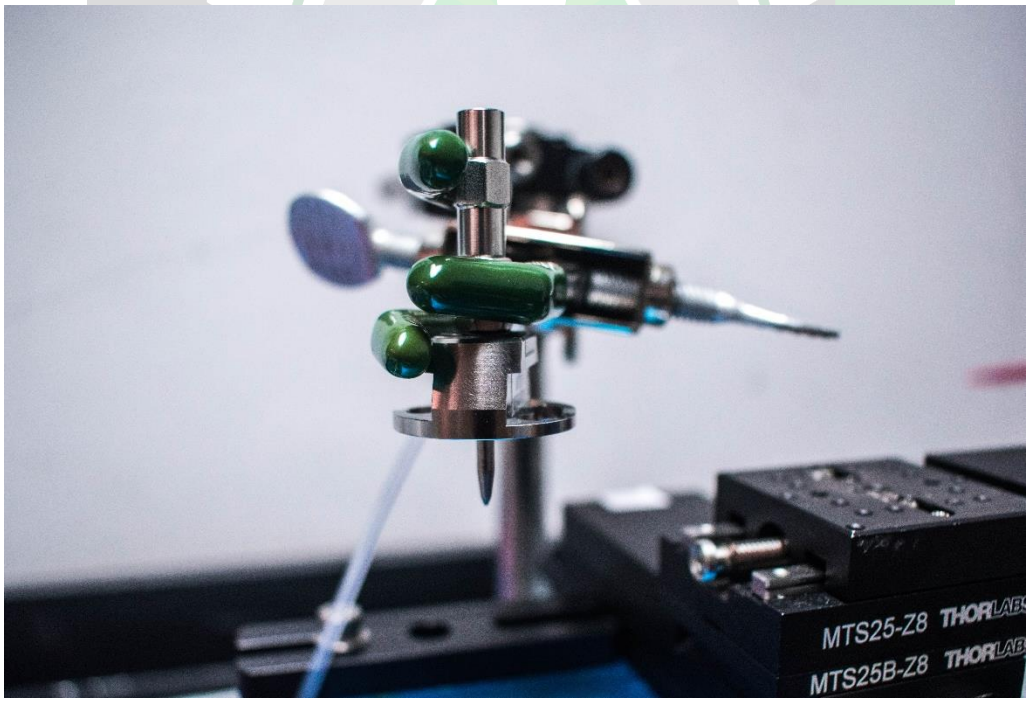
Depositing Sample using the ESI Needle Spray Deposition Technique



Created by: Marcos A. Barreto

Manager: Samuel Hernandez-Rivera

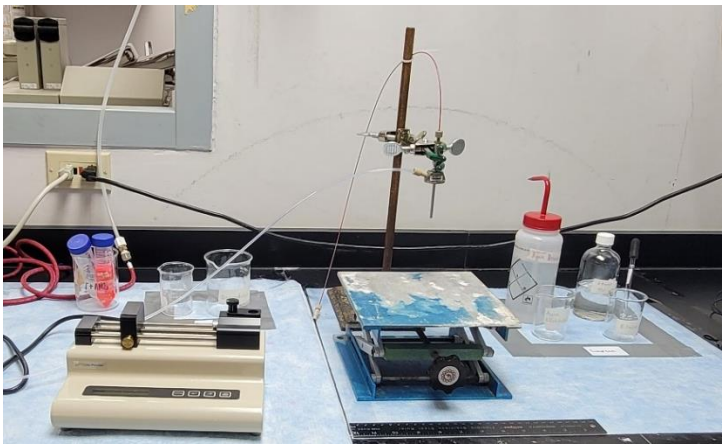
SOP-01	Marcos A. Barreto Naihomy Tirado Tatiana Vega Tatiana Serrano Steven Palmer Maria Villarreal Edwin Caballero		University of Puerto Rico at Mayagüez
Effectivity: March/23/2022	Depositing Sample using the ESI Needle Spray Deposition Technique		Revised by:
Revised:			Approved by:

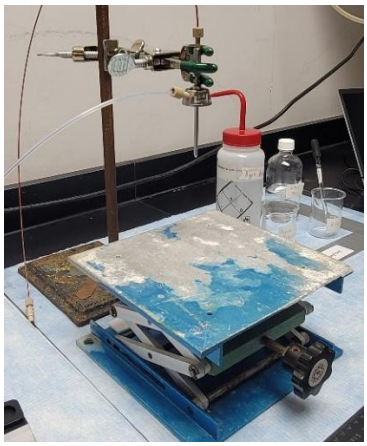
This SOP uses the following:

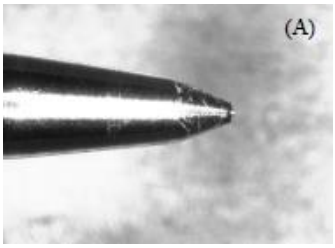
Personal Protective Equipment:
- Safety Glasses
- Gloves

Chemicals:
- Ethanol
- Methanol
- De-ionized Water

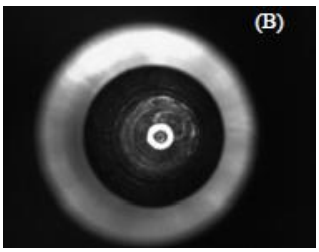
Equipment/Materials:
- Kim Wipes
- Syringe Pump 1-channel, 115-V
- 250, 500 μL gas tight Hamilton glass syringes
- Nebulizer support assembly
- Nitrogen gas (cylinder)
- Infusion PEEK tubing
- Laboratory jack







