Poseidon 210™

Introduction to Poseidon Microwell E-chips

Introduction

Conventional liquid chamber designs for *in situ* transmission electron microscopy (TEM) employ two ultrathin, electron transparent films, such as amorphous silicon nitride (SiN), sandwiched together in a dedicated sample holder. Due to beam charging effects and Brownian motion, the sample is able to move freely within the volume of liquid contained between the two films if no surface modification is used to tether and immobilize the sample. Such motion complicates *in situ* liquid imaging because it increases the time necessary to locate and focus on the sample, leading to beam damage, and causes loss of resolution due to blurring. To address these issues, Protochips has developed a new E-chip for the Poseidon *in situ* liquid system called the Microwell E-chip. This E-chip features an array of micron-sized wells etched into the surface of the SiN window. Liquid fills the microwells, which function like individual compartments of an ice cube tray, preventing the sample from moving along the length of the window.

Microwell E-chips enable very small, compartmentalized volumes of liquid to be imaged. Figure 1A shows a single 10 x 10 µm well that is partially filled with liquid. Liquid is pooled primarily along the sides of the well, or as single droplets in the center. Samples can then be imaged in very thin liquid layers, enabling better resolution and better contrast. Researchers using Microwell Poseidon E-chips have imaged low contrast macromolecular specimens such as liposomes (Figure 1B) and transcriptionally active rotavirus (Figure 1C). The thin liquid layers enable better contrast and higher resolution of low-Z elements compared to conventional SiN window chips. Individual microwells can be imaged independently of one another, which enables researchers to mitigate beam damage by sequentially imaging each microwell in the array. Processes such as electron beam induced nanoparticle growth or degradation, which are highly dependent on beam dose, can be systematically studied in individual microwells without disturbing the sample in a neighboring well. Figure 1D shows lead nanoparticles that were formed from a solution of lead nitrate contained in a microwell that was exposed to the electron beam.

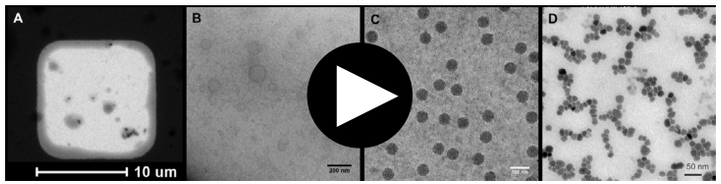
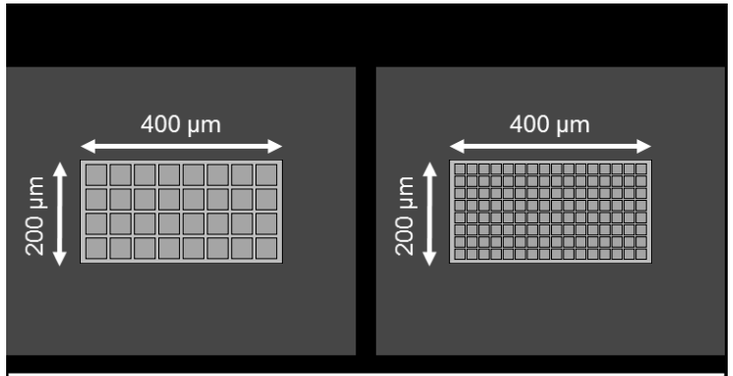


Figure 1: *In Situ* TEM Imaging of Liquid Samples with Poseidon Microwell E-chips
(A) TEM image of a partially filled microwell. Note that the liquid is primarily confined along the edge and walls of the microwell, or as droplets on the microwell surface. (B) TEM image of pegylated liposomes imaged in liquid. (C) TEM image of rotavirus particles imaged in liquid. (D) TEM image of lead nanoparticles imaged during beam induced growth from a solution of lead nitrate.

