CENTRAL SOPHISTICATED INSTRUMENTATION FACILITY

(CSIF)

Guidelines for Maintaining Sample Quality

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INDEX

GENERAL INSTRUCTIONS

1. Fill up the request form completely, forwarded and signed by the user/supervisor/ head of the institution or department (with seal) /in-charge of the equipment as mentioned in the form.
2. All samples must be labelled appropriately and the same must be mentioned in the request form as well.
3. Try to bring the samples in a container or vial.
4. If the samples have to be returned after analysis, you may collect the same within 2 weeks. Thereafter, the samples will be discarded without any notice.
5. If the sample is not qualified for analysis, the CSIF operators/technicians will reject them at once.

LIQUID CHROMATOGRAPHY-MASS SPECTROMETER (LCMS)

1. Filter the sample using 0.2 micron filter.
2. Avoid volatile samples for analysis using LCMS.
3. Use only MS grade solvents as mobile phase for analysis and also for sample preparation.
4. Avoid any salt based buffers as mobile phase or samples containing salts. Desalting of samples is a must before injecting into MS.

BET SURFACE AREA ANALYZER

1. Sample has to be in powder form and if it is solid, then the size should be less than 5 mm X 5mm.
2. Sample should be in dried condition.
3. For Pre-treatment -sample decomposing temperature should be made available.
4. Set the pre-treatment temperature less than decomposing temperature.

ATOMIC FORCE MICROSCOPE (AFM)

1. A "typical" size for the multimode is 8 mm x 8 mm. Thickness should be less than 4 mm. The maximum possible size for the multimode is a circle of 15 mm diameter, but only the central part will be available.

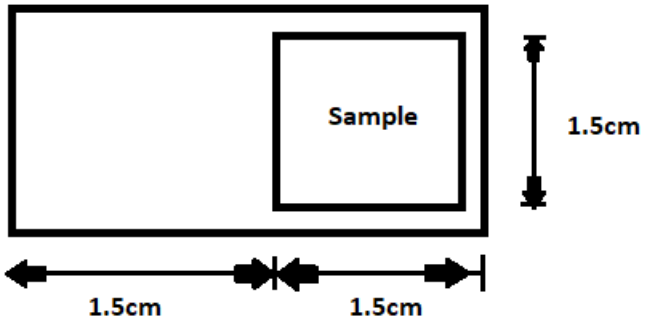
2. Dry powders are a problem for AFM. If your particles are loose on a flat surface, they will most likely move when the AFM tip bumps into them. Usually, re-suspending in water, followed by dilute dispersion onto freshly cleaved mica, then thorough drying will result in particles getting fixed to the surface. Other possible fixing techniques are fixing on a membrane, dispersion onto a glue that can be removed later and even-flaming (for bacterial cells) can also be done.
3. Sample practical height should be under your scanner Z limit.
4. Sticky type samples are not recommended.

CELL SORTER

1. You can use any cells which is around 20 µm as BD FACS Melody has 100µm Nozzle. Cell size should be 5 times less than the size of the Nozzle.
2. Use special precautions while using the Unfixed Bacterial/Yeast/Algae samples. Perform the 'Aseptic Sort' after using the Bacterial/Yeast/Algae samples.
3. After sample preparation the samples have to be filtered before acquiring/ sorting in BD FACS Melody equipment.
4. QC has to be run before every experiment, to check the performance of the equipment. Compensation & FMO may be done as per the experiment of the user.
5. One of the most important rules of sorting is to pass your cells through a cell filter before bringing it to the sorting lab. This will help to prevent clogs in the machines. Choose the correct size filter as per the size of the cells in your sample.

POWDER X-RAY DIFFRACTOMETER (XRD)

1. If physical treatment of sample like milling, grinding are required then proper care should be taken.
2. The optimal particle size of powder diffraction samples is in the range of 1 to 5 µm.
3. Powder samples: Sample should be finely powdered and the quantity should be at least 300 mg.
4. Samples coated on glass: The sample should be coated on one end of the glass slide as shown in the figure.



