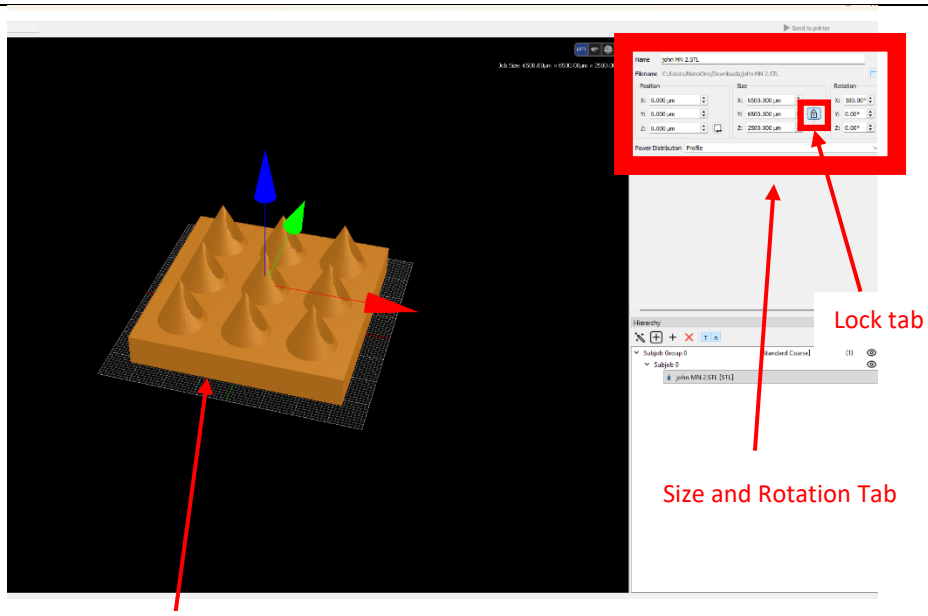
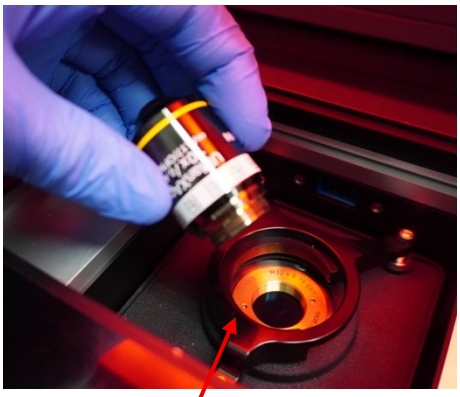
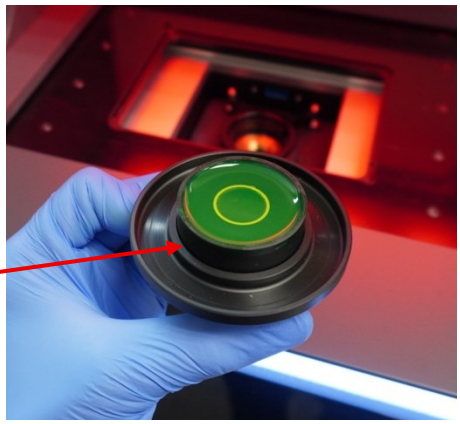
MANUAL OF THE _____**NanoOneBio Instrument from UpNano** _____

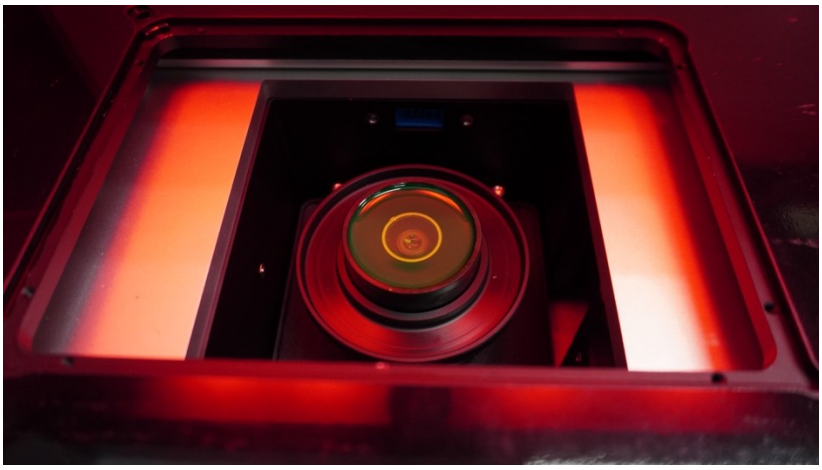
WRITTEN BY	John Nekaine Kwaku Opoku, Md Adil Hossain, and AKM Ahsanul Habib
PERSON IN CHARGE OF THE PROCEDURE	Dr. AKM Sawar Inam
VERIFIED BY	Dr. AKM Sawar Inam
APPROVED BY	HEAD OF THE LABORATORY: Prof. Dr. Shawana Tabassum

PRECAUTIONARY STEPS

DESCRIPTION
Always wear gloves when operating this machine.
Always exercise caution when assembling the components.
Clean the vet properly
Always be careful while installing the objective