Closer side view of spray needle

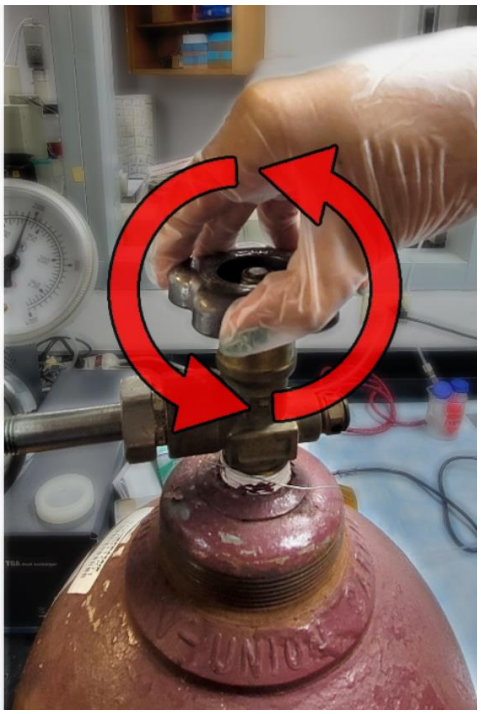


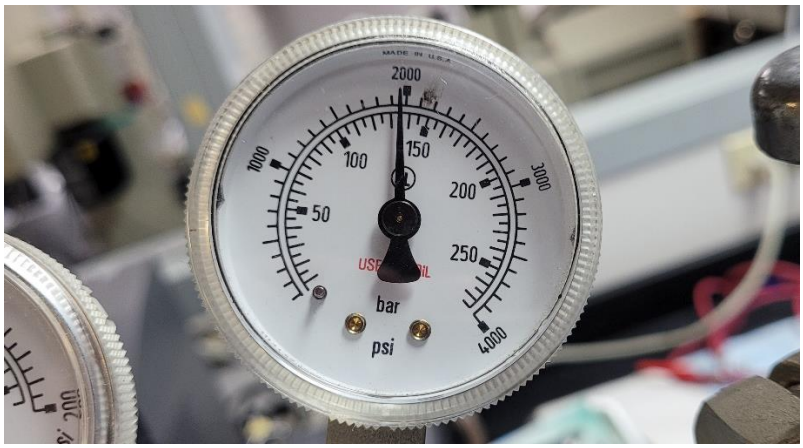
Closer top view of spray needle

WARNING: Exercise caution when handling the ESI needle to avoid pinching your fingers. When needle is not in use, always keep tip capped with plastic cover.

SETTING UP ESI NEEDLE

1. **Open** nitrogen gas cylinder by turning tank pressure valve. Pressure at 2200 psi.



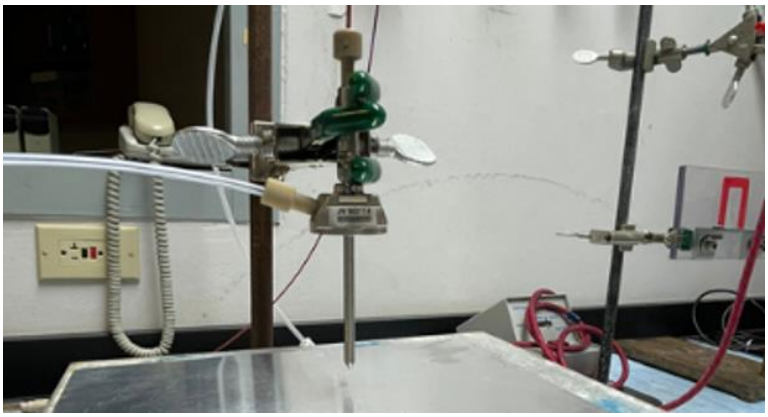


DO NOT USE IF PRESSURE IS BELOW 200 psi

2. **Adjust** nitrogen pressure to 15 psi using the two-stage regulator. Leave the valve of the 2nd gauge closed.

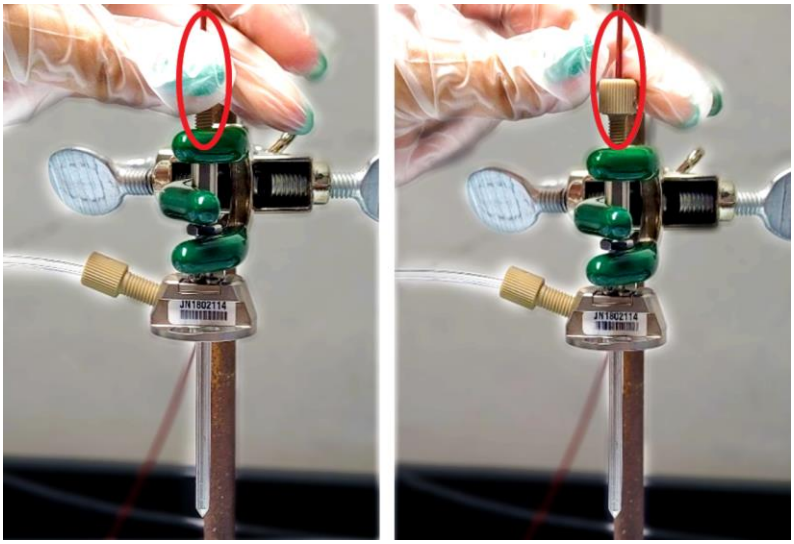
3. **Level** ESI needle adequately to stand using lab clamps.

Use bubble level.

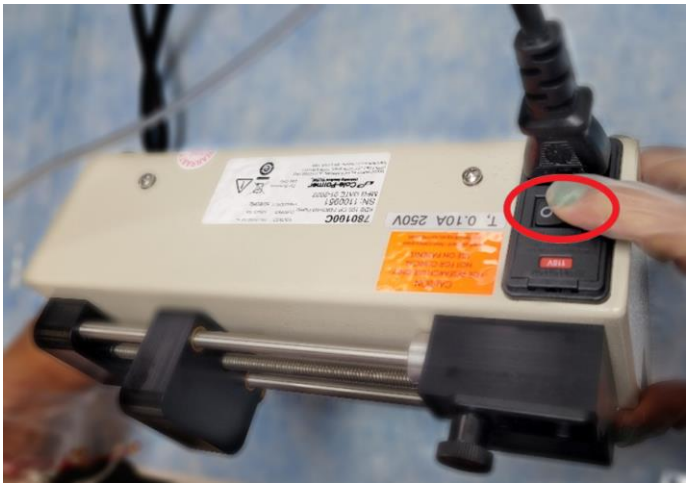


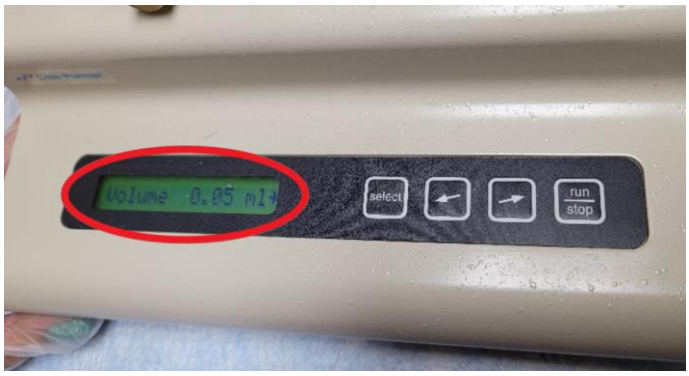
4. **Connect** nebulizing gas to the ESI Needle while holding the needle.

