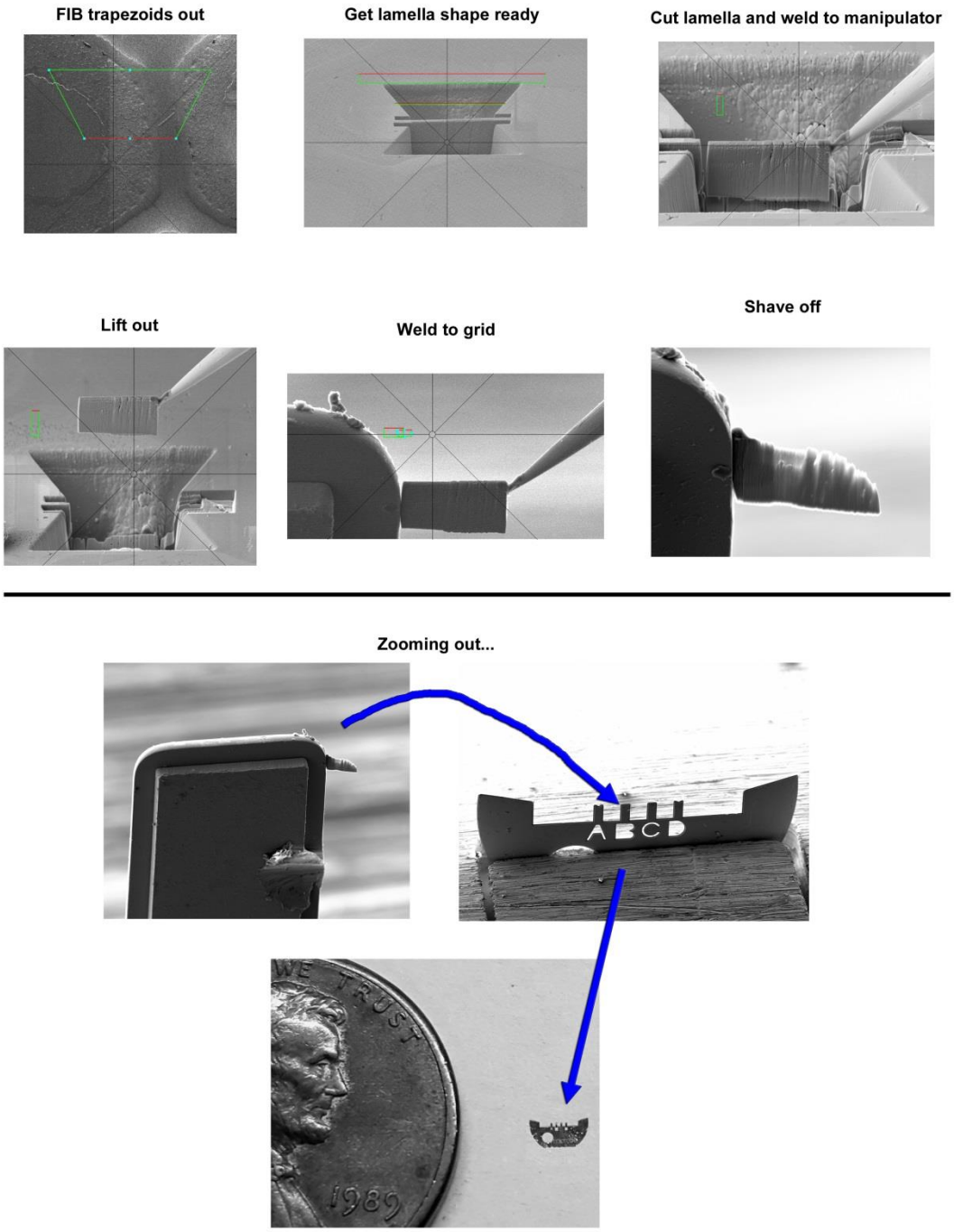

Figure 26 TEM sample preparation summarized.

We will FIB out the shapes listed in Table 1 (also shown in Figure 27):

Table 1 The different cuts needed to shape the lamella before picking up

Cut name	Shape used	Milling mode	Current	Stage Tilt
Top trench	Trapezoid	Mill for depth	2 nA	54°
Bottom trench	Trapezoid	Mill for depth	2 nA	54°
Top detail	Rectangle	Mill for depth	500 pA	52°
Bottom detail	Rectangle	Mill for depth	500 pA	56°
Right cut	Rectangle	Deposition mode	200 pA	19°

Left cut	Rectangle	Deposition mode	200 pA	19°
Bottom cut	Rectangle	Deposition mode	200 pA	19°
Needle spacer	Rectangle	Mill for depth	200 pA	54°

Notice that in Table 1 there are different shapes used, different milling modes, and different stage tilt values as well as currents. Below we will talk about the reasons behind these settings. Figure 27 shows all the cuts as seen with the "*E*" image mode.

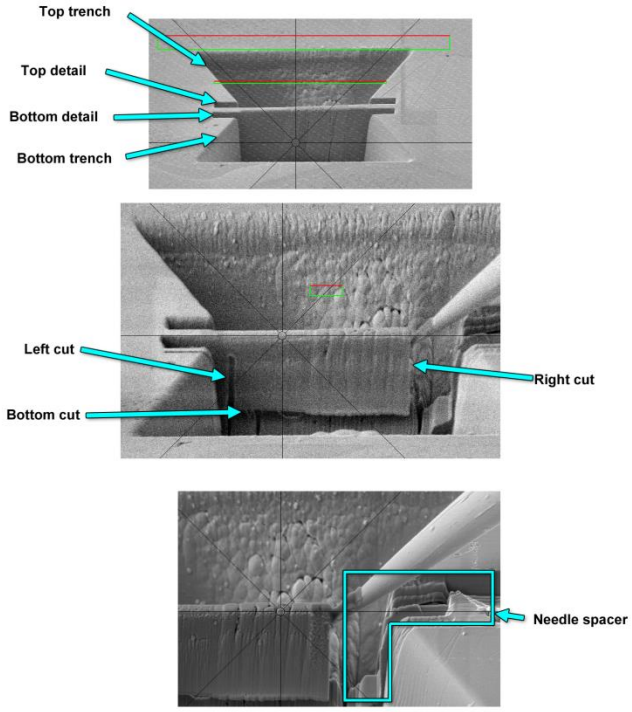

Figure 27 The different cuts made viewed by the "*E*" image mode.

This was my first lamella and probably doesn't look like a very efficient milling. I wasted a lot of time and space, but I guess is good enough for the teaching purpose of this document. Another thing that comes with experience is being able to judge the size of the lamella—if we think of my lamella as a cuboid (or a box), I was aiming for box with the following dimensions 15 µm×6 µm×1 µm. But as you will see my milled shapes are exceeding those measurements significantly because you tend to lose a lot of material at every cut.

Digging the first trench

Note: There is one step I'm skipping here which I will introduce later for simplicity. The step consists on making a protective layer on top of the area where the lamella will come out of (before digging the trenches) using the Gas Injection System. I just don't want to introduce a whole new system before we are fully familiarized with the FIB.

Ok, start by inserting a trapezoid, and making it an isosceles trapezoid where the smaller base (Width in Figure 28) is a few microns bigger than your target lamella length (in my case I made it 20 µm and the lamella ended up being 15 µm long).