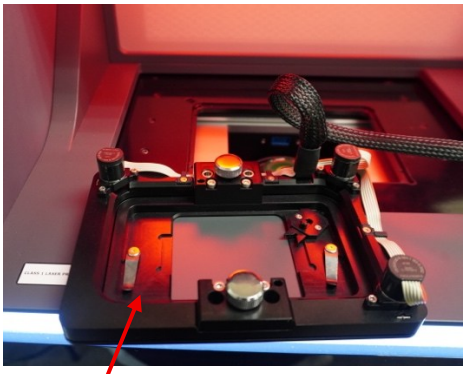
Printing Glass Substrate Manual

ACTIVITY	VISUAL DEMONSTRATION
1. Launch Think3d software. Ensure everything is correct under the **Job Settings** tab (Objective type, Substrate Type and Size, etc.). 2. Drag and drop the .STL file model into the workspace, and orient it upright using the X, Y, and Z controls in the **Size and Rotation tab.** 3. Ensure all dimensions of the design are correct, then click on the lock tab to lock dimensions. 4. Click on the 'Printer' tab, and choose the correct objective (UplanXApo 10x/0.40) from the dropdown menu.	
5. Carefully place the objective into its designated locking system inside the build room until it is fully seated.	 
6. Select the VAT (Air) containing the resin (e.g., UpPhoto) and place it carefully in the build room on top of the objective. Ensure that the magnetic lock engages securely.	

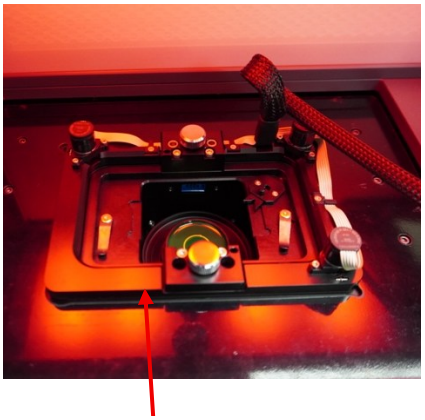


(VAT mounted into build room)

7. Fix the tilt frame/ stage insert tightly into the build room.
8. Reference the stage – Go to **Settings** and click **Reference Stage** from the dropdown menu. Wait for the process to complete.



Tilt frame (can be detached from the build room)

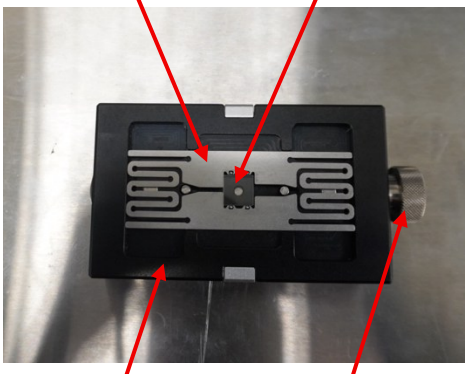


Tilt frame inserted tightly into build room

9. Mount substrate holder onto the mounting block. Place the glass substrate onto the substrate holder. Ensure that it is tightly connected to the substrate holder using the knob.
10. When the glass is tightly connected, detach the holder from the mounting block by unscrewing the knob.
11. Use a small air pump to remove any dirt or debris from the glass substrate.