5. **Connect** PEEK infusion tubing through the PEEK super flangeless nut to the ESI needle port. Hand tight the flangeless nut.



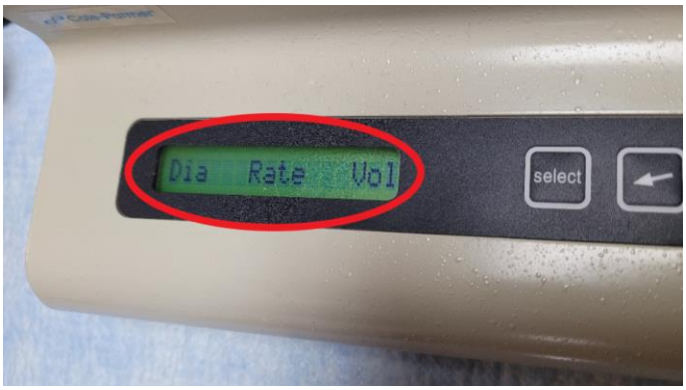
6. **Turn ON** the Cole Palmer infusion pump. ON/OFF button is located at the back side of the pump.



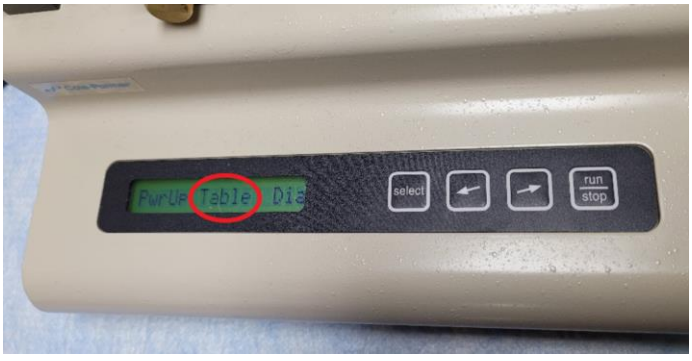


7. **Press** SELECT button on the pump menu.

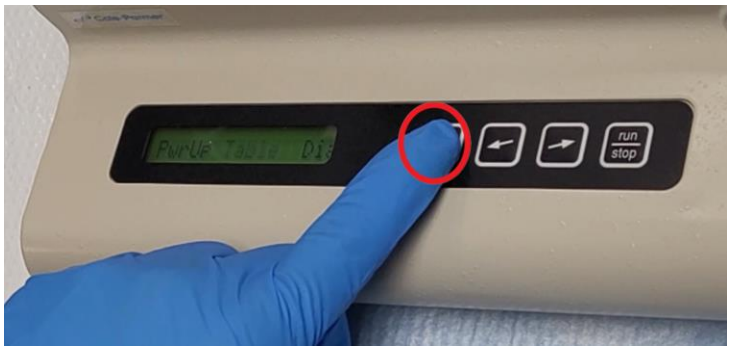
8. **Repeat** step 7 until Dia Rate Vol appear on the screen.



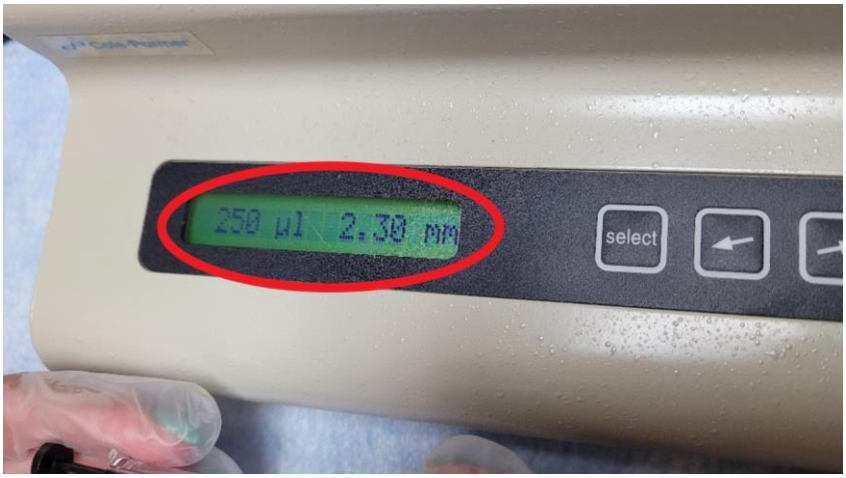
9. **Choose** “Table” by moving with left and right arrows.



10. Press SELECT button on the pump menu.



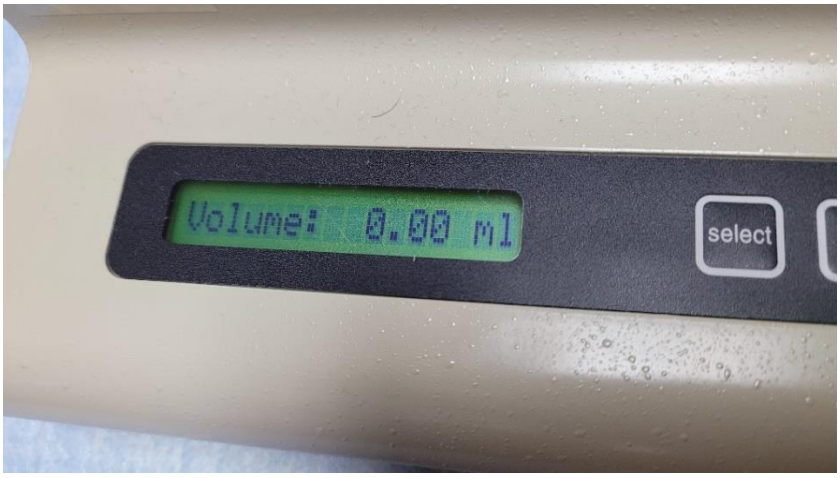
11. Choose syringe volume. Current syringe available in Q-042 is the Hamilton model 250 μL.



