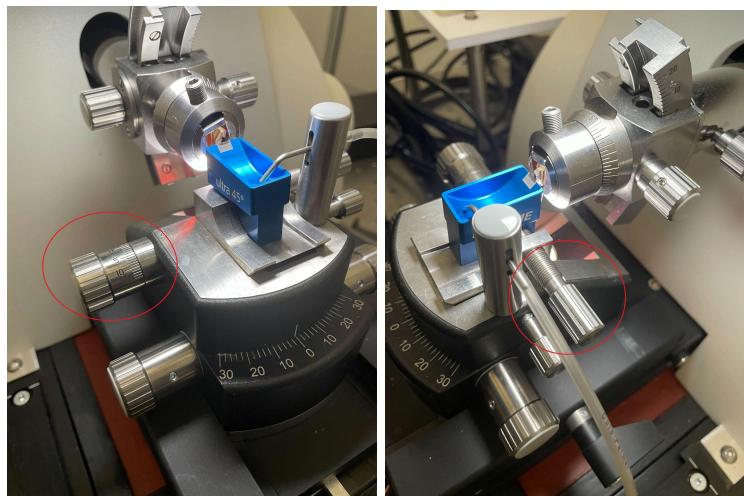


The purpose of this manual / checklist is to ensure that in the myriad of variables to control over the collection period that nothing is forgotten or overlooked.

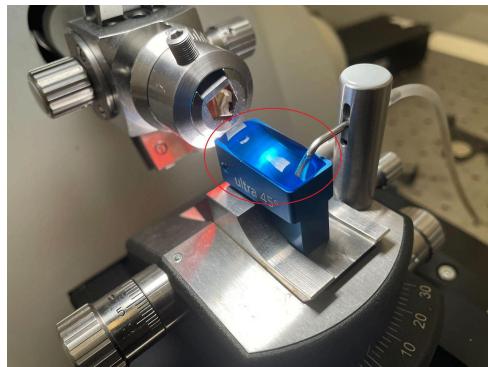
Setup:

Minimum 0.5-1 hour before collection:

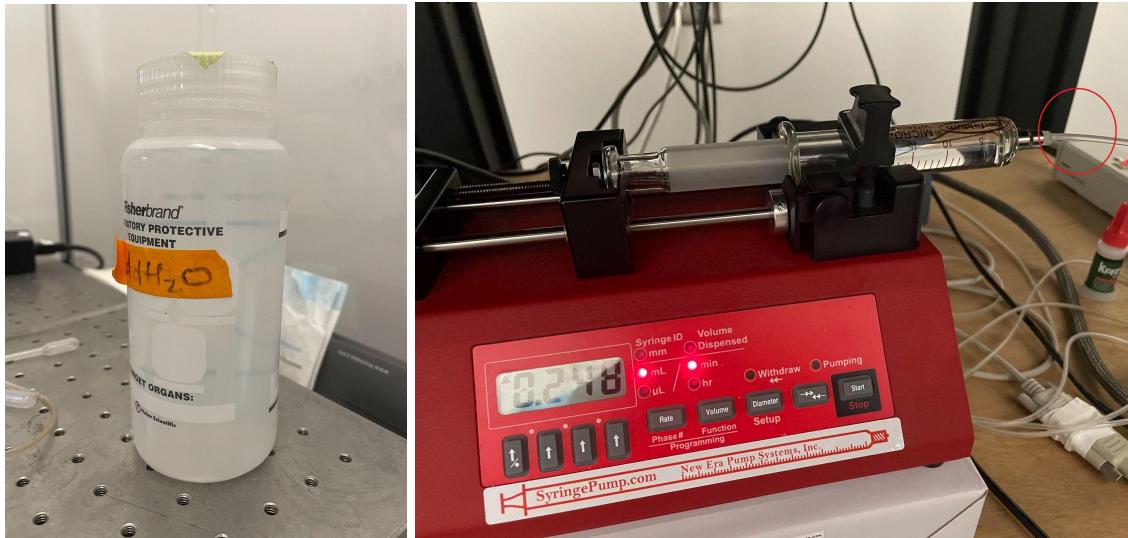
- Place the diamond knife on the stage, ensuring it is at the correct angle (usually 6°) and screwed on firmly.



- Insert the sample in a microtome chuck into the ultramicrotome (already done in above image)
- Add diH₂O and overfill the diamond knife boat (ensure water is covering the edge of the knife)



- Fill the autopump syringe with diH₂O (detach at red circle), pump through tubing (remove air bubbles), place the pump spigot into the diamond knife boat, draw water into the mini syringe, make sure no air bubbles there.



- If using the humidifier, fill with water, turn on using the highest fan setting.
(Remember to turn down the fan when ready to cut)

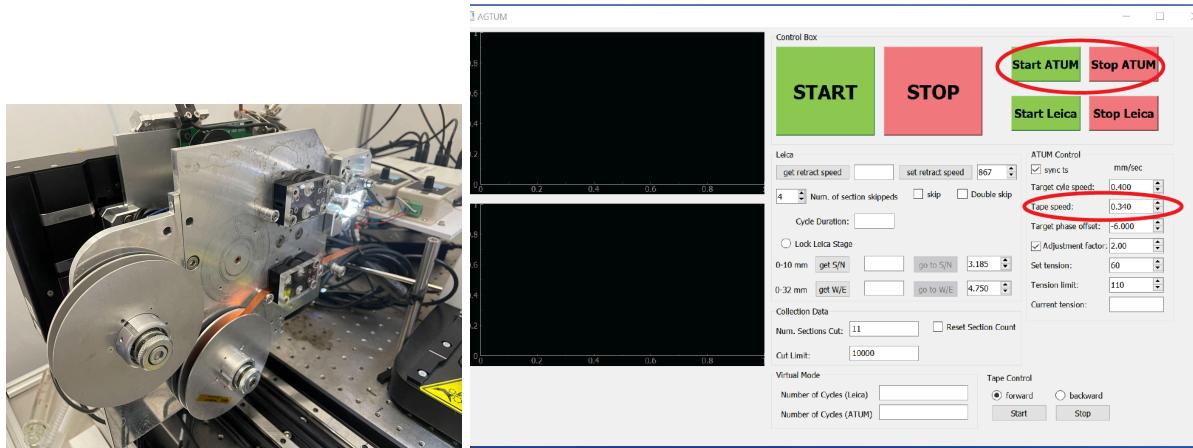


During this 0.5-1 hour wait you can do the following:

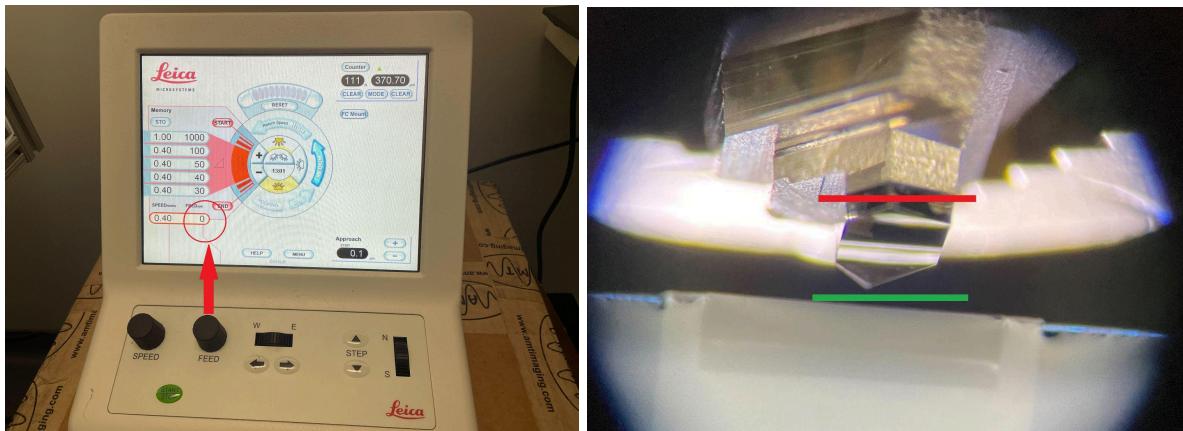
- Transfer the tape so that the copper side is up, and the barcodes are on the outside. NOTE: this may need to be done the day before depending on the length of tape.



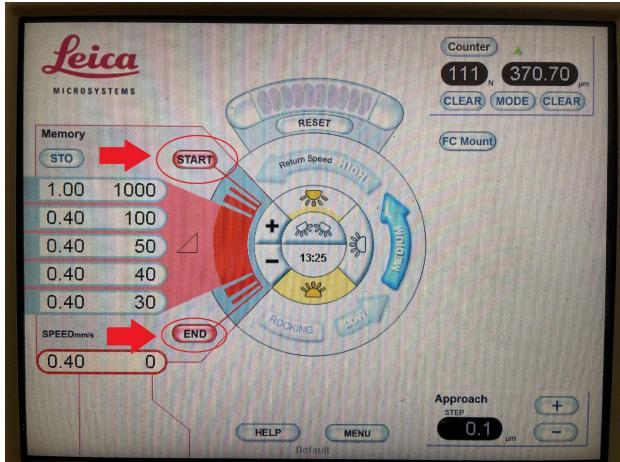
- Restart the Computer. Set up zoom window for recording collection. Send link to zoom meeting to yourself in slack.
- Open Skipcut_EWH program in Spyder. Load tape into the ATUM. Do this by putting the spool onto the reel hub and feeding the tape into the pinch roller (see image below).



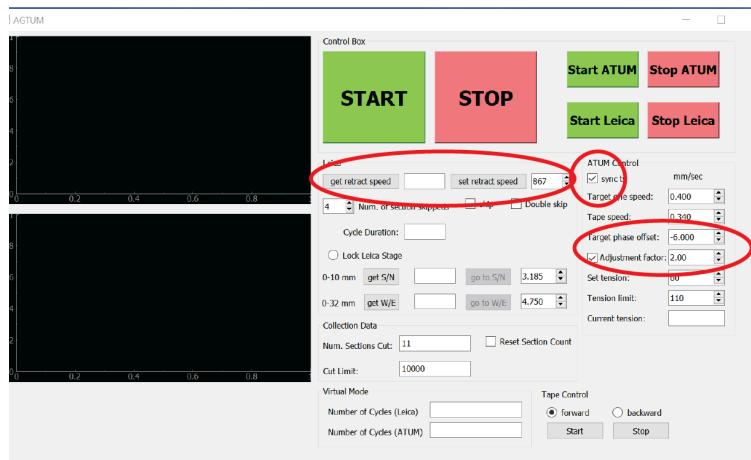
- Finding synchronization speed for block and apertures. Make sure to set the travel distance on the Leica to 0.



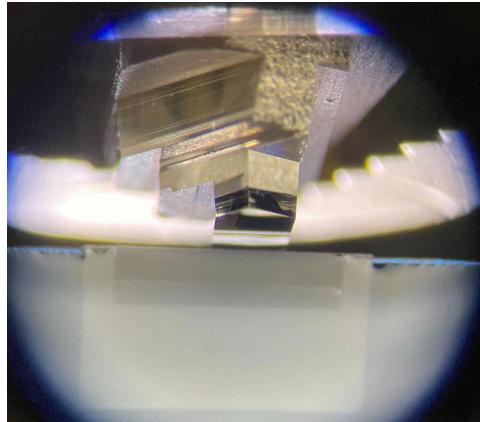
Note: this will require temporarily moving the knife semi-close to the sample block and setting the cutting window on the Leica interface (cutting window illustrated on microscope image). Press Start on Leica interface at position in image (slightly before when the block would hit the knife), Press End on Leica interface. You may need to draw the water down for a second to do this (see light reflection in image - should be gray). Make sure your knife angles are good enough that you will not accidentally cut any of the block! Move the knife back and reflood with water when done setting the cutting window.



Note: To set synchronization Press the synchronization check box, change “set retraction speed” and “tape speed” until synchronization listed in the terminal is in sync at zero. To increase how much the tape speeds up or slows down, increase or decrease the “adjustment factor”.

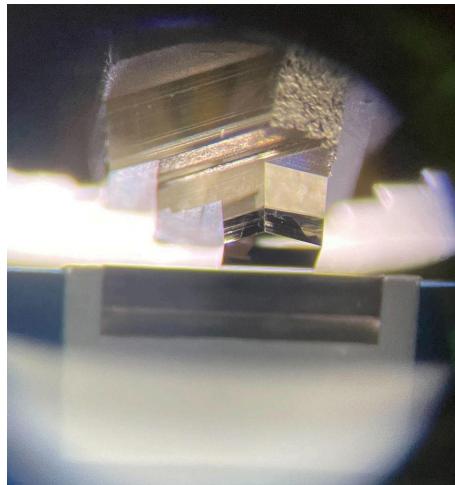


- Reset the leading portion of the tape back to the start after synchronization (or desired area).
- Manually move the knife close to the edge of the block (about 1 mm away)



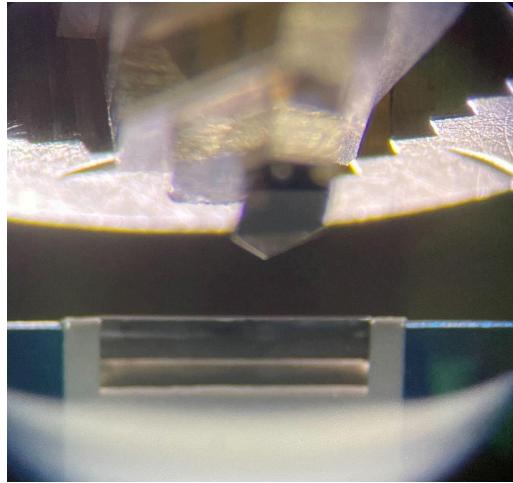
- Align the knife edge with the edge of the sample block.

