Poseidon™

SAMPLE PREPARATION GUIDE

Handling and Surface Preparation

The Poseidon system enables in situ imaging of liquid and hydrated samples inside the electron microscope. Liquid is isolated from the vacuum between two semiconductor specimen supports, called E-chips, that are loaded into the tip of the Poseidon TEM holder. Each E-chip contains a transparent silicon nitride (SiN) membrane for imaging. One E-chip contains an integrated spacer layer, which sets the liquid thickness (shown in yellow in Figure 1) and allows the chamber to be easily configured for flow or static (non-flow) operation. The second, larger, E-chip, which is flat, is then pressed against the spacer E-chip to form the sample chamber. The following material is provided to serve as a guide for preparing E-chips for use, and basic Poseidon sample preparation techniques.

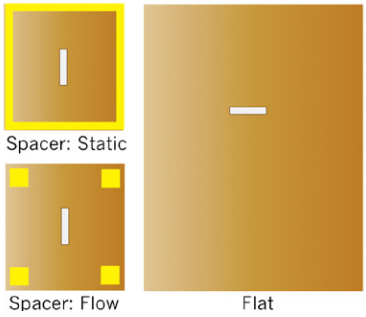

Figure 1: E-chip configuration

Recommended supplies and equipment

Stereoscope	Well Plate or Spot Dish
Carbon Fiber Tweezers	Hot Plate
HPLC Grade Solvent	Filter Paper
Beakers or Dishes (2)	Dry-Pump Station
Residue-Free Compressed Air	Pipet (0.5-10 µL)
Alcian Blue	Eyelash Tools
Glow Discharge or Plasma Cleaner	

E-chip handling and cleaning

Poseidon E-chips feature a very thin SiN membrane and should be handled carefully to avoid breaking this membrane. E-chips are supplied to customers in "gel-paks" with the membrane side of the chips facing upwards. Remove the E-chips from the gel-pak by gripping the edges of the chip with the carbon fiber, rather than metal, tweezers, to prevent damaging the silicon frame of the E-chip.

E-chip cleaning

Prepare the E-chips in a clean, low-dust environment, and use high purity, HPLC-grade solvents, to prevent particles from settling on the surface of the E-chips during and after the cleaning process.

1. Submerge the E-chips in a dish or beaker of acetone. Agitate the E-chips by swirling the dish in a circular motion for 1-2 minutes
2. Next, transfer the E-chips into a dish or beaker of methanol (Do NOT allow the E-chips to dry during the transfer) and swirl for 1-2 minutes

E-chip drying

To prevent drying residues and contamination from dust particles, the E-chips may be dried using residue-free compressed air.

1. Hold the E-chip by the edges with the carbon fiber tweezers and remove the E-chip from the final rinse
2. Gently wick off the excess liquid by touching the edge of the E-chip to a piece of filter paper.
3. Still holding the E-chip with the tweezers, gently direct a flow of air across the E-chip surface, until it is dry.

E-chip surface preparation

The SiN surface of the Poseidon E-chip, which is normally mildly hydrophobic, may be modified prior to experimentation to improve liquid flow and promote sample adherence to the E-chip surface.

Hydrophilic Surface Preparation

When working with aqueous or polar solvents, a hydrophobic surface can interfere with establishing flow through the chamber. Thus, the surface of the E-chip should be made hydrophilic prior to working with samples in aqeuous or polar mediums. This is easily accomplished by introducing either a negative or positive charge to the E-chip surface.

Negative Charge

A negatively charged surface can be introduced by briefly plasma cleaning the E-chip or exposing them to a glow discharge. This treatment also serves to eliminate any hydrocarbon residue which may be present on the E-chip.

1. Place the clean, dry E-chips on a clean glass slide and glow discharge or plasma clean the E-chips for 2-5 minutes.
2. The hydrophilicity can be tested by dispensing a 1-2 µL droplet of water onto the E-chip. Liquid should spread evenly across the surface of the E-chip, and will remain hydrophilic for several hours.

Positive Charge

As an alternative to glow discharge or plasma cleaning, the surface can be made hydrophilic by dipping the E-chips in alcian blue, a cationic copper phalocyanine dye.

1. Place a droplet (~25 µL) of alcian blue into one well of a 96 well plate or spot dish. Fill 4-5 of the remaining wells with water.
2. Dip the E-chip face down onto the droplet of alcian blue
3. Next, dip the E-chip consecutively in each of the wells containing water, until the water remains colorless.

Hydrophobic Surface

The hydrophobicity of Poseidon E-chips can be increased by heating the E-chips on a hot plate to bake off residual moisture from the surface. This treatment improves

adherence of lipid and other nonpolar materials to the SiN surface.

1. Place clean E-chips onto a glass slide and place the slide a hot plate.
2. Heat the E-chips at 150 °C for 1.5 hours.
3. Remove the E-chips from the hot plate and let cool.