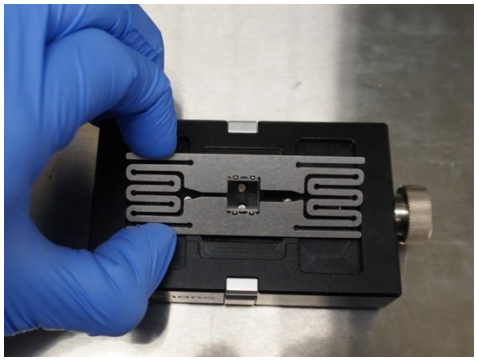
Substrate Holder

Glass Substrate



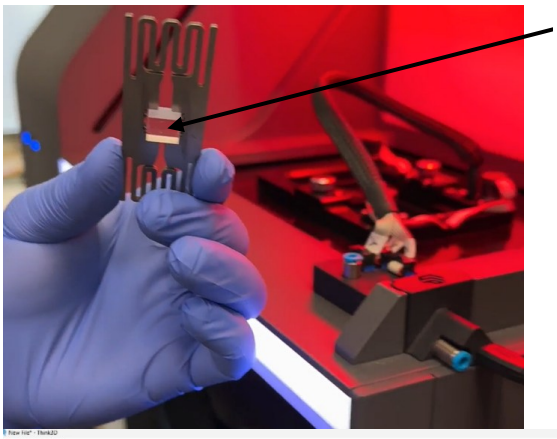
Mounting block

Knob



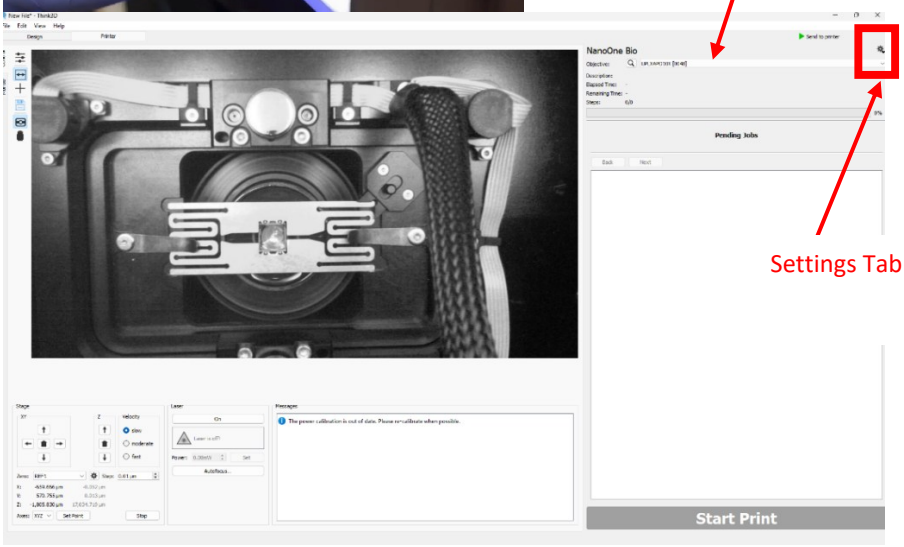
(Detach holder from the mounting block)

12. Install the substrate, secured in the holder, onto the build stage, ensuring that the printing surface of the glass substrate faces downward.
13. Also, make sure you don't move the build stage while doing this else you may need to reference the stage again.

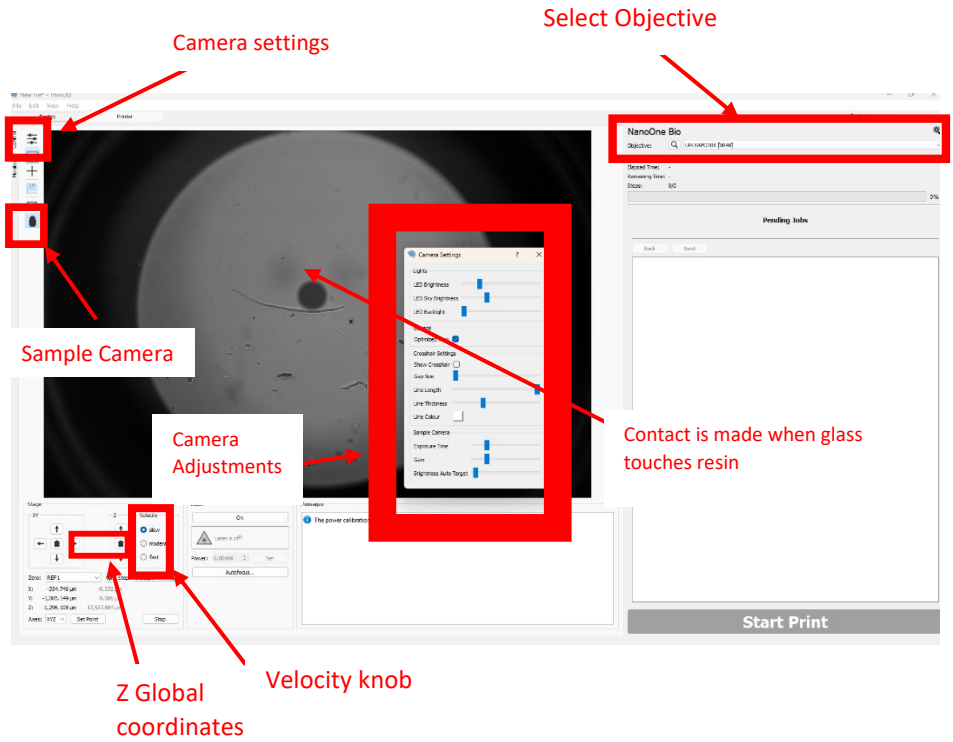


14. Verify on the computer screen that the correct objective has been selected.
15. Close the build room.
16. On the computer screen, select the Objective being used.

NB: *If you see a warning signal on the Messages bar that "One or more of the motors for tilt correction has exceeded it limits", click Tilt, Manual Tab, and Go to Zero*.

