Protochips
Quantifiably Better™

Poseidon Select

USER MANUAL

M-00001 v1.2

List Figures and Tables

Protochips, Inc.

Protochips, Inc. provides solutions to enable development and commercialization of revolutionary new materials and applications. Utilizing technology developed around miniaturized semiconductor devices, Protochips transforms one of the most widely used tools in nanotechnology – the electron microscope – into a complete nano-scale laboratory. *In situ* study of materials for the life and physical sciences in the electron microscope is now Quantifiably Better.

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Quick Start Guide

1) Clean the Poseidon Select E-chips.
   a) Gently swirl E-chip(s) in acetone (~2 minutes).
   b) Gently swirl E-chip(s) in methanol (~2 minutes).
   c) Dry the E-chip(s) by blowing compressed air parallel to the surface
   d) Plasma clean or glow discharge.
   e) Check membrane areas of the E-chip(s) for debris or breakage using a stereoscope.
2) Remove the Lid and Inspect the Tip
   a) Unscrew the three screws and remove the lid.
   b) Verify the tip is free from lint and other debris and the o-rings are properly seated.
3) Attach Tubing and Check for Clogs
   a) Attach the syringe to one of the PEEK tubes.
   b) Manually depress the syringe until a liquid droplet appears at the exit of the tubing.
   c) Attach the microfluidic tubing to the holder
   d) Prime each liquid line with liquid and verify that there are no clogs
4) Load the E-chip Pair
   a) Insert the spacer (small) E-chip into the pocket, with the membrane side facing upwards.
   b) Place the large E-chip on top of the first E-chip, such that the membrane is facing downwards.
5) Attach the Lid and Seal the Tip using the Torque Driver
6) Perform a visual inspection of both sides of the Poseidon Select TEM holdertip using a stereo microscope.
7) Perform a Leak Check.
   a) Check the holder using a dry pump. The holder should reach a base pressure of $5x10^{6}$ mbar
   b) Re-inspect the tip in a stereomicroscope after performing the leak check
8) Holder is now ready for use in the Electron Microscope
9) After use:
   a) Use the syringe pump to flow water through the system to flush holder and tubing
   b) Disconnect the tubing and manually flow distilled water through each tube with the syringe.
   c) Remove the lid and E-chips and manually flush each input/output port with water
   d) Rinse the tip with water and allow tip to dry.
   e) Reassemble the tip prior to storage.

I. Poseidon Select Platform Overview

Poseidon Select is a complete *in situ* platform centered on advanced semiconductor-based sample supports called E-chips (environmental chips), which provide both superior performance and allow user modification of the sample chamber on experiment-by-experiment bases. Each Poseidon Select is a complete *in situ* solution, which includes the Poseidon Select TEM holder, initial supply of E-chips, and components necessary to enable specific functionality.

Poseidon Select Sample Holder

The Poseidon Select holder is used to load samples into the TEM and maintain a liquid environment around the sample during imaging. The sample is contained between E-chips which are secured within the tip of the holder. Two o-rings located in holder tip provide a vacuum tight seal, and the assembly is held in place by a lid which is secured by three screws. The total volume of liquid that can be contained within the reservoir surrounding the E-chips (with o-rings in place) is approximately 1 microliter. This value does not include the volume contained in the internal tubing. There are two version of the Poseidon Select , the standard version and a JEOL UHR compatible version. The two Poseidon Select differ in the tip design due to the fit of the pole piece, as shown in Figure 1.

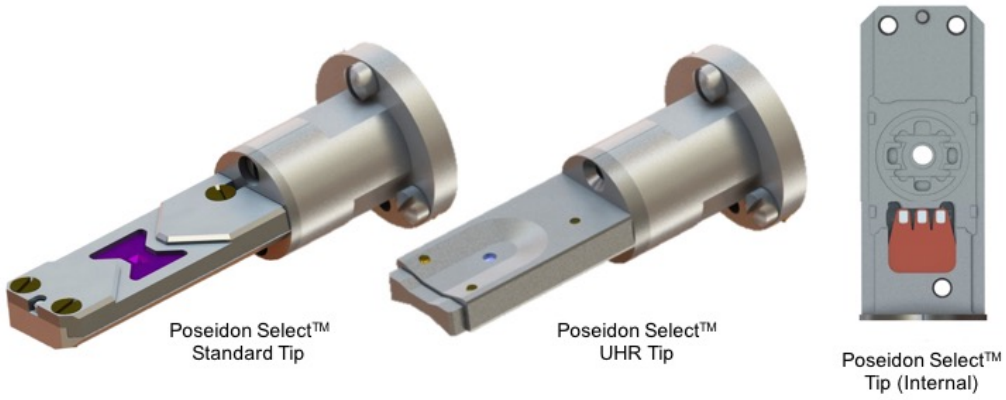


Figure 1: Poseidon Select Standard and UHR TIp Design

Poseidon Select Standard

EDS compatible liquid TEM holder. The Poseidon Select TEM system contains three integrated liquid ports, two input and a single output for flow for mixing applications. A

flexible electronic circuit contact pad is integrated into the tip for use with the optional electrochemistry or heating E-chips.

Poseidon Select UHR

Non-EDS compatible Poseidon Select holder designed to fit the confined space of the JEOL UHR pole piece.

Microfluidics

Internal microfluidic tubing is integrated into the shaft of the holder to allow for continuous liquid flow and/or the injection of sample during imaging via an external syringe pump. The Poseidon Select microlfuidic system consists of two components: permanent stainless steel outer tubing which runs the length of the holder shaft and replaceable capillary tubing inserts composed of polyether ether ketone (PEEK) thermoplastic polymer. The stainless steel outer tubing is not meant to come into contact with liquid and should never be used withought the PEEK capillary inserts in place. The outer diameter of all Poseidon Select capillary tubing and the corresponding fittings is 800 µm. PEEK tis compatible with temperatures up to 260 °C. PEEK tubing has a high chemical resistivity. However, it should not be used with the following chemicals: bromine, nitric acid >90%, sulfuric acid >95%. Solvents such as dimethylsulfoxide (DMSO) and tetrahydrofuran (THF) may cause PEEK to swell and therefore should be avoided. External lengths PEEK tubing are connected to input port(s) and the exit port of the holder to facilitate the circulation of fluid through the holder. The volume of liquid contained in the holder is given in

Table 1: Poseidon Select Liquid Volume

Model	Tip (µL)	Volume With Capillary Flow Insert	Volume Without Capillary Flow Insert
FEI	1.44	27.14	702.74
JEOL	1.44	29.48	742.43
Hitachi	1.44	32.74	839.99

Electrical Inputs

The Poseidon Select system is designed to interface with a Gamry 600+ potentiostat for electrochemical applications or a Keithley 2450 power supply for heating applications through the integrated electrical locking multi-connector (ELMC) located at the base of the holder. The Poseidon Select TEM holder contains an integrated 3-electrode flexible circuit in the tip of the holder which makes electrical contact with electrodes patterned onto the

surface of either electrochemistry or heating E-chips to enable electrochemical or heating experiments *in situ*. The location of the flexible circuit is such that the integrated electrodes are not exposed directly to electrolyte which reduces electrical noise and prevents damage to the electrical contacts due to corrosion from liquid contact. The external electrical connector is located on the base TEM holder directly blow the liquid ports.

Poseidon Select E-Chips

Refer to Section II.

Syringe Pump

An external Hamilton syringe pump is used to deliver liquid through the microfluidic tubing into the tip of the Poseidon Select TEM holder. Please refer to the Hamilton user manual for setup and operation.

Optional Package: Heating

The Poseidon Select heating system provides the safety, thermal stability and thermal uniformity of a reservoir heater with the proximity, serviceability, and thermal response of a MEMs heater. In addition to the standard Poseidon Select components, the heating package includes a power supply, software, laptop controller and cabling.

Optional Electrochemistry Package:

The Electrochemistry option provides low noise electrochemical measurements. In addition to the standard Poseidon Select components, a Gamry Reference 600+ potentiostat, software, cabling and calibration cell are included. The Poseidon *Ex-Situ* Cell, shown in Figure 2, is a light microscopy correlative tool designed to facilitate experiment development for the Poseidon product platform. The unit replicates the E-chip pocket and wetted materials of the Poseidon Select TEM holder and uses the same E-chips, gasket and microfluidics.

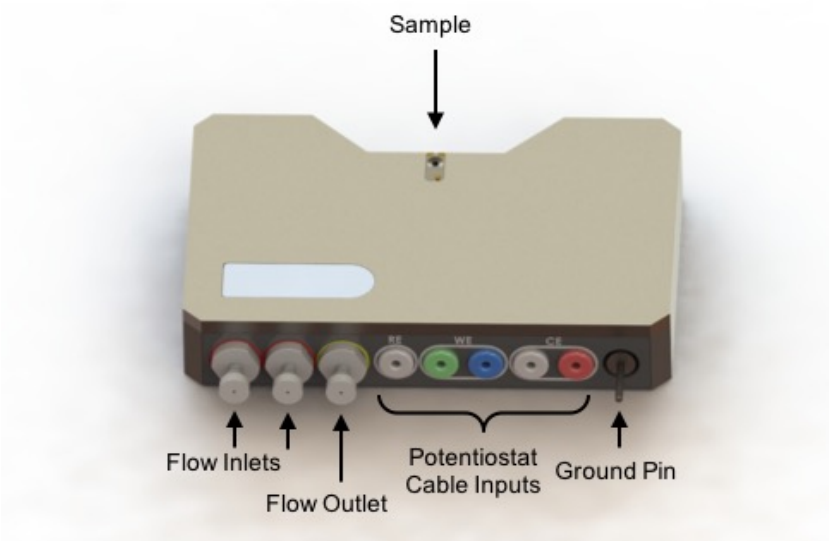


Figure 2: The Poseidon *Ex Situ* Cell

The *Ex-Situ* Cell is compatible with inverted, upright, stereo and confocal microscopes for imaging the enclosed E-chips. The Ex-Situ cell can be used in tandem with the Poseidon Select TEM holder to speed up experiment development or it can be used on its own as a research instrument to better understand samples on Poseidon Select E-chips. The geometry of the *Ex-Situ* Cell is compatible with 96 well plate stage clamps but can be used on any dissecting stage or table top. Bulkheads, microfluidic and electrical inputs are raised above the fixture height for standard 96 well plate stage clamps even if the unit is place upside-down for use with inverted microscope. The working distance from the top of the lid to the sample is 1 mm and the total height of the unit is 22.3 mm.

The tip design of the *Ex-Situ* cell maintains the same geometries of the E-chip reservoir used in Poseidon Select TEM holders. Please refer to the applicable sections of the manual for E-chip loading, connecting the microfluidic tubing, connecting the potentiostat or connecting the power supply.

II. Poseidon Select E-chips

Each experiment requires a pair of Poseidon Select E-chips which form the microfluidic enclosure and serve as the sample support. The E-chips integrate a very thin yet durable amorphous window, providing a strong barrier for containing liquid with minimum beam scatter. E-chips should be handled very carefully to avoid breaking this membrane.

To accommodate the wide variety of samples, E-chips provide the user with the ability to specify seven key parameters that optimize the system for their particular sample and experiment: (1) window size and orientation, (2) spacer height, (3) spacer material, (4) flow or static liquid environment, (5) heating, (6) electrode geometries, and (7) electrode composition.[1]

2.1 E-chip Parameters

Liquid thickness & Volume Contained Between E-chips

Each experiment requires two E-chips: a 2 x 2 mm E-chip, which typically contains an integrated spacer and a 4.5 x 6 mm E-chip, which forms a seal over the small E-chip. Liquid thickness is set by the spacer height of the E-chip combination. The volume of liquid between the two E-chips can be approximated by multiplying the area of the spacer E-chip by the nominal spacer thickness. (When using a heating or electrochemistry E-chip, remember to account for the spacer thickness of the insulating layer). Some variation in total volume is introduced due to the spacer and/or electrode configuration; however, this volume is negligible. In general, the volume of liquid ranges between 0.6-22 nL, depending on E-chip combinations.

