MANUAL

of

University Sophisticated Instrumentation Center (USIC)

Babasaheb Bhimrao Ambedkar University

(A Central University)

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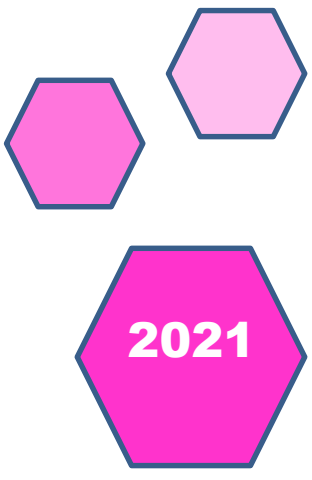

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Profile:

The USIC of the University has a Director/In-charge and an Advisory Committee consisting of 06 faculty members from the various departments of BBAU.

Individual researchers may not be able to generate huge research funds for research instruments, the University established University Sophisticated Instrumentation Center (USIC) with a mission to enrich the resources on a shared basis for promoting R&D. It is governed by the Advisory Committee members from various Schools and Departments of BBAU.

The objective of the USIC is as follow:

- To strengthen the technological infrastructure to carry out advanced research in various science disciplines under one roof.
- To provide guidance for acquisition of data and train personnel in operation and maintenance of Sophisticated Instruments.
- To organize short-term courses/workshops on the use and application of various instrument and analytical techniques for students, teachers and technical personnel from our University and outside of the Universities/Institutes.

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STANDARD OPERATING PROCEDURES

a) General Laboratory Safety Procedures:

To stay safe in a lab, users have to remember following important lab safety rules:

1. Only bring the things you need into the lab, it means users should leave all your personal items outside.
2. Food and drinks are strictly prohibited in the lab.
3. Wear gloves when handling corrosive chemicals. There are different types of gloves for different types of chemicals, so make sure to use the correct ones.
4. Unauthorized experiments or procedures must not be attempted.
5. Make sure the glassware users use always clean and dry.
6. Remember to put labels on the samples and make sure to write what it contains with specific coding in the sample vials.
7. Keep in view the lab safety symbols for safety point of view.

8. Chemicals need to be stored in designated cabinets and never just left on the floor.
9. If you accidentally spill a chemical, users should immediately warn everyone that something has been spilled and take necessary measures immediately.
10. Report all accidents, injuries, and breakage of glass or equipment to instructor immediately.

11. Never touch, smell or in any other way interact with the chemical before users know what they are dealing with.
12. Make sure to clean all used glassware and return reagents to the storage area. Apart from them, users dispose waste in the right containers and clean workbench before leaving the lab.

(b) Instrument Safety:

Some instruments have extremely hot surfaces and high voltage associated with them and precautions should take to avoid interacting with them. A proper training regimen and basic calibration are vital for safe usage and the generation of reliable data.

(c) Proper Labeling, Storage, and Management of Chemicals

Proper labeling and storage of chemical are essential for a safe laboratory work environment. In appropriate storage of incompatible or unknown chemical can lead to spontaneous fire and explosions with the associated release of toxic gases.

About the Center

The University Grant's Commission desires that every Central University must have Sophisticated Instrumentation Centre. The creation of such center is becoming obligatory for every university for the reasons that the modern day science demands the use of the precision equipment for the acceptability of the data. Such centers on one hand helps stop the duplication of the sophisticated equipment, which costs huge amounts of money and in turn helps in regulating the use of the sanctioned money in most judicious ways.

The University Sophisticated Instrumentation Centre (USIC) is established as a central facility and housed in the ground floor of the School for Environmental Science building. Its main objective is to provide services to all faculty, researchers and students of various departments in the University. This facility is also made available to the outside research as students on payment basis. Training programs and workshops/seminars are organized regularly for faculties, researchers and students to use and application of various instruments and analytical techniques for awareness among the users. The USIC has the following equipment.:

1. Scanning Electron Microscope (SEM), Model: JSM 6490 LV, Make: JEOL, Japan
2. X-Ray Diffraction (XRD), Model: D8 Advance Eco, Make: Bruker, Germany
3. Inverted Laser Scanning Confocal Microscope (ILSCM), Model: LSM900, Make: Zeiss, Germany
4. Preparative HPLC, Model: LC-20AP, Make: SHIMADZU, Japan
5. Fast Performance Liquid Chromatograph (FPLC), Model: AKTA 10, Make: GE-Healthcare Bio-Science, Hong Kong
6. Fourier Transform Infra-Red Spectrometer (FTIR), Model: Nicole 6700, Make: Thermo-Scientific, USA

Apart from the above equipment the following minor equipment are available in USIC:

1. High Speed Centrifuge Machine (CM12-Plus Remi)
2. Vortex Mixer (CM101-Plus Remi)
3. Double Distillation Unit
4. Hot Air Oven, Deep Freezer (-40^{o}C)
5. Electrophoresis, UV Lamp
6. Ultrasonic cleaner
7. Microwave oven

8. Magnetic Stirrer with Hot Plat
9. Photoresist Spinner
10. Research Microscope and Refrigerator

Scanning Electron Microscope (SEM):

A Scanning Electron Microscope (JSM 6490) scans is a focused electron beam over a surface to create an image. The electrons in the beam interact with the sample and produce various signals that is used to obtain information about the surface topography and composition. It can be use both under low & high vacuums that depending on the nature of the specimens. Low vacuum with low kV (energy) provides the user the longer duration for scanning the materials, organic specimens, before it is charged. The instrument has been coupled with an optional accessory, viz., EDS 133, EV Dry Detector (INCA x-act) of OXFORD instruments, UK, which has enhanced the application range in a manner that any metal associated with the biological material or in isolation or in the form of alloy may be identified and also quantified. The sample preparation for SEM facility has also been supported with the two important units, viz., Sputter Coater of JEOL, Japan (JFC 1600, Auto Fine Coater) and Critical Point Dryer (CPD) Emitech K 850 of Quorum Technology of UK.

(a) SOP for using the JEOL JSM6490LV Scanning Electron Microscope:

- Switch on system and open the JEOL Scanning Electron Microscope software (6490 SEM) from the desktop.
- Open the Stage window in the SEM software. Confirm that X, Y, R and T are set to 0. Confirm that Z is set to 40mm. If they are not, click in one of the windows corresponding to the settings and change them to 0,0,0,0, and 40, then press Go and wait for the stage to stop moving.
- Push and hold the Vent button on the SEM cabinet until the button lights up, bringing the specimen chamber to atmospheric pressure. While the chamber is venting, put on gloves and load your samples in the proper sample holder insert. Tighten the samples in place with set screws, then load the sample holder insert into the proper sample holder. Measure the height of your samples above the top of the sample holder and record this height. Make note of your sample order in the sample holder.
- Close and latch the chamber door. Press and hold the Evac button on the microscope cabinet until the button lights up. This will start pumping the air out of the chamber.
- Set up initial operating parameters for using the microscope before moving your sample or switching on the High Tension (HT). For biological samples, set the voltage (kV) to 15kV, set the spot size to 30, and select SEI for viewing the sample.
- Return to the Stage window, and enter 0,0,0,0, and 10mm plus the height in millimeters from the top of the sample holder to top of tallest sample.
- Focus the microscope on the sample by either hitting the AFD button on the software or using the Focus knob on the knobset. Readjust the contrast and brightness if necessary.
- Examine your samples, and photograph them. It may be necessary to change the accelerating voltage to reduce charging of a sample (lower kV) or to increase resolution (usually, higher kV).
- Photography is done with either the Scan 4 button, which will take an 80 second scan of the sample and then freeze the frame, or with the Photo button, which will take a 160 second scan of the sample and then freeze the frame.
- If you are finished using the microscope for the day, after repumping the chamber remove your samples from the sample holder.
- The filament on the microscope will last for only about 30 hours, so if you need to leave the room for more than a few minutes, please shut the filament off.