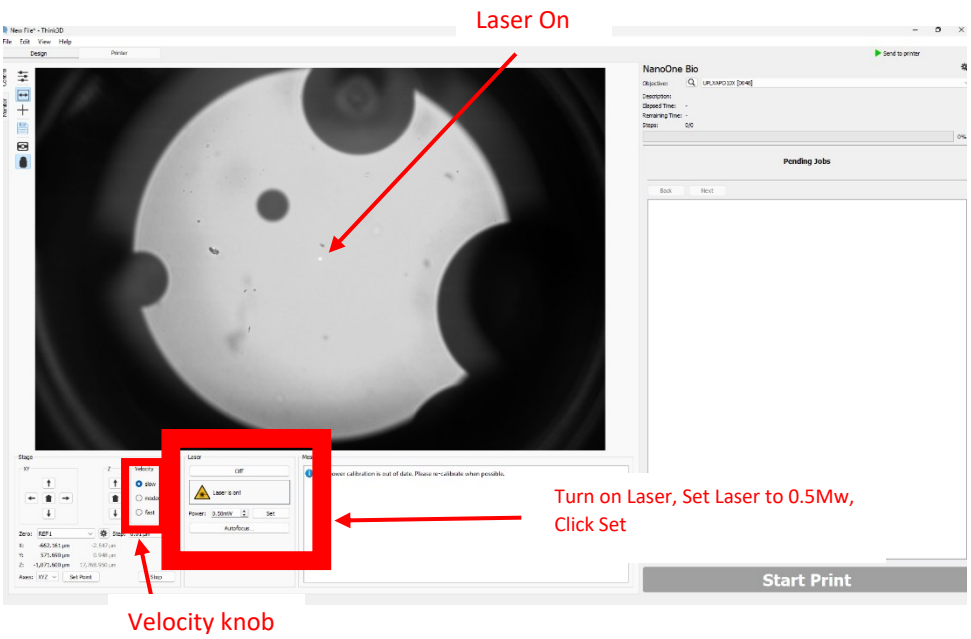


17. Adjust the substrate position relative to the objective by closing the gap between the Z-axis and the stage at a **moderate** velocity. The objective typically makes contact with the substrate when the **Z Global coordinate** is slightly above **16,000 µm.**
18. At a Z position of 16,000 µm, switch to a Slow velocity and continue increasing Z **until contact between the substrate and the objective is observed** (you will notice visually that the glass has touched the liquid resin).
19. Immediately after contact has been established, navigate to the **Sample Camera** and adjust **Camera Settings** by

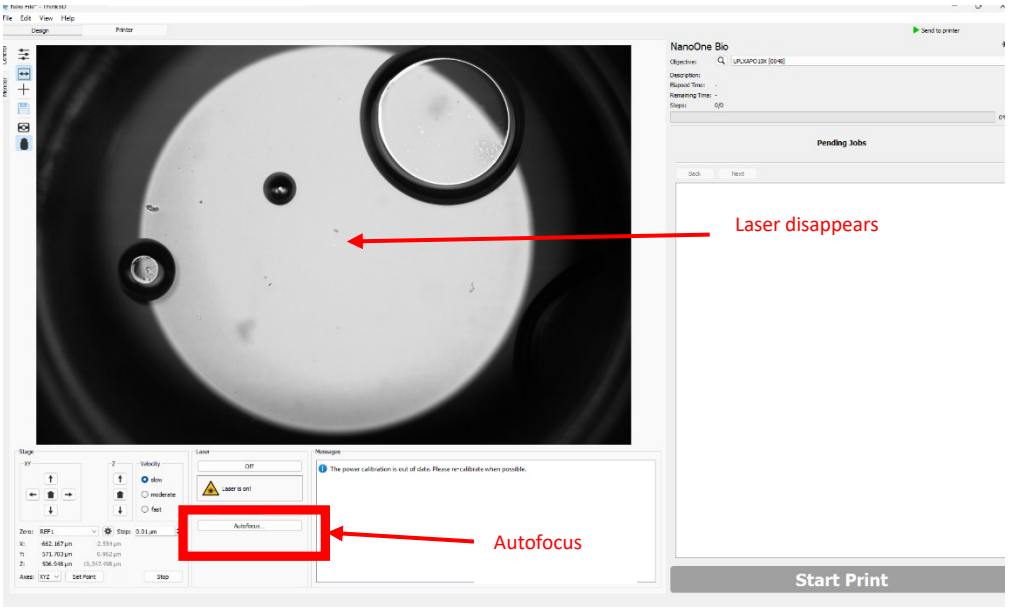


increasing the exposure time, gain, and LED brightness.

20. Turn on the laser and allow 15–20 seconds for warm-up. Set the laser power to **0.5 mW** and click **Set.** The laser (faint white light) will show in the middle of the substrate on the computer screen.

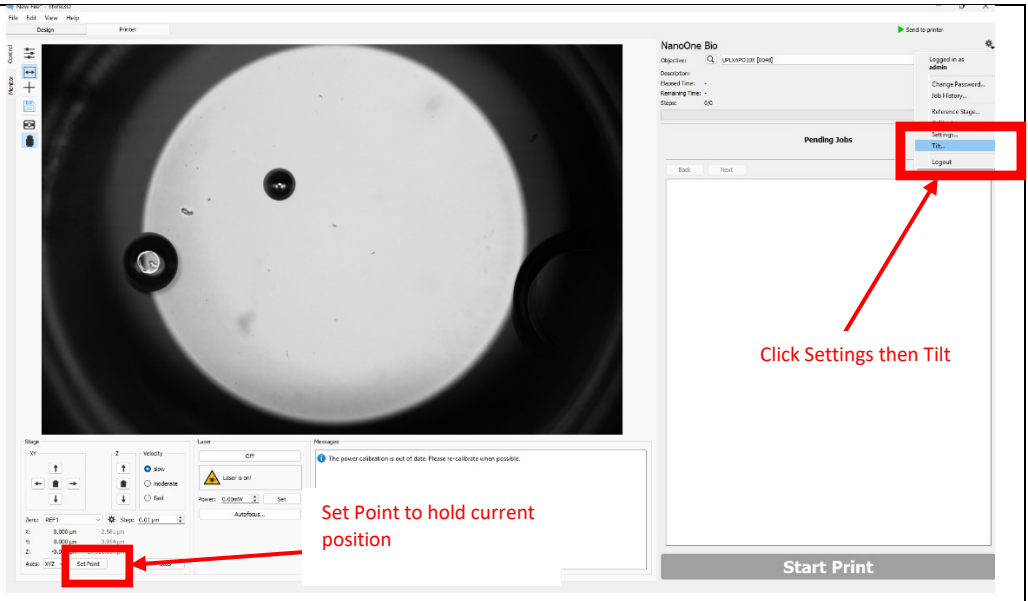


21. While operating in *Slow* velocity, gradually increase the velocity until the **laser spot disappears**.
22. Click **Autofocus** and verify that the Material, Profile, and Focus Target settings are correct. Then click Start. The substrate will temporarily disappear and reappear, indicating that the autofocus process has completed successfully.