Heating E-chips

Heating E-chips contain integrated metal heaters patterned onto the 4.5 x 6 mm E-chip which mate with the electrical contacts pad in the tip of the holder. The heating wires are outside of the viewing area and protected by an insulating coating (typically SU8) that also doubles as an integrated spacer, thus these E-chips can be paired with spacer-less small E-chips. Please consult the Poseidon E-chip ordering guides for individual E-chip specifications.

[1] Parameters 5,6 and 7 require the purchase of optional heating or electrochemistry packages

Electrochemistry E-chips

Poseidon electrochemistry E-chips contain integrated electrodes patterned onto the 4.5 x 6 mm E-chip which mate with the electrical contacts pad in the tip of the holder. The electrodes are designated as the working electrode (WE), reference electrode (RE) and counter electrode (CE). The electrodes interact with the sample/electrolyte. To reduce noise, the electrode-containing E-chips contain an insulating coating (typically SU8) that also doubles as an integrated spacer, thus these E-chips can be paired with spacer-less small E-chips. Please consult the E-chip ordering guides for individual E-chip specifications.

2.2 Handling

E-chips are supplied to customers in gel-packs with the membrane side of the chips facing upwards. E-chips should be removed from the gel-pack using the carbon fiber tweezers supplied with the Poseidon system. To remove the E-chip from the gel-pack, grip the sides of the E-chip with the carbon fiber tweezers, as shown in Figure 3, gently twist back and forth, then pull the E-chip off of the gel material. When removing the large E-chips from the gel-pack, it may be necessary to slide the tip of the tweezers underneath the E-chip to loosen it from the gel-material. Always handle the E-chips by gripping them at the edges. Metal tweezers can chip the edges of the E-chips and should not be used to remove the E-chips from the gel-pack.

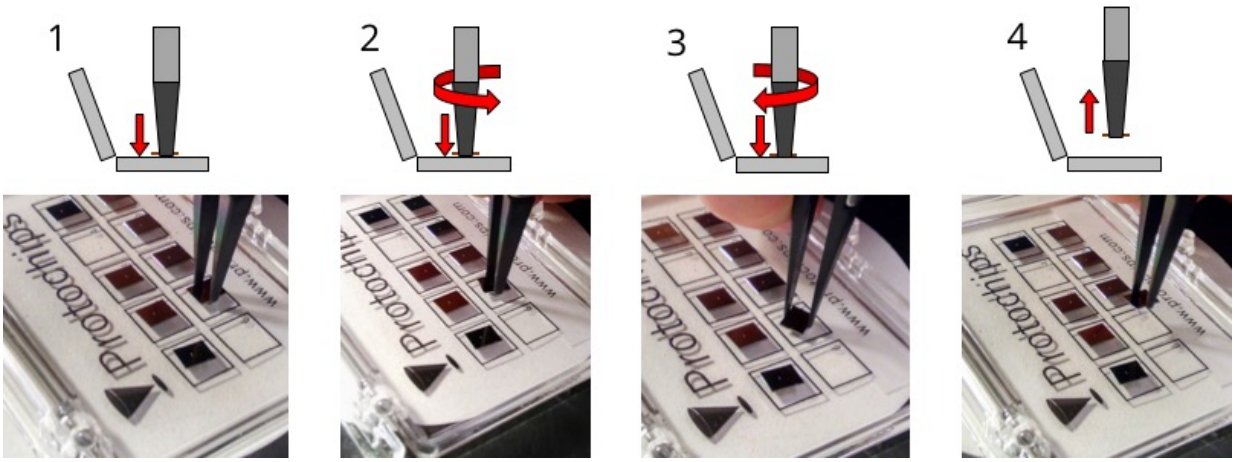


Figure 3: Removal of E-chips from the Gel Pack

2.3 Removal of the Photoresist Protective Coating

The SiN membrane of new E-chips is coated with a protective photoresist coating to prevent damage to the membrane during dicing process. This protective coating must be removed prior to use. Only the top surface receives the protective coating. The bottom of the E-chip will appear to be a different color prior to removal of the coating. After cleaning, both the top and bottom surfaces will have the same shiny appearance. A comparison of coated versus cleaned E-chips is shown in Figure 4.

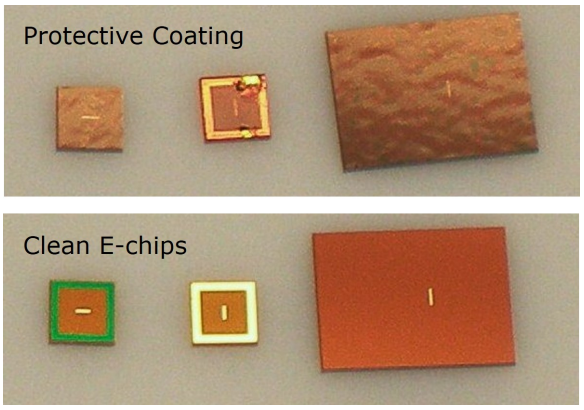


FIGURE 4: E-CHIPS BEFORE AND AFTER THE REMOVAL OF THE PROTECTIVE COATING

To remove the protective coating follow the steps outlined below and shown in Figure 5. For best results, use wide-bottom beaker or shallow dish that is 3-4 inches in diameter. Add enough solvent so that the E-chips will be submerged in at least 0.5 cm of liquid. Do not allow the E-chip(s) to dry until the last step (drying). The E-chip surface must remain wet to prevent dicing debris from resettling on the surface prior to the final methanol rinse. Never use ultrasonic bath to clean the E-chips as this will break the membrane.

1. **Acetone rinse**: To dissolve the protective coating, immerse the E-chips in a clean beaker filled with acetone and agitate gently for a minimum of 2 minutes.
2. **Methanol Rinse**: Immediately transfer the E-chip(s) into a new beaker filled with methanol and agitate gently for a minimum of 2 minutes.
3. **Dry**: Remove the E-chip(s) and quickly blot it (SiN side up) on a lint-free paper towel or piece of filter paper to remove excess liquid. Dry the surface of the E-chip by directing a flow of air across (parallel) the surface of the E-chip. (Note: nitrogen, argon, and compressed or canned air may be used as the drying agent).
4. **Visual Inspection**: After the E-chip(s) are dry, check that the membrane is intact using an optical microscope. After stripping the protective coating, check the E-chips for debris and damage to the window or spacer that would cause the E-chips to function improperly. Small drying residues do not generally interfere with use. Do not use devices which have drying residue or debris directly over the SiN window. Also, avoid using devices if large particles of debris are located on the spacer itself, since these will interfere with obtaining the proper liquid thickness.

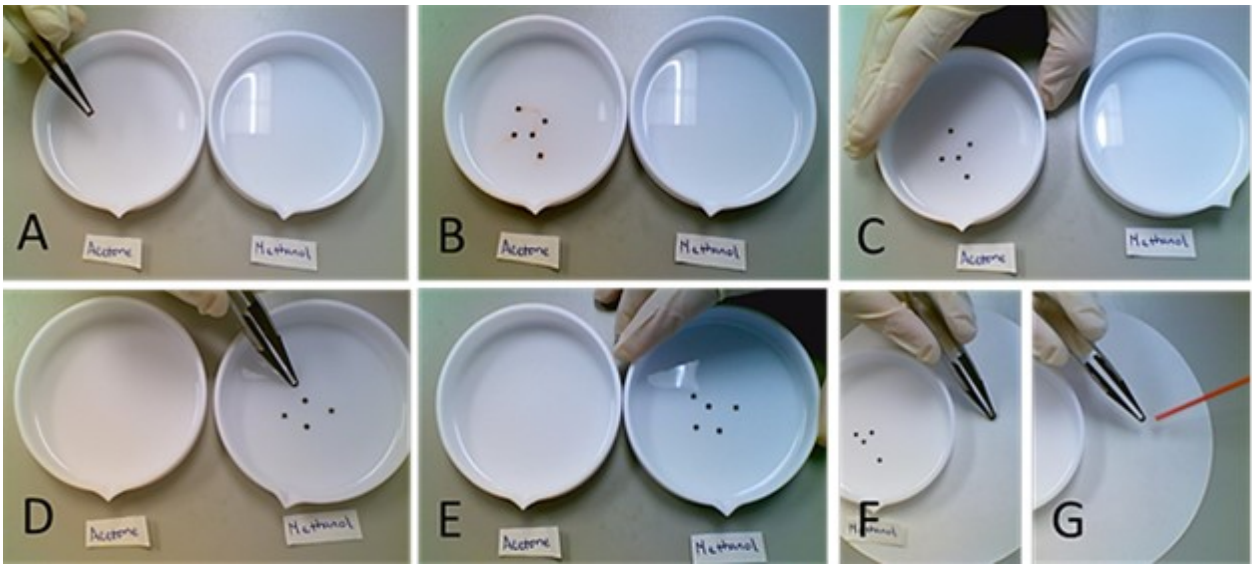


Figure 5: Removal of the Protective E-chip Coating

2.4 Plasma Cleaning E-chips

The surface of the E-chip is mildly hydrophilic after removal of the photoresist coating. When using aqueous based liquids it is necessary to increase the hydrophillicity of the E-chips by plasma cleaning or glow discharge treating them prior to use. Plasma cleaning the E-chips will also reduce contamination during imaging.

E-chips may be plasma cleaned or glow discharged using a variety of gas mixtures and plasma intensities. In general, the membrane side should be plasma cleaned for 2-5 minutes to render it hydrophilic if using either an air or argon only plasma. Once plasma cleaned, the surface will remain typically remain hydrophilic for several hours. However, for best results plasma clean the E-chips immediately prior to loading them in the Poseidon Select holder. Most E-chips can be plasma cleaned multiple times without damaging the surface of the E-chip, the exception being Electrochemistry E-chips with a glassy carbon electrode. To reduce contamination during imaging, both the top and bottom side of the E-chips may be plasma cleaned.

Glassy Carbon Electrodes

Excess plasma cleaning on E-chips with a glassy carbon electrode will wear away the glassy carbon. Limit the time and intensity that these E-chips are plasma cleaned. Visually inspect the glassy carbon after plasma cleaning to check for a uniform electrode layer.

III. Poseidon Select Holder Setup

The Poseidon Select TEM should be cleaned thoroughly between every use. The following section assumes that the user is starting with a clean Poseidon Select TEM holder.

- *Do not touch the holder on or below the o-ring on the shaft of the holder. Touching the holder in this area, and especially at the tip, can cause organic contamination in the microscope, and reduce the performance of the holder and microscope.*

3.1 Connecting the External Tubing

Capillary PEEK tubing inserts, with an outer diameter of 800 µm, is used to connect the syringe to the TEM holder. This tubing is very flexible and easy to connect. The flexibility will prevent unwanted external vibrations from coupling into the holder during an experiment. The microfluidic tubing is connected to both the liquid reservoir (glass syringe) and the capillary insert in the Poseidon Select TEM holder using a PEEK zero dead volume (ZDV) fitting as shown in Figure 6. The input tubing is colored red with an internal diameter of 127 microns and the output tubing is colored yellow and has an internal diameter of 178 microns.

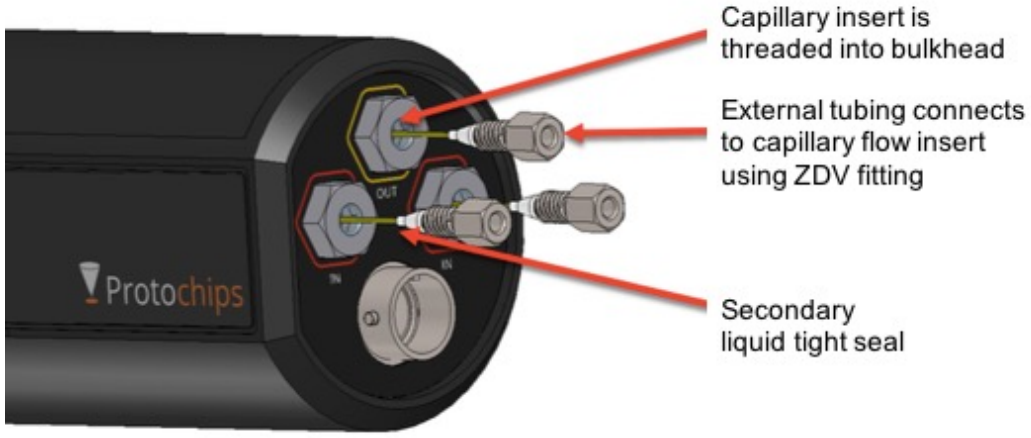


Figure 6: Tubing Attachment

Connecting the Syringe

The syringe is connected to the microfluidic tubing using the same type of fittings as the TEM holder.

