Standard Operating Protocol (SOP) for Microscopy and Imaging Facility at CCRF

Objectives for use:

- **a.** 3D and 4D image acquisition
- **b.** Multi-time series live cell-imaging (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 of 3D stacks
- **k.** Image 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. Users are required to check the CCRF website and navigate to the Microscopy and Imaging sub-facility tab that has the booking form:
   The following sample details need to be provided:
    - i. Sample type
    - ii. Purpose
    - iii. Type of imaging/experiment
    - iv. Required time slot
    - v. Other equipment required- gas cylinders, cell incubator, centrifuge, refrigerator, live imaging setup
2. Sample preparation guidelines are mentioned below, and users must strictly adhere to these guidelines:
    - i. Only confocal grade glass slides, cover slips, plates and dishes are to be used; cover slips mandatorily must be of 1 or 1.5 number with approximately 0.17 mm thickness.
    - ii. In case one is not sure about the glass slides, coverslips, etc. please consult the scientist before you perform the experiment.

iii. Users must be fully aware of the excitation and emission wavelengths of the fluorophores/ dyes that they have used in their experiments.
iv. It is the responsibility of the user to collect the data after acquiring the images. Data in the main system is periodically deleted to free up the system space. The facility will not be responsible for any loss of data.

3. The scientist in-charge should be consulted before the experiment is planned to discuss the reagents, experimental paradigms, sample size, etc. for the planned experiment.
4. Consultation for experiment (before booking) can be done verbally by visiting the facility, through mobile/ telephone or via e-mail.
5. The user should bring all the reagents required (The facility has basic requirements like gas cylinders, refrigerator, and stage-incubators for incubation. Basic dyes like toluidine blue, methylene blue, cell-counting chamber, shakers, 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).
6. The booking window is open 45 days and 30 days in advance for the confocal and the image processing system respectively; it is the responsibility of the users to plan their experiments and book the slots ahead of time.
7. At least one-week prior notice should be given for cancellation, so that the slot may be offered to another person; otherwise, charges will be levied.
8. Analysis is to be conducted on the dedicated offline system, so that the online system may be kept free for users. Data will be provided only in formatted hard drives. No pen drives should be used on the system unless prior formatting is done in view of/by the scientist/ technical officer.

User logs are maintained both electronically and as hard copy. Users should sign the register once their experiments are complete and they have received their data.