(b) Sample Preparation for Scanning Electron Microscope:

1. Specimen Collection
2. Primary Fixation: with 2.5% Glutaraldehyde/Karnovsky's fixative
3. Washing: sample will wash with 0.1 M Phosphate Buffer for 3 changes
4. Post Fixation: 1% Osmium tetroxide will be used as a post fixation.
5. Washing: sample will wash with 0.1 M Phosphate Buffer for 3 changes
6. Dehydration: Specimen will be dehydrate using increasing concentration of Acetone: 30%, 50%, 70%, 90%, 95%, 100% (Dry Acetone).
7. Drying: Specimen will be dry by Critical Point Dryer.
8. Specimen Mounting: Specimen will be mounting on to the Aluminum Stubs with the help of Carbon tape.
9. Coating: Sample will coat using Sputter coater to make the sample conductive.
10. Specimen will be observe in Scanning Electron Microscope

(c) Precautions while working in the LAB

- Never handle the SEM chemicals with bare hands. Always use disposable gloves.
- The glassware should be kept clean and dry before starting the tissue processing protocols.
- Never bring the solution of glutaraldehyde, formaldehyde and osmium tetroxide close to nose and eyes. They may cause permanent damage to the membrane of these organs.
- Any SEM chemical that comes in the contact with skin should be flushed with water.
- Always use hand wash to clean hands.
- Use fume hood while dealing with osmium tetroxide (OSO_4).
- Never dispose the used SEM chemicals into wash basin. Collect them separately into bottle or boxes and dispose into a pit or incinerator.

X-ray Diffraction (XRD):

X-ray Diffraction (XRD) is a powerful technique for characterizing crystalline and polycrystalline materials. It provides information of structures, phases, texture, and other structural parameters, such as average grain size, crystallinity, strain, and crystal defects. The system is equipped with Goniometer, X-ray source, Tube housing, Mount, Sample holder and Detector with Optics and Slit systems. The system has DIFFRAC (EVA evaluation software), DIFFRAC (TOPAS software) for diffractogram analysis and ICDD PDF-4 Axiom 2020 database.

(a) Sample Preparation for XRD:

1. Solid samples are prepared for X-ray diffraction by grinding to powder form/talc (<0.062 mm).
2. Place a sufficient amount of sample in the center of the sample holder.
3. Spread the sample to a thin layer with a glass rod (rolling it over the sample works well).
4. Place sample in desiccator to dry.
5. Desiccate and transfer sample to sample holder for XRD analysis.

(b) Loading a sample

- If the green light on the tube is on, the shutter is shut down then no X-rays are being emitted (the desired case for sample insertion).
- Press the green “open door” button on the right side panels to unlock the glass doors.
- Choose the one appropriate sample holder.
- Before scanning the sample check the machine calibration.
- After putting your sample in the appropriate holder, fit the holder into the machine’s sample mount by inserting from below until a magnetic attraction locks the sample holder in. Close doors and make sure to ”jiggle” the black handles click into the closed position.

(c) Scanning

- At the bottom of the commander screen, enter in starting and stopping 2ɵ values. If you do not know what to enter, then use 2° and 70° as default start and stop values, respectively.
- Between those two, set increment (step) by which machine advances in degrees. If you don’t know what to enter, then use 0.02 ɵ as a default value.
- Set seconds per step (or you can select that label to change to degrees per minute). These four inputs (start, stop, increment, and seconds per step or degrees per minute) will determine how long it will take to run your scan. If you do not know what enter, then use 10 DEG/MIN as the default value.
- If you are performing a quick reconnaissance scan, and then you want higher quality data, you can select a region of your reconnaissance pattern and hit “use zoom” below to input that region as the start and stop of a next, more detailed, scan. Modify the scan step increment and rate as desired for a more detailed scan.
- Once you scan is complete, there is a comment line on the top of the pattern on the screen. Enter details about the sample and any other relevant information (you may want to do this before you start the run).
- Save your pattern, if desired, using “Save as” in the standard Windows menu at upper left of computer screen.
- Save to an appropriate location within the folders for class work, students, faculty, et al. Commander will customarily save in a “.raw” format. All data should be saved in the C: drive User_Data folder, in a sub-folder with your name.

- EVA are applications with which to manipulate data. Start with using EVA. Use "open" or "import" to access your pattern. Use the toolbox then to work with the pattern, choosing tasks in the tool box and using the cursor to outline areas of interest on the pattern.