1. Load the syringe with liquid. The syringe comes in two parts – the plunger and the glass barrel. The plunger must be moistened with water before insertion into the

barrel. Fully insert the plunger into the barrel, and then draw liquid into the syringe by pulling back on the plunger while the tip of the syringe is submerged in a beaker of liquid.

2. Assemble the microfluidics. The syringe provided with the system has a Luer lock fitting.
3. Locate the Luer lock-to-ZDV fitting and screw this onto the syringe.
4. Thread the microfluidic tubing through the ZDV fitting, then screw the assembly into the end of the syringe.
5. Check the connection, manually press the plunger on the syringe and look for liquid to emerge on the opposite end of the tubing. Verify that there are no leaks at the adapter.

Connecting the Input Tubing to the Holder

The input tubing delivers liquid from the syringe into the holder. After confirming that liquid is flowing through the tube, connect the free end of the tubing to the Poseidon Select holder.

1. Connect the free end of the red input tubing (127 µm inner diameter) to the inlet of the Poseidon Select TEM holder.
2. Once the tubing is connected to the TEM holder, remove the lid from the tip and manually press the plunger again to check flow from the syringe to the holder tip. Verify that no leaks are present at the microfluidic connections, and that water emerges at the holder tip.
3. Once flow is verified, load the syringe into the syringe pump as described in the syringe pump manual.
4. Connecting the yellow output tubing (178 µm inner diameter) to the holder
5. Connect a short length (3-6 inches) of yellow tubing (178 µm inner diameter) to the output port. The length of tubing at the output port is shortened to prevent a build-up of pressure that can result in membrane ruptures.

Priming the Tubing with Fluid

When operating Poseidon Select in flow mode the internal capillary tubing should be purged with fluid to remove trapped air and prime the lines before loading the E-chips and sample. (When operating in static mode the internal tubing may be purged with fluid as described here, or operated with air in the internal tubing). The priming liquid should be the same or compatible with the liquid used during the experiment.

To purge with liquid, attach a short length of PEEK tubing to the syringe filled with fluid and connect the tubing to the Poseidon Select holder. Remove the lid and gasket or o-rings from the tip and purge each line with liquid by manually depressing the plunger of the syringe with the thumb, until liquid is observed exiting the port in the holder tip. Wick the

excess liquid away from the tip as it exits with a piece of filter paper to prevent contamination from building up in the tip of the holder.

Flush several microliters through the line to ensure adequate flushing of the tubing. Repeat for each input and output tube. After flushing, rinse the tip of the holder with water (to prevent salt crust from forming is using buffer or electrolyte) and dry the tip portion of the holder before inserting the gasket or o-rings and loading the E-chips. Figure 7 shows the location of the fluid input and output ports relative to the locations that the fluid enters and exits the tip of the Poseidon Select holder.

- *This same procedure should be repeated after use to clean the fluid lines of the holder, but the fluid volume that is forced through each line should be increased. The fluid may be forced through either manually or with the accompanying syringe pump. The amount of fluid that is necessary to clean the fluid lines is dependent on the type of fluid, sample concentration and viscosity. For cleaning use a minimum of 300 microliters per line, but up to several mL may be required. If a large volume of fluid is required to clean the lines, it may be simpler to flush the system with the E-chips using the syringe pump, then followed by manual flushing of each fluid line without the E-chips inserted.*

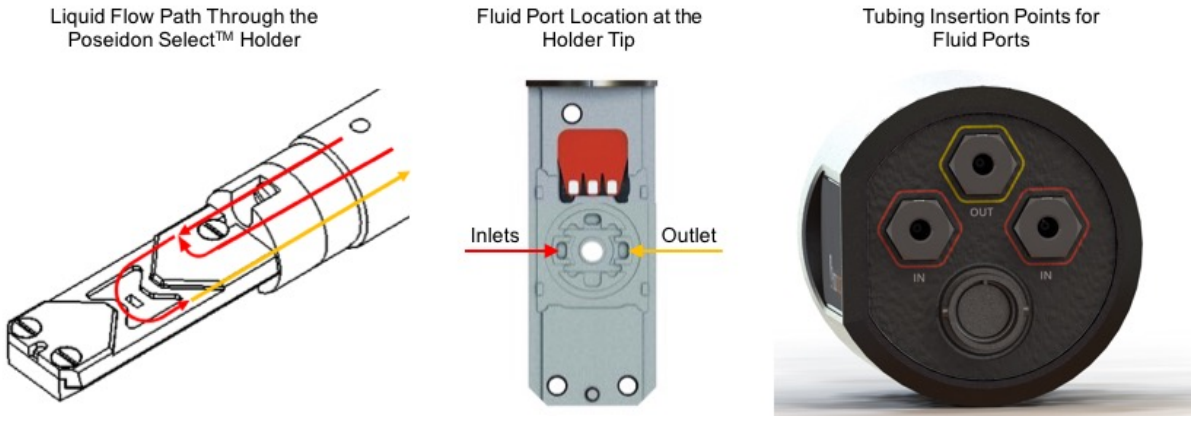


Figure 7: Location of Poseidon Select Liquid Ports

3.2 Preparing the Holder Tip

Before use, carefully check the inside of the tip under a stereoscope and remove any lint or debris which may interfere with the seating of the E-chips or the sealing of the o-rings. It is

particularly important to remove any lint or particles that are on or under the o-rings, as the presence of debris on the o-ring can cause the holder to fail a leak check.

Sealing Gasket & O-Rings

The sealing gasket and/or o-rings play an essential role for the holder as they provide the vacuum seal around the holder in the microscope. The Poseidon Select holder tip can accommodate either a sealing gasket or a pair of o-rings as shown in Figure 8. It is important to match the o-ring material to the liquid that is used during each experiment. Consult the Chemical Compatibility Guide to select the correct o-ring material for your experiment.

Gasket/O-ring Insertion

Insert the sealing gasket or o-ring pair in the tip of the holder before inserting the E-chips.

1. Inspect the sealing gasket under a stereoscope to check for any debris such as fibers which will interfere with sealing.
2. Remove any debris in the tip that could interfere with proper sealing. Residue-free, compressed air can be used to clear the tip surface of dust and other small particles. Extensive buildup of debris in the o-ring pocket may require ultrasonic cleaning, if so, please refer to Section 6.3.
3. Place the gasket into the grooves in the tip of the Poseidon holder.
4. Re-inspect for fibers or debris before inserting the E-chips.

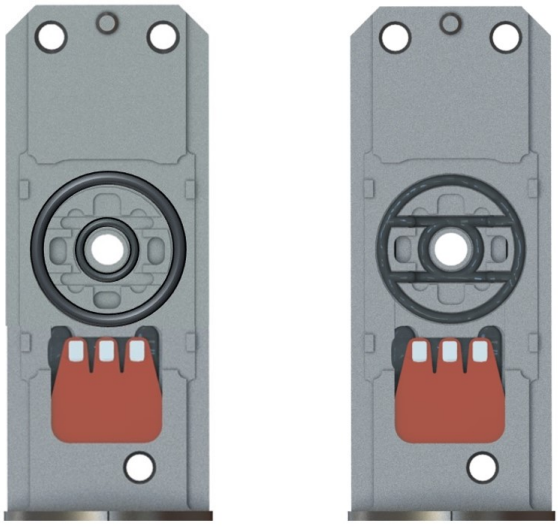


Figure 8: Poseidon Select Accomodates O-rings or A Sealing Gasket

3.3 Tip Assembly

Two E-chips are required for each experiment: a small E-chip which contains an integrated spacer to set the liquid thickness and/or flow path, and a large E-chip rests on top of the small E-chip and seals the sample chamber from the vacuum. When performing electrochemistry or heating experiments the large E-chip is the active chip, and electrodes or heating elements are patterned directly onto its surface. Consult the E-chip Ordering Guide to for available heater options, electrode configurations and spacer sizes. Because the small E-chip is square the windows of the two E-chips may be positioned in the tip of the holder, such that the windows are oriented either parallel with, or orthogonal to one another.

Samples can be loaded into the holder in a number of ways. Solvent or buffer dispersed samples can be placed directly on the bottom (spacer) E-chip using a pipette, or introduced via flow. Biological specimens can be grown directly on either chip prior to insertion in the pocket. Samples can also be applied to an E-chip and dried prior to loading. The basic E-chip loading process is shown in Figure 9 and is the same for each method, which vary only in when and how the sample is applied during loading.

- *When handing Poseidon E-chips do not allow tweezers or pipette tips to come into contact with the SiN membrane as it could cause a rupture of the window.*
- *Note Because the lid is notched, the back screw may be left in place before inserting the Poseidon E-chips. The back screw serves as a pivot point for positioning the lid.*

1. Insert Gasket **2. Insert Small E-chip** **3. Prime with Liquid** **4. Insert Large E-chip**

Figure 9: E-chip Loading

3.4 E-chip Loading

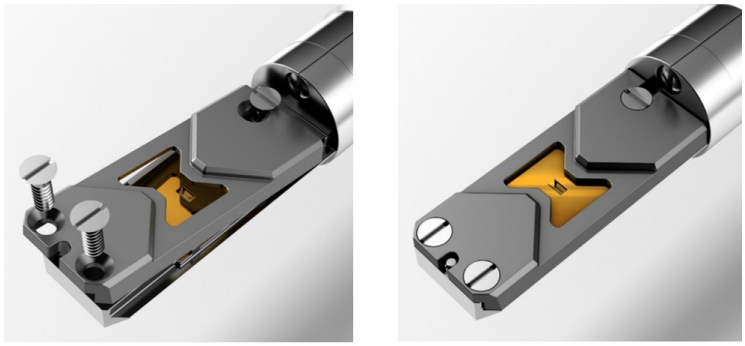
Before loading the Poseidon Select TEM holder, prepare the E-chips for use by removing the protective photoresist coating and plasma cleaning/glow discharging the membrane

surface (if using aqueous liquid, non-polar solvents such as hexane do not require plasma cleaning for adequate wetting).

1. Insert the small E-chip into the pocket with the membrane and spacer side facing upwards.
2. Using a micropipette, containing 2-3 µL of liquid, dispense some of the liquid from the pipet, such that only a small bead of liquid is present at the end of the pipet (you will not dispense the entire volume of liquid onto the E-chip). Gently touch the droplet onto the surface of the E-chip without touching the tip of the pipet to the membrane. Wetting the first E-chip prior to loading the second E-chip will enable liquid to flow more easily between the E-chips. To reduce the volume of liquid on the E-chip, excess liquid may be removed using a small triangle of filter paper. Use the filter paper to gently wick off excess liquid from the edge of the E-chip (do not touch the filter paper to the SiN window) until only a small amount of liquid remains covering the window area.
3. Insert the second E-chip into the pocket. This E-chip should be inserted with its membrane side facing downwards. Hold the E-chip at a 30-45 degree angle and align the back of the E-chip with the pocket of the holder (this will prevent the liquid from sticking the small E-chip and dislodging it). Once the E-chip is aligned, allow it to fall into place. Adjust its position by nudging it along the edges with the tweezers until it is properly aligned.
4. The membranes of each chip are now facing inwards, containing the sample. Verify that the E-chips are seated properly in the pocket, lying flat with respect to the tip.
5. Check that the two windows are aligned with an optical microscope.

3.5 Lid Attachment

When the holder is closed, the o-rings form a vacuum tight seal. The lid is secured to the tip using three screws, as shown in Figure 10.