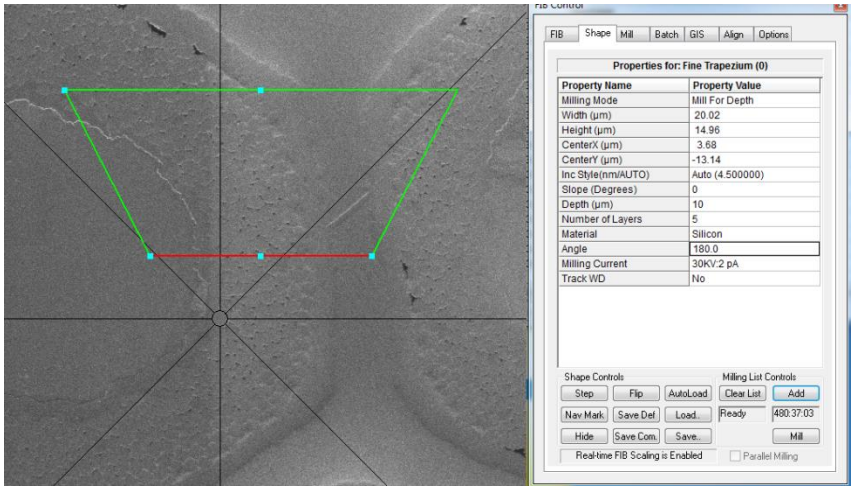

Figure 28 shape parameters
Image obtained using ZEISS SmartSEM software
www.zeiss.com/smartSEM

These are the trench settings of Figure 28:

1. Use "milling for depth" instead of time.
2. Adjust the shape parameters (by dragging the shape points with your mouse) so it looks similar to the trapezoid in Figure 28
3. Set the depth. In my case I'm doing 10 µm despite my lamella being about 6 µm tall in the end.
4. The deeper you mill the more layers you want[22], I used 5.
5. You can tell the software what kind of material you have, and it will calculate how long the fibbing will be depending on the depth. In most cases you want to use silicon, since it's a good reference material (but that's all it is, a reference, you can use whatever you want).
6. You definitely want coarse milling (2 nA) for such a big shape.

Once you have checked all parameters push Clear List, Add and Mill. It will tell you how long it will take. In my case this took about 30 minutes.

Smoothing out the trench

If you think of fibbing as digging a hole on the ground, you may end up with a hole that is larger at the top (the surface), and much narrower at the bottom—and if you are digging two holes next to each other to create a lamella, you might end up with a lamella that is thicker at the bottom (see Figure 29).

[22] The layers are the number of passes, just like when you use and end mill to cut through a piece of metal. In my case I wanted to dig 10 µm deep trenches and therefore I used 5 layers. If you do fewer layers you may get re-deposited material. Just like digging a hole on the ground, the deeper you go the more chances you have of it collapsing on you, therefore you have to be more careful as you go deeper (using more layers).

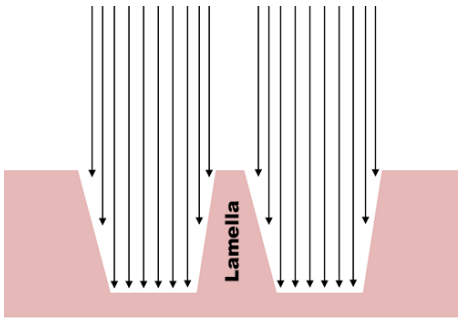

Figure 29 Lamella made at a fixed tilt angle.

To account for this effect, you may want to tilt the stage so that the FIB comes in at an angle as shown in Figure 30. This is done by tilting the stage 2° more for the top part of the lamella and 2° less for the bottom (meaning, 56° for the top and 52° for the bottom).

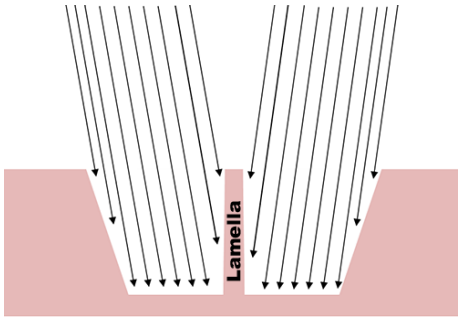

Figure 30 Lamella made by adjusting the tilt angles of the detail cuts.

These two cuts are done using rectangles, and they should be about 10 µm wider (5 µm on each side) than the width of your trapezoid. You also want to use a finer milling current such as 500 pA, and a depth slightly shallower than your lamella was. The settings in Figure 31 took about 9 minutes for me.

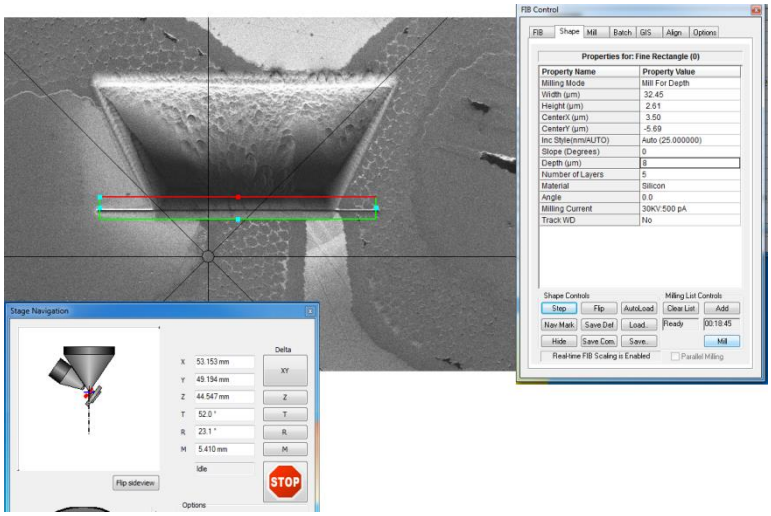

Figure 31 Top detail shape parameters. Notice the stage tilt of 52°.
Image obtained using ZEISS SmartSEM software
www.zeiss.com/smartSEM

Digging the second trench

There is something you have to take into account when digging the second trench. As you may have noticed, fibbing takes time. And in electron microscopy longer times means longer drift. If the grounding of your sample is poor you will have a lot of problems with drifting. Now, depending on the drifting direction you may want to dig your trench so that it doesn't drift towards your lamella[23]. Therefore when digging the next trench, you have one of two options:

1. If the drift is acceptable, flip the trapezoid electronically using the Angle under Property Value in the Align tab.
2. If you suspect the drift could affect your lamella, rotate the sample so the drift is always away from your lamella. Use Stage Navigation controls so you can rotate exactly 180°.[24]

The settings for the second trench are the same as the first, except for the detail cut angle (if you didn't rotate the stage). You can see a picture of my lamella at this point in Figure 32.

