

**Worksheet for grid coating – PEI film**

NAME: \_\_\_\_\_

Date: \_\_\_\_\_ Temperature: \_\_\_\_\_ °C

Relative humidity: \_\_\_\_\_ %

Glass microscope slides: Gold Seal (#3050), Lot \_\_\_\_\_, cleaned on \_\_\_\_\_

PEI solution: batch \_\_\_\_\_; in 1,2-dichloroethane (conc. = \_\_\_\_\_ % w/w)

Grids: SynapTek, Be-Cu, notch-dot, 1×2 mm slot (Ted Pella 4516); cleaned on \_\_\_\_\_

Also, all glassware were rinsed with 1,2-dichloroethane on the previous day and air dried in a fume hood.

**Film characteristics:**

Slide	Side	Interference color	Notes (Uniform thickness? Blemish? Spots? Dust?)	Used?
1	A			
1	B			
2	A			
2	B			
3	A			
3	B			
4	A			
4	B			
5	A			
5	B			
6	A			
6	B			
7	A			
7	B			

**Film quality check with tSEM**

- The main purpose of this procedure is to check the quality of newly cast PEI film at the imaging condition (i.e., magnification, brightness, and contrast) actually used for serial section imaging.
- Suggested format for film batch number = [Date film was cast in the format YYYYMMDD]-[Slide Number][Slide Side]; e.g., 20150125-3A
- Adjust magnification to ~2,800× so that the width of the field is ~40 μm. While imaging a test thin section, optimize brightness and contrast so that the histogram is centered and spread well. Note the values here:
  - Brightness = \_\_\_\_\_ % ; Contrast = \_\_\_\_\_ %
- Use the values above to image the film (frame size = 3,072 × 2,304; scan speed = 8; with line averaging n = 1).
- You may also want to image the film at a reduced brightness level to visualize its flaws – if these flaws (except for holes) are not visible at B/C values at which the test thin sections are imaged, then they should not affect the quality of ultrathin section images. Thickness variation of the film can be checked by imaging it at a lower magnification (width of the field should be 100-500 μm) and lower brightness level. If it shows many holes (several small holes are acceptable) or many streaks or tracks, then discard this batch of grids (They can be reused).
- Suggested format for the image file name = GridQC\_[Film batch number].tif; e.g., GridQC\_20150125-3A.tif
- Note the film quality on the table below and petri dish holding grids with newly cast PEI film.
- Scan this worksheet and save along with the image files.

**Film quality**

Batch	Image files	Notes (Uniform thickness? Blemish? Holes? Good enough for series?)