1. Position the Lid **1. Secure the Screws**

Figure 10: Attaching the Poseidon Select Lid

1. Verify that the E-chips are aligned within the tip of the holder using a stereoscope.
2. Carefully place the lid on to the holder, being careful not to displace the E-chips.
3. Insert the back screw, and tighten it until it catches. This will prevent the lid from slipping while inserting the remaining two screws.
4. Insert the two remaining screws into the tip. Gradually tighten each screw; moving from screw to screw so that pressure is applied evenly during this process. Finish sealing the tip using the torque driver to prevent over-tightening. When the set torque value is reached, the driver will "slip" preventing further tightening

➢ *For simplified attachment of the lid, the back screw may be left in place when removing and replacing the lid. Loosen the back screw, such that the lid, when angled slightly can be slid away from it. When replacing the lid, angle it such that the slot at the back of the lid can be slide onto the back screw.*

5. When fully tightened, the lid should appear to sit flat against the tip. Inspect the tip using a stereoscope, and be certain that the E-chip membranes are intact. Remove any excess liquid from the holder. Wipe the tip with a lint-free wipe or cloth. DO NOT touch the E-chips, as this could break the membrane.
6. After loading inspect the top and underside of the loaded Poseidon tip under a stereomicroscope and verify that the viewing region where the windows overlap is transparent. Rotate the holder very slightly so that the light is reflected off the top window to verify that the window is intact. Rotate the holder 180 degrees and do the same for the bottom window. A diagram and cross section of the fully assembled Poseidon tip is shown Figure 11.
7. (Optional) After verifying that both the top and bottom windows are intact, inject a small amount of liquid into the tip by depressing the plunger of the syringe with

your thumb while simultaneously observing the windows through the stereoscope. If you are using windows configured for flow, you should see the membranes deflect outwards slightly as liquid passes through the channel. Check that no droplets of liquid have appeared on the outside of the top or bottom E-chip. If droplets of liquid are visible, it indicates a leak due to either the E-chip windows breaking or debris on the o-rings preventing proper sealing.

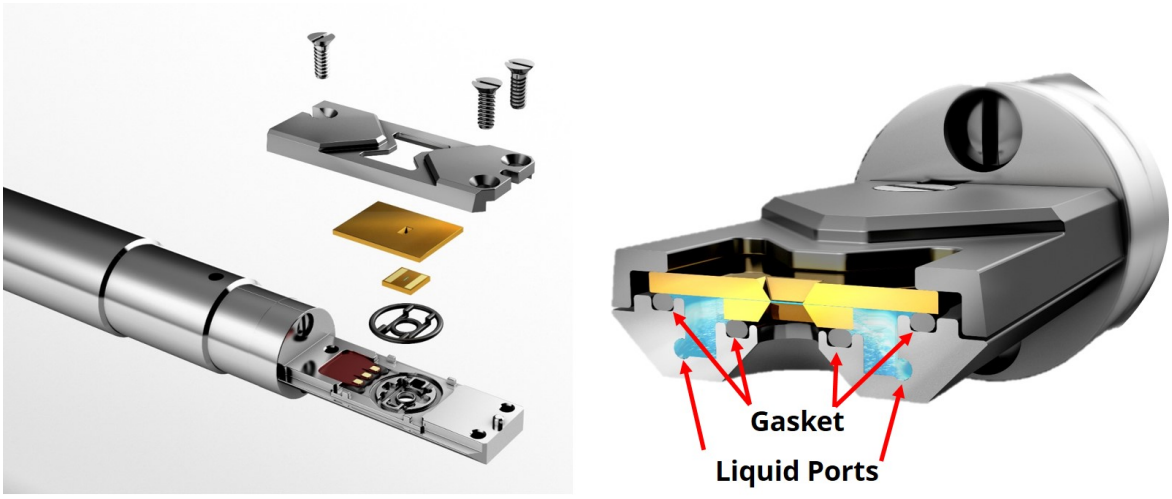


Figure 11: Exploded View and Cross Section of the Assembled TEM Holder

3.6 Leak Check

A thorough leak check is required prior to loading the Poseidon Select TEM holder into the microscope. A dry pumping station is necessary to pre-test the holder vacuum and remove any residual liquid from loading prior to insertion into the microscope. The leak check should proceed as follows:

1. Inspect the loaded tip under a stereomicroscope and verify optically that both the top and bottom membranes are intact.
2. Place the holder into an approved leak check station and ensure that the ends of the tubing are open to atmosphere. DO NOT perform a leak check with the flow port plugs inserted.
3. The Poseidon Select TEM holder has passed leak check if it obtains a vacuum level of 5.5×10^{-6} mbar or better within twenty minutes. Observe the vacuum gage carefully for the first few minutes. Look for the needle to decrease at a steady rate, without jumping back and forth. Once vacuum has equilibrated, remove the holder and check the windows under the stereoscope as described in step number 1.
4. Re-inspect the loaded tip under a stereomicroscope and verify optically that both the top and bottom membranes are intact.

Over time the o-rings and the o-ring pockets can collect debris. If the pumping system is taking longer than normal to achieve the proper vacuum level when the holder is in the exchange position or when inserted in the column, it may be an indication that the o-rings need to be cleaned or replaced. Note: If the external ports are sealed (flow port plugs in place), small leaks such as those caused by debris on the internal o-rings may not be identified. Always remove the flow port plugs before leak testing the holder or operating it in the microscope.

- The Poseidon Select TEM holder may be plasma cleaned after it has been loaded before it is inserted into the microscope to reduce contamination (optional).

IV. Operation

After a successful leak check and optical inspection the Poseidon Select TEM holder may be inserted into the load lock of the TEM. If possible, monitor the vacuum level in the load lock to verify that the holder is pumping down to the desired pressure. Once the holder has reached high vacuum in the load lock, insert the holder into the column. Watch the vacuum level and confirm that high vacuum is maintained with the Poseidon holder in the microscope. Excess water on the holder can appear to be a "virtual leak" as the water evaporates. If the vacuum rises significantly, retract the holder back into the load lock and wait for several more minutes.

4.1 Liquid Flow

Verify that the pump is plugged in and that the switch at the back of the pump is set to "on." To set the flow rate, ensure that the Hamilton syringe pump is plugged in and turned on. Secure the syringe in the pump. The controls for the syringe are located on the front panel. During imaging, the recommended flow rate is 120-240 µL/hr. This can be varied as needed, but the maximum flow rate should not exceed 300 µL/hour while the holder is in the TEM. The maximum safe flow rate is dependent on sample viscosity and spacer thickness. Users should optimize flow rates *ex-situ*.

Basic Pump Operation[2]

- *Before use, ensure that the the Harvard Apparatus syringe pump "FORCE" is set to a value of to 50% or less.*

1. On the syringe pump touch screen, select option QUICK START.
2. Choose the following settings in the boxes on the left side of the screen.
3. For METHOD SELECT, select INFUSE ONLY.
4. For SYRINGE SELECT, select the size of syringe (we supply a Hamilton 5 mL syringe, and 10.3 mm is the only choice possible for this syringe).
5. For INFUSE RATE SELECT, enter 300 µL/hr (the recommended flow rate to flush/fill the microfluidic tubing).
6. Start the flow by pressing the triangular PLAY button in the lower right corner. The screen will display how much liquid has been dispensed during the session, and the PLAY button will become a PAUSE button.
7. Check for any leaks at the microfluidic connections or at the tip, and wait for the liquid to exit the outlet tubing. It can take several minutes for the flow to reach the

[2] Please consult the Hamilton Syringe Pump user guide for model specific features such as infuse/withdraw and programming.

end of the outlet tubing. Once again, inspect the tip using a stereoscope and verify that the E-chip membranes are intact.

8. Establish a stable flow with this system can take 5-15 minutes for the liquid in the syringe to reach the sample region and be visible through the membrane. However, once proper flow is established the flow rate responds linearly to changes in pump speed and injection. Suggested flow rates for most imaging during imaging are 1-2 µL/min.

4.2 Fluid Effects on Pressure

Many factors affect the fluid pressure on the membrane in a Poseidon liquid cell including:

- Liquid viscosity
- Length and size of exit tubing
- Flow Rate
- Liquid specific gravity
- Clog or occlusion in the exit tubing

If the membrane pressure exceeds the strength of the membrane, the membrane will break. It is recommended that users proceed with caution if the estimated membrane pressure is greater than 14.7 psig. Table 2 shows effects of viscosity, tubing length, and flow rate.

Table 2: Fluid Viscosity and Flow Rate

	Exit tubing Length	Flow Rate	Membrane Pressure (psi)	(mBar)
H20 *Viscosity: 1 cp*	3 cm	100 ul/hr	0.5	34
		300 ul/hr	1.5	102
	670 cm	100 ul/hr	1.6	113
		300 ul/hr	4.9	339
50% Glycerine / 50% H20 *Viscosity: 5.29 cp*	3 cm	100 ul/hr	2.6	179
		300 ul/hr	7.8	538
	670 cm	100 ul/hr	8.7	600
		300 ul/hr	26.0	1794
Olive Oil *Viscosity: 43.2 cp*	3 cm	100 ul/hr	15.5	1073
		300 ul/hr	46.6	3214
	670 cm	100 ul/hr	51.8	3571
		300 ul/hr	155.5	10729

* above data are estimates and are not valid if there are clogs or occlusions in the exit tubing
** above data assumes 100 ul diameter exit tubing

To reduce pressure on the membranes:

- Minimize the length of the exit tubing. A shorter length will reduce the pressure at the membrane.
- Use 150 um diameter tubing on the exit tubing instead of 100um.
- Reduce the flow rate as much as possible for the experiment.

- *Very low vapor pressure fluids (e.g. ionic liquids) are recommended to be used in static mode only, because the low vapor pressure may note trigger the gun valve of the microscope to close in the event of a membrane rupture.*

4.3 Heating

This feature is only available as part of the Heating add-on package.

The Poseidon Select TEM heating system provides the safety, thermal stability and thermal uniformity of a reservoir heater with the proximity, serviceability, and thermal response of a MEMs heater.

Heating System

The system is made up of: Holder, Electronic Hardware, Software Controller, Syringe Pump and the frame heating E-Chip.The core of the heating technology is the frame heating E-Chip. The heating E-Chip is a consumable which features a patterned heating element that is also an on-chip temperature sensor. The heating element is not directly exposed to the fluid reservoir, but it is highly responsive to changes in flow rate or environmental conditions. The highly conductive silicon frame is used to spread heat evenly across the liquid reservoir and smooth thermal deltas across the in-tip liquid reservoir with an unmatched thermal stability and uniformity regardless of the flow conditions. The heater element is housed on a robust substrate. This allows for safe heating over the entire chip and the entire liquid reservoir without affecting the integrity of the more delicate window. The heating chip is controlled using a software PID specifically tuned for these heating E-chips for in-situ control.

Heating Cables and Connections

The Poseidon Select TEM heating system utilizes a Keithley 2450-NFP SMU to source and measure the stimuli to the heating chip. The following cables are included with the Poseidon heating system:

- Controller (laptop) AC adapter
- Keithley 2450-NFP power cable
- Communications cable, which connects the controller to the power supply
- Grounding kit, which facilitates sharing the same earth ground between the SMU and the TEM.
- TEM: Triax to banana cable, which connects the power supply to the TEM holder.

Heating Software

The Protochips-designed software features an easy-to-use graphical interface to the power supply. Heating experiments with precise closed-loop temperature control can be

performed in manual and programmed waveform modes. The Poseidon software includes a tuned PID temperature controller that can be operated in either closed or open-loop mode. To get started using the Poseidon software please consult the Poseidon Select TEM Software Manual.

Heating Setup and Operation

Setting up the Poseidon Select TEM holder heating for operation is easy and can be completed in a few simple steps. The holder is connected to the power supply using the

1. Plug in the power cord into the back of the power supply to an electrical outlet that shares the same electrical ground as the microscope. Make sure to use the grounding kit to share the same ground with the microscope. Verify a low resistance of less than 5 ohms with a multimeter. If the power supply does not share the same electrical ground noise will be introduced, and images will become blurry and distorted.
2. Insert the communications cable into the Ethernet plug indicated in the picture below. The other end plugs into the Ethernet port on the controller.
3. Insert the triax cable into the power supply indicated in the picture below. The other end of this cable plugs into the TEM holder.
4. On the front of the power supply depress the POWER button to turn ON the Poseidon Power Supply.
5. Plug the cable into the external electrical connector on TEM holder.

4.4 Electrochemistry

This feature is only available as part of the Electrochemistry add-on package.

Potentiostat

The Gamry Reference 600+ manual provides the specifications, first time setup instructions and operational instructions for the potentiostat.