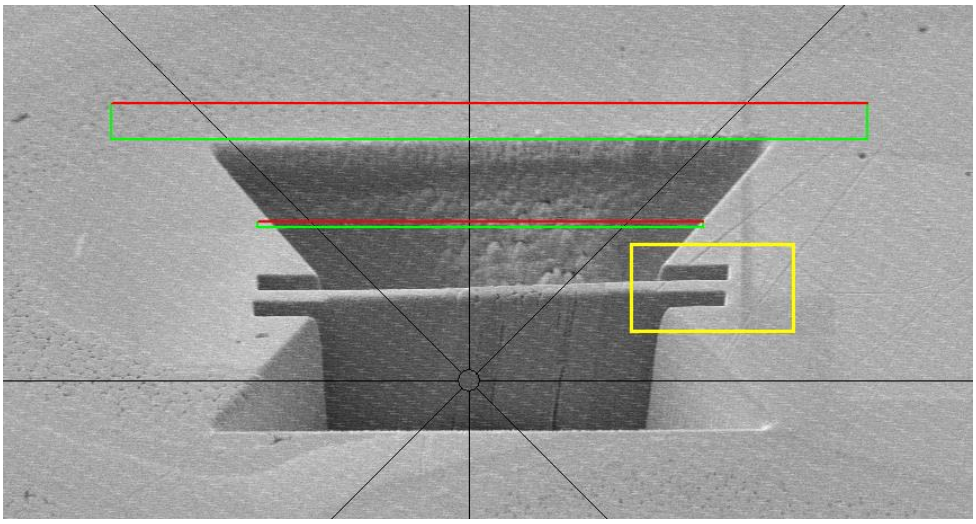

Figure 32 My lamella once both trenches and both detail cuts were made.

Other cuts

At this point the lamella is ready for the side and bottom cuts. If we recall, our goal is to detach the lamella from the sample, and attach it to a manipulator needle. The problem we face at the moment is that the "*I*" image (the FIB gun) is seeing the lamella from its top view so we need to tilt the stage in order to perform the side and bottom cuts of the lamella. These three cuts are done with a Tilt of 19°[25], using rectangular shapes in deposition mode, and a probe current of 200 pA.

When performing the side cuts you want to leave a thin piece (close to the surface) still holding the lamella or else it would fall into the trench before it is attached to the needle. You also want this thin piece to be at the left side of the lamella, since the needle will be coming from the right, as seen in Figure 27. The bottom cut should be the last cut, since it is the most prone to re-deposition. Also, keep in mind that the bottom cut may be hard to see[26].

After these three cuts, you probably want to go back to 54° tilt and make some room so the needle can access the side of the lamella. Use a rectangle and perhaps a current of 500 µA keeping in mind that too much fibbing time could redeposit material on your bottom cut. You can see the way this cut looks from the "*E*" image in Figure 27, notice the angle and the

[23] In my case the drifting was upwards, hence why I fibbed the top trench first.

[24] I'm not sure how much this will affect your alignment. It depends on how close your trenches are to the center of the stage.

[25] Notice that you may have to adjust your Z and you may have to realign your probes (I know, it sucks!).

[26] I had to tilt the stage to see if it had gone through.

thickness of the needle and make sure your cut is big enough. I've also added a yellow square on Figure 32 where this shape is supposed to go.

Picking up lamellae

There are two instruments you will be using for picking up your lamella, the Gas Injection System and the Omniprobe.

Gas injection system (GIS)

The Gas Injection System or GIS[27] is used to deposit material. You can either use it to deposit a protective layer on your sample (which I didn't) or to weld two pieces of material together. It works by injecting a gas into the chamber (or rather, a spray) which contains particles of a certain desired material such as carbon platinum or tungsten. When the spray is attacked by the ions from the FIB gun, it decomposes and the remaining particles (of the active ingredient) deposit onto the area below the beam. The cool thing is that the shape and size of the deposition matches your fibbing shape! Meaning that all you have to do is create a shape just like you have done for regular fibbing, except that you tell it to use a certain gas[28].

Carbon, platinum, and tungsten are very desirable for FIB-use because once deposited, they are very hard—and therefore resistant to unintended fibbing effects.

GIS initialization

In order to initialize the GIS you are going to have to move your sample back to the exchange position. Naturally you don't want to lose your current position given all the time and effort you spent getting the eucentricity and coincident points. So, to save your current position, go to Panels Tab →Stage points list → Add to add your current position in the Stage Points List window. This allows you to come back to it once you are done initializing the GIS.

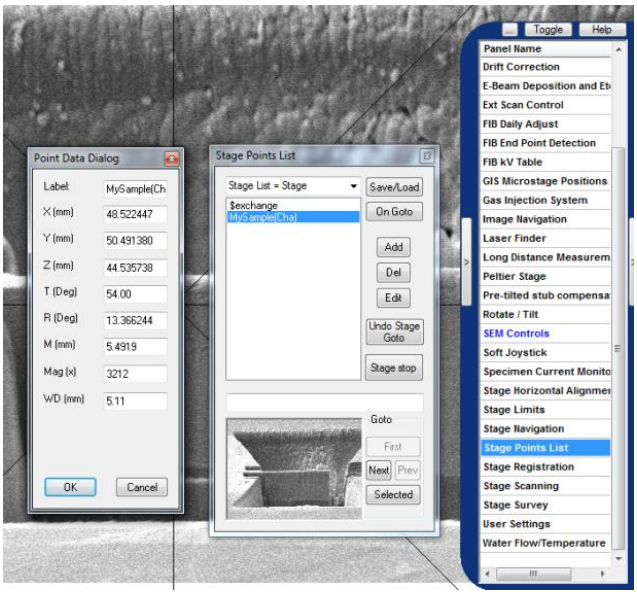

Figure 33 By clicking the Add button in the Stage Points List I can save my own position. DO NOT delete the $exchange position! Also notice that when you click Edit, the Point Data Dialogue comes up.
You can copy the stage values from here and paste them in the Stage Navigation Window.
Image obtained using ZEISS SmartSEM software
www.zeiss.com/smartSEM

[27] Once again spell out the three letters G.I.S, instead of saying the word "gis".

[28] Using the Gas ID option in the Shape tab as it will be described later

To recall the $exchange[29] position you can double click on it. However, make sure you set the Tilt back to zero and move the z down considerably before recalling $exchange since it sometimes could move other axes before z and may end up hitting the gun.

Heating up and initializing

You may want to pull up the detailed GIS panel (Gas Injection System) from the Panels Tab since it shows the temperatures and other GIS properties in much more detail (see Figure 34 below).

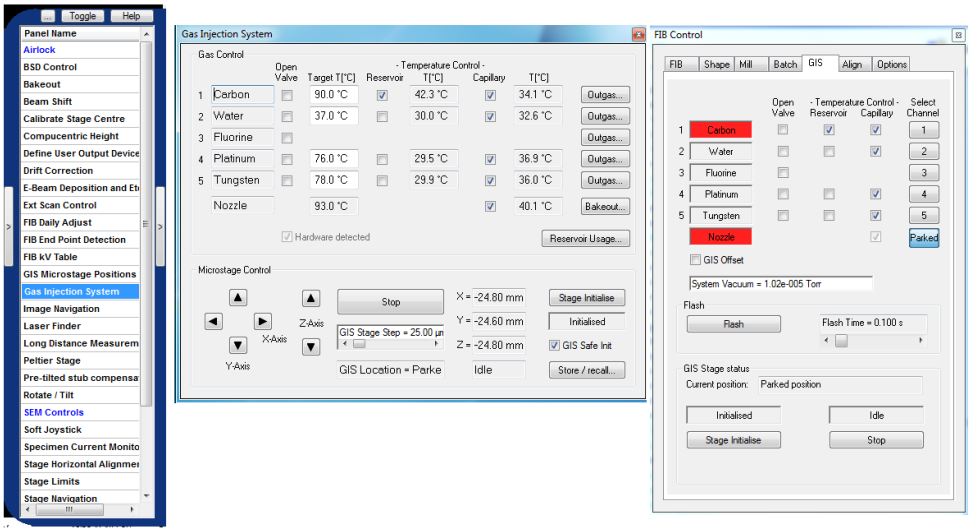

Figure 34 GIS tab in the FIB Control (right) and the Gas Injection System panel (middle) showing much more detail. Notice the current temperatures are not near the target temperature, therefore the GIS is not ready to be used. Hence the red boxes
The Gas Injection System panel is obtained from the Panels Tab (left).
Image obtained using ZEISS SmartSEM software
www.zeiss.com/smartSEM

Now, the first thing you need to do is to heat up the GIS. Do this by checking the reservoir and capillaries boxes of the material you are using (carbon is the most commonly used). Since the capillaries are tubes in parallel it might be a good idea to check all the capillaries so they help keep the carbon capillary temperature more stable (see Figure 34 for the boxes I checked). Also notice that although the capillary target temperature is 100°C, it will rarely go above 95°C. Either way, keep an eye on these temperatures—you don't want them to fluctuate too much. If they do, you may have to contact one of the SEM frequent users/technicians.