12. Press SELECT button on the pump menu.

13. Choose desired volume to transfer per run.

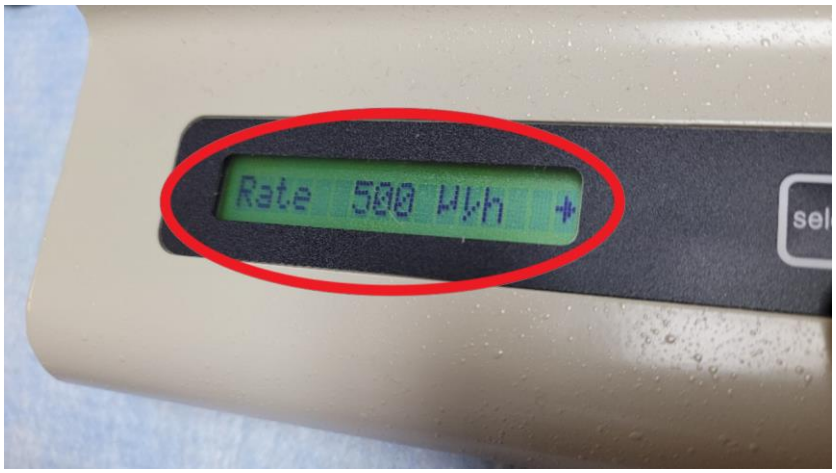
Click left or right arrow until the desired volume is chosen.



OPTIONAL: If 0.00 mL volume is chosen, there is no limit on the amount of volume transferred. User may manipulate manually the pump by pressing the gold button on the pump fixture.

14. Press SELECT button on infusion pump menu.

15. Set flow rate to 500 $\frac{\mu L}{hr}$ (default) by pressing the SELECT button on select flow rate.



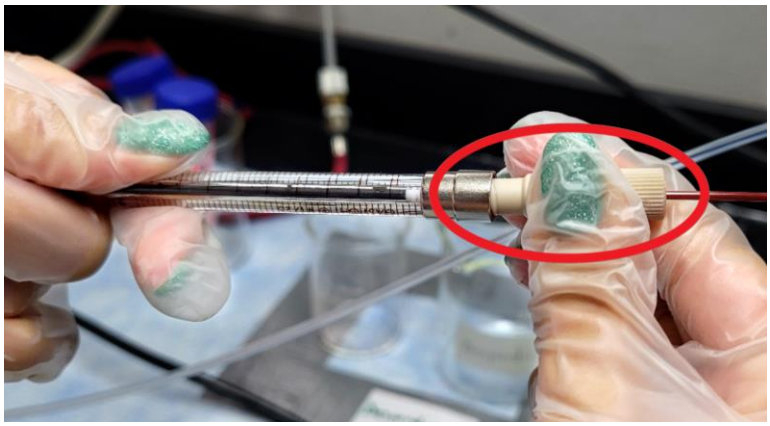
Adjust flow rate depending on the substrate surface.

16. Press SELECT on infusion pump menu.

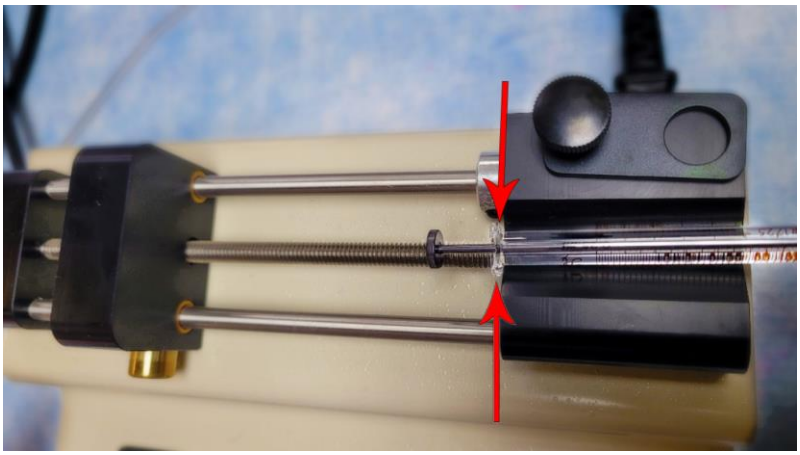
DEPOSITING MATERIAL ON SUBSTRATE

1. **Collect** desired volume of the solution using gas tight syringe.

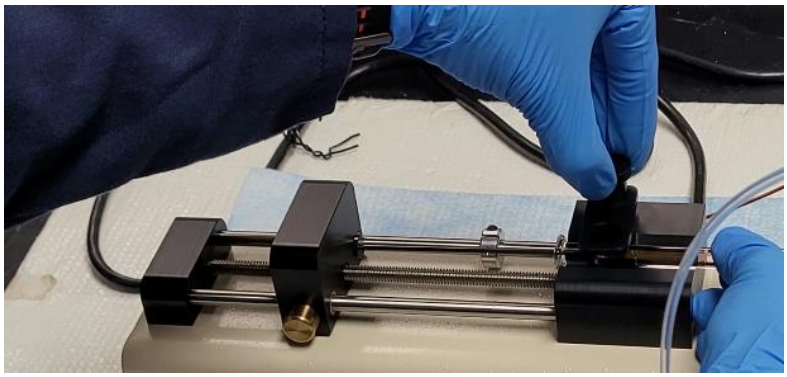
2. **Connect** pump screw.



