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# Sample Preparation of "Soft" Matter Materials for EDS Analysis in Both SEM and TEM

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Soft materials present a difficult problem when preparing them for EDS analysis for both SEM and TEM. The majority of soft material samples are not stable under a constant condensed probe for EDS on a specific area. For general morphology images most of the soft materials for SEM are normally imaged at 2kV or less. Care must be taken when switching over to a higher kV (i.e. generally 20 or 30kV) for EDS spectra to be collected. This tends to burn the area of interest and possibly destroy it as well.

Most of the SEM data has been taken using an S-5200 low voltage-high resolution SEM, and the EDS spectra collected using a Noran Vantage EDS system (Fig.2). The detector is tilted $18^{o}$ toward the sample and the collection time is 100 seconds with an average dead-time between 20 and 30% of the count-rate. All SEM samples are coated with 2.5-5.0 nanometers of Tungsten (W) using a Southbay Technology high vacuum coater. Samples are pumped down in the coater to a vacuum of $10^{-6}$, coated using argon gas, while the sample is continually rotated and tilted throughout the coating process to ensure an even deposition. W is the preferred metal used for the coating because you cannot image the W grains until extremely high magnifications. Au and Au-Pd can be seen on the samples at magnifications much lower than the W. Carbon is avoided, mainly because most of these soft materials have carbon in them to begin with, so it is preferred to avoid that additional presence on the samples. In order to image the cross-section of these samples, the film is sandwiched between two pieces of sticky carbon tape and mounted onto a sample stub using another piece of carbon sticky tape. Then the entire bottom of the sandwich and tape are painted with Ag paint to assure good conductivity of the sample to the stub (Fig.1a). When dry, the sample is placed into $LN_2$ and the film above the carbon sandwich is removed using a thin edge screwdriver or scalpel also at $LN_2$ temperature. After the sample is back up to room temperature it can now be coated with W. The planar samples are just mounted flat on sticky carbon tape on the SEM stub and surrounded by Ag paint to make a good connection and assure conductivity to the stub (Fig.1b).

The sample preparation for most TEM samples is very similar to biological samples. For biofilms, the samples are fixed with glutaraldehyde and $OsO_4$ and go through a dehydration series of different grades of acetone and then embedded into Embed 812. The samples are then left overnight in a $60^{o}$ oven to polymerize. Samples are then removed from the molds and microtomed with an RMC PowerTome Ultramicrotome using a $35^{o}$ Diatome diamond knife. Ribbons of 70nm thick sections were picked up and air dried. The grids were then stained with Uranyl Acetate for 15 minutes, thoroughly washed with ultrapure $H_2O$ and air dried. The samples were then imaged in a Philips CM200 using a 4Pi Revolution imaging and EDS system (Fig.3). For the soft matter materials (films, etc.) the samples can be immediately embedded into either EpoFix or Embed 812. These samples are also microtomed using the same equipment and parameters above, but the grids are vapor stained for 1 hour using $RuO_4$. Then they are ready for imaging and EDS in the TEM. For acquiring EDS spectra, the sample holder is tilted ~$18^{o}$

toward the detector and the spot size reduced to achieve an acceptable count rate with sufficient dead time.

Drawings of sample orientation and a couple examples of the types of samples imaged and EDS spectra acquired from are shown below. More examples will be shared at the presentation.

1(a) 1 (b)

**Figure 1(a).** Cartoon of mounted cross-section for SEM prior to freeze-fracture of the extended film.
**Figure 1(b).** Cartoon of mounted planar section for SEM.

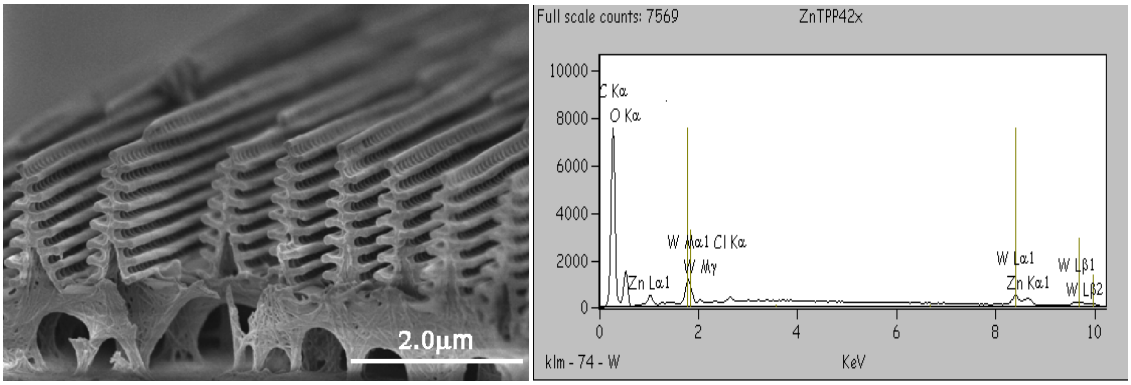


**Figure 2.** SEM image of Morpho-Butterfly scales and corresponding EDS data from it. Bar = 2.0µm.

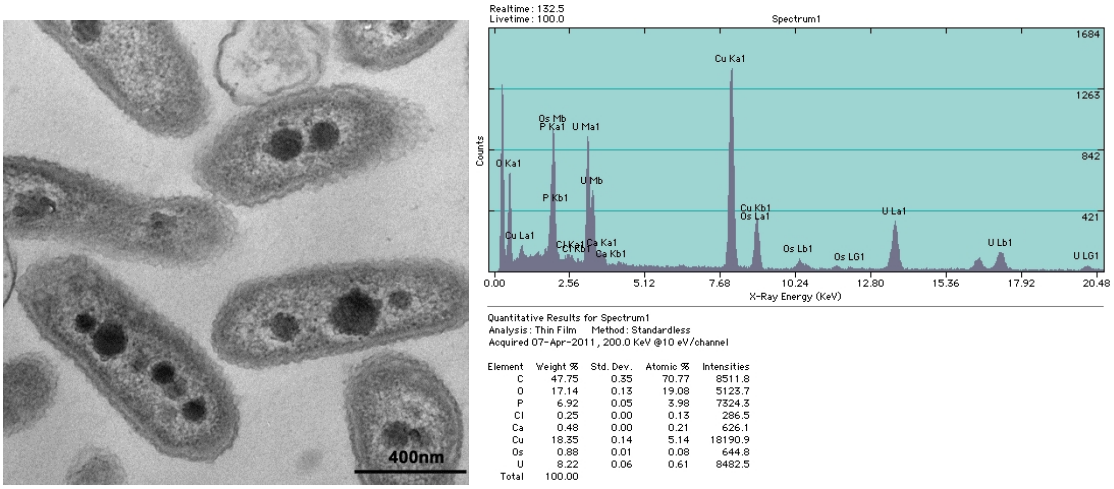


**Figure 3.** TEM image of polyphosphates appearing in a biofilm and the corresponding EDS data from that area of the image. Bar = 400nm.

In summary, it is necessary to remember that soft matter samples require a little extra care if EDS spectra is going to be acquired from the samples. Adjustments are necessary to both kV and selection of areas as you proceed. A new Bruker SDD and EDS package is being installed at this time, so all future EDS work on the Hitachi S-5200 will be carried out using this new system and compared to this data.

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