

Standard Operating Protocol (SOP) for Confocal Facility at CCRF

Objectives for use:

- a. Image acquisition for 3D, 4D
- b. Live cell-imaging control for multi-time series (with control of gas environment and moisture around the culture plate/slide etc.).
- c. FRAP
- d. FRET
- e. Photo activation and conversion
- f. Co-localization of 2-5 signals. (NOT Double or triple labelling that can be observed and imaged on a regular fluorescent imaging microscope)
- g. Spectral analysis for:
 - i. Spectral imaging and unmixing
 - ii. Auto-fluorescence separation by online spectral un-mixing
 - iii. Histogram analysis of emission spectrum
- h. Obtaining z-stacks
- i. 3D analysis to evaluate & display 3D image data stacks with measuring tools,
- j. 3D Visualization & multichannel volume rendering 3D stacks
- k. Reconstruction
- l. Measurements across z stack
- m. Movie co-localization with intensity profiles for quantification
- n. Real time ratio-display
- o. 2D and 3D image deconvolution

Method:

1. Website for imaging facility including LASER confocal should have:
 - a. User registration cum booking form
 - b. Details of:
 - i. Sample
 - ii. Purpose
 - iii. Type of imaging/experiment
 - iv. Requirement of time slot
 - v. Other equipment required- gas cylinders, cell incubator, centrifuge, refrigerator, live imaging
2. Sample preparation guidelines
3. Booking
 - a. Time of the scientist in-charge
 - b. Instrument use
4. Consultation for experiment (before booking), verbal, telephonic, e-mail
5. The scientist in-charge should inform the user about the reagents that may be required for the planned experiment.
6. The user should bring all the reagents required (CCRF has basic requirements like gas cylinders and stage-incubators for incubation. Basic dyes like toluidine blue, methylene blue, cell-counting chamber, shakers, refrigerators etc. are to be provided by the user; the user can get in touch with the General Facility, CCRF to see if these reagents/equipment are available).

7. At least one-week prior notice for cancellation, so that the slot may be offered to another person, otherwise charges will be levied.
8. Analysis to be conducted on the offline system, so that the online system may be kept free for users. Data to be provided in CDs/DVDs brought by the user. No pendrives to be used on the system unless prior formatting is done in view of/by the scientist/ technical officer.
9. Completion report to be provided to PI/registered user by e-mail. Log to be maintained electronically.