5. Thin films (for GIXRD): Samples should be coated on the substrate as shown in the figure. The size and thickness of the substrate should be 3 cm x 1.5 cm and at least 1 mm respectively.
6. Coating should be uniform and thin (~50 µm).
7. For identification and characterization of unknown samples like minerals, rock etc. the diffraction pattern should match with the pattern available in our database.
8. So, in the case of biological and other unknown samples, we recommend doing XRF prior to analysis by XRD.

X-RAY FLUORESCENCE SPECTROMETER (XRF)

1. Always provide neat, clean dust free pellets /samples.
2. 20 mm diameter, dust free and pressed pellets are recommended.
3. The pellet must be homogeneous.
4. For liquid samples, avoid carcinogenic/corrosive/ infectious/ highly acidic materials.

INDUCTIVELY COUPLED PLASMA MASS SPECTROMETER (ICPMS)

1. Sample must be in liquid form.
2. For fresh water samples a minimum of 50 ml is required. Saline/brackish or water samples with high TDS will not be taken for analysis, and will be rejected.
3. Samples must be prepared by acid digestion method.
    - Weigh out 0.2 g of sample material and transfer it into a beaker and add 15 mL conc. Nitric acid to it.
    - Heat it on a hot plate at a temperature not exceeding 120 °C. Keep the beaker closed while heating.

- Add 2-3 drops of hydrogen peroxide to the above solution and boil it till the solution becomes clear.
- Add more Nitric acid if there are still residues in the solution.
- Add 2-3 drops of hydrogen peroxide. Hydrogen peroxide is added to oxidise the organic contents in the sample.
- Conc.Sulphuric acid is also added to remove traces of plastic in the sample. Temperature of the hot plate can be reduced after the solution gets boiled.
- The digested solution is then filtered using a Whatman-42 filter paper (or 1µm filter paper) and transferred into a 50mL standard flask.
- The solution is made up to the mark using high purity 'milli Q' water. This solution can be used for measurements using ICPMS.
- If the element concentration goes above 200 ppb, dilute the present solution and take the measurements again.

CHNS/O ANALYZER

1. Any kind of powdered sample can be tried to analyze but avoid samples that are explosive in nature.
2. Liquid samples cannot be analyzed.
3. Samples should be homogeneous and finely powdered.

FT RAMAN SPECTROMETER

1. Any kind of solid or liquid samples can be analyzed but avoid carcinogenic/ corrosive/ infectious/ highly acidic or alkaline samples which may affect the optical components.
2. Samples should be homogeneous and well packed in the sample holder so that it will not spill out and fall on the lens.

FIELD EMISSION SCANNING ELECTRON MICROSCOPE (FE-SEM)

A. POWDERED SAMPLES

1. Samples must be crushed properly to ensure that the particles does not form agglomerated structures.
2. Measure conductivity of samples before submitting them, depending upon the conductivity, operator can fix appropriate gold sputtering time.

3. Powdered sample must be calcinated to ensure 0% water content in it.
4. Quantity of sample should not be less than 1 mg and try to bring the sample in small containers rather than choosing folded papers.
5. All samples must be named appropriately and the names must be mentioned in the request form as well.

B. <u>THIN FILMS/SHEET LIKE SAMPLES</u>

1. For FE-SEM, the film must be coated on thin glass plates having thickness of not more than 1 mm.
2. Samples coated on metal discs or metal surfaces gives high quality images.
3. Drop casted samples must be dried using IR lamp or other facilities for at least 2 hours to ensure proper dehydration.
4. Film/glass plate dimension should not exceed more than 0.8 cm x 0.8 cm.

C. <u>BIOLOGICAL SAMPLES</u>

1. For plant and plant related samples, fixation is required.
2. Applicant must prefix the sample in appropriate dehydration medium like gluteraldehyde or formaldehyde.
3. Fixation procedures vary from sample to sample, it is the sole responsibility of the applicant to choose appropriate method.
4. For fresh samples, Critical point drier (CPD) is required even after fixation.
5. Since fixation does not give good results for plant samples like seed or fruits, we suggest proper oven drying for at least 24 hours before analysis.
6. Applicant must ensure that the sample section thickness must be less than 1mm in order to avoid charging.
7. For animal/animal parts, the sample must be fixed in proper dehydration medium. Thickness must not exceed 1mm and therefore we suggest to take sections of the parts of which the image is required.
8. For glands/animal parts that contain oil, oven drying is required, at least for 24 hrs.
9. Any samples without proper drying or fixation will be rejected and the slot will be given to the next person.