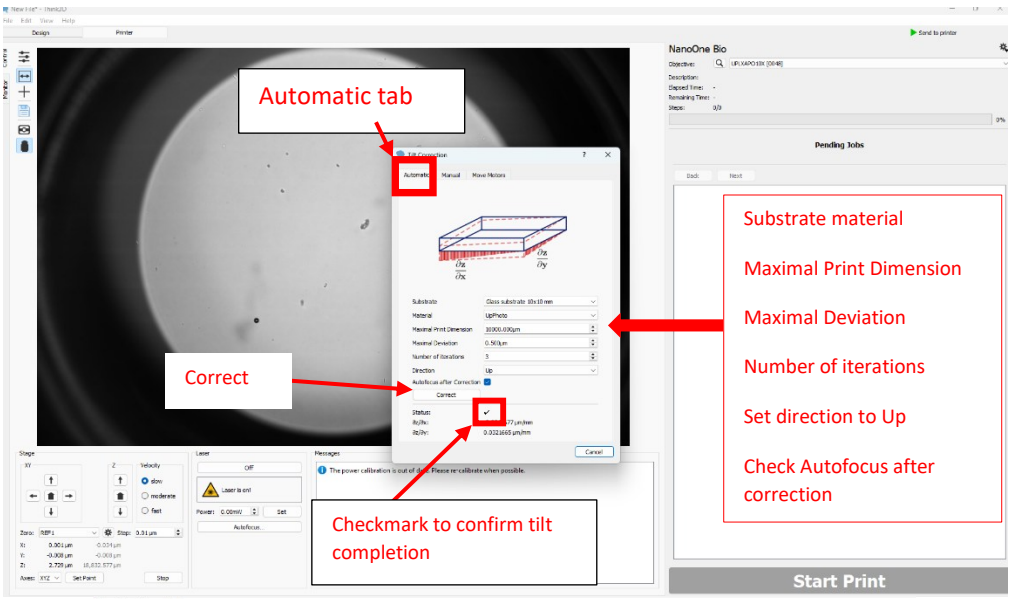
23. Close tab and click **Set Point** to hold the current position.
24. Click Settings button and select **Tilt** from the dropdown menu.

There are 2 types of Tilt Correction: Manual and Automatic Correction. You can do either one of them.



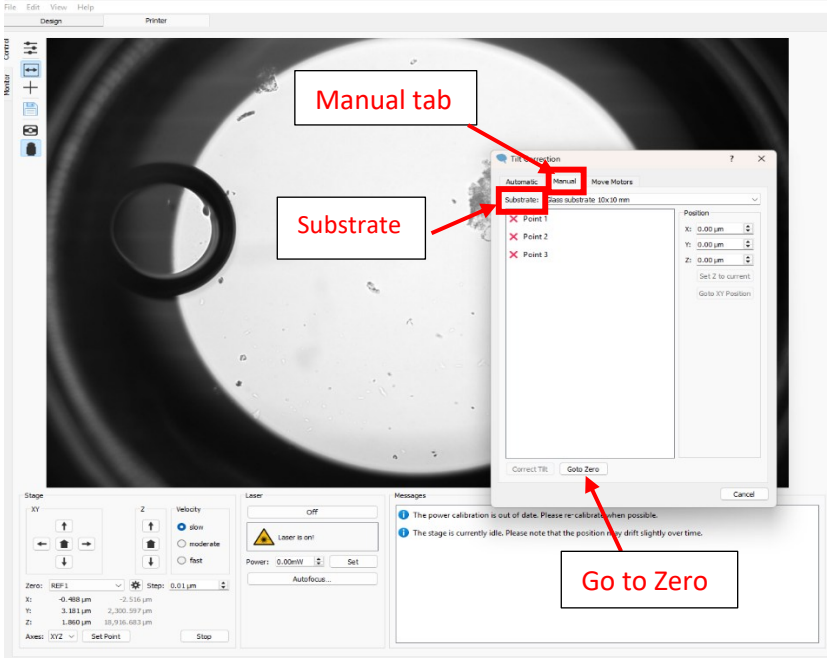
For Automatic Correction:

25. Click the Automatic tab on the Tilt Correction pop-up menu.
26. Select substrate material and set Maximal Print Dimension (size of substrate) and Maximal Deviation (0.5µm)
27. Set Number of iterations to 3 and Direction to Up, then check **Autofocus after correction**
28. Click Correct.
29. Check for the checkmark on **Status** to confirm whether the tilt correction has successfully completed.