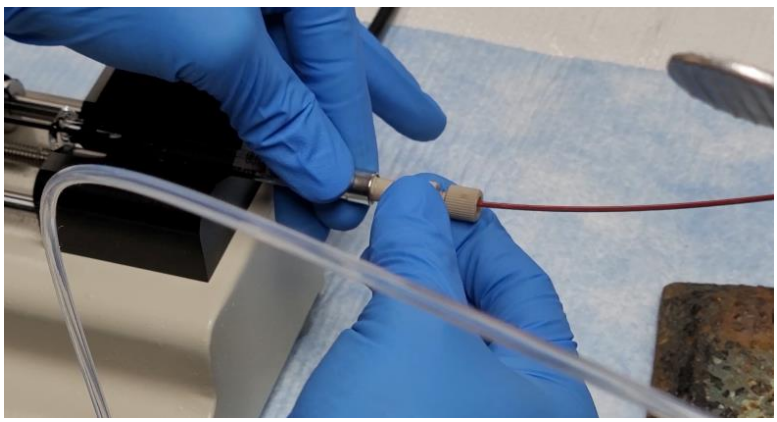
3. **Position** gas tight syringe on the syringe pump.



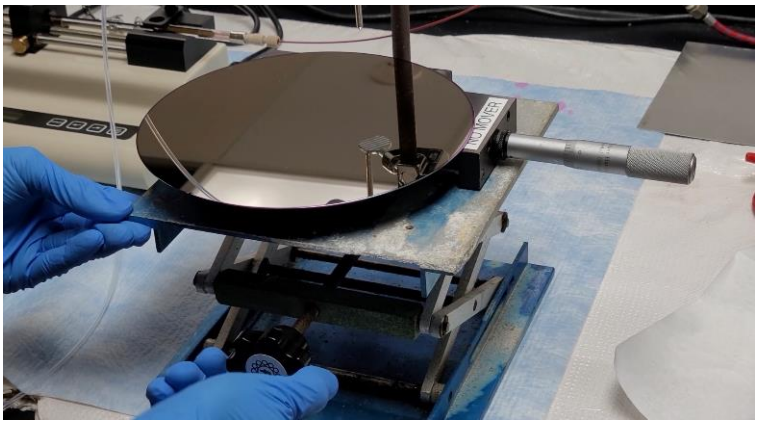
4. **Fix** gas tight syringe using the pump fixture. Adjust pump screw as needed to properly fit the syringe in place.



5. **Connect** syringe to the PEEK infusion tubing via luer lock connections.

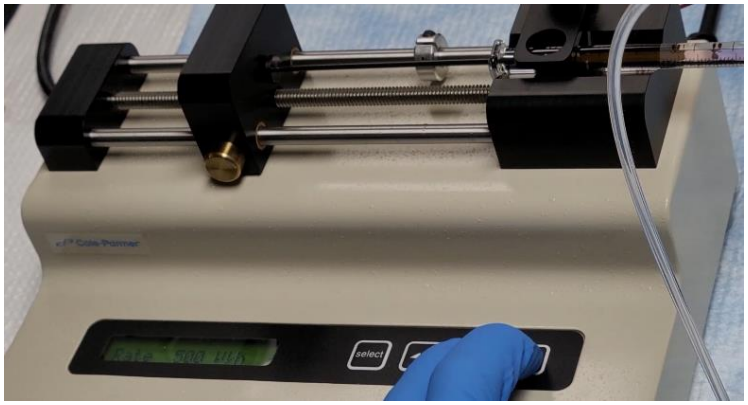


6. **Place** substrate at 2-cm to the tip of the ESI needle **using a lab jack if necessary**.

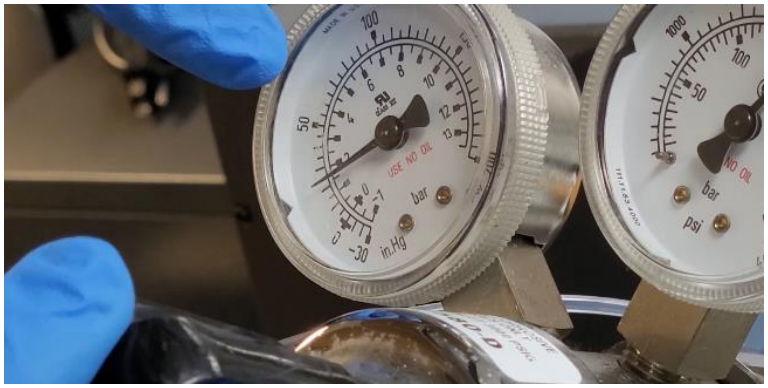


DO NOT TOUCH THE NEEDLE!!!!!!

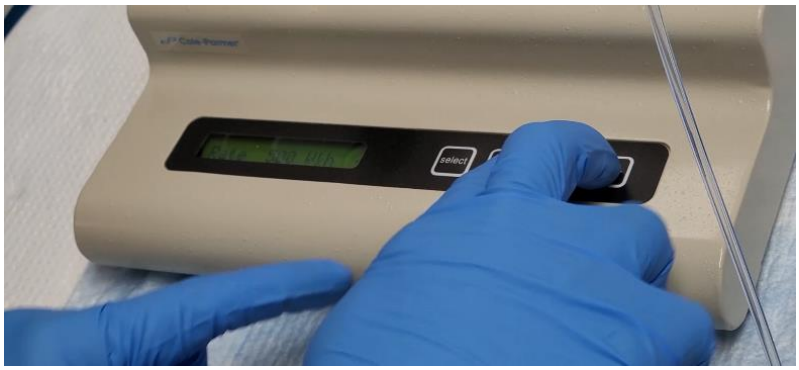
7. **Fill** PEEK tubing by holding down the right arrow (→) and RUN/STOP button. The fast forward action is enabled. Release buttons when infusion tubing is filled with the solution.



8. **Open** outlet valve of the nitrogen cylinder (15 psi).



9. **Infuse** material by pressing RUN/STOP button. The material is ready to be dispensed on the substrate.