FOURIER TRANSFORM INFRARED SPECTROMETER AND MICROSCOPE (FTIR IMAGING)

1. Any kind of solid sample, powdered samples and thin films can be analyzed but avoid carcinogenic/corrosive/ infectious material/highly acidic or alkaline samples which may affect the optical components.
2. Samples should be homogeneous and well packed in the sample holder so that it will not spill out and fall on the lens.

FAST PROTEIN LIQUID CHROMATOGRAPHY (FPLC)

1. When filtering proteins, be sure to select a low protein binding membrane such as PVDF or PES. Nylon filters often bind protein and will cause a loss of yield.
2. Samples should be 0.45 or 0.2 µm filtered to remove particulates and small contaminants.
3. If your sample does not flow easily through one of these filters, try a 0.8 µm filter. This size often works well for proteins with visible particulates and proteins are not amenable to smaller pore filtration.
4. When preparing your sample for ion exchange, dilute or desalt it to bring its conductivity below 10 mS/cm. This will ensure optimal binding.
5. Use proper protein storage methods after purification to protect proteins.
    - Short term (24 hr or less): 4°C
    - Long term: –80°C. Include 5–50% glycerol, albumin (10 mg/ml), and/or reducing agents like DTT or BME in the storage buffer. Freeze protein in small aliquots in micro centrifuge tubes. Flash freeze tubes using liquid nitrogen, or dry ice/ethanol bath.
6. Can also store protein as ammonium sulfate precipitate at 4°C.

REAL TIME PCR (RT-PCR)

1. Design amplicons that are 70–150 bp long.
2. Design primers that have a GC content of 40–60%.
3. Avoid sequences with long (4+) repeats of a single base.
4. Make sure your primers have a melting temperature between 50 and 65°C.
5. Avoid designing primers for regions with secondary structures. Use programs like MFOLD to map predicted structure of your target sequence.

6. Design your primers to span an exon-exon junction if you are assessing gene expression in eukaryotic cells to avoid amplifying any contaminating genomic DNA that may carry over from the purification step.
7. Check the sequences of the forward and reverse primers for 3' complementarity. This can result in primer-dimer formation.
8. Verify specificity by using any online tool, such as Primer-BLAST, to confirm the target of interest is unique.
9. Design probes with melting temperatures 8–10°C higher than that of the primers.
10. Probes should be shorter than 30 nucleotides, for most applications. If the probe is longer consider using an internal quencher.
11. Make sure your probe does not have a G at its 5' end. 5' G's can quench fluorescence even after hydrolysis.
12. Design your probe, or one of your primers, to span an exon-exon junction, if you are assessing gene expression in eukaryotic cells.
13. Run the probe sequence through a Primer-BLAST alignment to ensure that the sequence is unique to your target of interest.

SYSTEM MICROSCOPE

1. Never place a wet sample on the stage to image a sample that is wet or contains a strong acid, solvent or base. This may damage the objectives.

THERMOGRAVIMETRIC ANALYZER (TGA)

1. Any kind of powdered samples can be analyzed but try to avoid samples with explosive nature.
2. Liquid samples cannot be analyzed.
3. Samples should be homogeneous and finely powdered.

DIFFERENTIAL SCANNING CALORIMETER (DSC)

1. DSC is not designed for decomposition studies. Stop the Measurement before decomposition.
2. TGA or STA could be used to find out decomposition temperature of sample prior to analysis by DSC.
3. Films, sheets and membranes should be cut into discs according to the diameter of the crucible.

4. Powder sample should be homogeneous in nature, if not then other sampling techniques like coning and quartering should be adopted for heterogeneous samples.
5. For polymorph study, samples should not be crushed.

UV-VIS-NIR SPECTROPHOTOMETER

1. Any kind of solid or liquid sample can be analysed but avoid carcinogenic/corrosive/ infectious /highly acidic or alkaline samples which may affect the optical components.
2. Samples should be homogeneous and well packed in the sample holder so that it will not spill out and fall on the lens.