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Recipes: Electron Microscopy sample preparation

Date Updated: October 30, 2015

Tool Name: Electron microscopes

Manufacturer: FEI Company

Model: FEI/Aspex Explorer SEM, FEI Nova NanoSEM430, FEI Helios NanoLab650 FIB/SEM, and FEI Tecnai Osiris S/TEM

Recipe 1: SEM/TEM Sample preparation from powders using a solvent

Preparation of SEM/TEM samples from a powder is prohibited in any of the microscope rooms.

1. Perform the sample preparation under the fumehood on Wetbench #9, or in an appropriate area of your own lab.
2. Wear appropriate personal protection equipment, e.g. gloves, face mask, etc.
3. Find a suitable solvent for the powder, e.g., distilled water, isopropanol, ethanol, etc. (not reactive to the powder but capable of forming a suspension solution with the powder)
4. Make a suspension solution with the selected solvent and powder at a roughly 10:1 (v:v = solvent:solid) ratio in a small disposable vial.
    a. When necessary, sonicate the solution for a minute with the vial capped to assist the suspension of particles in the solvent.
5. Let the suspension solution rest for a minute or two.
6. Pipette the decant of this solution and then put a drop on an SEM stub (note: sticky tape is not needed here) or a TEM grid.

   a. Such samples contain scattered smaller particles which are useful for analyzing the size distribution and might be in the suitable size range (100 nm or less) for TEM work.
   b. Generally, higher population of particles can be found near the edge region of the drop.
   c. Pipette the bottom thick/concentrated region of the solution to prepare an SEM sample if large particles and clusters are of interest.
7. Dry the samples for minimal 30 minutes. **Drying samples is prohibited in any of the microscope rooms, including the use of Ag and carbon pastes, super glue, etc**.
8. For SEM samples, hold the stub pin firmly and use an air-duster or nitrogen gas gun to thoroughly blow the sample side.
   a. Make sure all the flows go under the fumehood.
   b. The blowing may likely damage the TEM grid, so this step doesn't apply to TEM samples.
9. Bag the waste, used gloves, any spills, and wiping towel, and label (with the expected composition at the user's best knowledge) on the bag, and dispose the bag in the designated nanoparticle waste bin.
10. Adjust the solvent to powder volume ratio and perform the above steps if the resultant particles on the surface (of SEM stub or TEM grid) are over-populated (piling up and clustering), under-populated (<1particle/10μm^2), or at the user's discretion, when examining the sample under SEM or TEM.

<u>Recipe 2: SEM sample preparation from powders without using a solvent</u>

This recipe is not ideal for most of our scopes. However, we realize that a solvent might react with certain powder materials, and could possibly bring contamination or material surface modification although trace amount. Users are allowed to prepare SEM samples from powders without using a solvent, as long as they agree to follow this recipe at their best effort.

1. **TEM sample preparation from a powder has to follow Recipe 1.**
2. Perform the sample preparation under the fumehood on Wetbench #9, or in an appropriate area of your own lab

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3. Wear appropriate personal protection equipment, e.g., gloves, face mask, etc.
4. Spread a small amount of powder on a clean surface, e.g., Al foil, or filter paper.
    a. Always minimize the amount of powder used for sample preparation.
    b. A spread of scattered powder within 5 mm^2 range is more than enough.
    c. When you can see the scattered powder on the clean surface, there is already too much for SEM imaging. Therefore, don't pile up too much powder.
5. Place a piece of double-sided conductive carbon sticky tape on an SEM stub.
6. Hold the stub pin and press the sticky tape against the spread powder.
    a. Apply reasonable pressure to make sure the powder is well adhered.
    b. Some grinding and rubbing of the tape against the powder can be useful as well.
7. Drop/tab the stub with the sample side (on the sticky tape) against another clean surface, e.g., filter paper, for multiple times, and observe the surface until no more noticeable amount of powder falling off the stub.
8. Hold the stub pin firmly and use an air-duster or nitrogen gas gun to thoroughly blow the sample side.
    a. Make sure all the flows go under the fumehood.
9. Bag the waste, used gloves, any spills, and wiping towel, and label (with the expected composition at the user's best knowledge) on the bag, and dispose the bag in the designated nanoparticle waste bin.
10. Powder spill in any of the microscope rooms is prohibited, and if it ever happens, users must report immediately for appropriate remediation.

<u>Recipe 3: Magnetic test for SEM/TEM samples</u>

1. All SEM/TEM samples need to pass our magnetic test before use on our electron microscopes.
2. Remove your mechanical watch(es) and anything else that can be influenced by strong magnetic field.
3. Place a piece of clean filter paper on our testing magnet in the EM sample preparation room.

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4. Hold the SEM stub or TEM grid with its sample side facing our testing magnet at 1~2 mm distance (or place the sample side on the filter paper) for 2 minutes.

5. If the sample passes the test, i.e., no noticeable change (e.g. particle transfer) on the filter paper, no detachment of the sample, etc., the sample should be magnetic safe in our electron microscopes. However,
    a. **If the sample is known to be magnetic, user should write on the logbook regarding the known magnetic property.**
    b. **When a nickel TEM grid is used, the user should not use the double-tilt and dual-axis TEM holders.**
    c. **If the sample is prepared from a dry powder without using a solvent, user should not use a working distance shorter than 5 mm with FEI Nova NanoSEM430 and 4 mm with FEI Helios NanoLab650 FIB/SEM.**
6. If the sample doesn't pass the test,

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a. If some particles are absorbed to the filter paper, abandon the sample when it is a TEM sample and re-prepare the sample with a better method.
b. When it is an SEM sample, go back to the fumehood on Wetbench #9 and blow the sample thoroughly with an air-duster or a nitrogen gas gun. This sample can be only imaged under the field-free mode with FEI Nova NanoSEM430 and Helios NanoLab650 FIB/SEM, and user should never switch on the immersion mode when such a sample is in the chamber. It is fine to study the sample with the FEI/Aspex Explorer SEM.
c. When a bulk sample (e.g. film, parts, or blocks) tries to escape from the SEM stub, make sure the sample is solidly fixed on the SEM stub (with metal clips or strong glue) and the SEM stub is solidly locked on the SEM stage. This sample can be only imaged under the field-free mode with FEI Nova NanoSEM430 and Helios NanoLab650 FIB/SEM, and user should never switch on the immersion mode when such a sample is in the chamber. It is fine to study the sample with the FEI/Aspex Explorer SEM.
d. In case of b) and c) above, user must write on the logbook regarding the observed magnetic behavior of the sample and consult with the tool owner before performing EDX analysis (since EDX analysis on NanoSEM430 and Helios involve formation of magnetic trap in the microscopes).

Recipe 4: Working with user's own non-standard SEM sample holders

1. Users must report to the tool owner if they bring their own non-standard SEM sample holders/adaptors.
2. SEM sample holders are mostly made of aluminum and copper. Use of steel in making SEM holders/adaptors should be avoided, as some steel has very poor conductivity and interferes with the immersion mode of high resolution SEM.
3. Even with holders/adaptors made of aluminum and copper, users should still be careful when picking up locking screws. Steel screws can create problems when using the immersion mode of high resolution SEM.

4. If a steel holder/adaptor has to be used, the user must let the tool owner be aware of and write down in the logbook with regard to the usage of a steel part in the microscope, and make sure all parts are well secured.