FREQUENTLY ASKED QUESTIONS (FAQs)

1. Is it mandatory to fill out the booking form for using the confocal system?

   Yes, absolutely! It takes around 10 minutes to fill out the form and makes it easier for the scientist in-charge to schedule your appointment and plan the experiment. Importantly, it saves time as you will be already providing the required information before coming with your samples.

2. Will the facility and the scientist in-charge help in designing the experiments?

   Yes, you are welcome to discuss and trouble-shoot your experiments with the scientist in-charge. The facility is here to support all the users from designing experiments, capturing images and processing data.

3. Can I book the system / cancel booking at short notice?

   It is preferable not to do so but we do understand there could be circumstances beyond one's control. The decision will be made depending on the availability of the system and at the discretion of the scientist in-charge.

4. I am new to microscopy and imaging with limited knowledge and experience on microscopy. How can I learn more about confocal microscopy and its applications in the biomedical field?

   You can learn more about microscopy and its applications from our website ccrf.aiims.edu/ccrfconfocal/ and navigating the "Learning Resources" tab. You will find webinar and demo videos from CCRF-AIIMS under "Tutorials" tab and a list of books, links to videos and websites for learning the basics of microscopy under "Other Important Resources" tab. You are welcome to speak to the scientist in-charge of the facility who can help you get started with the basics of microscopy.

5. I can visualize the sample well under an epifluorescence microscope in my lab but I can't see it here. Why is it so?

   Fluorescence imaging is very sensitive to the stability of the fluorophores. Do not panic if this happens! There could be multiple reasons; the sample you prepared could be old or you could have kept it exposed to light at room temperature and bleached it in the process. Every microscope is different and the settings/parameters for visualizing the sample are also not the same. If possible, try to get an image that you have captured previously on another microscope with the acquisition settings. This will be helpful in figuring out if there is any particular issue with your sample or the microscope.

6. Why is it important for me to know about the excitation/emission spectra of the dyes/fluorophores that I am going to use/have used in my samples?

   At the heart of the confocal microscope systems are the lasers. These lasers are coherent light sources that are used to excite the fluorophores in your sample. Therefore, without prior knowledge of the excitation/emission wavelengths of the dyes/fluorophores, you cannot decide which lasers to use to excite your samples. You may not know the exact excitation/emission wavelengths but you should be

aware of the wavelength range at which the dyes/fluorophores get excited and emit fluorescence.

7. How much help is provided by the microscopy facility in terms of offline processing, data quantification and making image panels for manuscript submission?

   Every user has different experimental needs. Some users might want to do quantifications/data analyses and making image panels themselves. We are happy to provide all the required support and assistance in terms of offline processing, data quantification and making image panels on a case-to-case basis.

8. Who should we contact or talk to for discussing/designing experiments?

   Please write to us at confocal.ccrf.aiims@gmail.com to discuss/design your experiments or to fix an appointment. You can also walk-in to the facility and talk to the scientist in-charge; however it is better to schedule a Zoom/Webex/Skype/Google meeting in this era of social distancing.

9. Can I book the microscope system for the entire day if I need to perform some time-sensitive experiments or developmental assays?

   Yes, this can be done after discussion with the scientist in-charge. Usually, the preferred time slot is a maximum of 3 continuous hours in a day but depending on the demands of the experiment, arrangements can be made for longer use of the system. Please reach out to the scientist in-charge well ahead of time for a better planning and execution of your experiments.

10. Am I allowed to use the system on my own?

    No, you are not allowed to handle the system on your own. You will be allowed to focus your sample and select a region that you want to image. You are free to ask questions and learn the theory behind what is exactly going on during image acquisition. You may be allowed under certain circumstances to handle the system only under supervision of the scientist in-charge at his/her discretion.

11. What are the norms for authorship and acknowledgement in case of publications, reports and any other scientific content resulting from the use of the confocal system in CCRF?

    ***Acknowledgement** is mandatory for any publications, reports and scientific content resulting from the use of the confocal system in CCRF. This helps us to track how well the system has been utilized for publication purposes. The standard sentence to be used is "We acknowledge the Confocal Microscopy Facility, CCRF, AIIMS New Delhi for help with image acquisition and analyses." Please get in touch with the scientist in-charge to get details on the microscope model, details of the objectives, N.A., etc. before writing the Materials and Methods section of your paper.*

    ***Authorship** in a manuscript is subjective and depends on the effort, time, inputs for the experimental design, troubleshooting, image processing, quantification and statistical analyses including help with writing up specific section(s) of the manuscript that the scientist has contributed to. This should be discussed upfront*

and depending on how much help is required from the facility/ scientist, a consensus can be reached on whether an authorship is warranted.

For general authorship guidelines, please refer the following:

https://oir.nih.gov/system/files/media/file/2021-08/guidelines-authorship_contributions.pdf

https://www.icmje.org/recommendations/browse/roles-and-responsibilities/defining-the-role-of-authors-and-contributors.html