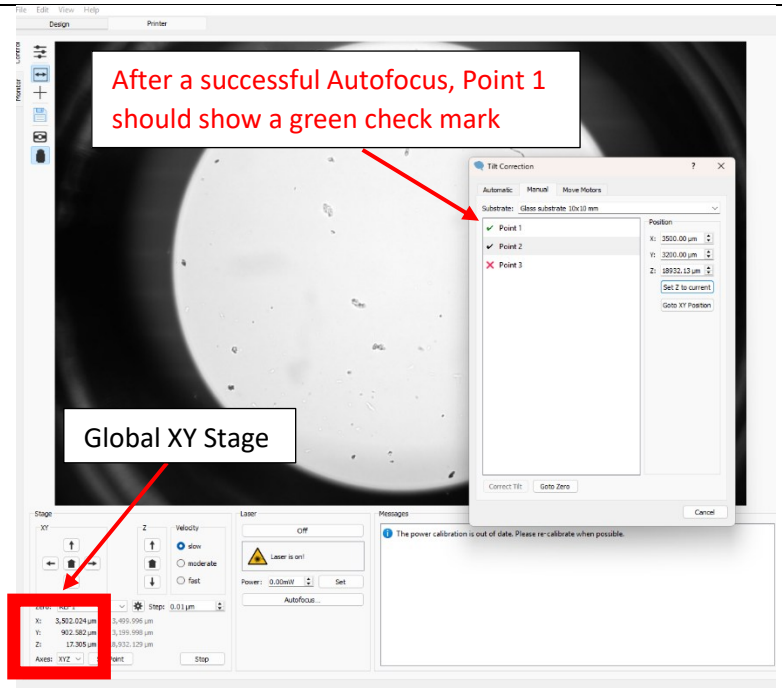
For Manual Correction:

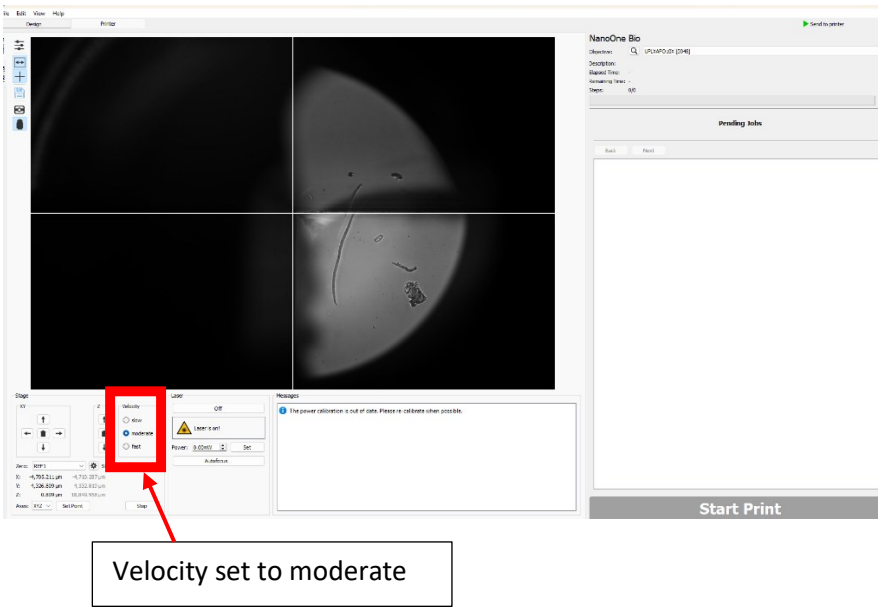
- Click the Manual tab on the Tilt Correction pop-up menu.
- Select your substrate type from the drop-down menu next to “Substrate:”
- This should then generate 3 points (Point 1, Point 2, Point 3) where you will have to autofocus at.
- Click Goto Zero and select Yes to zeroing of motors.



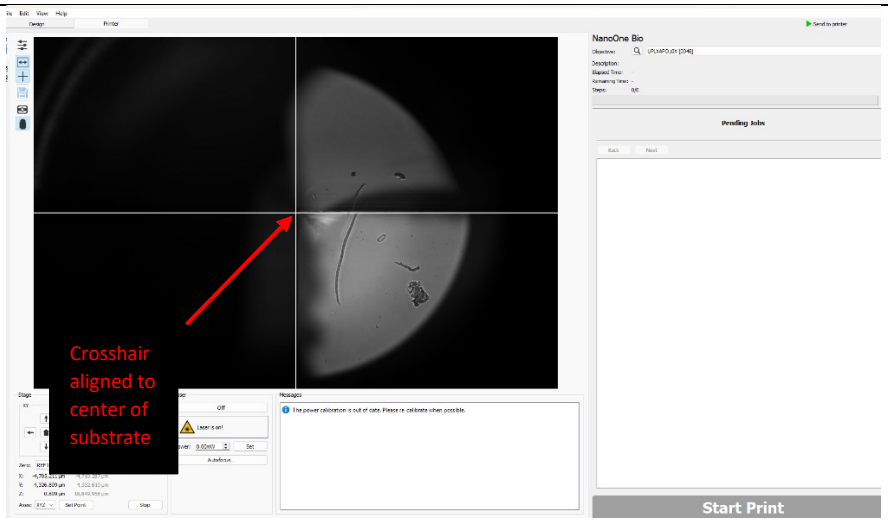
- Click on Point 1, it should highlight. You will see the XY values on the right. Then click “Goto XY Position”. The stage will move to that corner.
- Once the stage stops, click Autofocus. After this is successful, click “Set Z to current.” Point 1 should show a green check mark.
- Repeat this for Point 2 and Point 3.
- When you click on each point, the XY values on the Tilt Correction menu should be the same as the global stage XY values.
- The Z global coordinates for all 3 points should not be more than 100µm apart from one another.
- Once all 3 points are checked, click “Correct Tilt.” If tilt is successful, there will be no warning signal at the messages bar.
- Be alert to see if there are any warning messages on the Messages bar. If there are any messages regarding the motors of the tilt correction, then the tilt correction was not successful, and you need to troubleshoot before proceeding.