By pressing the Stage Initialize button in the GIS tab (see Figure 34), you will see the GIS nozzle translate to a certain calibration position (very close to where your sample would be if you had not retracted it!) and then retreat back to the safer Parked position.

[29] Make sure not to delete or overwrite $exchange otherwise all users will no longer be able to do "sample exchange"

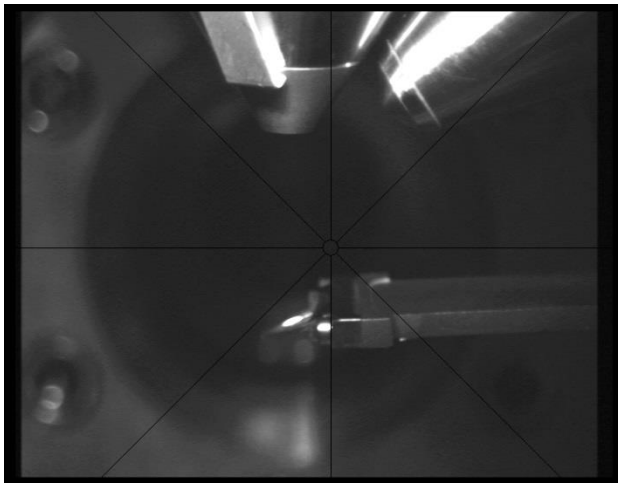

Figure 35 GIS in the middle of the screen before retracting to the Parked position

Once the nozzle is initiated you will be able to deposit material onto your sample. We will return to how to do this later, for now let's leave the nozzle in its Parked position and let's see how the Omniprobe is initialized.

Omniprobe

To pick up your lamella you are going to have to weld it to the manipulator needle. Below I go into the details on how to initialize and work the Omniprobe. Note: this whole initialization should be done with your sample still in the exchange position. Therefore it is recommended to initialize both the GIS and the Omniprobe at the same time.

Omniprobe initialization

The manipulator probe (or needle) works with an external computer that instructs the needle how to move using the network[30].

Figure 36 Omniprobe computer screen

In order to operate it you first have to make sure the control box is on

[30] This means that if the internet is down you may not be able to work with it.

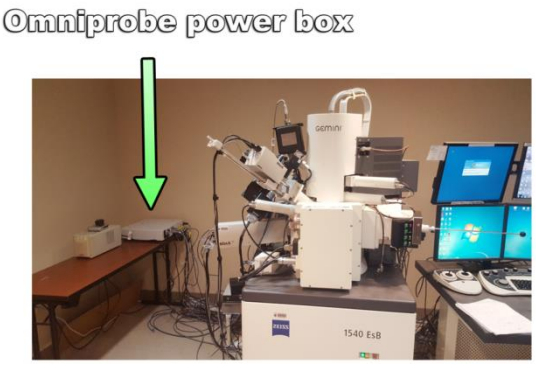

Figure 37 omniprobe power box

Then, launch the Omniprobe software (there should be a shortcut on the desktop) and log in using supervisor mode. The password is "super". Once you open the software it may require you to "home" the axes as seen in Figure 38. Don't click Start yet…

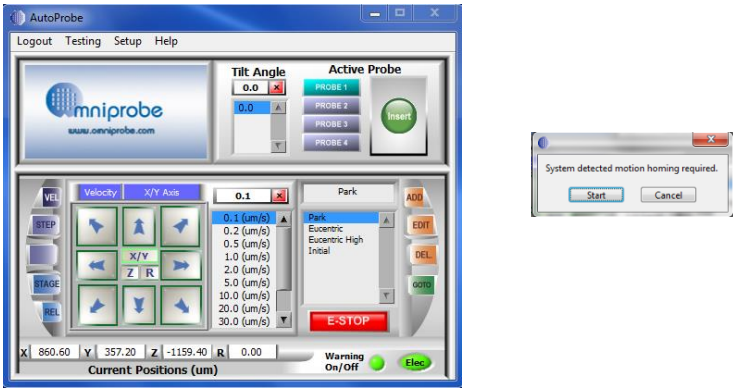

Figure 38 The Omniprobe window (left) and a very common question asked when the system has not been initialized (right).
Image obtained using the AutoProbe® software
For more information visit https://www.oxford-instruments.com/

There is a little issue we have with the probe system/software, and it requires us to work around it. You see, the rotation control (R, seein in Figure 38) is not available, and the software cannot initialize unless all axes are checked for functionality. So we need to trick the software into thinking R is functional. The software checks the functionality of all axes as soon as you open it, and as it does so, it writes a code in a text file called AutoProbe® settings.txt[31]. If this code says all functions are good, the software has the permission to "home" all axes (which is what the Start button in Figure 38 will do, homing).

The problem is that if R is not available the homing will not be done adequately and the rest of the axes will be frozen. For this reason we need to modify the AutoProbe® settings.txt after it is written. To do this, open the AutoProbe® settings.txt file (before homing!) and notice that next to the axis names there is a YES or a NO. Most likely the R has a NO beside it[32], so type "Yes" to match all the others and save the file.

At this point you can click on Start or (if you didn't get the message in Figure 38) you can go to Setup → User Setup→Home Axes. A new window should pop-up, check all boxes except for R and click Begin Homing (see Figure 39). This will do a series of calibrations so that the AutoProbe® software knows where the needle is.

[31] There is a shortcut on the desktop of this computed that takes you to the location where this text file is, look for Autoprobe - Shortcut

[32] If all say YES then someone may have already done this and you may home the axes without any issues.

Figure 39 Home Axes widnow.
Image obtained using the AutoProbe® software
For more information visit https://www.oxford-instruments.com/

Alright, the software now knows where the needle is. The issue now is that it doesn't know where your sample *will be* (once we recall our position). So next I will explain how to calibrate the needle to your sample surface.

Omniprobe calibration

If you haven't moved any of the SEM or FIB settings, both your guns should be focused on your sample surface (or at least where it was before you retracted it[33]), so all we have to do is move the needle around until it shows up at this "coincident point". And (voila!) just save those coordinates to an Omniprobe position called Eucentric[34] so the needle can come back to that once you recall your sample position.

To do all of this, first of all let's insert the needle. Press the Insert button (see Figure 38). You will hear a popping noise and you may be able to see the needle (in TV view). Then, highlight Park (in the text box above the red E-STOP button) and click the green GOTO button. This Park position is just a convenient idle position.