(d) XRD General Safety Protocol:

X-ray beams from XRD units are hazardous. Serious injury can result if any body part is exposed to this high radiation even for a very short time. Furthermore, X-ray beams are often small and difficult to locate and/or measure so extreme caution must be exercised when working with an XRD.

- Unauthorized and untrained visitors/observers are not allowed to use the X-ray machine.
- Make sure X-ray tube shutter is closed before changing samples and verity there is no active beam present.
- Do not bypass safety interlocks. Use the main switch to turn off power to the x-ray tube.
- Accurately maintain the XRD log book that include date, operator name, beam voltage, current and time ON and time turned OFF.

(e) Emergency Instrument Shutdown:

- Hit the red Emergency Stop rounded button on front corner of the XRD instrument cabinet. X-Rays are no longer being generated.
- Turn off power to other parts of the instrument by depressing the red "0" button of the rounded right-had corner for the instrument.

Inverted Laser Scanning Confocal Microscope (ILSCM):

Inverted Laser Scanning Confocal Microscope is a fluorescence-imaging technique that produces exquisitely sharp optical sections through biological specimens by exciting a laser over the sample and collecting emission data, point by point, and reconstructed with a computer, allowing three dimensional reconstructions of topologically complex objects. The ILSCM (ZEISS, LSM 900) equipped with multichannel fluorescence imaging Z-stack, colocalzation, time-lapse imaging. FRET, FRAP, Photo activation and conversion. It is capable of highly sensitive spectral confocal imaging of fixed as well as live biological samples.

Specification:

- Solid-state Laser module for UV405 nm and Visible 488nm, 552/561nm, 638nm with AOTF.
- XY motorized Scanning stage with 160mm and 110mm.
- Motorized 6 position DIC nosepiece, and Motorized Universal Condenser.
- 6 position mot fluorescence turret for accommodating fluorescent filters for sample visualization and camera based imaging.
- High resolution confocal grade Semi Apo objectives of 10x, 20x, Plan Apo 40x/0.95, 60x/63x/1.4.

- DIC prisms for 20X & 60x/63x objectives.
- Fluorescent filters for DAPI, FITC & Rhodamine.
- Eyepieces PLAN 10/22 BR M.

Dos or don't for ILSCM Laboratory:

(a) When you come for imagining your sample should be READY to put on the microscope stage;

(b) Unauthorized and untrained visitors/observers are not allowed to access the ILSCM machine;

(c) Unauthorized and untrained visitors/observers are not allowed to remove objectives of the microscope or attempt any maintenance;

(d) When focusing on your sample PLEASE MAKE SURE TO CHECK THE HEIGHT OF THE OBJECTIVE LENS SO THAT YOU DON'T CRASH INTO THE SAMPLE.

(e) Avoid wrong immersion contact (oil on water lens, water on oil lens).

(f) If you accidently place water on an oil objective lens or vice versa, wipe it off with lens tissue and notify immediately to USIC staff.

(g) Data generated from microscope will be provided on CD (Compact Disc) or DVD (Digital Versatile Disc) only;

(h) When completed your studies and imaging work please switch off the lasers and wait until they are properly shut down before exiting the ILSCM software;

(i) Maintaining a clean workspace. Food and drink are NOT allowed;

Preparative HPLC System:

Prep HPLC is used to separate and refine high-purity target compounds from a mixed solution. It is used to fraction high-purity compounds required for subsequent evaluation, analysis, and processes in the shortest possible time. The Preparative HPLC (SHIMADZU, Model: LC-20 AP) equipped with SPD-M 40A Photodiode Array Detector which wavelength rage is from 190-800nm, CBM 20A system controller, Fraction collector FRC-10A, C18 & C8 column and LabSol ADD PDA connect CS ready software.

(a) SOP for Prep HPLC:

- Ensure that the instrument connected to power supply and all communication cords between the units are connected properly.
- Ensure that the appropriate solvent mixture (water: Methanol) is pumped for seal wash.
- Ensure that the outlet tube from the detector is dipped into a waste bottle.
- Prepare mobile phase, fill it in the reservoir bottle and ensure that the filter is below the level of mobile phase.
- Double click the lab solution icon on desktop screen to open the Lab Solution software & Logging in.