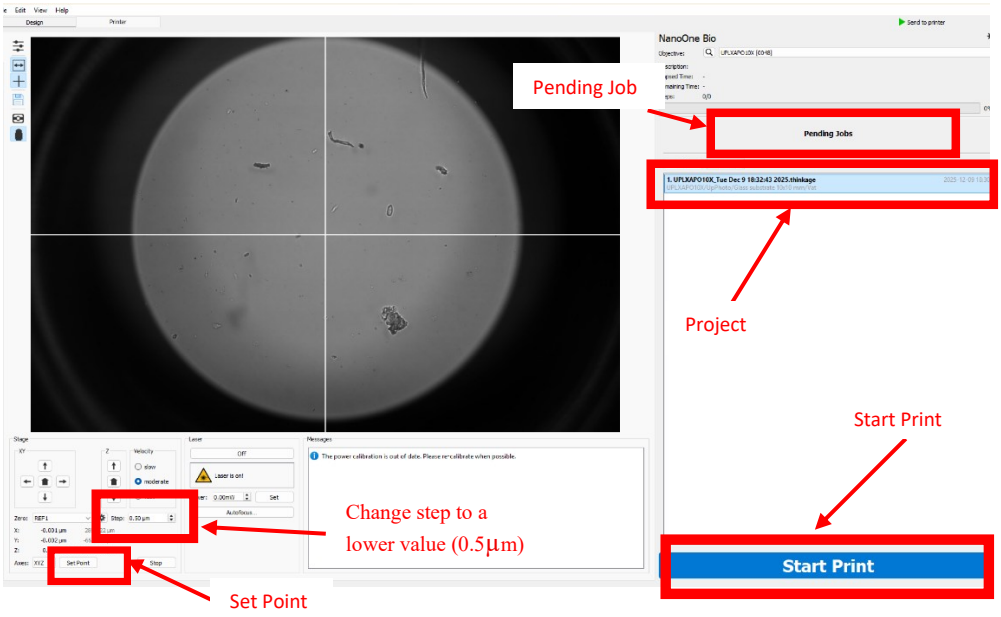
30. After tilt has completed successfully, Set the velocity to moderate. Using the arrow keys, move to the edge of the substrate (left top corner) and then you need find the center of the substrate.

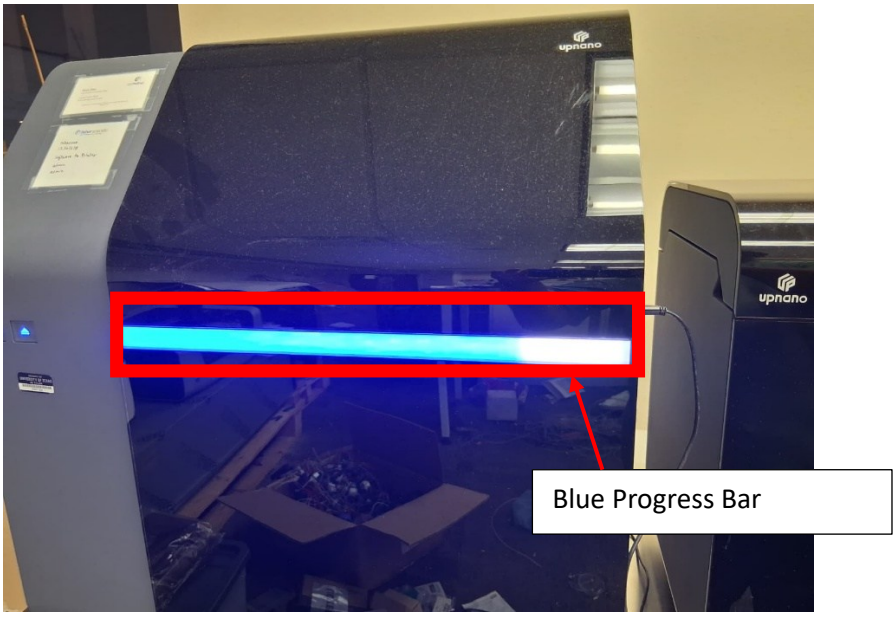




31. After finding the top left corner of the substrate and aligning with the Crosshair center in Sample Camera, Click on Step and insert half the length of substrate (5000µm) then move right side. To do that Use moderate velocity, click Shift + left direction and then using same step (5000 µm), click Shift + down direction. This will take you to the center of the substrate.
32. Change step to a lower value (0.5µm)
33. Do another Autofocus then click **Set Point** after it has completed. This is the origin (0,0).
34. Select **Send to Printer.**
35. Return to the Printer tab. Under pending jobs, click on your project and click Check. This is to ensure the printing will only happen on the substrate, not outside of it.
36. Click on **Start Print.** Now, printing has begun.
37. The printer displays a blue progress bar to show the printing progress. A full bar signifies that printing is complete.
38. Once printing is complete, increase the Z in the global coordinates, open the build room, remove the frame and the glass substrate holder. Your printed design will be on top of the glass substrate.
39. Remove the VAT and the objective, place them back carefully in their respective safes and close the build room.
40. Now the design is ready for cleaning.