Potentiostat Calibration

If the potentiostat has traveled to a new location, calibrate the Gamry potentiostat according to the Gamry manual. This is covered in the Gamry manual. Calibration of the potentiostat is required infrequently. All calibration routines can be accessed through the

Gamry Framework software in the Utility selection under Experiment. A Universal Dummy Cell is provided for calibration of the Reference 600+[3].

Recalibration is required under the following circumstances:

- First time setup.
- One year or more since the last calibration.
- The potentiostat has been serviced.
- Breaks or discontinuities in the data curves have been recorded with the system.
- Operating environment of the system has dramatically changed since the last calibration.

Poseidon Electrochemistry Cabling

The Reference 600+ potentiostat connects to the Poseidon Select TEM holder through the miniature banana patch adapter (MBPA) dongle which connects the ELMC at the back of the TEM holder to the Gamry cable as shown in Figure 12.

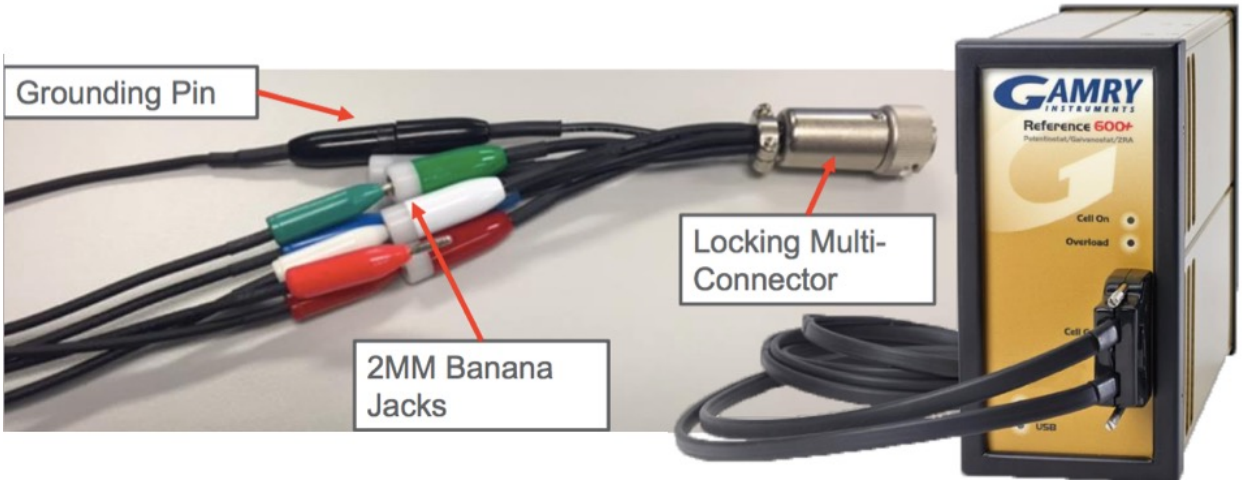


Figure 12: Electrochemistry Cabling

The miniature banana plugs on the Gamry cable are categorized into three categories: RE (Reference Electrode), WE (Working Electrode) and CE (Counter Electrode). Active electrochemical E-chips connect with the holder with three pads that are also designed with a reference electrode, working electrode and counter electrode.

1. Connect the Gamry cell 25 pin D-connector end of the Gamry cable to the front of the Reference 600+ potentiostat.

[3] Please consult the Gamry Reference 600 + user guide for model-specific use and calibration instructions.

2. Plug the color coded miniature banana plugs at the opposite end of the Gamry cable into the miniature banana connectors on the end of the MBPA dongle. (For most applications the orange banana plug will be left floating).
3. Plug the other end of the MBPA dongle into the ELMC port located at the base of the Poseidon Select TEM holder.

Power on the Gamry Reference 600+ and start up the associated laptop controller. Attach the Gamry unit to the laptop controller with the supplied USB cable.

Most electrochemical experiments can be run in one of two configurations: the standard three electrode configuration; and the standard two electrode configuration. In each configuration, the electrical input ports on the holder match the color of the cable termination of the provided cable of the Reference 600+.

Table 3 and Table 4 list the cable connections for standard three and two electrode experiment configurations:

Table 3: Three Electrode Cabling Configuration

Reference 600+ Cable Termination	Holder Electrode Input
Green "Working" Banana Connector	Green Working Electrode Miniature Banana
Blue "Work Sense" Banana Connector	Blue Working Electrode Miniature Banana
Red "Counter" Banana Connector	Red Counter Electrode Miniature Banana
	Grey Counter Electrode Miniature Banana **
White "Reference" Banana Connector	White Reference Electrode Miniature Banana
Black "Ground" Banana Connector	Black Holder Ground Pin Connector
Orange "Counter Sense" Banana Connector	Float this connection and keep isolated
** Leave this connection isolated in this configuration	

Table 4: Two Electrode Cabling Configuration

Reference 600+ Cable Termination	Holder Electrode Input
Green "Working" Banana Connector	Green Working Electrode Miniature Banana
Blue "Work Sense" Banana Connector	Blue Working Electrode Miniature Banana
Red "Counter" Banana Connector	Red Counter Electrode Miniature Banana
White "Reference" Banana Connector	Grey Counter Electrode Miniature Banana
	White Reference Electrode Miniature Banana **
Black "Ground" Banana Connector	Black Holder Ground Pin Connector
Orange "Counter Sense" Banana Connector	Float this connection and keep isolated
** Leave this connection isolated in this configuration	

Connection between the potentiostat and the holder is not limited to the two proposed configurations above. It is common to use either the counter or the reference electrode as the working electrode depending on which electrode is required for imaging during the experiment. The Gamry Reference 600+ is a floating potentiostat. In order to avoid parasitic capacitance while working *in-situ*, the black grounding dongle of the holder must be plugged into the black pin connector on the Gamry cable. This pulls the floating potentiostat to the goniometer of the TEM. Additional shielding may be required around the exposed connections.

Ex-situ, the system can be used in floating mode, and it can also be pulled down to an earth ground by using the binding post on the back of the potentiostat. It is important to verify that nothing is connected to the binding post of the potentiostat while working in-situ.

Basic Single Frequency EIS Operation[4]

The electrical performance of the system can be tested through a single frequency EIS. This is the most preferred system test because it allows the user to electrically characterize the experimental solution at the sample with respect to a contrast liquid. The test will show that the experimental solution has replaced the contrast liquid between the E-chips and that the active E-chip has a good connection with the flexible contacts in the tip of the holder.

Single frequency EIS is a potentiostatic impedance technique where impedance at a constant frequency is measured and plotted versus time. With the Gamry Framework software the operator can control the frequency, AC amplitude, DC offset and repeat time.

[4] Please consult the Gamry Reference 600+ user guide for model-specific instructions.

Single Frequency EIS is used in this case to sample the impedance and phase of a contrast liquid compared to the desired electrolyte. The contrast liquid should be a liquid that has a higher impedance and lower phase angle than the desired experimental solution. The single frequency EIS analysis should proceed as follows:

1. Prime the sample, microfluidic chamber, internal and external microfluidic lines with the contrast liquid such as water. Continue to flow this contrast liquid through the system for a couple minutes to guarantee that there are no air bubbles.
2. Check for any leaks at the microfluidic connections or at the tip and wait for the liquid to exit the outlet tubing. It can take several minutes for the flow to reach the end of the outlet tubing. Once again, inspect the tip using a stereoscope and verify that the E-chip membranes are intact.
3. Electrically connect the holder to the potentiostat in the standard three electrode configurations.
4. Set up the Single Frequency EIS experiment in the Gamry Framework software. The setup screen can be found under “Experiment > (D) Electrochemical Impedance > (6) Single Frequency EIS”. Set the monitor Frequency to 5100Hz, the AC voltage to 10 V, the DC voltage to 0 V, the repeat time to 0.05 min, the total time to 2 hr. and the estimated Z to 10000 ohms. Do not press “OK” yet.
5. Replace the contrast liquid (such as electrolyte) in the Hamilton Syringe with the desired experimental solution.
6. Using the Harvard Syringe Pump set the pump infuse rate to 300 µL/hr for ex-situ work or 120 µL/hr for *in situ* work.
7. After starting the flow of the new contrast liquid, press “OK” on the Gamry Framework Single Frequency EIS Setup. The experiment will begin.
8. Observe the impedance and the phase angle of the plot in the Gamry Framework Software. As the desired experimental solution replaces the contrast solution at the working electrode of the active E-chip, the impedance will decrease and the phase angle will increase. It will be noticeable, but it could take between 15-20 minutes to flow through the system and diffuse between the two E-chips.
9. When the phase angle and impedance have plateaued after the change from the contrast liquid to the experimental solution at the working electrode, the test is complete. At this point, the experimental solution is between the two E-chips at the working electrode on the electron transparent window and the electrical conductivity of the system, indicating a properly prepared sample.

Cyclic Voltammetry[5]

A cyclic voltammetry sweep, in either the standard two electrode or standard three electrode configurations, will have different ideal potential limits with each E-chip pair because the system is without a fixed true reference potential. This requires the user to begin scanning a smaller potential window than expected. Expand the potential scan limits of each cyclic voltammetry sweep slowly, keeping the current level inside of the bulk oxygen/hydrogen generation reaction.

To Set up a Cyclic Voltammetry experiment. The setup screen can be found in the Gamry Framework Software under “Experiment > (C) Physical Chemistry >(5) Cyclic Voltammetry”.

The current level necessary to begin the reaction will change depending on the nature and concentration of solution in the cell. Too high of a current will drive the bulk reaction and cause the generation of bubbles and alter of the electrode surfaces on the E-chip. If this happens, assuming that the electrode surfaces were not damaged, liquid will need to be flowed through the cell to flush out the liquid between E-chips.

To obtain a curve with pronounced peaks, start small and edge out, making a note of when you see the current increase sharply. You will not see the peaks of interest without going a little past the reaction point (oxide formation is very broad and goes out towards the positive potential limit and you won't see a well-defined oxide reduction without approaching the limit, for example), but too far will generate bubbles. When the curve begins to peak in either direction, gently push the limit by 10-20 mV increments to attempt to obtain the same limit current shape on both the positive and negative extremes.

[5] Please consult the Gamry Reference 600+ user guide for model-specific instructions.

V. Imaging

For best results, it is important to use the lowest electron dose (illumination intensity) possible to reduce perturbation of the liquid by the electron beam. Do not align the electron beam with the Poseidon Select TEM holder inserted. All microscope alignments should be performed prior to inserting the Poseidon TEM holder using a standard TEM specimen holder.

5.1 Locating The window

Use low magnification mode (low mag) to search for the window region. Depending on the E-chip combination and orientation the window regions will look like an illuminated square or rectangle on the phosphor screen, and its size will range from 20 x 20 μm^2 to 50 x 500 μm^2. Regions where the liquid layer is the thinnest will appear brighter, as it is in the corners of the window. The best resolution will be obtained near in the corners of the window. The liquid thickness is greatest at the center of the window due to outward bowing of the silicon nitride membranes in the vacuum.

5.2 Focusing

Once the region is located, immediately reduce the beam intensity (either by spreading the beam or increasing the spot size) to the lowest feasible dose and increase the magnification.

- *Using low mag mode to set the Z-height and focus can cause the liquid to move away from the window (dewet).*

Set the eucentric height (Z-height) using the edge of the window as a reference and continue to adjust the beam illumination to maintain a low electron dose. It is best to optimize imaging conditions within a small region to prevent sample damage. Adjust focus so that there is a defined edge to the window as Continue to increase magnification and adjust the focus and/or Z-height until you have reached the magnification at which the sample can be visualized. Once sample is visible, bring the sample into focus.

- *To save time, store the coordinates for each corner of the window in microscope software. In general, the window region for a give holder will be located at approximately the same stage position for a given microscope*

5.3 Imaging

- *For JEOL microscopes equipped with a high contrast aperture, the in gap objective aperture should not be used. Use the high contrast aperture to increase contrast.*

Image using the highest number spot size with which you can obtain good results (high number spot size = less illumination). If the microscope is equipped with a beam blank, it should be inserted when not actively imaging to prevent specimen damage and or bubble formation. Localized bubbling is often observed under high electron dose conditions, especially when operating at magnifications above 100,000X. Bubbling occurs less readily when imaging under flowing liquid conditions. Bubbles can often be removed by reducing the illumination (either by spot size or reducing magnification) or by blanking the beam, to allow the liquid layer to recover.