Note: (Lateral angle of knife, yaw of sample block, vertical angle of sample block) Ensure it will be a straight cut (not cutting a tilted sample). Reiterate this process, moving the knife forward every time until very close to the edge of the block (small light bar visible between knife edge and sample block)



Note: water may need to be temporarily drawn down using small syringe

Note: you may also need to leave the sample block far above the knife to make sure when you re-overfill the knife boat you do not wick water onto the sample.



- Open Ulhardware program in Spyder. Move the ATUM to the knife.

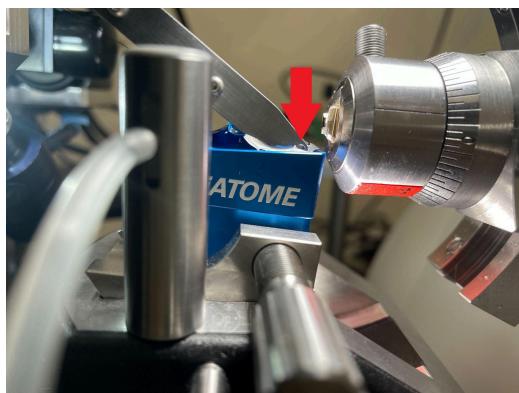
Note: Start with big movements (X: 100, Y: 10 Z: 2), but decrease as you get closer to the knife edge (X: 0.05, Y: 0.01, Z: 0.01)

- Open LeicaWaterCam program in Spyder.

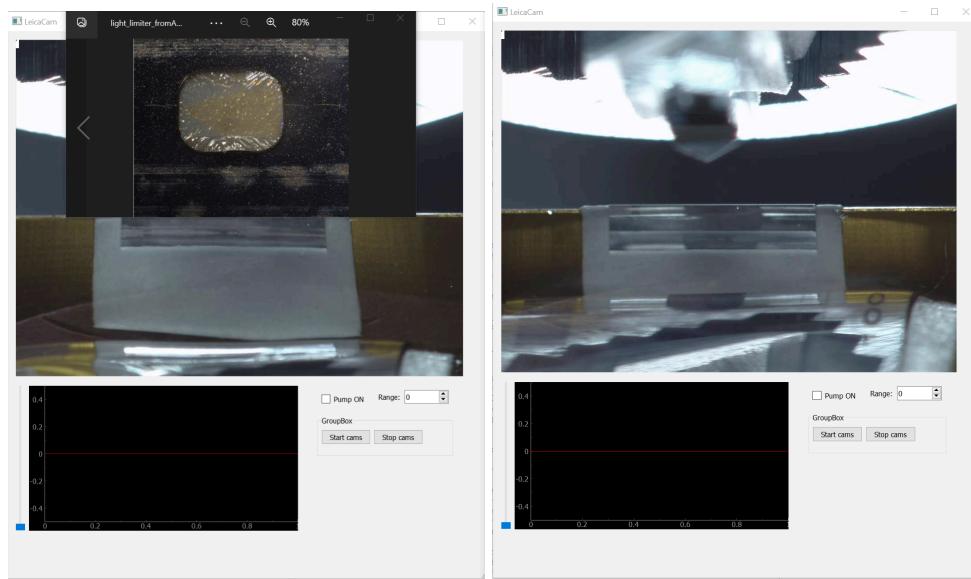
Note: Make sure it is broadcasting the video, if not, turn off the camera and close the program. Turn on the camera after a minute (should hear a beep when it connects to the computer) then open the program again.

- (New) Open GridtapeCameras_EWH_220730.py program in spyder.
- Place the nose of the ATUM into the boat and make sure the aperture aligns with where the section is being cut.

Note: A good rule is that the pin for the roller on the end of the nose (visible from the side) should be halfway in the water (**Z**). There are also images of previous collections screenshot on the desktop of the computer to see optimal distances from the block (**X / Y**). Overall a distance where the tissue will be picked up by the tape before it is done cutting, but not too close to hit the boat or cause vibrations. Next page...



Note: To make sure you do not hit the boat you can open a window and place it over the knife edge and make sure you do not see any change in the relative movement. If you do hit it, back up the nose, and back up the knife and reposition the knife relative to the sample block.



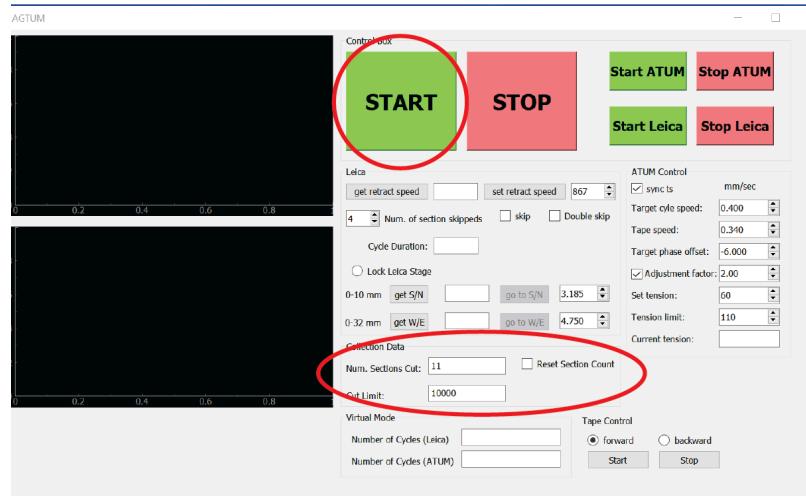
Note: these images of the LeicaCam are old

- Draw the water down to level (grayed). Lower the sample and move the knife closer to the sample (nearly cutting, but do not hit the block!). Move the ATUM nose forward if necessary, but shouldn't usually be unless your block was far back still. **Important! Remember to turn the under-light off!**

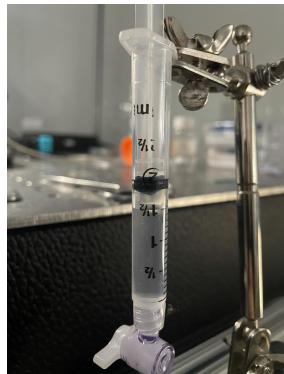
Collection:

Note: It is recommended at this point to make sure you use the bathroom, fill up some water to drink, or do anything else you need to before starting.