E-chip Description:

The window region of the Microwell E-chips consists of 200 nm thick SiN, which is 200 by 400 µ in size, into which the array of wells is fabricated. The SiN membrane in each well is 30 nm thick with a depth of 170 nm. The microwells are optimal for samples that are <100 nm in size. Although the entire window is beam transparent, only the microwell regions are meant for data acquisition. Currently, two well sizes are available: 20 x 20 µm and 10 x 10 µm, each chip containing 24 and 128 wells respectively, as shown in Figure 2.

For best results, a Microwell E-chip should be paired with a normal, spacer-less (spacer height: 0 nm) Poseidon E-chip containing a 50 x 500 µm SiN window. The E-chip pair may be used in either the parallel or crossed configuration. Note that our standard E-chips have a smaller window than the Microwell E-chips, so only a subset of the microwells will be visible for S/TEM imaging when the E-chips are assembled in the Poseidon holder. The number of visible microwells depends on the window overlap area of the E-chip pair.



Array	Microwell Size	Window Geometry	Well Depth
4 x 8	20 x 20 µm	400 x 200 µm	170 nm
8 x 16	10 x 10 µm	400 x 200 µm	170 nm

Figure 2: Geometry of Microwell E-chip Arrays

Poseidon 210™

Introduction to Poseidon Microwell E-chips

Selecting the Correct Microwell E-chip:

Microwell E-chips are available in both the large and small E-chip form factor. However, because the microwells are designed with 30 nm thick SiN in the viewing region, rather than the standard 50 nm thick SiN, for the best resolution it is important to match the Microwell E-chip to the point of electron beam entry or exit. 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 fully inserted?

- If the lid faces 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.
- If lid faces 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.

When imaging in TEM mode the Microwell E-chip should be located closest to the point of beam exit for the best resolution. When imaging in STEM mode, best resolution is obtained when the Microwell E-chip is located at the point of beam entry. Use Table 1 to select which Microwell E-chip is best for your experiment.

Table 1: Determiming E-chip Orientation

Holder Orientation	TEM	STEM
Not Inverted	Small	Large
Inverted	Large	Small

Table 1: Determining E-chip Orientation

Sample Loading:

The Microwell E-chips provide excellent resolution for small, beam sensitive, low contrast samples because they confine small amounts of liquid in discrete compartments within the viewing window of the E-chip. In contrast to a standard liquid cell, the liquid chamber should not be completely filled with liquid when using a microwell E-chip, as shown in Figure 3.

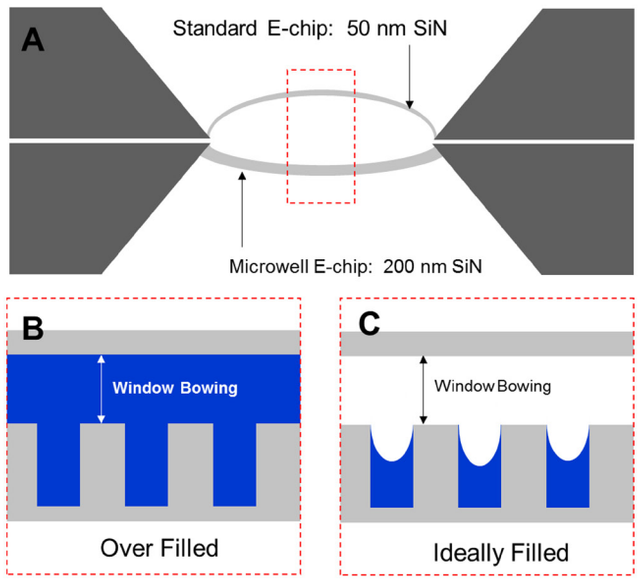


Figure 3: Cross Section of Over and Ideally Filled Microwell E-chips
(A) Cross section indicating membrane bowing of a paired Microwell E-chip and standard E-chip. (B) Cross section of an overfilled liquid chamber. Liquid will fill the space between the E-chips that is present due to inherent bowing of the 50 nm thick SiN window. (C) Cross section showing a liquid chamber with the ideal amount of liquid. Liquid is contained only in the microwells. Excess volume caused by window bowing is filled with air.