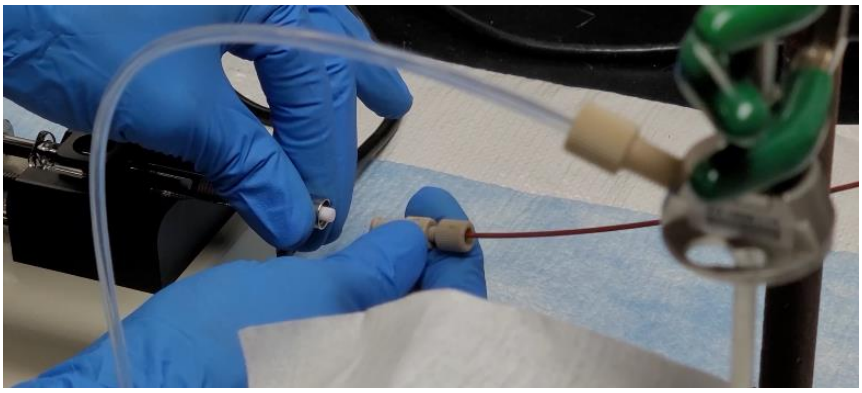
10. **Determine** amount of deposit by using a timer recording the elapsed time between start and finish stage of deposition.

11. **Stop** deposition when completed by simultaneously pressing the RUN/STOP button and closing the valve of the two-stage regulator.

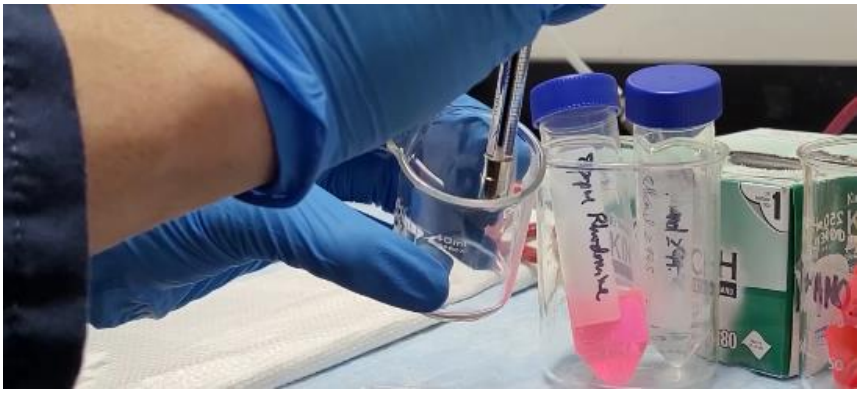
12. Remove substrate and store in a suitable container (e.g., petri dish).

POST-DEPOSITION CLEAN UP

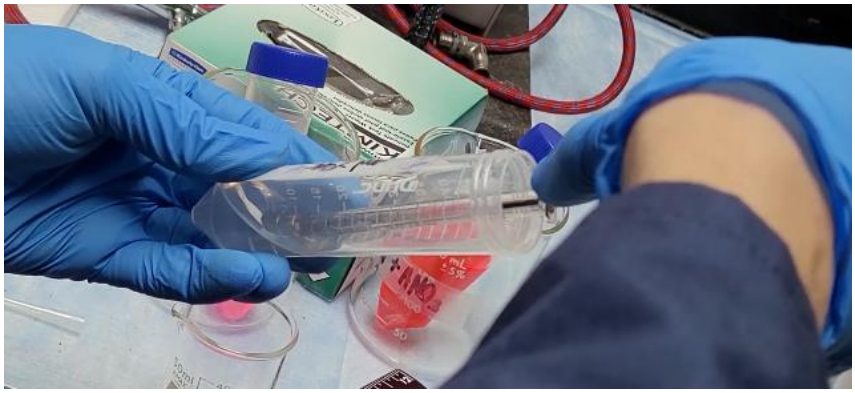
1. **Disconnect** syringe from the infusion tubing.



2. **Discard** residual material inside syringe.



3. **Wash** syringe with water four (4) times.



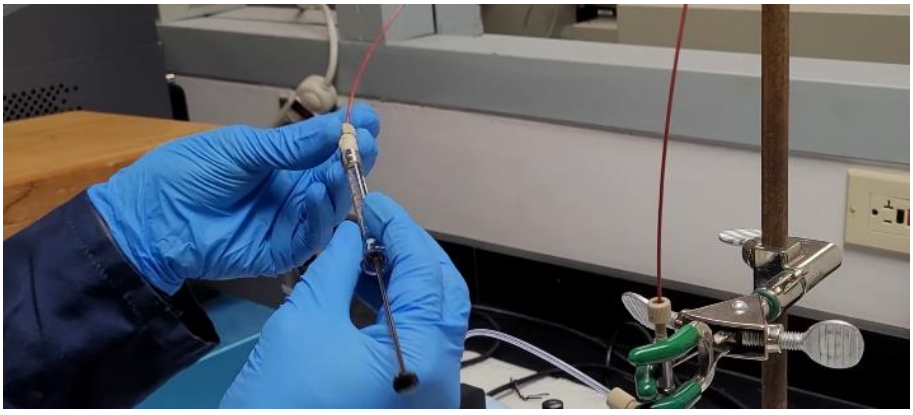
4. **Wash** syringe with ethanol four (4) times.

5. **Clean** infusion tubing with ethanol using Pasteur pipette.

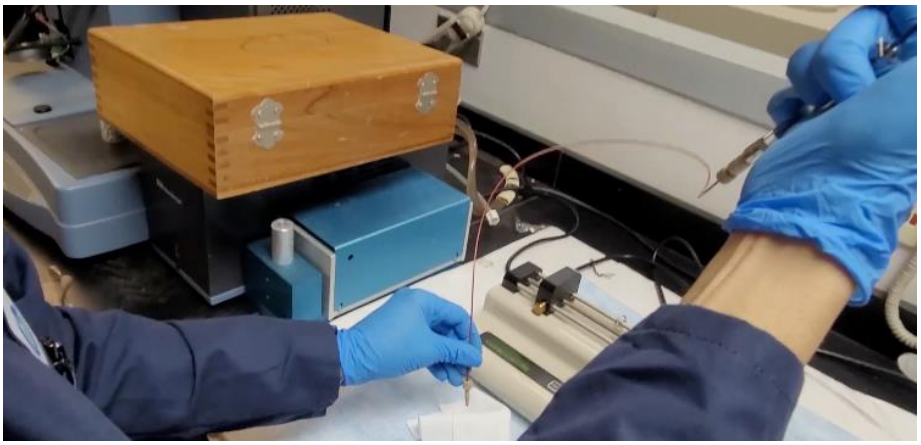
6. **Clean** extremely gently the needle tip with ethanol with Kleen wipes.