- Start recording the zoom meeting.
- In the Skipcut_EWH GUI, press Start (cutting depth should still be 0!) Also good to reset the collection num to 0 if it is not already by checking "Reset Section Count". If you only want to cut a set amount of section change the "Cut Limit" to the desired amount.

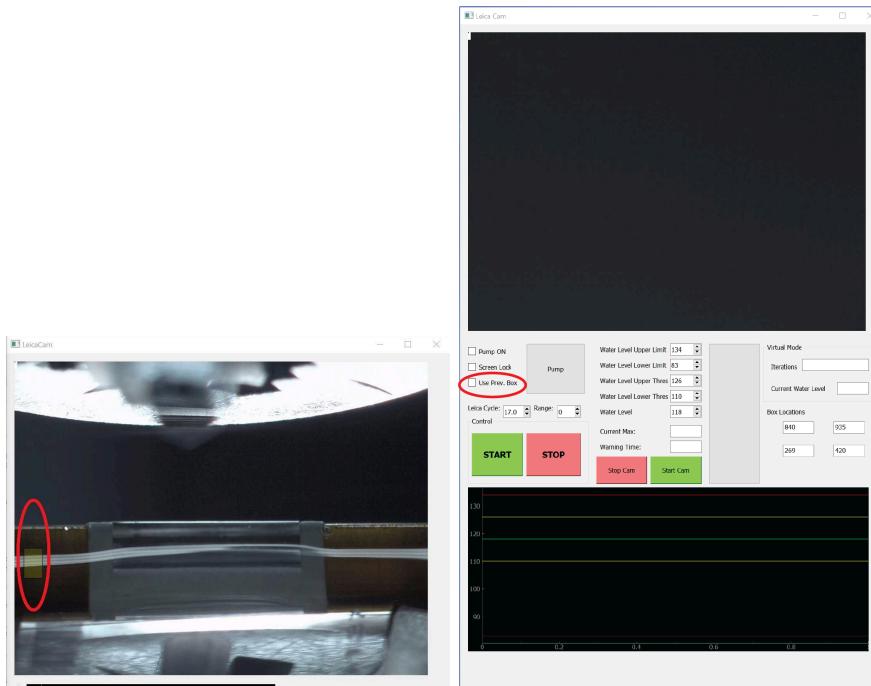


Note: this will allow you to see how much water is drawn up by the ATUM during collection. Add water with the small syringe if it seems it will go below the knife edge.

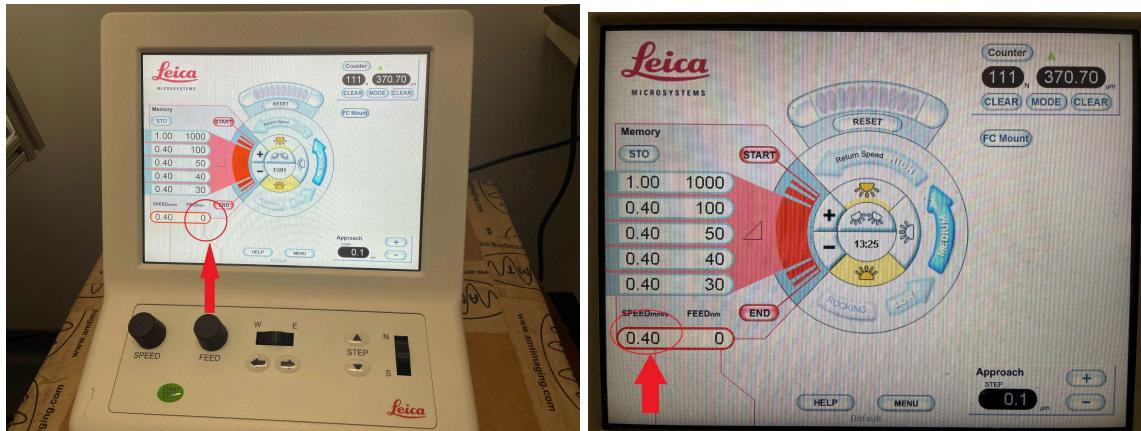


- Draw the water down to slightly below level (using the small syringe), but critical to make sure you do not draw too much water and have it no longer on the edge of the diamond. (see next image)
- On the LeicaWaterCam GUI, draw a box around the band of light reflection and press Start. Or press the “Use Prev. Box” checkbox to use the last box.

Note: It is recommended to set the box going from the top of the knife boat to as close to the ATUM nose as possible. This will prevent the water reflection from leaving the box and being misinterpreted.



- On the Leica interface, set the cutting depth to 45 nm and change the cutting speed to 0.3 mm/s and then back to 0.4 mm/s. (Sometimes it needs this)



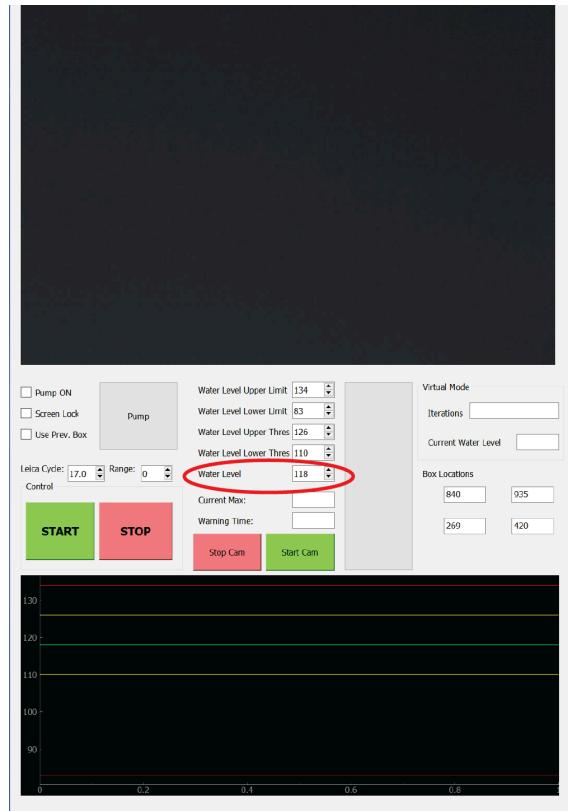
Note: if you are starting with a new block and your ROI won't be hit with the first cut you can start at ~100 nm and work down to 45 nm, this is a lot easier and less time consuming.

Note: it may take a while to start cutting, you can increase the cutting depth to speed this up, but it risks having a large first cut which is undesirable. The knife will last longer with only thin cuts (presumably).

- Once cutting sections, observe how they land on the gridtape, ensure it is optimal in X (tissue will land over the aperture and not on the sides, resin on the sides is fine)

Note: If it is not optimal, stop, move the knife back, and reposition the ATUM nose. Or if cutting is very stable, move the X, but only when not actively cutting.