5.4 Alpha Tilt

All Poseidon Select TEM holders are single tilt only. Use Table 5 to determine the maximum amount of alpha tilt for the Poseidon model with respective to the microscope pole piece.

Table 5: Poseidon Select Tilt Allowance

Manufacturer	Pole Piece	Poseidon Model	(°) In-Gap Aperture	(°) No In-Gap Aperture
FEI	X-Twin	Poseidon Select Standard	± 29	± 29
	Twin / Supertwin		± 37	± 39
JEOL	ARM 300F FHP	Poseidon Select UHR	NA	±5
	ARM 300F WGP	Poseidon Select UHR	NA	±23
		Poseidon Select Standard	NA	±51
	UHR	Poseidon Select UHR	± 2	± 2
	HRP	Poseidon Select UHR	± 7	± 20
		Poseidon Select Standard	±3	±18
	HT	Poseidon Select UHR	± 24	± 26
		Poseidon Select Standard	±19	±43
Hitachi	HT7700 Std. Lens	Poseidon Select Standard	8	
	HT7700 High Tilt	Poseidon Select Standard	40	

VI. Holder Cleaning & Maintenance

After removal from the microscope, the Poseidon Select TEM holder should be thoroughly flushed and cleaned prior to storage. This section reviews how to clean the platform components.

6.1 Routine Cleaning

Although PEEK tubing is very resistive to clog formation, precipitation of material inside the tubing can occur if the solution is allowed to dry inside the tube. After use, the holder should be flushed thoroughly with a cleaning solvent, such as water in order to maintain the clog-free operation of the Poseidon holder. Flushing can be done manually or with the syringe pump.

1. Flush the entire system with water. Using the syringe pump, flush the system with distilled water for 30 minutes or longer using a flow rate of 300 µL/hour.
2. Remove the lid and internal o-rings from the tip and purge each line with liquid individually until liquid is observed exiting the port in the holder tip. Flush several microliters through each line to ensure adequate flushing of the microfluidics.
3. Dry the tip portion of the holder thoroughly and reinsert the two internal o-rings. The holder can be stored for short intervals without drying if the tip remains sealed and the import/exit ports are blocked using the included set of flow port plugs.
4. Manually flush the PEEK tubing with water after disconnecting it from the holder. For longer storage, after the holder is fully flushed, disconnect the tubing and remove the lid and E-chip stack.

- *Rinse the inside of the tip and lid thoroughly with water and allow it to dry. For best results store the holder with the lid secured to the tip.*

6.2 Cleaning the Microfittings

The ZDV fittinggs through which the PEEK tubing is connected to the holder and/or syringe come in contact with the liquid that injected into the holder tip. Thus, it is necessary to rinse the ZDV fittings between uses when changing liquids. For more thorough cleaning, or to remove material buildup, the ZDV fittings may be immersed in a water or ethanol and cleaned in an ultrasonic bath for several minutes.

6.3 Ultrasonic Tip Cleaning

Thorough flushing of the tubing and rinsing of the Poseidon tip after each use will generally be enough to keep the system clean. However, ultrasonic cleaning may be used when

simple rinsing is not sufficient. The integraded flexible electrical circuit (which contains EPDM and Kapton components) is immersible in water, ethanol or acetone for cleaning.

To clean the Poseidon holder ultrasonically, immerse only the tip of the Poseidon Select TEM holder in a small beaker of distilled water, ethanol or acetone and sonicate for 30 seconds to 1 minute. Note the location of the vent holes on the shaft of the holder. Do not allow liquid to enter the vent holes. Figure 13 shows the maximum safe level of liquid immersion for the Poseidon Select TEM holder tip.

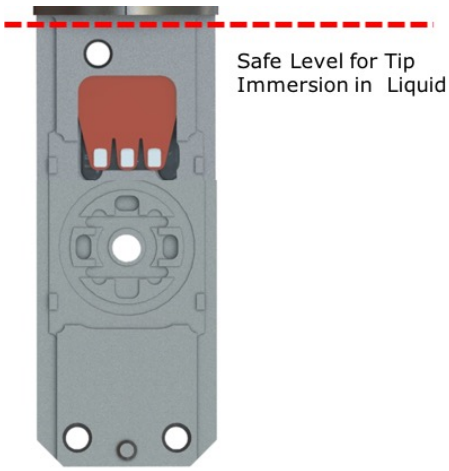


Figure 13: Ultrasonic Cleaning of the Poseidon Select Tip

6.4 Exchanging the Internal Tubing

The Poseidon Select TEM has a unique, patent pending “tube-in-a-tube” design for liquid delivery to the tip of the holder, as shown in Figure 1. A permanent outer tube of 316 grade stainless steel, which is does not come into contact with the liquid, is hermetically attached to the titanium tip of the holder. Liquid is then fed to the tip of the holder via a PEEK capillary tube insert that seals against the stainless steal tube. A liquid tight o-ring attached to the tip of the PEEK capillary insert forms a liquid tight seal at the tip of the holder and prevents backflow of liquid into the permanent stainless steel tube. The user can easily change the inner tube, wetted tube, without disassembling the holder. This tube-within-a-tube design which is the safest, most effective method to replace tubing. Unlike other designs, the Poseidon design seals the inner tube against an outer tube that is hermetically sealed to the tip of the TEM holder and extends to the handle of the holder. This design allows the liquid to be sealed with an o-ring against atmosphere, rather than the vacuum of the TEM column. In the unlikely event the o-ring becomes damaged or

unable to hold vacuum, a leak would only occur within the outer tube which extends to the handle.

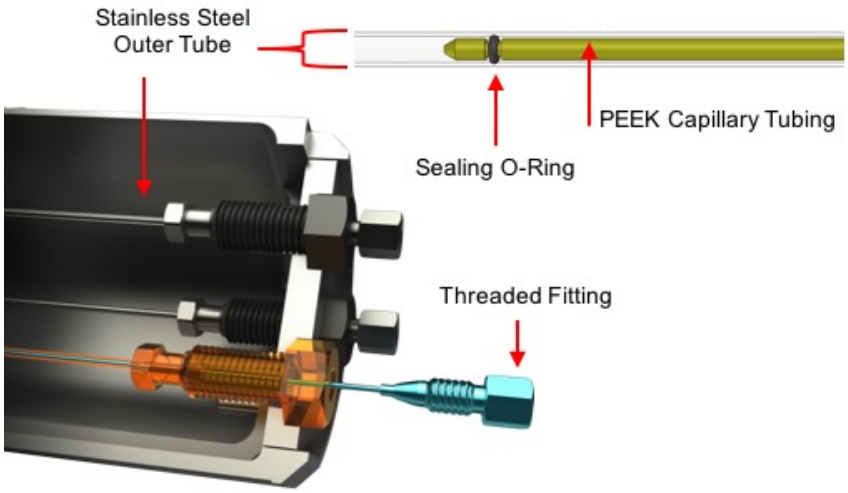


Figure 14: Replaceable Microfluidic Tubing

Replacing the internal tubing is straighforward, as shown in Figure 15

1. Take the new PEEK capillary insert and insert the tube into the fitting at the holder base.
2. Push the tubing in, about 12 inches, to fully insert.
3. Screw the threaded fitting into place to secure the capillary tubing insert.
4. To remove a PEEK capillary insert, unscrew the threaded fitting located on the base of the holder, and pull the insert out of the stainless steel outer tubing.

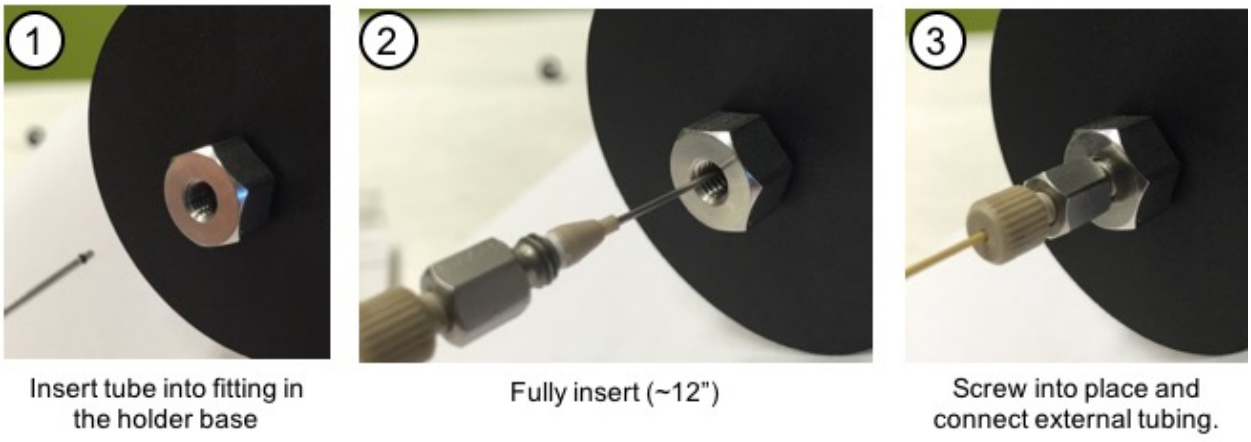


Figure 15: Insertion of the PEEK Capillary Insert

6.5 Plasma Cleaning the Poseidon Holder

Like most TEM holders, the Poseidon TEM holder should be plasma cleaned periodically to remove any residual organic material from the holder tip. The Poseidon holder is fully compatible with commercial plasma cleaners, and depending on usage the holder should be plasma cleaned on a regular basis. The more the holder is used, the more it should be cleaned. Generally, 5 to 15 minutes in argon and/or oxygen plasmas should suffice. It is also recommended that the holder be stored under vacuum when not in use.

1. Remove the lid and internal o-rings from the tip of the sample holder.
2. Ensure that the liquid ports are flushed with water, then flush the tubing with air to remove any liquid from the internal tubing (simply “fill” the syringe with air to push any remaining liquid.
3. Insert the flow plugs in the liquid ports.
4. Insert the Poseidon TEM holder into the plasma cleaner and perform a plasma cleaning cycle. The specimen tip lid may be cleaned using the same procedure with plasma cleaners that contain a shelf for cleaning smaller parts.

- *When plasma cleaning the Poseidon holder without E-chips in place it is necessary to close the liquid input and output ports by inserting the flow port plugs. If plasma cleaning a holder with E-chips in place it is not necessary to close the liquid ports.*

VII. Sample Preparation

7.1 Contrast and Imaging Conditions of Low-Z Specimens

For organic-based samples, there is significant variety in the electron density, mass thickness etc., from sample to sample. This can make it difficult predict what types of organic materials are suitable for imaging with Poseidon, particularly because the thickness of the liquid and the beam acceleration voltage can significantly impact the results. A good rule of thumb is that the closer the phase of the material is to the liquid that it is in, the less contrast it will have with Poseidon.

Dense, cross-linked, or highly conjugated organic materials such as polymers, carbon black, and latex nanoparticles are relatively easy to image with a variety of acceleration voltages due to their high intrinsic contrast. Very small and/or low contrast samples usually give better contrast when imaged with low acceleration voltages (i.e. 120 kV) since lower energy electrons have a higher chance of interacting with the sample and being scattered. Examples of different types of samples and their recommended imaging conditions in Table 6. Hollow or semi-permeable vesicle samples usually have extremely low contrast, and are very fragile with respect to the electron beam. However, this does not necessarily preclude using imaging in liquid. Low dose conditions help reduce sample degradation due to electron exposure and increases the contrast that can be obtained. Because these particles are often designed to allow loading of material inside them (as drug delivery vehicles) so their contrast can be manipulated by either loading a high contrast material inside them or integrating high contrast materials into the membrane itself.