The thin SiN windows undergo bowing as a result of the vacuum differential between the microscope column and cell, as indicated in 3A. Microwell E-chips are fabricated using a 200 nm thick SiN film to strengthen the window and reduce bowing given its large area compared to a standard Poseidon E-chip. Although bowing is reduced it is not eliminated, and the normal window E-chip, which is 50 nm thick SiN, will still bow significantly. It is essential when adding the sample to minimize the volume of liquid applied, as too much liquid will negate the benefits of the Microwell E-chips. If too much sample is added, excess liquid will fill the region between the E-chips, as shown in 3B, increasing the liquid thickness through which the beam must travel. In a correctly filled Microwell chamber the liquid is confined primarily in the microwells, as shown in 3C. In general, a sample volume of 0.3-0.5 µL is preferred, although excess liquid may be removed by wicking it off with a small triangle of filter paper.

Before loading a sample, always remove the protective coating from the E-chips, following the instructions included with the E-chip package. If using an aqueous sample, the E-chips must be plasma cleaned or glow discharged to create a hydrophilic surface. The time required will depend on the power of the plasma cleaner or glow discharge unit, but in general, 5 minutes is recommended. Use a longer time for low powered units.

Poseidon 210™

Introduction to Poseidon Microwell E-chips

For easy assembly of the E-chips in the Poseidon holder, the sample should always be applied to the small E-chip. When using a large E-chip containing microwells, apply the sample to the spacer-less small E-chip. The liquid will be forced into the microwells of the large E-chip upon assembly of the liquid chamber.

Helpful Hint: It can be difficult to dispense small volumes (<0.5 µL) directly onto the small E-chip when it is positioned in the Poseidon holder. The best way to apply very low liquid volumes is to place the clean, plasma treated, small E-chip onto a gel pack (such as the ones they are shipped in). The gel pack will hold it in place and prevent it from sticking to the tip of the pipet once the liquid is dispensed. Be sure that the SiN membrane is facing up. Using a micro-pipet with a fine tip, dispense the liquid onto the surface of the small E-chip. After dispensing the liquid, grip the edges of the E-chip with carbon tipped tweezers and remove it from the gel pack. Position the E-chip in the tip of the holder, and place the large E-chip on top. Assemble the Poseidon tip as normal.

If you are unable to load the E-chip outside of the holder, 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. Assemble as usual.

Because of the small volume of liquid necessary to load a Microwell E-chip, not all microwells will be completely filled.

The Microwell E-chips may be oriented in either parallel or crossed configuration; however, due to the width of the microwells, it is recommended to pair them with a corresponding E-chip containing a window size of 50 x 550 µm.

Conclusions

Poseidon Microwell E-chips enable small volumes of liquid to be confined in micron-sized wells fabricated in the electron transparent surface of the SiN window. This enables thinner liquid layers for high resolution and high contrast imaging which is essential for imaging low-Z materials. With traditional silicon nitride, windows resolution is easily lost due to sample motion and small scattering cross sections. Data can be acquired from independent microwells, reducing sample damage over time and streamlining experiments. The Poseidon Microwell E-chips will fit all existing P200/210 and P500/510 holders, which use the large/small E-chip design. Contact us to discuss the full range of capabilities of Poseidon. We can be reached at (919) 341-2612 or contact@protochips.com.

References & Acknowledgements

Protochips would like to thank Dr. Deborah Kelly (Carillion Institute, Virginia Tech, Roanoke, VA) for providing the images of the liposomes and rotavirus particles, and Dr. Albert D. Dukes, III (Lander University, Greenwood, SC) for providing the image of beam-induced growth of lead nanoparticles. Further details for the samples highlighted may be found in:

- Dukes et. al., (2014) Applications and Design of Reinforced Silicon Nitride Windows for *In Situ* Liquid Transmission Electron Microscopy. Microscopy and Microanalysis, 20 (Suppl. 3), pp 1090-1091.
- Dukes et. al., (2014) Improved Microchip Design and Application for *In Situ* Transmission Electron Microscopy of Macromolecules. Microscopy and Microanalysis, 20, pp 338-345.