Now remember the goal is to put the needle at the coincident point and overwrite the Eucentric coordinates. You may want to check if someone had used the needle recently and their Eucentric was close to your current coincident point. So highlight Eucentric High and click GOTO[35]. This Eucentric High position is supposed to be a few hundred microns above the Eucentric. If you can see the needle in the "*E*" or "*I*" images you are in luck and you may only have to do a couple of adjustments. If not, it may be trickier but as long as you understand the way the needle moves you should be able to find it.

There are two orientations to the needle (*i.e.* two x, y and z systems) one of them is called the PORT and the other one is called the STAGE. You can switch between these two using the STAGE button in the lower left corner of Figure 38. If it says STAGE it means the STAGE system is active and vice-versa.

When you move the needle in the PORT mode, the z axis matches the needle's axis, so this can be very useful when retracting the needle straight back on its own axis. However, when you move the needle in the STAGE mode the axes match those of the stage at 54° tilt—which is very useful since the x and y match the "*I*" image and the z is normal to your sample surface.

The PORT mode is actually very difficult to use and you shouldn't need it (except for retracting the needle). Also notice that in STAGE mode you won't be able to see any z movement if you are looking only at the "*I*" image. So you need to be

[33] And it should stay as is, retracted

[34] I guess the Omniprobe software developers decided to refer to the coincident point as the Eucentric which is slightly confusing for us since we defined eucentricity as a stage property and coincident point as the place where the guns meet. So, when talking about the probe, Eucentric means the coincident point.

[35] You can't recall the Eucentric for safety, since it will be dangerously close to your sample.

clever about what axis to move when looking at which mode. Another thing you need to be aware of is your speed, you can move the needle between 0.1 µm/s and 30 µm/s[36]. Make sure you use the lower speed values whenever you are close to your sample, you don't want to slam into it! And last but not least, notice that pressing the X/Y button or the Z button gives you different sets of arrows (see Figure 38 for x/y controls and Figure 40 for z controls).

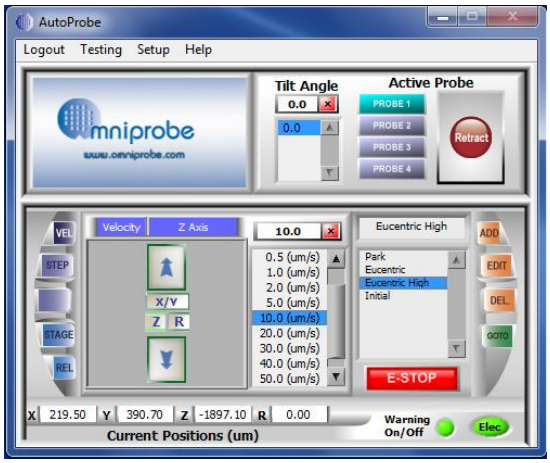

Figure 40 Omniprobe window with the z axis selected. Also notice that the needle in this case is inserted therefore pressing the red circular button would Retract it.
Image obtained using the AutoProbe® software
For more information visit https://www.oxford-instruments.com/

It might take you a couple of frustrated attempts to learn how to move the needle around, but as usual, be patient! Now, once you have the needle tip at the canter of both the "*E*" and "*I*" images, highlight Eucentric and click EDIT to overwrite it. Overwriting the Eucentric position automatically sets the Eucentric high a couple of hundred microns above it (in the STAGE z axis) and it will only allow you to recall the Eucentric high for safety.

Ok, there is one last position we recommend you to save. This position is called Initial and the reason we want to save it is because the needle gets dangerously close to the GIS system when moving from Eucentric high to Park. For this reason we want Initial further back (in the PORT z axis) than Park. Doing this is actually very simple. Go to Park and using the PORT mode move the z axis up until its value gets to about - 2000 µm (I think the limit is something like - 3700 µm but there is no need to go that far). Highlight Initial and click EDIT to overwrite it.

Alright, you are set! Retract the needle by pressing the round red button (Figure 40) and you are ready to bring your sample back to the position you saved.

Working together (GIS and Omniprobe)

Bring your sample back by recalling the position you saved previously in the Stage Points List window (see Figure 33). But be careful when recalling these parameters, make sure you do the M and the Tilt manually by copying and pasting the values into the Stage Navigation window. Once these are recalled, you may just double click the position to fully recall all axes.

After your sample is back in place, and the GIS temperatures are optimum, the nozzle is ready to be placed above your sample surface, you do this by pressing the Select Channel buttons next to the material you want to use—in the GIS tab. Don't be afraid, as long as your sample is at 54° Tilt and 5.11 WD, the nozzle won't hit your sample. But it does come really close, as seen in Figure 41.

[36] I never thought I would ever say the term *microns per second*…

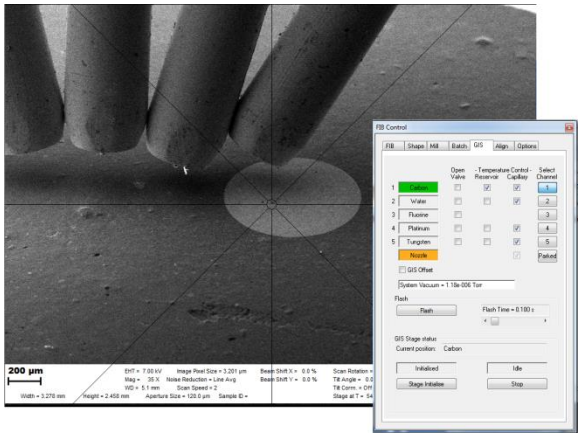

Figure 41 The GIS viewed at low magnification in the "*E*" image. The rightmost tube is Carbon (1) and the numbers move from right to left, ending on the other side with Tungsten (5). We never use Water (2) or Florine (3) therefore are most likely empty.
Image obtained using ZEISS SmartSEM software
www.zeiss.com/smartSEM

If you feel like the nozzle is still far from your sample you may perform minor adjustments to its positions using the arrow buttons in the Gas Injection System window (see Figure 34). The adjustments (if any) will most likely need the z and the y axis only. Be careful! Don't slam the GIS against your sample!

Once the GIS is at an adequate position, use the AutoProbe® software to Insert the needle in. Highlight Eucentric High and click GOTO. If you zoom out all the way in the "*E*" image you may be able to see the needle on the top of your screen. Slowly bring the needle down using the STAGE z axis (still AutoProbe® software) and carefully position it next to the lamella using the rest of the axes. See Figure 27 for the needle position (already welded).

You are ready to perform your sample deposition. Make a rectangle that covers the tip of the needle and a small piece of your lamella, use deposition mode and a FrequencyY of 100 (see Figure 42). Don't forget to change the Gas ID option in the Shape tab. You don't need very high current for this, a current of 10pA should be enough (however, you may have to calibrate this probe). Press Clear List, Add and Mill in the Shape tab.