Table 6: Sample Type and Liquid Imaging Conditions

Sample Types	Examples	Preferred Conditions
High-Z Metal Materials	Gold, Platinum, Lead	100 - 300 kV TEM or STEM
Low-Z Metal Materials	Calcium Carbonate, Iron Oxide, Silver	120 - 300 kV TEM or STEM
Biological Particles < 150 nm	Proteins, Virus	120 kV low dose
Vesicles	Micelles, Liposomes	120 kV low dose TEM or STEM
Prokaryotic Cells	Bacteria	200 kV TEM or STEM
Eukaryotic Cells	Yeast, Mammalian Cells	STEM
Organic Nanoparticles	Carbon Black Latex Beads, Carbon Nanotubes, Dendrimers, Polymers	120 kV - 300 kV TEM or STEM

7.2 E-chip Orientation for TEM/STEM Imaging

For applications in which the sample is immobilized to the surface of the E-chip window, it is important to attach the sample to the E-chip that is closest to the point of electron Beam Entry or Exit for best resolution.

The order in which the E-chips are inserted into the holder does not necessarily correspond to their orientation once the holder is inserted into the microscope. First, determine the orientation of your Poseidon holder when it is inserted into the microscope goniometer. Does the lid face upwards (toward the electron beam) or downwards when it is fully inserted?

- If the lid is facing upwards (Not Inverted): The large E-chip is closest to the point of electron beam entry and the small E-chip is closest to the point of beam exit. Poseidon Select Standard TEM holders are not inverted.
- If lid is facing downwards (Inverted): The small E-chip is closest to the point of electron beam entry and the large E-chip is closest to the point of beam exit. Poseidon Select UHR TEM holders are inverted.

When imaging in TEM mode the sample should be attached to the E-chip closest the point of Beam Exit for the best resolution. When imaging in STEM mode, best resolution is

obtained when the sample is located at the point of Beam Entry. Use Table 7 to select which E-chip the sample should be attached to obtain the best resolution for a TEM/STEM.

Table 7: Determining E-chip Orientation

Holder Orientation	TEM	STEM
Not-Inverted	Small	Large
Inverted	Large	Small

7.3 Hydrophilic/Hydrophobic E-chip Surface Preparation

In order to reduce the instances of dewetting of the liquid chamber during an experiment it is best to match the hydrophilicity of the solvent used with the surface hydrophilicity of the E-chip.

Hydrophilic Surface

For samples that require a hydrophilic surface, it is recommended to plasma clean the E-chip using a glow-discharge or plasma to make the surface hydrophilic.

1. Remove the E-chip(s) from the gel pack and place them on a glass slide.
2. Place the slide and E-chip(s) into the plasma cleaner or glow discharge unit.
3. For E-chip(s) without glassy carbon electrodes it is good to clean the E-chip(s) for 2-5 minutes. For E-chip(s) with glassy carbon electrodes do not clean the E-chip(s) for more than 1 minute. Plasma cleaning has an adverse reaction with the glassy carbon under extended times and intensities.
4. Inspect the integrity of the glassy carbon electrode under a stereoscope after plasma cleaning. E-chips are ready for further surface preparation or immediate use.

The surface of the E-chips will remain hydrophilic for several after plasma cleaning. Plasma cleaned E-chips will become more hydrophobic over time. They may be re-plasma cleaned to restore surface hydrophilicity.

Hydrophobic Surface

Certain samples require a hydrophobic substrate in order to adhere properly. The SiN surface of the E-chip can be made hydrophobic by heating them on a hotplate.

1. After removing the photoresist coating transfer the E-chips onto a glass dish containing clean filter paper.
2. Heat the E-chips at 150 ˚C for 1.5 hours.

3. Remove from the hotplate and let cool.

7.4 Drying sample onto the E-chip

For many applications, particularly electrochemistry, it is convenient to dry the sample onto the surface of the E-chip. Determine which E-chip the sample should be dried onto using the guidelines in Section 7.2.

Samples Dissolved in High Vapor Pressure Solvents

For samples dissolved in quick drying solvents, such as methanol or hexane, the liquid will generally evaporate quickly enough for a good dispersion of sample to be found across the window of the E-chip.

1. Place the E-chip that sample will be deposited onto on the sticky surface of the gel-pack to keep them in place (make sure the E-chips are membrane side up; do not allow the membranes to contact the sticky gel surface).
2. Dispense a small volume of sample onto the E-chip and allow the liquid to dry. A heat lamp may be used to reduce the drying time.
3. Check the sample deposition with an optical microscope. If more sample is needed repeat Step 2 until an adequate amount of sample has been deposited.
4. (Optional) Plasma clean or glow discharge the E-chip a briefly for 10-15 seconds to adhere the sample. This step should only be performed for samples that are robust to the plasma.

Samples Dissolved in Aqueous Solvents

1. Plasma clean the E-chips so that the SiN membrane is hydrophilic.
2. Dispense a small volume of sample onto the surface of the E-chips and let the droplets sit for 5 minutes. (Note, do not let the droplets dry out on the E-chip).
3. Remove the excess droplet from the E-chip. This can be done by wicking off the remaining liquid with filter paper or drying it quickly with compressed air or gas.
4. Check the sample deposition with an optical microscope. If more sample is needed repeat Step 2 until an adequate amount of sample has been deposited.
5. (Optional) Plasma clean or glow discharge the E-chip a briefly for 10-15 seconds to adhere the sample. This step should only be performed for samples that are robust to the plasma. Note that this will remove organic surfactants or passivating molecules from the surface of the sample.

7.5 Creams and Gels

Thick or viscous samples, such as oils, emulsions, creams, and gels can be imaged by placing a thin layer of the material on either the small or large E-chip. Because of their viscosity, gels and creams should not be flowed through the tubing.

1. Spread a thin layer of sample on clean surface.
2. Invert the E-chip, and set the membrane side down, on top of the thin layer of sample. Avoid pressing down on the E-chip to prevent over-loading of sample.
3. Check that the window is intact.
4. Transfer the E-chips into the Poseidon holder. Assemble the tip.

7.6 Adherent Cell Preparation

Cells may be grown, transformed, labeled or processed directly on an E-chip as shown in Figure 16. The E-chip material is compatible with standard sterilization and tissue culture protocols and equipment. Thus, E-chips can be easily incorporated into current practices, streamlining laboratory procedures. The following steps outline the process of culturing cells directly on the Poseidon E-chips[6].

1. Immerse the E-chips in a solution of 0.01% poly-L-lysine for five minutes at room temperature. Remove excess poly-L-lysine by soaking the E-chips briefly in fresh HPLC grade water. It is not necessary to allow the coated E-chips to dry.
2. Transfer poly-L-lysine treated E-chips into new well of the 96 well plate. Hold the E-chips so that they remain upright (flat side up) at all times, and do not allow the tweezers to come into contact with the surface of the microchip, except at the edges.
3. Immerse each E-chip in a well containing ~150 µL of growth media.
4. Add a droplet of cell suspension to each well.
5. After ~1 minute the E-chip were inspected with an inverted light microscope to verify that cells had begun to adhere to the windows.
6. An E-chip should have no more than ~1 adhered cell per 50 x 50 µm2 area. The presence of more cells than this inhibits them from flattening against the window.
7. Check the cell density on the window of the E-chips after ~5 minutes. If the desired amounts of cells are not on the window, another droplet of the cell suspension may be added. If too many cells are present, transferring the E-chip into a new well with fresh media may remove cells which are not tightly adhered to the window surface.
8. Once the desired amount of cells is observed on the window, transfer the E-chip to a new, media filled well and incubate as normal.

[6] Protocol is adapted from: PNAS 106(7): 2159-2164

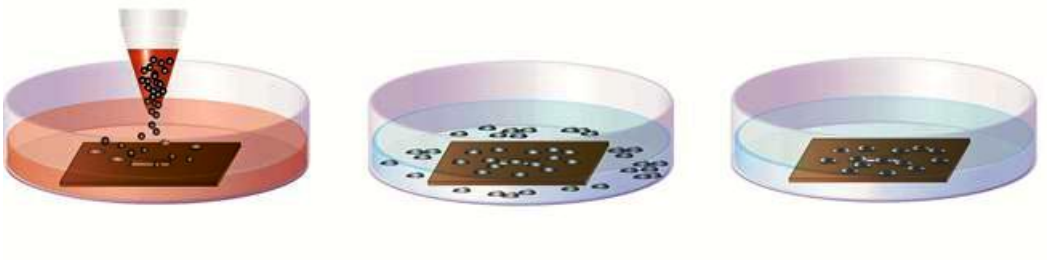


Figure 16: Preparing Adherent Cell Samples

VIII. Miscellaneous

8.1 Chemical Compatibility

Care should be taken to avoid chemicals that could etch the materials used to manufacture the Poseidon platform. These materials include titanium (holder tip), kapton and EPDM (electrical contact pad), silicon, silicon nitride, SU-8, gold (E-chips), stainless steel (permanent tubing), PEEK (microfluidic tubing), and glass (syringe). Approved chemicals must be used with the appropriate o-ring material.

Please consult the Protochips Poseidon Customer Portal for chemical compatibly questions.

8.2 Ground Test for Available Outlets:

The ground for an available outlet is considered to be good if it meets the following criteria:

- The resistance (Ω), as measured with a multi-meter, <1 ohm from the grounding plug on the outlet to either a bolt on the TEM column or TEM zero point grounding plate.
- Voltage potential from ground plug on the outlet to TEM column or TEM zero point grounding plate should less than <1 volt. The Initial voltage value can be higher than 1 volt so as long as the final potential drops to less than 1 volt.

If the available outlet does not meet the ground test outlined above, it is necessary to ground the Gamry Potentiostat directly to the microscope using the ground wire supplied in the ground kit. If the available outlets fail this test, the ground plugs on the Gamry and the computer must be defeated using the ground lift kit supplied with the system.

- ***WARNING***: The equipment should not be double grounded or you will cause a ground loop. If a piece of equipment has a 3 prong outlet, its chassis should NOT be grounded to another ground without first defeating the grounding plug on the outlet.

Grounding directly to the TEM:

1. Defeat the grounds on the Gamry & computer using cheater cables or plugs.
2. Ground Gamry and computer to TEM zero point grounding point, or other grounding point that meets the resistance and voltage criteria: Resistance < 1 Ω and Voltage < 1 V using the supplied ground wire.

Special considerations when grounding directly to the TEM:

- *If the computer does not have 3 port outlet, use of cheater plug is not required.*

- *In certain situations, computers that do not have a grounding plug (3 port outlet) may require they be grounded manually to the TEM ground as indicated above ether by grounding the USB cable or by grounding the computer chassis.*

Grounding issues with laptops can be verified by floating the computer (running on battery), and checking to see if issues clear up.

8.3 Diagnosing Poseidon Holder Leaks

If the Poseidon holder fails a leak test the most common sources of failure are broken/defective E-chip windows, improperly seated E-chips, debris in the tip pocket or defects/debris on the o-rings (both external and internal).

The following steps will enable the user to diagnose if the holder has developed a leak that requires a repair. First compare the vacuum level that the holder reaches with the fluid ports open to the vacuum level that the holder reaches with the fluid ports closed (flow port plugs in place).

1. Record the vacuum level of the holder in the leak check station with the fluid ports open. Remove any microfluidic tubing and make sure that the flow port plugs are not inserted.
2. Record the vacuum level of the holder in the leak check station with the fluid ports closed. Close the fluid ports by inserting the flow port plugs.
3. **Results: Vacuum Level Drops**: If the vacuum level drops after switching the fluid ports from open to closed, the leak check failure probably due to either a broken window or a debris in the tip or on the internal o-rings. Replacing the E-chips or cleaning the holder tip will most likely correct the issue. Note that if the window is broken, the holder will not generally reach a pressure below $5x10^{-5}$ torr with the flow port plugs in place. **Proceed to Step 3.**
4. Results: The Vacuum Level Stays the Same
5. If the vacuum level stays the same, the leak is likely due to debris/defects on the external o-rings, debris or defect on the pump flange, or a leak at the rear vacuum wall of the holder. **Proceed to Step 7.**
6. Remove the E-chips and inspect them for broken windows or damage. Check the back of the small E-chip to verify that there are no defects or imperfections in the silicon frame which could cause the E-chip not to seal against the small o-ring.
7. Remove the internal o-rings and check for any debris in the o-ring groove.
8. Clean the tip of the holder thoroughly to remove any debris in the pocket.