(b) Procedure for method creation:

- Click on Instrument → LC-20 AP →Go to File →New Method File → Create Method as per Monograph → Download & Close → File → Save Method File → Save in Specific Product Folder.
- Open the purge valve of the instrument and purge the corresponding solvent line (s) in which mobile phase with flow rate of the pump as 1.0 ml/min until the solvent line is completely filled with new mobile phase.
- Ensure that no air bubbles are noticed in the solvent line.

(c) Procedure for Single Run:

- Click on Acquisition at Left Assistant Bar → Start Single Run → Input Require Detail → Press OK → Data Acq dialoge box PoPs up → Insert sample through manual injector in load position and pull down after injecting the sample → LC and PDA running will show on screen and base line can be observed in PDA tab.

(d) Procedure for System Shutdown:

- After completion of analysis, wash the column by downloading washing method & keep it back in the relevant place.
- Click on 'Instrument ON' at down assistant Bar to disconnect instrument form Pump, Lamp & Column oven.
- Close the panel by click on file & then exit.
- Switch off small round button & switch off the mains of system controller. After completed switch off the PC.
- Enter the column & instrument usage details in the logbook.

Fast Performance Liquid Chromatograph (FPLC):

Fast Performance Liquid Chromatograph (FPLC) is a form of medium pressure chromatography that is used to analyze or purify mixture of proteins and other biomolecules. The FPLC (main AKTA 10) has been provided with other accessories which include Sephadex G75, Sephacry1 S – 200, Deae Sepharose Fast FL, CM Sepharose fast flow short & long column holders, AKTA user kit etc. to make the facility of broader usage.

(a) SOPs for ion exchange or hydrophobic chromatography:

1. Connect chosen column to the system.
2. Wash with 1-2 column bed volumes (CBV or CV) of starting buffer.
3. Apply sample. Collect unbound material in the separate container or start to collect fractions. Collect full tubes on this stage.
4. Elute proteins using either continuous or stepwise gradient of elution agent. The duration of continuous gradient should be 10-20 CBV. With stepwise elution apply 3-4 CBV of each concentration. Collect fractions (25-50% of CBV).
5. Clean column with 1-2 CBV of high concentration elution agent (1-2M NaCl for ion exchange matrix or water for hydrophobic matrix).
6. Check protein concentration in fractions by method of Bradford.
7. Analyse protein containing fractions using SDS-PAGE.
8. Combine fractions containing TP, check volume and protein concentration then calculate yield of protein after the step.

(b) Cleaning of chromatography system:

1. It is extremely important to keep clean all parts of system (tubing, pumps, sample loops etc.)
2. Always pump water through all part of system after chromatography to prevent salt to be crystallised in the tubes and channels.
3. To prevent bacterial growth 20% ethanol wash is usually recommended.
4. EDTA solution to keep AKTA systems between purifications. It never failed.

Fourier Transform Infra-Red Spectrometer (FTIR):

Fourier Transform-Infrared Spectroscopy (FTIR) is an analytical technique used to obtain an infrared spectrum of absorption or emission of organic (and in some cases inorganic) materials. The FTIR has been supplemented with an accessory called Attenuated Total Reflectance (ATR) to enhance the use and application of the instrument in way that with this facility any form of material, i.e., filmy/pellet; liquid, including liquids of flammable nature may be studied; rather that its limited use of getting spectra of the solids on the powdered form through making pellet by mixing the same with the KBr under a hydraulic press.

(a) <u>SOPs for FTIR:</u>

1. Take a small amount of powder sample, about 0.1-2% of the KBr amount. The powder sample and KBr must grind the mixture for 3-5minutes.
2. Assembling the die and add the powder to the 7mm collar. Put the die together with the powder into the hydraulic press.
3. Press the powder for 2 minutes to form a pellet. A good KBr pellet is thin and transparent.
4. White spots in a pellet indicate that the powder is not ground well enough, or is not dispersed properly in the pellets.
5. Disassemble the die set and take out the 7mm collar. Put the collar together with the pellet onto the sample holder.
6. Fix the sample holder in a FTIR spectrometer (Thermo-Nicolet 6700) and analyzed in the spectral region 4000-400 cm^{-1} against KBr background.
7. Please clean the die after you finish your experiment.