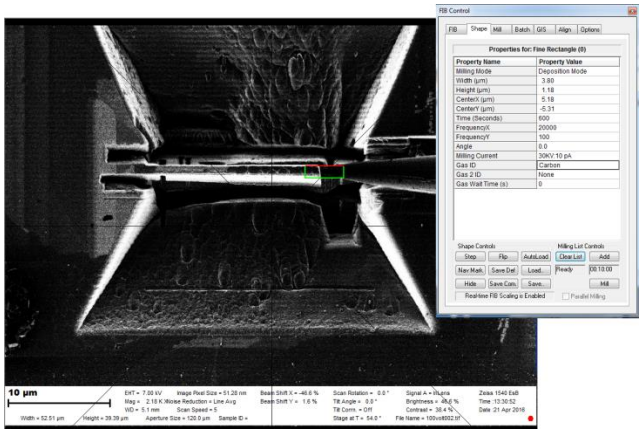

Figure 42 "*I*" view of my lamella and the shape parameters for welding. Notice the deposition mode and a FrequencyY of 100 (normally 2).
Image obtained using ZEISS SmartSEM software
www.zeiss.com/smartSEM

While depositing material, switch to "*E*" and make sure the deposition is being done adequately, and if there are ANY signs of milling STOP immediately using the stop button in the Mill tab, this could happen when the gas is running low. You may stop the welding once you consider the welding is good.

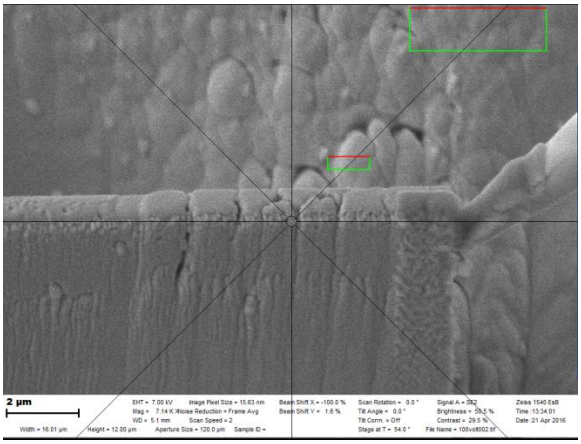

Figure 43 "*E*" view of my lamella welded to the manipulator needle using carbon.

Once welded you may FIB out the piece that is holding the lamella to the sample using the same settings you used for the side cuts. Figure 44 shows the "*E*" view of my last cut.

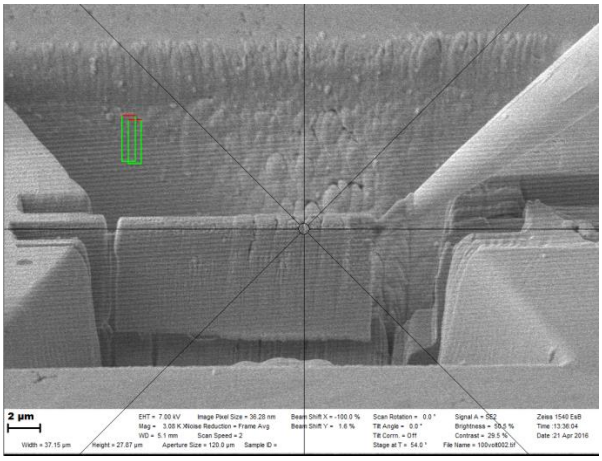

Figure 44 "*E*" view of my last cut.

Alright, your lamella is free! You may use the STAGE z axis (up) in the AutoProbe® software to move the needle away from your sample—slowly—and once it is a few microns away from your sample, recall the Eucentric High position. Then, recall the Initial position and Retract the needle.

Put the GIS in the Parked potion using the GIS tab. You may take your sample out of the SEM now and forget about it—as well as its stage coordinates—you will not need them anymore. Congratulations, this is a pretty big step actually (or at least it felt for me).

Using the GIS before making the lamella

There is one thing I didn't do, which now we have enough knowledge to do. Before digging your trenches, you want to deposit a rectangle of carbon above the area where your lamella is going to be (exceeding slightly the lamella size[37]) to protect it. To do this, make a rectangular shape that covers the area, and use the 100 pA probe with a FrequencyY between 20 and 100. Don't forget to change the Gas ID option in the Shape tab, and press Clear List, Add and Mill. This should give you a layer about 150 nm thick.

Your area is now protected, poroceed to dig your lamella out.

[37] About 4 µm by 25 µm

Welding lamella to grid

What we need to do now is weld the lamella to a TEM grid (seen in Figure 26). You may have to ask Dr. Kametani about acquiring a grid, and how to mount it for SEM use.

Inserting and calibrating the grid

Once your grid is in, you may perform the same steps as if you were going to FIB near the tip of one of the grid prongs[38]. Meaning:

1. Find eucentricity.
2. Match your gun's coincident points.
3. Calibrate relevant probes (10 pA, 50 pA and 200 pA[39]).

As well as re-calibrating your probe's Eucentric position in the Omniprobe software, which requires retracting your grid to the $exchange position, moving the needle to the coincident point and re-writing Eucentric. At which point, you may Retract the needle and recall your grid position in (don't forget to recall the M and the Tilt first before you double click on $exchange).

Alight, after all of this, you may be thinking all we have to do is:

1. Bring the GIS in,
2. Bring the needle (lamella) in,
3. Weld one side of the lamella to the grid,
4. FIB out the needle weld, and
5. Celebrate!

And you are right, but is not as straight forward as we would like. You see, when the GIS activates it seems to pop-out a burst of gas that is **often strong enough to rip the lamella off the needle**, therefore the GIS needs to be activated before the actual welding/fibbing happens for the popping to happen into empty space.

Welding steps

So here is how it is done. We want to bring the GIS in (as we did before) by pressing the Select Channel button next to the material you are using—on the GIS tab. Then, insert the needle by pressing the green Insert button in the Omniprobe software. Highlight Eucentric High and click GOTO.

This is where we want to **start the GIS before we move the needle down**, so check the box that says Open Valve (next to the material of choice in the GIS tab, see Figure 34). Once it is flowing, slowly bring the needle down using the STAGE z axis, and carefully position it next to the grid using the rest of the axes.

Once adequately placed, make a rectangle that covers a small piece of the lamella and a small section of the grid. Use deposition mode and a FrequencyY of 100, and remember that you don't need very high current for this—a current of 10pA should be enough.

One thing you have to keep in mind is that this time it may take much longer because the gas is freely flowing in all directions instead of being stopped by the surface of your sample. And as always if there is any fibbing happening, stop immediately!

[38] The grid sticks out normal to your stage x, y plane and your lamella will be welded to the side of the prong (see Figure 26)

[39] You will not need to perform any coarse milling.

Separating the needle

After the weld is done, you may close the GIS valve by unchecking the Open Valve box[40]. And now we are just one FIB operation away from having our lamella right where we want it. Use a small rectangle perhaps with the 50 pA probe or the 200pA probe to FIB out the deposition that is joining the needle to the lamella. Make sure you do this as quickly as you can because **the needle can move slightly over time and it may rip your welding off**. And once free, use the STAGE z axis (up) to move the needle away from your sample—slowly. Recall Eucentric High position, recall the Initial position and Retract the needle. Put the GIS in the Parked position. You won't need these anymore (phew!).

Shaving the lamella

The final step is fibbing out the needle on both sides to make it thin enough so that it is electron transparent. This is easily done by using shapes on either side of the lamella since we are seeing straight from above on the "*I*" image. What we want to do is use a series of trapezoid shapes to take off slices in a gradual way.

Figure 45 shows a sketch on how this should be done. Notice the bases of the trapezoids need to be pointing outwards and that they have to be angled (~0.2°) in order to avoid the lamella becoming too thin close to the weld. A picture of my lamella getting shaved and the final product is seen in Figure 46.