9. Reinsert or replace the o-rings and verify that there is no lint or fibers on the o-ring surface.
10. Insert a pair Blank (windowless) E-chips into the holder to eliminate window breakage as a factor. If Blank E-chips are not available, insert a new, clean pair of E-chips. Do not add sample or liquid.
11. Repeat Steps 1 & 2.
12. **Results**: Holder passes the leak check: it is functioning properly
13. **Results:** Vacuum drops below original value, but does not pass: **Proceed to Steps 9-15.**
14. Inspect the external O-rings for debris or defects.
15. Clean or replace the external o-rings as needed. Ensure that the external o-rings are adqueately greased.
16. Inspect the pump flange for debris or scratches, and if necessary clean the flange with compressed air and an alcohol swab.
17. Leak check the holder.
18. **Results:** Holder passes the leak check: it is functioning properly
19. **Results:** Vacuum drops below original value, but does not pass: **Repeat Steps 4-8 and Retest.**
20. **Results:** Vacuum level does not change: **Contact Protochips.**

8.4 Setting the Torque Driver

A torque driver is supplied with each Poseidon platform to ensure an accurate seal and to prevent the E-chips from breaking due to uneven pressure across their surface. Protochips recommends a torque setting of 0.1 - 0.14 ft-lbs/in.

1. Assemble the torque driver pressing the flathead tip into the driver, until it clicks and remains locked in position, as in Figure 17A-B.
2. To input the torque limit, twist the black plastic ring counter-clockwise so that it is in the free position, as shown in Figure 17C.
3. Because “0.1” is not listed on the torque scale, first set the torque value 0.20 ft-lbs/in, as in Figure 17D.
4. Turn the handle counter-clockwise six steps to give a final value of 0.14 ft-lbs/in, as in Figure 17E.
5. Finally, set the torque value by rotating the plastic disc clockwise from “free” to “lock” as in Figure 17F.

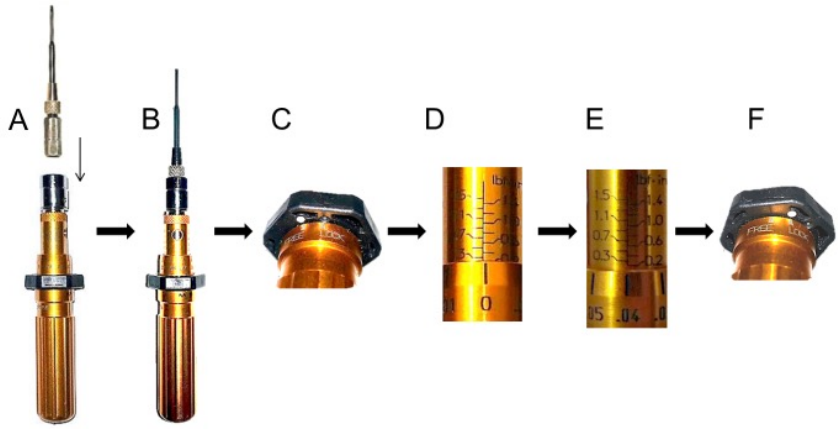


Figure 17: Setting the Torque Driver

8.5 Determining Liquid Thickness

Electron Energy Loss (EELS) is the most common way to measure liquid thickness. However, the thickness of the liquid layer for a given region can be also be determined from the parallax shift. This method has the advantage that it does not require calibration and does not require energy filtering. The fluid thickness (T) is determined by parallax shift (p) over a tilt angle of ±γ, using the following equation:

$$T = \frac{p}{2 \sin \gamma}$$

In order to measure the parallax shift it is necessary to have distinguishable features on both the top and bottom membranes. Furthermore, these fiducial markers must be in close proximity to one another, and located in the region of interest. Note that the collection of parallax data is often not possible or convenient without altering the sample or introducing fiducial markers (such as gold nanoparticles) onto the membranes.

- *For further information please refer to: L. Reimer and H. Kohl, Transmission Electron Microscopy, Springer, New York, 2008, page 259*

When imaging with scanning transmission electron microscopy (STEM), the liquid thickness between the SiN windows at the position of the beam can be calculated by the ratio of electrons scattered onto the detector, N, to the electrons in the probe, N0, as shown in the equation below:

$$T = -I(\beta) In(1 - \frac{N}{N_0})$$

Where T = liquid thickness, I(β) = The mean free path length of an elastically scattered electron at opening angles β or larger. (For water, this value is 10.4 μm).

8.6 Window Bulging

For all liquid in-situ TEM experiments, window bulging, due to the pressure differential of the vacuum in the TEM column, is an inherent issue. The effect of window width on bulging is shown graphically in Figure 18.

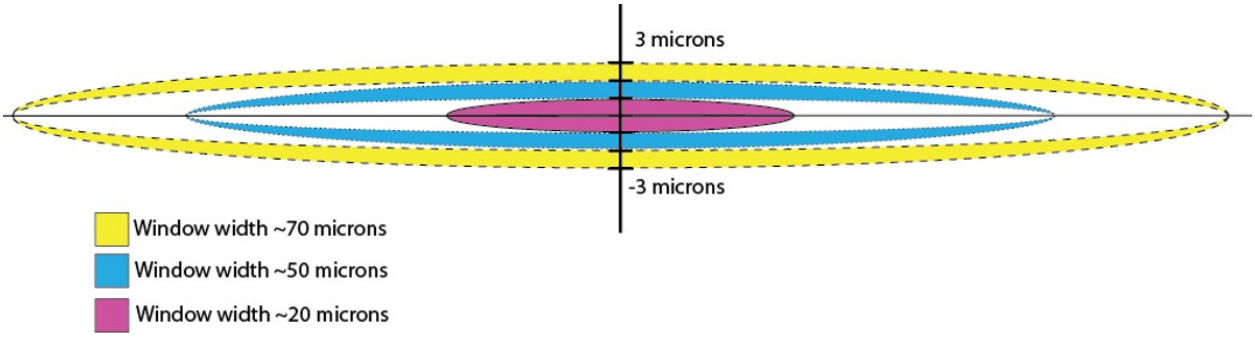


Figure 18: Window Bowing

This bulging effectively increases the thickness of the liquid layer and creates a variable liquid layer thickness across the width of the cell. This increase in liquid thickness is substantial, often several microns. As shown in the equation below, for a fixed applied pressure P, the amount of bowing (displacement Wo) is directly proportional to the square of window edge length a, and inversely proportional to membrane thickness t.

$$W_o = \alpha(Pa^2)/4t$$

In short, the amount of bulging for is proportional to the width of the window, provided that the thickness of the SiN film is the same.

8.7 Window Orientation

In addition to the width of the window, the relative orientation between the top and bottom windows can also affect the liquid thickness across the in situ cell. Aligning both windows parallel to one another yields a large rectangular viewing area. Positioning the windows in parallel configuration provides the maximum sample viewing area. However, due to inherent size variation among windows, parallel configuration typically results in miss-alignment of the windows as shown in Figure 19.

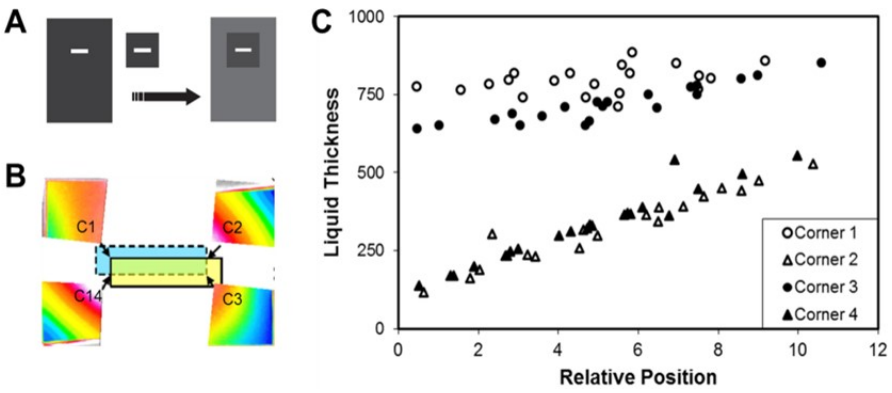


Figure 19: Parallel E-chip Orientation

Crossing the windows perpendicular to one another yields a smaller, square viewing area, but provides more accurate window alignment. Crossed windows exhibit a more reliable thickness profile than using the windows in parallel configuration. Although the sample viewing area is reduced in comparison to parallel window orientation, crossed windows provide a more uniform liquid thickness regardless of window alignment as shown in Figure 20.

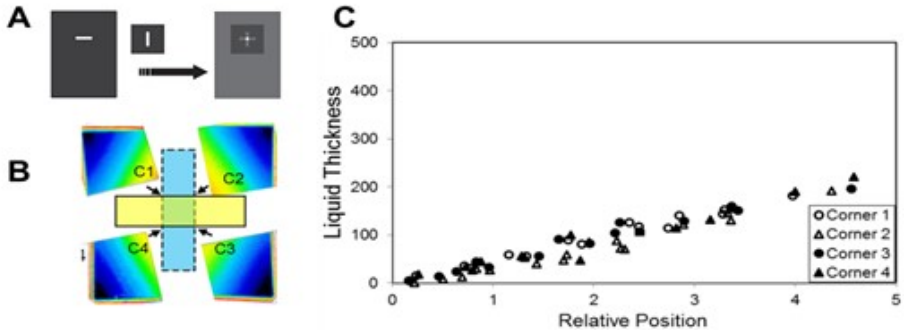


Figure 20: Crossed E-chip Orientation

IX. Frequently Asked Questions

What is the range of liquid thickness if Poseidon is used?

Liquid thickness is set using the spacer E-chip. Spacers are fabricated directly on the spacer E-chip and do not require any user assembly. Consult the most recent E-chip Selection Guides for available spacer sizes.

Can liquid be adjusted to any thickness we want with Poseidon? How about the accuracy of liquid thickness?

The integrated spacers on the E-chips set the nominal liquid thickness. However, because the silicon nitride membrane bulges in the vacuum of the microscope there is a gradient of liquid thickness across the window. Thus the liquid thickness is the nominal spacer thickness at the edges and corners of the window, but may be thicker at the center. Please see the following paper for information about liquid thickness and bowing: Microsc.Microanal., 2013, 19 (4) pp.1027- 1035

Based on your experience, is there any suggested value of thickness for most kinds of liquid to get good TEM image quality?

The liquid thickness must be large enough to enclose the sample. In general, the thinner the liquid the better the imaging quality. Thin liquid layers cause less scattering, and provides better resolution. Scanning Transmission Electron microscopy (STEM) can image through much thicker liquid layers than TEM. We recommend that the 5 micron spacers only be used for STEM imaging**.**

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In this manual provide a general guide for liquid thickness parameters.

Won't particles move too fast to focus well during TEM observation?

Particle movement depends on many factors including: flow rate, sample charging, liquid thickness and electron dose. In general, Brownian motion is limited due to confinement of the sample between the windows of the liquid cell, however, some movement is inevitable. Customers are urged to consult the scientific literature to see what types of resolution/images can be obtained.

Is there any outgassing to TEM column during observation?

No, if the system is properly sealed and leak-checked prior to insertion into the microscope no outgassing will occur.

Can the Poseidon E-chips be cleaned with an ultrasonic bath?

No. Sonication pf the E-chips will result in rupture of the silicon nitride membranes.

Can Poseidon E-chips be re-used?

Poseidon E-chips are designed to be consumables. In order to ensure reliable performance and prevent cross-contamination of samples, E-chips should not be reused. However, used E-chips can be saved to test sample dilutions, surface chemistry, ex-situ, or to demonstrate/practice loading the holder.

Can Poseidon E-chips be sterilized?

E-chips may be sterilized by autoclave, UV-light, and chemically, such as with ethanol or formaldehyde.

What is the minimum/maximum pump speed?

Liquid can be delivered using a pump speed as low as 1.28 pl/min. The pump motor has been observed to stall when using speeds above 510 uL/hr due to the small diameter of the PEEK tubing. Protochips recommends using a flow rate of 120 µL/ hour (2 µL/minute) or less during imaging. The flow rate should not exceed 300 µL/hour (5 µL/minute) when the holder is in the microscope.

Can I use Poseidon without flow?

Yes. Poseidon can be operated with a flow rate of zero or without the pump attached.

Where can I get replacement screws, gaskets, o-rings, and exchangeable tubing?

Replacements should be purchased from Protochips, Inc., to ensure compatibility.