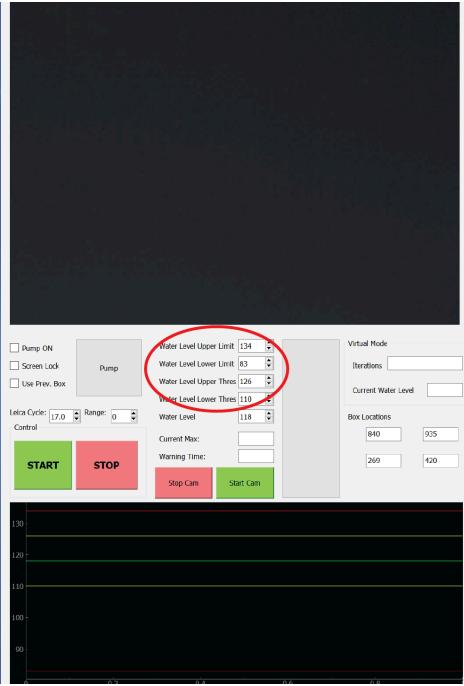
- After a few passes of the sample block, set the threshold on the LeicaWaterCam GUI by changing the number of the “water level” (green line) to be at about the height of the highest peak of the water level.



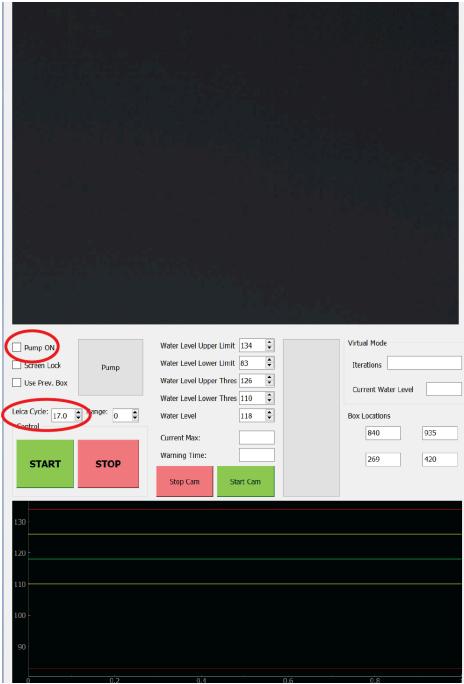
Note: there is no rule of thumb regarding where to set the threshold, but slightly below the highest point on the blue line in the graph.

- Change the numbers of the other lines (“Water Level Lower Thres.”, Water Level Upper Thres.”, etc.). The thresholds should be about 5 - 10 pixels above and below the water level and the limits should be about 15 - 30 pixels above or below the water level.

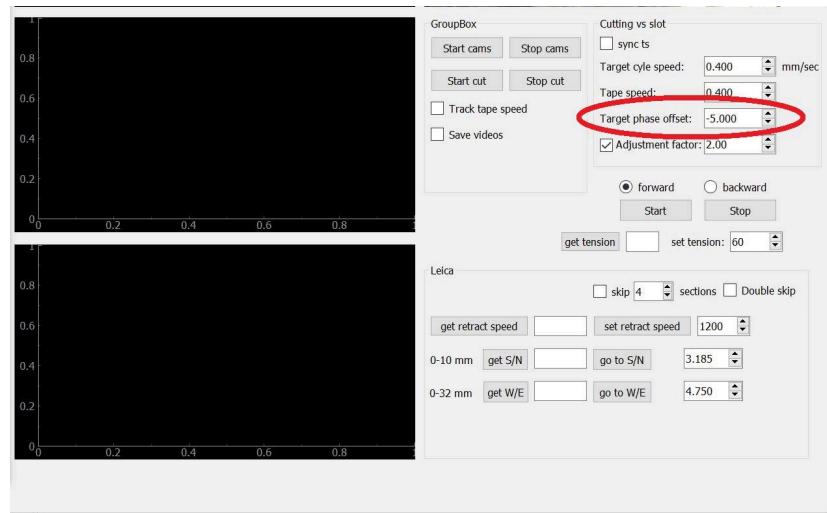
Note: it is important to pay attention to this for the first 10-15 minutes at a lot can change during that time and may require the water level and thresholds being changed.



- Change the “Lecia Cycle” time to the number being output in the console of SkipCut_EWH or on the AGTUM Gui. Turn on the pump.



- Once sections start appearing in the Post-Collection camera on the SkipCut_EWH interface, observe where they are landing, change the offset accordingly to move the sample placement forward or backward relative to the aperture.



Note: If the section lands too far behind you will need to apply a NEGATIVE offset (for example -1.300). For a section landing too far in front you will need to apply a POSITIVE offset (for example: 1.300). Adjustment factor is by how much it will correct (multiplier).

Cleaning the Knife:

Note: cleaning should only be done in an emergency if the sections are hanging or there are other noticeable problems that cannot otherwise be alleviated by moving to a fresh knife edge.

- Turn off Pump on LeicaWaterCam GUI
- Press Stop Cams and Cut simultaneously in AGTUM GUI.

Note: It is best to stop when the cutting arch is highest (furthest away from the knife edge).

- Turn off synchronization (unchecked synch box on AGTUM GUI).
- Using a blade, cut a sliver of foam from the piece of foam in a scintillation vial of ethanol. (Should already be on the air table)
- Carefully rub the diamond edge of the knife with the foam, taking care not to touch the diamond with anything but foam.

Note: it is important to really make sure it is clean or it will not go well. Look through the microtome lenses and ensure nothing is on the edge of the diamond or in the water.

- Flood the boat with water
- Let the water adhere to the diamond for 5 minutes

- Reduce the water level to collection level (see above).

Note: this next part is very tricky and requires being quick and focusing on multiple variables simultaneously.

- Resume Cams / Cut by Pressing Start for Cams and Cut.

Note: watch the water level to ensure it does not get too low from resuming movement of the tape.

Note: make sure no water drops get onto the block, this is a very common issue and can be really bad! If this happens it is best to stop and start it all over again.

Note: seems best to set it to 50 nm and sacrifice a little bit of z resolution to ensure a good first cut.

Note: watch synchronization and make sure it does not go crazy.

- Check Synchronization to turn it on.

- Check the pump checkbox to turn on the pump once it has settled and you reset the threshold if necessary.

Note: an issue of the flow from the pump going into the small syringe has been noticed and can be solved by closing the stopcock on the small syringe.

Post - Collection:

- When done, in the Skipcut_EWH press “Stop Leica”

Note: only stop cutting when it is not actively cutting a section!

- Using the UIHardware program, move the ATUM away from the knife, start with Z up! Can also use park.

- Continue moving the tape through the ATUM onto the uptake reel..