Sample loading

Samples can be loaded into the Poseidon holder in several ways. It is recommended that sample loading and tip assembly be done under a stereoscope, with a magnification of at least 10x. Prior to loading the E-chips into the holder, prime the lines with either water or solvent to ensure proper sealing and to maintain hydration. Place the spacer E-chip into holder tip and add a droplet of sample solution or buffer. Next, position the flat E-chip on top to form a sandwich. If there is too much liquid between the E-chips it may prevent correct alignment of the E-chips. Excess liquid may be wicked off from the sides of the E-chip sandwich using filter paper. Once the E-chips are seated, attach the lid, using the pin at the front of the tip to guide placement and secure it with the three brass screws.

Suspended Samples

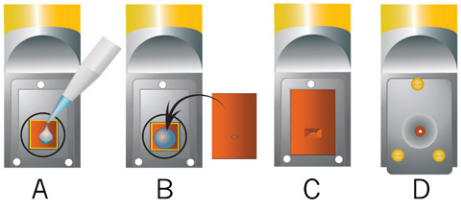

Figure 2: Loading the Poseidon holder

Samples suspended in solution, such as nanoparticles, virus, proteins, macromolecules, and bacteria, can be applied using a small volume pipet (0.5-10 µL).

1. First, clean and inspect the tip of the Poseidon holder.
2. Next, position the spacer E-chip in the holder tip and dispense a 0.5-2 µL droplet of sample onto the surface (2A).
3. Invert the large E-chip (2B) and position it over the spacer E-chip (2C).
4. Secure the lid to the tip using the three screws.

Select samples can be applied to the E-chip, and allowed to dry, after which the can be stored for imaging at a later time. Samples need to be resistant to drying artifacts, free from aggregates, and sufficiently dilute so as to prevent formation of salt crusts, which will lead to improper seating of the two E-chips. It is necessary to rehydrate such samples by applying a droplet of buffer or solvent between the E-chips during flow. This will also serve to prime the sample chamber for establishing flow.

Viscous Samples

Thick or viscous samples, such as oils, emulsions, creams, and gels can imaged in an undiluted state using a static cell configuration.

1. Place the spacer E-chip in a gel pack to prevent it from moving.
2. Carefully apply a thin layer of sample over the E-chip so that the sample is positioned across the SiN membrane using an eyelash tool.
3. Transfer the E-chip into the Poseidon holder.
4. Position the second E-chip and assemble the tip.

Adherent Cell Samples

Adherent cells can be grown directly on a flat E-chip using typical tissue culture conditions. For the following steps, place each E-chip into a well (of a flat bottom culture dish) that has been pre-filled with fluid. Then, transfer the E-chip between wells during each solution change or rinse step as described below.

1. Glow discharge or plasma clean the E-chips prior to use.
2. Soak the E-chip in a solution of 0.01% poly-L-lysine for 5 minutes, then rinse 3 times in water.
3. Place each E-chip into a well containing cell growth media with the E-chip oriented such that the flat surface is facing up.
4. Add a droplet of cell suspension to each well containing an E-chip .
5. Place the well plate in the incubator and allow cells to attach for several minutes
6. Check periodically until 4-5 cells are observed sticking to the SiN window.
7. Wait 5-10 minutes, then transfer each E-chip to a new well containing media (300 µL).
8. Allow the cells to adhere and grow under normal incubation conditions.
9. Transfer the E-chips between wells to rinse, label, fix, or stain the cells as desired.
10. Load the flow E-chip into the Poseidon holder (Live cells can be loaded in the Poseidon holder for immediate imaging, or fixed with glutaralehyde, for imaging at a later time). Plasma clean the spacer E-chip and load it into the holder. Add a droplet of cell-compatible buffer, and then place the flat E-chip containing the cells on top. Secure the tip, and operate with continuous flow to maintain hydration.

Gold fiducials

Fiducial markers can be introduced onto the E-chip surface by applying gold nanoparticles to the surface of the E-chip prior to sample loading.

1. Glow discharge the spacer E-chips (2 minutes in either a glow discharge or plasma cleaner is fine)
2. Place the spacer E-chips back onto the gel-pack to hold them in place (make sure the E-chips are membrane-side up, do not allow the membranes to contact the sticky gel surface).
3. Dispense 0.5-1 microliter of the aqueous gold solution onto the surface of the E-chips and let the droplets sit for 5. (Note, do not let the droplets dry out on the E-chip).
4. Remove the excess droplet from the E-chip (either by wicking it off with filter paper or removing it with the pipet).
5. Dispense 0.5-1 microliter of the water onto the surface of the E-chip and let sit for 5 minutes.
6. Pick up the E-chip from the gel-pack and hold it by the edges with the carbon fiber tweezers.
7. Use compressed air to blow the excess liquid off the E-chip, and dry the surface.
8. Plasma clean or glow discharge the E-chip a second time to stick the gold nanoparticles to the surface.