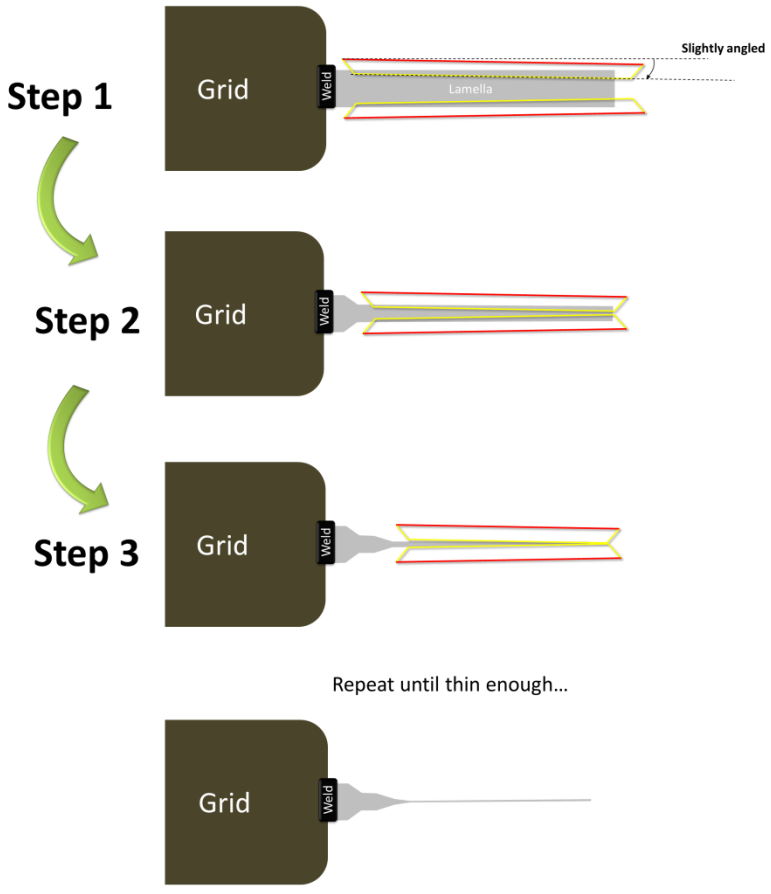

Figure 45 A sketch of the way the trapezoids need to be placed in order to get a good lamella. Notice they are angled inwards and the trapezoid bases are always on the outside.

[40] Actually I think it closes automatically when the fibbing is done.

Done! What next...? TEM away!

Alright, this sample is ready now for TEM. You may want to etch some of the surface since it may have become amorphous due to the FIB ions. This is done using our PECS ion milling machine. TEM away!

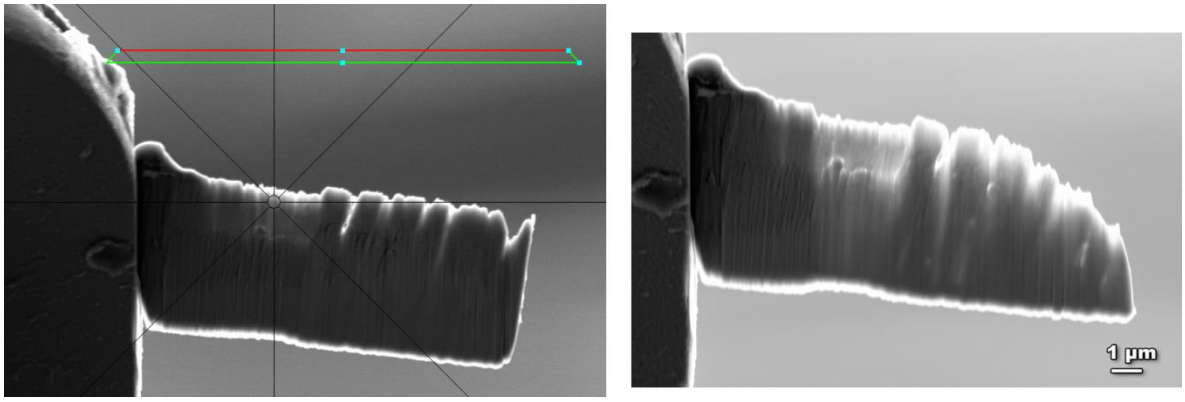

Figure 46 My lamella as it was getting shaved and the final product about 0.1 µm thick.

Step-by-step

Aligning the guns

1. Insert your sample and prepare the SEM for imaging—just like you would if you were taking basic images at a comfortable working distance of 10 mm or larger.
2. Set the gun voltage of 7 kV and an aperture of 120 mm in "high current" mode.
3. Still in the SEM imaging mode, find a spot that is easy to see and keep it as reference (at 400X or lower).
4. Tilt the sample using the stage navigation window to 54°. Notice that your point of reference has moved away.
5. Use M to find that spot again.
6. Go back to 0° Tilt, re-center your spot, and repeat steps 4 and 5. Notice that x, y and z still remain unchanged, you have only altered M.
7. Go back to zero Tilt and set the working distance (on the screen) to 5.11 mm.
8. Move the stage in the z direction (make sure you uncheck track-z) until the image is focused.
9. Repeat steps 3 to 6 but at a higher magnification (1500X should do). If at the end of this, with your stage tilted at 54°, the working distance is not 5.11 mm you may have to adjust z and re-adjust M again.
10. Turn the FIB gun on using the FIB Control panel (if the aperture was not initialized, initialize it).
11. Make sure the imaging current is set to 50pA using the Mill tab.
12. Switch to the FIB image by pressing the E or I button, set the magnification to match the one you currently have in the SEM image, and roughly focus the image.
13. Set the scan speed to 5 and keep it that way for the rest of your FIB use. InLens helps too.
14. Use the z **axis** (not x and y) to move the "*I*" view frame so that the reference spot matches its place in the "*E*" view frame. The x axis (of the screen) may not match, don't worry.
15. Use the beam shift knobs on the "*E*" image to match your reference spot perfectly in both view frames.
16. After all of this alignment you may freely navigate around your sample using x and y or pressing Ctrl+Tab and clicking on a specific point on the screen. Keep in mind that the "*E*" gun may go out of focus if you move in the y direction, but just focus it—there is no issue with this as long as you don't move too far. Stay close.

Adjusting the FIB

1. Go to the GIS tab and make sure all the valves are closed! Sometimes a mysterious macro opens them. Keep checking them every once in a while.
2. Try getting a sharp and well-focused image with the 50 pA (imaging) probe. Go to Align tab, and click Save. To save the focus settings for the current probe.
3. Focus the 200 pA, 500 pA and 2 nA probes in the same way.
4. Align the 200 pA, 500 pA and 2 nA probes to the 50 pA (imaging) probe repeating steps 4(a) through 4(k) for all.
    a) Draw shape, center and adjust size using Shape tab.
    b) Select all milling parameters (Figure 20).
    c) Press Clear List, Add and Mill.
    d) Go back to 50 pA.
    e) Check the location of the electronic square with respect to the milled square.
    f) Move the electronic square (click and drag) so that it matches the milled square.
    g) Switch the probe to the one you just used (under Align tab).
    h) Check the location of the milled square with respect to the electronic square.
    i) Use the FIB Abs Beam Shift sub-window in the Align tab to match the milled square to the new position of the electronic square.
    j) Press the Beam Shift Corr button.
    k) Press Save.
5. If you have done all the probes, remove the rectangle by selecting it and pressing Delete on the keyboard. Alignment is done!