DO NOT THE NEEDLE WITH HANDS!!!!!

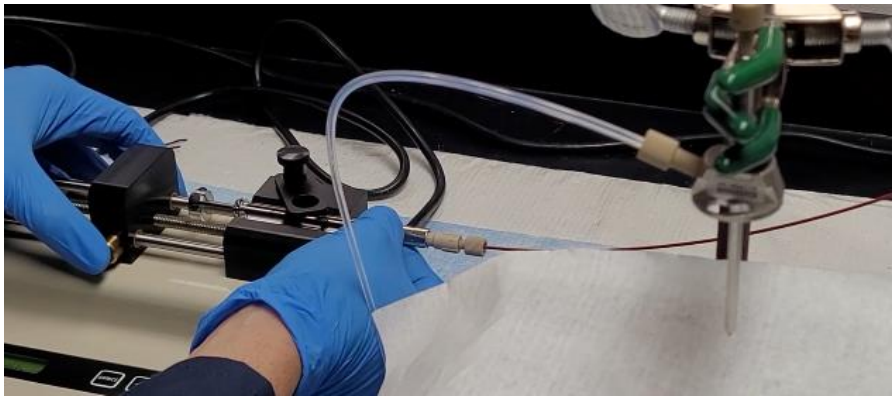
7. **Connect** syringe to the infusion tubing.



8. **Rinse** tubing with 250 μL of ethanol.



9. **Place** syringe with ethanol on infusion pump and deposit ethanol on a Kim wipe to clean the ESI needle.



10. **Close** main valve of the nitrogen cylinder.

11. **Clean** areas and dispose materials following established laboratory practices.

12. Power off the pump.

VERIFYING ESI SPRAY SYSTEM PERFORMANCE

1. **Weigh** 0.02919 g of Rhodamine 6G/640.

2. **Place** Rhodamine 6G/640 into a 50-mL PP vial.

3. **Fill** 50-mL PP vial with Ethanol while mixing the solution until the solid completely dissolved.

4. **Collect** 250 μL of marker solution using a Hamilton 250 μL gas tight syringe.

5. **Fix** syringe in place in the syringe pump.

6. **Connect** the ESI needle using an infusion setup (PEEK).

7. **Open** nitrogen gas cylinder.

8. **Adjust** nitrogen pressure to 25 psi using the two-stage regulator. Leave the valve of the 2nd gauge closed.

9. **Place** Kim Wipe at 1-cm to the tip of the ESI needle using a lab jack if necessary.

10. **Press** SELECT on the pump menu.

11. **Choose** the syringe type.

12. **Set** flow rate to 1000 $\frac{\mu L}{hr}$ by pressing the SELECT button on select flow rate.

13. **Fill** PEEK tubing by holding down the right arrow (→) and RUN/STOP button. The fast forward action is enabled. Release buttons when infusion tubing is filled with the solution.

14. **Open** outlet valve of the nitrogen cylinder.

15. **Infuse** material by pressing RUN/STOP button. The material is ready to be dispensed on the substrate.

16. **Determine** amount of deposit by using a timer recording the elapsed time between start and finish stage of deposition.

17. **Stop** deposition until color in Kim Wipe is evident by simultaneously pressing the RUN/STOP button and closing the valve of the two-stage regulator.

18. **Remove** substrate and store in a suitable container (e.g., petri dish).

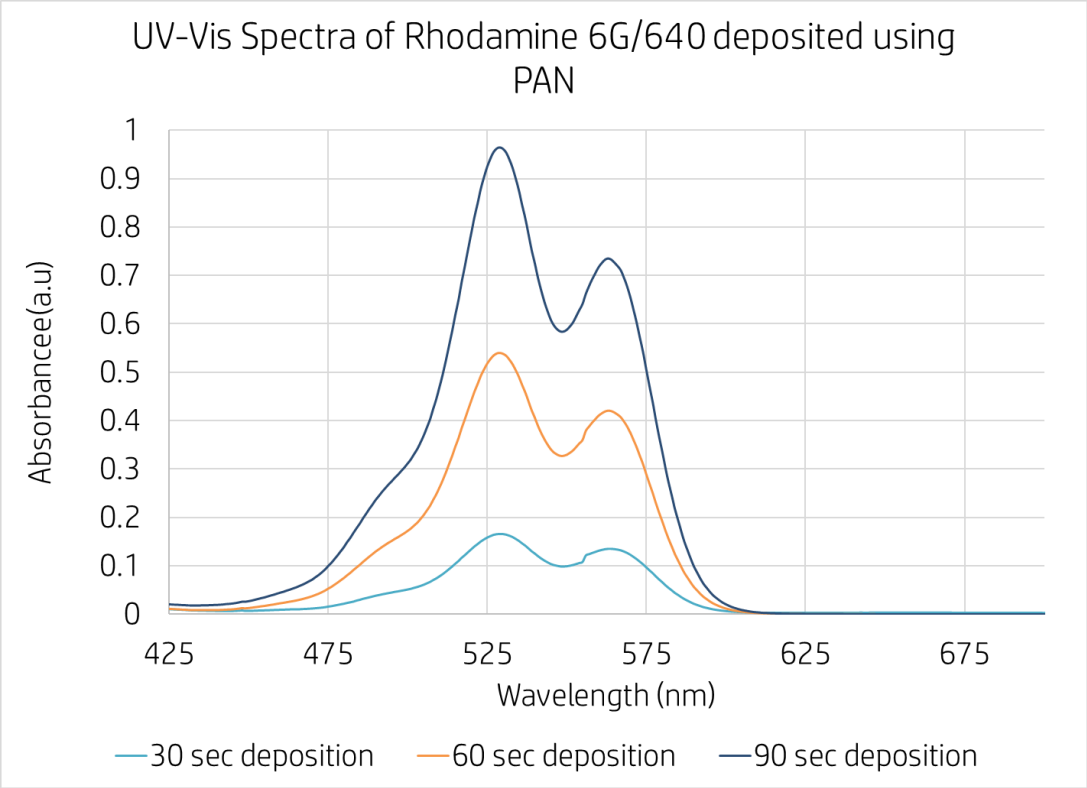
19. **Place** vial at 1-cm to the tip of the ESI needle using a lab jack if necessary.