- Once the tape is almost off the feed roller, remove it from the reel and remove the tape (do not let the tape go through the ATUM!)

- Remove the reels, press reset on the Leica Interface.

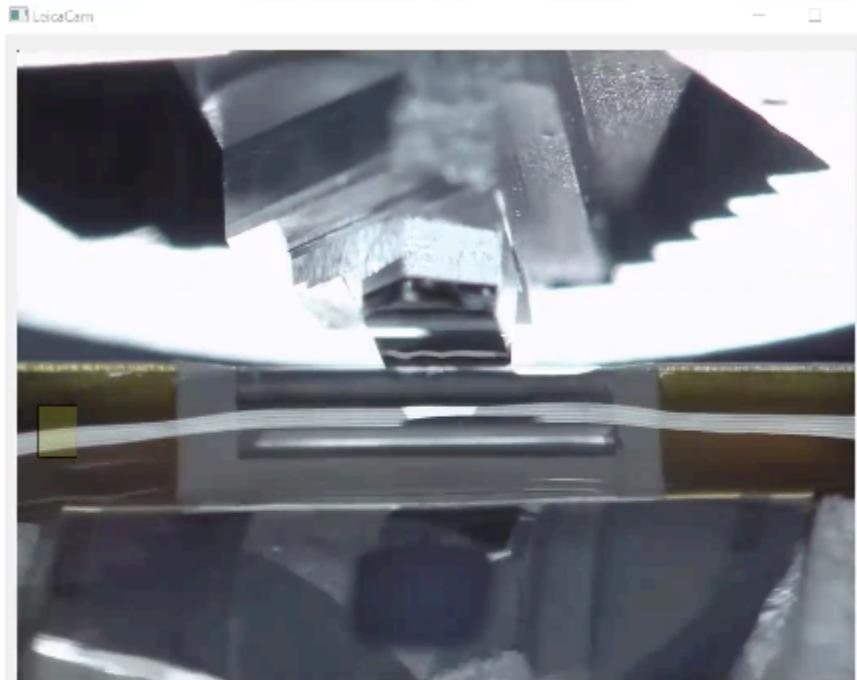
- Clean the knife edge, store the gridtape, shutdown Sypder, turn off the Leica, turn off all lights, drink an alcoholic beverage or equivalent activity to relax.

Post - Collection:

Section Collection 7.28.2022

1. Good collection example:

Time: 28:00

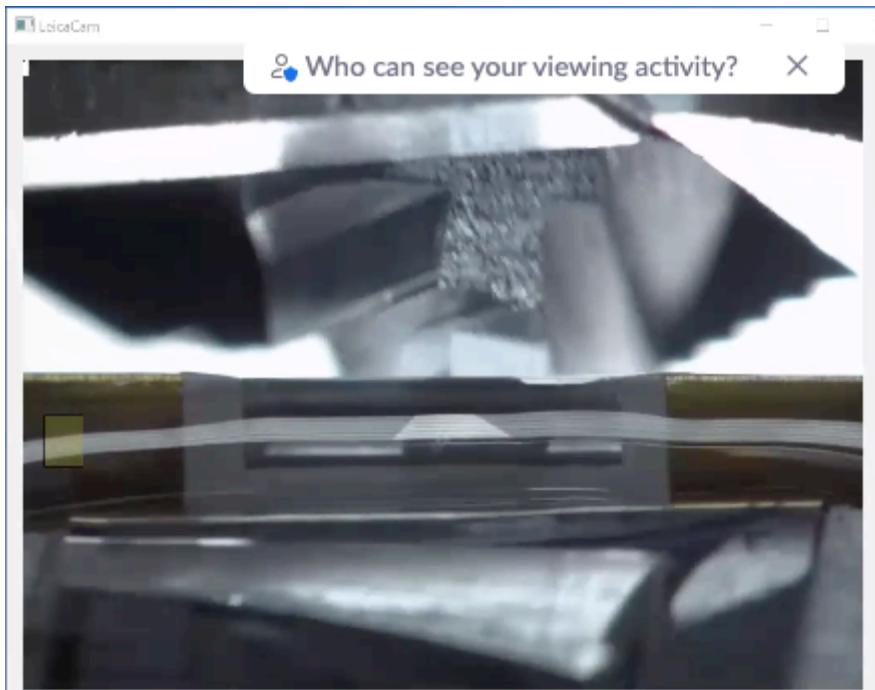


Beginning of Cut:



Middle of Cut:

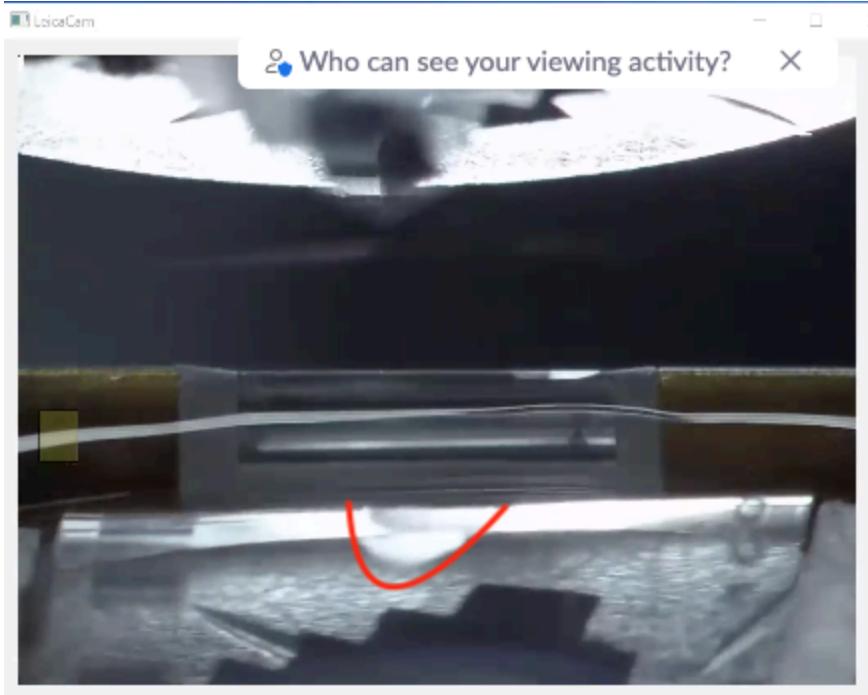
Last Revision: EWH - 2.09.23



End of Cut:



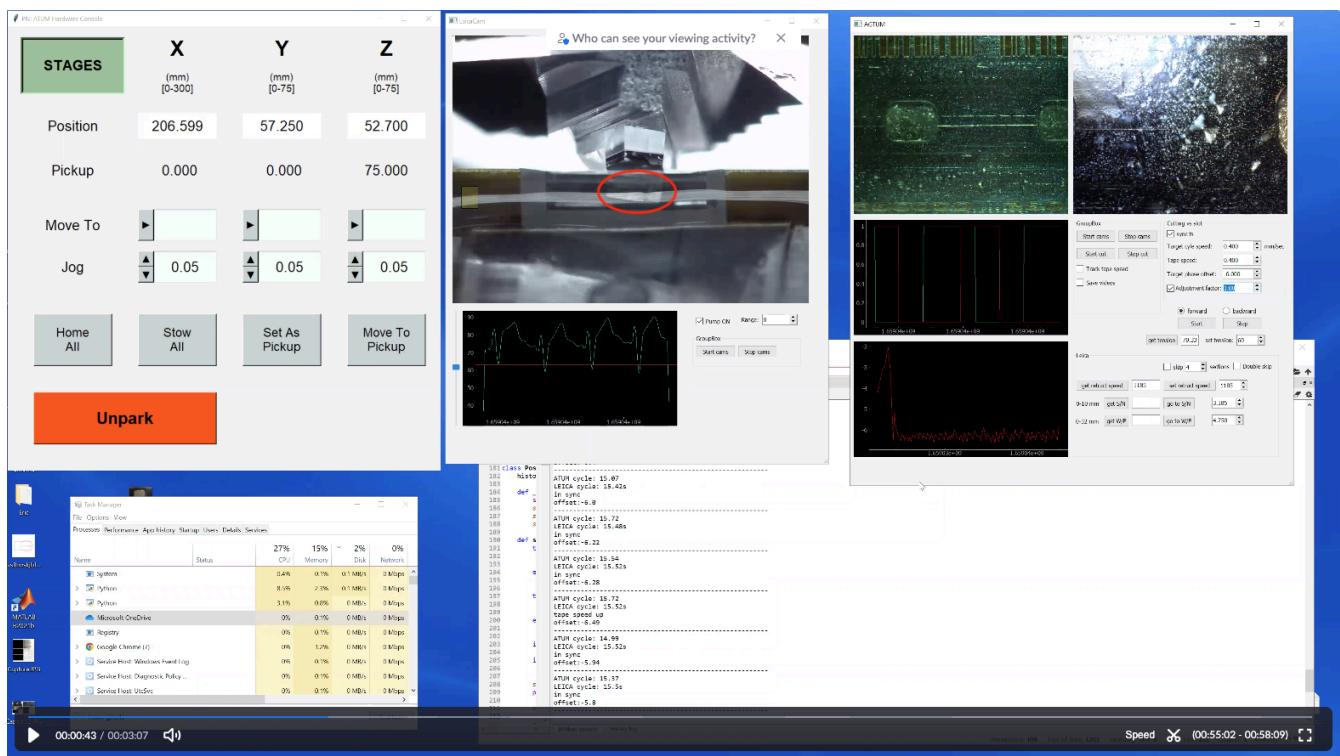
Good Section Placement:



- Notes: 1. sections coming off the knife are light gray ([interference pattern of thin section](#)), 2. Sections are complete (not cut) and appear free of chatter, 3. sections are hitting the knife at an appropriate location, 4. Water level is good, 5. Water pump is pumping consistently, 6. Synchronization is good.
2. Bad section collection example: Low Water Section Sticking / Cut



Section Cut in Half



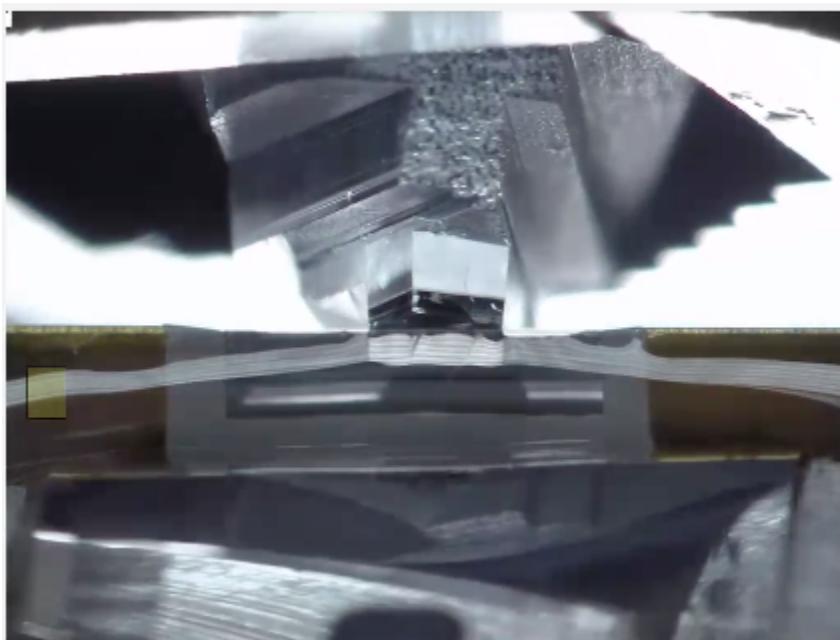
Time: 56:44

- **Problem Level:** High
- **Notes:** The water level has become too low, the section is becoming stuck on the knife mid-cut and / or being cut in half on the following sections.
- **How to prevent:** close monitoring of the water level at all times (the position of the light reflection), changes can occur slowly over time, but usually hit a threshold and then suddenly become a problem. Do not trust the water level monitor. Trust your own eyes.

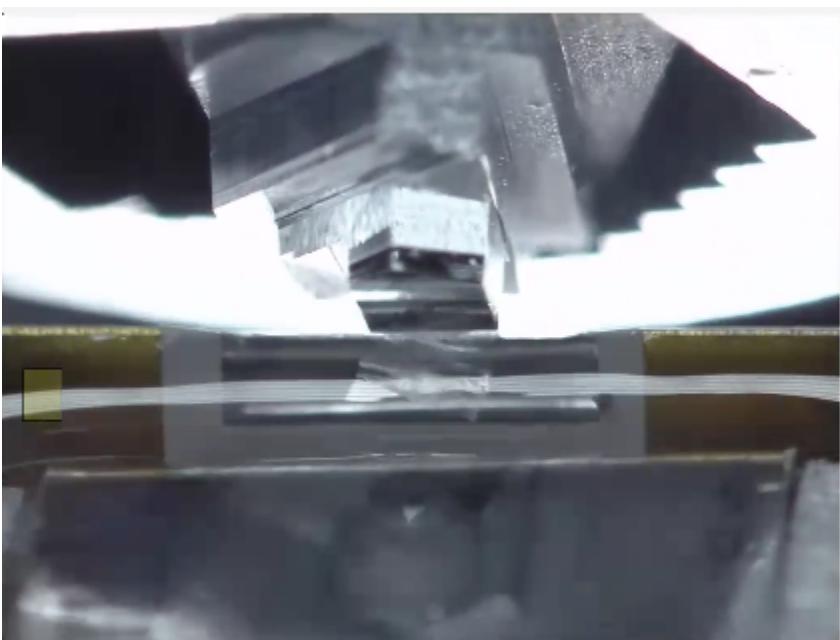
3. **Bad section collection example:** Too much Water