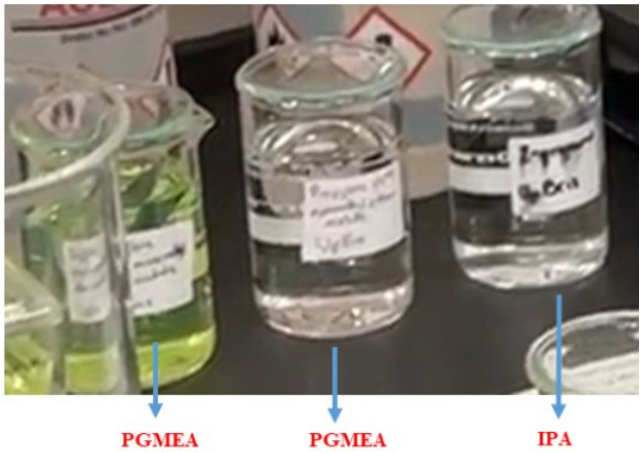




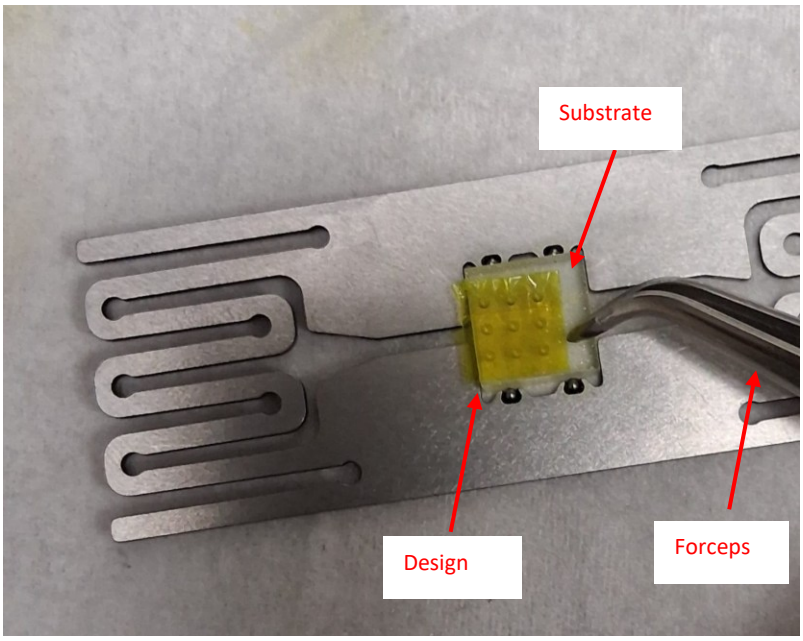


POST-PRINTING CLEANING PROCESS

41. Clean three beakers.
42. Fill two beakers with PGMEA (Propylene Glycol Monomethyl Ether Acetate) and one with IPA (Isopropanol).
43. Put the substrate with the design in the 1st beaker with PGMEA for 30 minutes and in the 2nd beaker with PGMEA for another 30 minutes.
44. Lastly, another 30 minutes in the 3rd beaker of IPA.



45. Finally, using the forceps, with a gentle push separate the design from the substrate.
46. For the UpPhoto resin, instead of PGMEA, three beakers of IPA can be used, and each bath will be for 30 minutes.
47. For UpBricks resin, 1st two baths should be of PGMEA
48. Pour the remaining resin in the IPA and PGMEA into a storage bottle. (This can be reused after purifying)
49. Soak the tissue in IPA and clean the whole surface of the vet in one go.
50. Then, carefully clean the lens area of the Objective without scratching it.
51. After that, clean the outer area of the Objective thoroughly with the IPA-soaked tissue.



SILANIZATION PROCESS

Normally this step is needed for chemical bridge between two fundamentally incompatible materials: an inorganic substrate (like glass) and an organic material (polymer). Best practice is to do silanization before printing.

52. Silanization solution: Take 50 mL of DI water in a beaker and start magnetic stirring. Then put 48 mL of Ethanol in the DI water. After that, put 300 µL of acetic acid. Finally, put 2000 µL of silane (3-(Trimethoxysilyl)propyl methacrylate) drop by drop in the solution and stir the solution for 30-35 minutes.
53. Properly clean all the glass substrate using tissue paper.
54. Then put all the glass substrates inside the Glass box.
55. Finally, pour the silanization solution into the glass box and close it.
56. After 30 minutes, dispose of the solution by pouring into a waste collecting bottle and clean the substrates using acetone one by one.