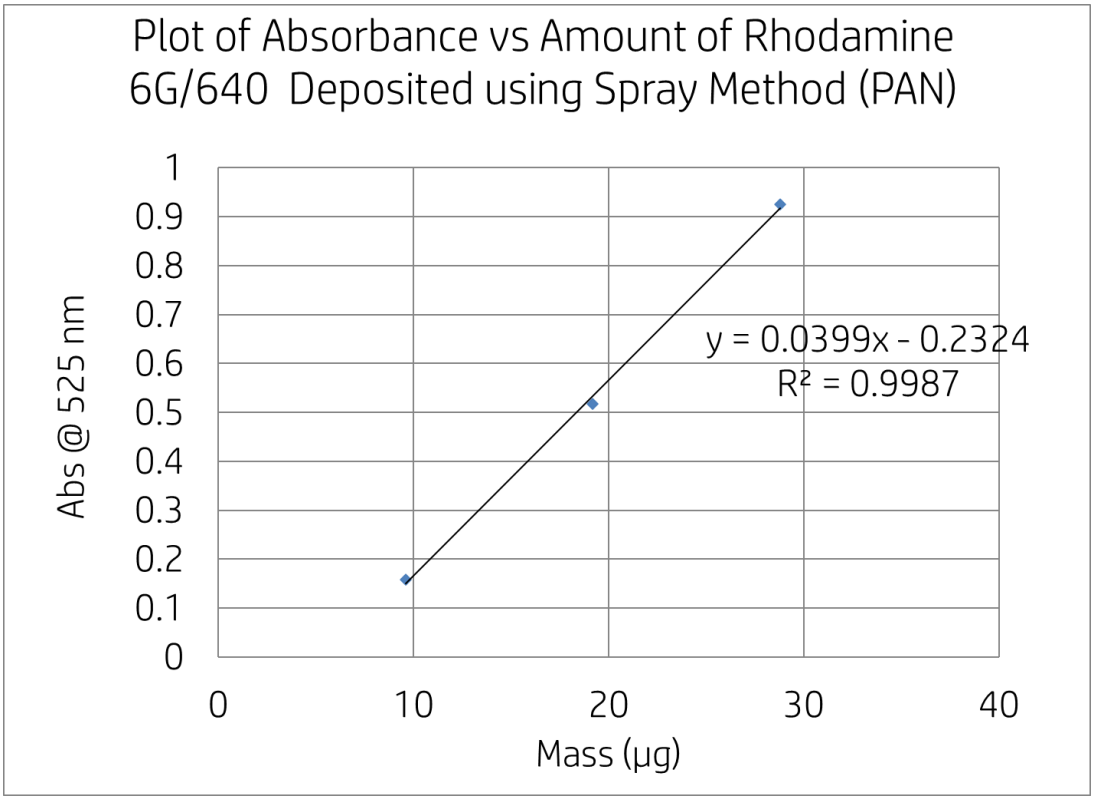
20. **Repeat** steps 14-16.

21. **Stop** deposition after a defined period.

22. **Reconstitute** samples with 2.1 g of ethanol.

23. **Analyze** samples using a DU800 UV-VIS spectrophotometer without further treatment.


UV-Vis Spectra of Rhodamine 6G/640 deposited using PAN
Absorbancee(a.u)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
425
475
525
575
625
675
Wavelength (nm)
30 sec deposition
60 sec deposition
90 sec deposition


Plot of Absorbance vs Amount of Rhodamine 6G/640 Deposited using Spray Method (PAN)
Abs @ 525 nm
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
10
20
30
40
Mass (µg)
y = 0.0399x - 0.2324
R² = 0.9987

CALCULATION FOR DOSAGE

1. **Collect** data.

Solvent density	0.789 $\frac{g}{mL}$ ethanol 99.5%
Marker concentration	$\frac{29190\ \mu g\ Rho}{1.99951x10^7\ \mu g\ soln}$
Flow Rate	1000 $\frac{\mu L}{hr}$
Period of first deposition	30 s
Period of second deposition	60 s
Period of third deposition	90 s

2. **Calculate** dosing rate.

$$1000\ \frac{\mu L}{hr} \cdot \left(\frac{1\ mL}{1000\ \mu L}\right) \cdot \left(\frac{0.789\ g}{1\ mL}\right) \cdot \left(\frac{1x10^6\ \mu g}{1\ g}\right) \cdot \left(\frac{1\ hr}{3600\ s}\right) = 219.7\ \frac{\mu g\ soln}{s}$$

3. **Calculate** amount of marker dispensed at different periods.

$$30\ s \cdot \left(\frac{219.7\ \mu g\ soln}{1\ s}\right) \cdot \left(\frac{29190\ \mu g\ Rho}{1.99951x10^7\ \mu g\ soln}\right) = 9.6\ \mu g\ Rhodamine\ 6G/640$$

$$60\ s \cdot \left(\frac{219.7\ \mu g\ soln}{1\ s}\right) \cdot \left(\frac{29190\ \mu g\ Rho}{1.99951x10^7\ \mu g\ soln}\right) = 19.2\ \mu g\ Rhodamine\ 6G/640$$

$$90\ s \cdot \left(\frac{219.7\ \mu g\ soln}{1\ s}\right) \cdot \left(\frac{29190\ \mu g\ Rho}{1.99951x10^7\ \mu g\ soln}\right) = 28.8\ \mu g\ Rhodamine\ 6G/640$$

Advisor Signature _____

Co-Advisor Signature _____