Last Revision: EWH - 2.09.23

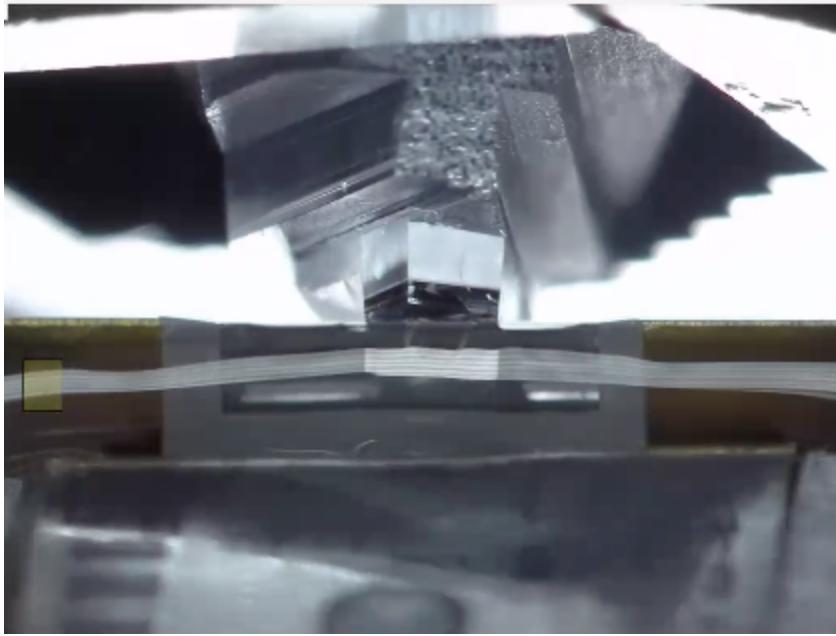
High Water Level:



Low Water Level:



Normal Water Level:



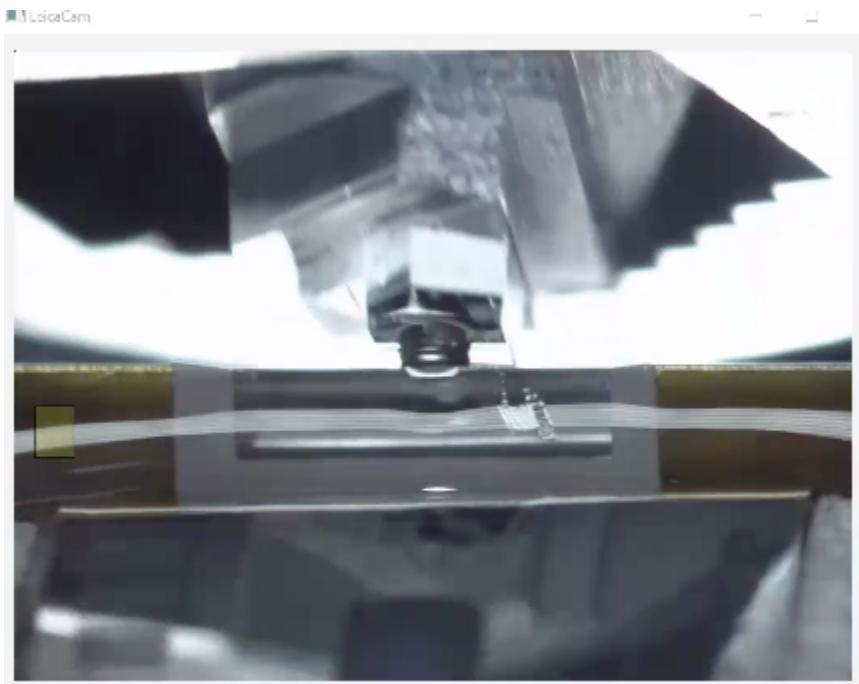
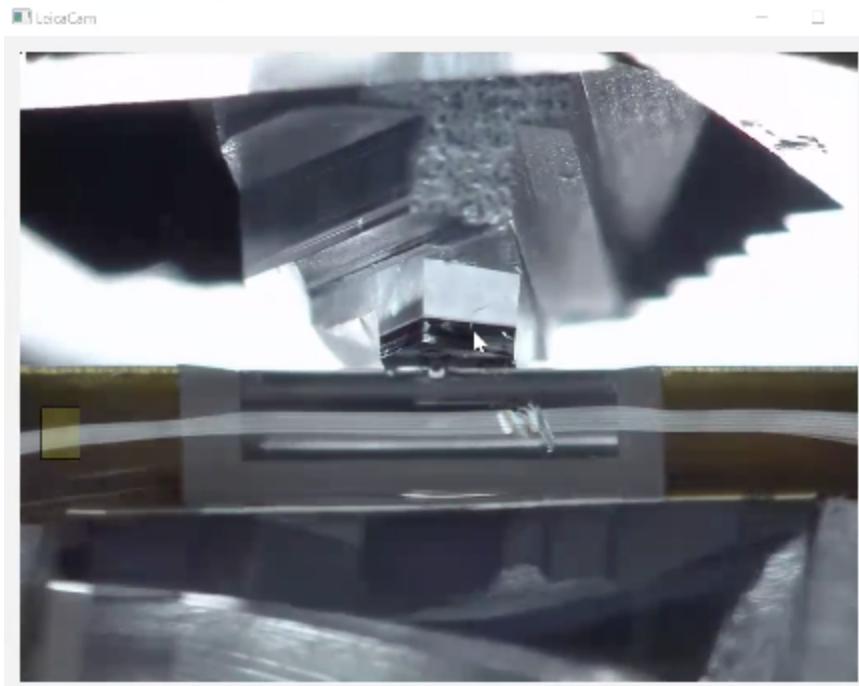
(For future reference, while keeping the yellow box away from the diamond is necessary I would put it closer to the cutting edge to capture the change in water level sooner and over the entire water surface (from knife edge to ATUM nose edge)

- Problem Level: Low
- Notes: The water level is becoming precariously high, it is not a problem yet, but I would go into the room and lower the threshold for pumping water. I would not lower it to the point where it does not pump, but where it pumps less.

4. Bad section collection example: Too much water

Time: 8:00

Water on Wicking onto Block Face:



- Problem Level: High
- Notes: the water level is too high causing the water to wick onto the block. This can prevent sections from being cut, cause uneven cutting, cause sections to sink, cause uneven cutting resulting in continued issues even after the water is back to normal.