Making a lamella

1. Find the spot you want the lamella to come out of. Keep in mind that you don't want to move too far from where your alignment was done, and where your eucentricity is.
2. FIB a protecting layer (see initialize FIB in the next page and perform those steps before this):
    a) Make a rectangular shape that covers the area
    b) Use the 100 pA probe with a FrequencyY between 20 and 100. Don't forget to change the Gas ID option in the Shape tab,
    c) Press Clear List, Add and Mill. This should give you a layer about 150 nm thick.
3. FIB the first trench:
    a) Insert a trapezoid
    b) Use "milling for depth" instead of time.
    c) Adjust the shape parameters by dragging the shape points with your mouse.
    d) Set the depth.
    e) Set the number of layers depending on your depth. The deeper you go the more layers you need, for a 10 µm deep trench I used 5 layers.
    f) Set the material. Silicon is a good reference material.
    g) Set probe to 2 nA.
    h) Press Clear List, Add and Mill.
4. Tilt the stage to 52° or 56° depending on which trench are you on.
5. FIB the corresponding detail rectangle:
    a) Insert the rectangle.
    b) Use "milling for depth" instead of time.
    c) Adjust the shape parameters with your mouse on the shape points.
    d) Set the depth (in can be slightly lower than the trench depth settings).
    e) Set the number of layers depending on your depth.
    f) Set the material. Silicon is a good reference material.
    g) Set probe to 500 nA.
    h) Press Clear List, Add and Mill.
6. If drift is acceptable use the same trapezoid settings as before and rotate it by 180°. Repeat steps 3 through 5 for the bottom trench.
7. If the drift is not acceptable rotate the stage by 180° (you may have to check alignment). Repeat steps 3 through 5 for the bottom trench.
8. Tilt the stage to 19°.
9. Check your z and gun alignment and re-adjust if it is too far off.
10. Using a rectangle with the 200 pA probe in deposition mode, perform the side cuts followed by the bottom cut.
11. Check your z and gun alignment and re-adjust if it is too far off.
12. Tilt back to 52° and make the spacer cut. Lamella is ready to be picked up.

Picking up lamellae

Initialize GIS

1. Go to Panels Tab →Stage points list → Add to add your current position in the Stage Points List window.
2. Recall the $exchange position. You can double click on it, but make sure you set the Tilt back to zero and move the z down considerably before recalling $exchange.
3. Check the reservoir and capillary box of the material you are using. It might be a good idea to check all the capillaries so they help keep the used capillary temperature more stable.
4. Press the Stage Initialize button in the GIS tab and wait for it to go back to the Parked position.
5. If you are only using the GIS you may recall your sample position. Make sure you do the M and the Tilt manually by copying and pasting the values into the Stage Navigation window. Once this is done, you may double click the position to fully recall all axes. If not, initialize the Omniprobe.

Initialize Omniprobe

1. Make sure the Omniprobe control box is on.
2. Launch the Omniprobe software and log in using supervisor mode, the password is "super".
3. Open the AutoProbe® settings.txt file and type "Yes" next to the R axis to match all the others and save the file.
4. Go to Setup → User Setup → Home Axes, check all boxes except for R and click Begin Homing.

Calibrate Omniprobe

1. Press the Insert button.
2. Highlight Park and click GOTO.
3. Highlight Eucentric High and click GOTO to check if someone had used the probe (or needle) before. If you see the needle in the screen you are in luck.
4. Knowing how the PORT and STAGE orientations work, and using both the "*E*" and "*I*" images, move the needle tip to the coincident point.
5. Once you have the needle tip at the canter of both the "*E*" and "*I*" images, highlight Eucentric and click on Edit to overwrite it.
6. Go to the Park position and using the PORT mode move the z axis up until its value gets to about - 2000.
7. Highlight the Initial position and click EDIT to overwrite it.
8. Retract the needle.

Working together (GIS and Omniprobe)

1. Recall your sample position. Make sure you do the M and the Tilt manually by copying and pasting the values into the Stage Navigation window. Once this is done, you may double click the position to fully recall all axes.
2. After your sample is back in place, and the GIS temperatures are optimum, press any of the different Select Channel buttons in the GIS to get the material of preference.
3. If you feel like the nozzle is still far for your sample you may perform minor adjustments to its positions using the arrow buttons in the Gas Injection System window.
4. Using the Omniprobe software, Insert the needle (if it is not in already).
5. Highlight Eucentric High and click GOTO.
6. Slowly bring the needle down using the STAGE z axis and carefully position it next to the side of your lamella

(the side that is not connected).

7. Make a rectangle that covers the tip of the needle and a small piece of your lamella.
8. Use the 10 pA probe in deposition mode and a FrequencyY of 100. You may have to calibrate this probe.
9. Don't forget to change the Gas ID option in the Shape tab.
10. Press Clear List, Add and Mill in the Shape tab.
11. Switch to "*E*" and make sure the deposition is being done adequately and stop it whenever you feel it is satisfactory (using the stop button in the Mill tab).
12. If there are any signs of milling make sure you stop immediately, this could happen when the gas is running low.
13. Once welded, you may FIB out the piece that is holding the lamella to the sample using a small rectangle with a current of 200 pA.
14. Your lamella is free! You may use the STAGE z axis (up) to move it away from your sample—slowly.
15. Once it is a few microns away from your sample, recall Eucentric High position.
16. Recall the Initial position and Retract the needle.
17. Put the GIS in the Parked potion.
18. Take your sample out of the SEM.

Welding lamella to grid

1. Insert your grid in the SEM and get it ready for FIB operations (near the tip of one of the grid prongs):
2. Find eucentricity.
3. Match your gun's coincident point.
4. Calibrate relevant probes (10 pA, 50 pA and 200 pA).
5. Save the grid's coordinates.
6. Retract your grid to the $exchange position and re-calibrate your needle's Eucentric position.
7. Once the Eucentric is calibrated(at the coincident point), Retract the probe.
8. Recall your grid position in (don't forget to do the M and the Tilt first).
9. Bring the GIS in by pressing the Select Channel button next to the material you want to use in the GIS tab.
10. Insert the needle in by pressing the green Insert button in the Omniprobe software.
11. Highlight Eucentric High and click GOTO.
12. Start the GIS before moving the needle down by checking the box that says Open Valve next to the material of choice in the GIS tab.
13. As the gas is flowing, slowly bring the needle down using the STAGE z axis and carefully position it next to the grid.
14. Once adequately placed, make a rectangle that covers a small piece of the lamella and a small section of the grid.
15. Use the 10 pA probe with deposition mode and a FrequencyY of 100.
16. When done, close the GIS valve by unchecking the Open Valve box (if it didn't stop automatically).
17. Use a small rectangle perhaps with the 50 pA probe or the 200pA probe to FIB out the deposition that is joining the needle to the lamella. Be quick, the needle tends to move slowly with time.
18. Once free, use the STAGE z axis (up) to move it away from the grid—slowly.
19. Recall Eucentric High position.
20. Recall the Initial position, and Retract the needle.
21. Put the GIS in the Parked position.

Shaving the lamella

1. Use a series of trapezoid shapes to take off slices off each side of the lamella until it is about 0.1 µm thick.
2. You may etch the surface using the PECS (not in this tutorial).
3. TEM away!