Proforma for availing the USIC Facility:

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Vidya Vihar, Raebarely Road, Lucknow – 226025
(Email: usicbbau@yahoo.com)

PROFORMA FOR INTERNAL / EXTERNAL USERS

Requisition No._____(For Office Use Only)

Requisition Form for

SEM	XRD	ILSCM	Prep HPLC	FPLC	FTIR	OTHER

(Tick required facility)

a. Name, Designation & Address of Supervisor with Telephone Number and **Email ID** _____

b. Name & Designation of user with Telephone Number and **Email ID** _____

c. Name of the Specimen / Tissue _____ from Animal / Plant / Others

d. Name of the fixative used _____ duration _____ Orientation required for viewing (SEM).

e. Number of specimen to be processed for the proposed thesis / research work. Total _____ Current _____

f. Name of the Project or Title of the proposed Thesis / Dissertation work _____

g. Name of the Funding Agency (in case of funded Project) _____

h. Please give details of previous publications, if any, emerging out of the work carried out at the USIC by your group. The chief investigator / supervisor is requested to provide information regarding publications in due course as it is require to maintain the record.

S. No.	USIC Facilities Utilized	No. of Samples Analyzed	Output / Publication
1			
2			

Signature of Supervisor/ Head
Date & Official stamp

Note: 1. In case of immersion fixation, minimal time should elapse between animal biopsy & fixation. Tissues should be fixed in 2.5% glutaraldehyde & 2% paraformaldehyde (PF), in 0.1 M phosphate buffer (pH 7.4) for 6-12 hr (depending on tissue type) at 4°C (for SEM only).
2. A maximum of 07 samples per performa will be accepted. Samples will be received between 11.00 A.M. – 03.00 P.M. Booking for SEM will be done at the time of depositing the samples.
3. It is mandatory to acknowledge the **USIC, B. B. A. University, Lucknow** in all the research publications emerging out from the work carried out through USIC facilities.

Declaration: I agree to all the terms & conditions of Data policy of USIC, BBAU, Lucknow.

Signature of Applicant

FOR OFFICIAL USE ONLY

Requisition No. _____ Date_____

A. Work Report: SEM/XRD/ILSCM/Prep HPLC/FPLC/FTIR/Other

S. No.	Sample received	Number of Sample	CPD		Sample prepared		Image Analysis / EDS	
	Date		Date	No.	Date	No.	Date	No.
1.								
2.								
3.								

C. Allotment of time: SEM/XRD/ILSCM/Prep HPLC/FPLC/FTIR/Other

S. No.	Date	Register Ref.	Number of Sample	Hours.	No of Images	Technical Support
1.						
2.						
3.						
4.						
5.						

Date_____

Staff-in-Charge (Technical)
USIC, B.B.A. University
Lucknow

Director
USIC, B.B.A. University
Lucknow

Fees/Charges for availing the USIC facility:

S. No	USIC Facilities	For Internal Users	For External Users
01	SEM without EDS	Sample preparation, Coating and Analysis Rs. 300/- per sample	Rs. 800/- per sample for National Laboratory and University
02	SEM with EDS	Sample preparation, Coating and Analysis Rs. 350/- per sample	Rs. 900/- per sample for National Laboratory and University
03	XRD	Rs. 400/- per Sample	Rs. 700/- per sample
04	ILSCM	Rs. 350/- per Sample	Rs. 700/- per sample
05	Preparative HPLC	Rs. 250/- per Sample	Rs. 600/- per sample
06	FPLC	Rs. 200/- per sample	Rs. 400/- per sample for National Laboratory and University
07	FTIR	Rs. 150/- per sample	Rs. 400/- per sample for National Laboratory and University
08	Spin coater	Rs. 50/- per sample	Rs. 100/- per sample

Note: Consultancy charges for data interpretations of XRD: Rs. 300/- per sample (Optional)