

Confocal Core Procedures

1. Contact Confocal Core to discuss experiment

- Meet with Susan and/or Stephen to discuss experiment
- Determine feasibility for confocal

2. Design experiment

- Appropriate fluorophores for specific system
- Proper fixation or necessity for live cell imaging
- Appropriate sample preparation (mounting media, substrate, coverslips, etc.)

3. Procedure for the facility (fig.1)

- Instructions for setting up the calendar for each system
- Instructions for mapping the terabyte
- Submission form (<http://elsie.nci.nih.gov>)

4. Perform experiment

- For fixed specimen, appointment can be made anytime
- For live cell, appointment needs to be made in advance

5. Make appointment

- Check calendar for times available (fig.2)
- Send email to Susan or Stephen with appointment request
- Facility confirms appointment by email
- Waiting list

6. Fill out submission form (fig.3)

- Samples automatically numbered for facility database
- Completed copy sent to user and facility by email
- Reminder email sent out day before appointment (fig.4)

7. Hands-on training on selected system

- Training occurs during appointment
- User works independently once fully trained (usually several appointments)

8. Back up images/data

- All original images backed up on terabyte and CD/DVD
- "Where is it" software to track location

9. Set up terabyte on the user's computer

- Help users access terabyte from their computer
- Help users set up software for their computer
- Teach users to convert images for use in other software packages (i.e. PhotoShop, PowerPoint, Illustrator, etc.)

10. Process images through software packages

- Bio-Rad LaserSharp2000 5.1
- Zeiss LSM 3.2
- Imaris 4.0.4
- PhotoShop 7.0

11. Help with analysis

- Co-localization
- FRET measurements
- FRAP measurements
- 3D reconstructions, surface renderings, etc.
- Intensity measurements and tracking
- Generation of movies (2D, 3D, 4D)
- Publication quality prints

fig.1

Confocal Procedures:

#1
Here's the instructions for setting up the calendars on your computer. If you have problems, email Stephen or me and we should be able to help you get them set up.

I have set up three calendars in Microsoft Outlook. One calendar is for scheduling time on the NCI Zeiss LSM 510, one for the NCI Zeiss 510 NLO Meta and the other is for scheduling time on the NCI Bio-Rad MRC 1024. These calendars will allow you to see the schedule of appointments for either of the Zeiss systems or the Bio-Rad confocal. You can then send an email to me to request an appointment for time that has not already been taken. I will update these calendars and send you an email to confirm your new appointment. If you have any problems with these calendars, please let me know. You will be able to view these calendars, but you will not be able to write in appointments.

Instructions for opening these calendars from your computer:
 1. Open Microsoft Outlook 2000. If you have an earlier version, you should have the NIH Help Desk update yours to the latest version.
 2. At top of screen, click on "File", then click "open", then click "other users folders".
 3. The "Open Other User's Folder" box will appear.
 4. Where it says "Folder", the default "inbox" appears. Use the drop down arrow to select "calendar" and then type in the "Name" space: "NCI Zeiss LSM 510" or "NCI Zeiss 510 NLO" or "NCI Bio-Rad MRC 1024".
 5. Click on OK and the calendar should open for the instrument you selected in the name space.
 6. Note: after opening the calendar once, you won't have to do #4 again. You will get the calendar displayed as an option at the "File/Open" prompt.
 7. Once in the calendar, using "view", you will be able to see a day at a time, a week at a time or a month at a time.

#2
When your samples are ready, you should fill out the submission form which can be found at <http://elsie.nci.nih.gov/confocal>. Your user ID is the first 7 letters of your last name and first initial. If you're not sure which confocal you need, check with me. Be sure to fill in all the required fields and then click on the "click to proceed" button. Only click once, otherwise duplicate forms will be sent. A message will appear after a few minutes indicating that your form has been submitted. If the form remains on the screen, it means that there is a mistake (usually one of the required fields has been left blank). Any problems, just call me or send an email.

#3
No one uses the confocal unless they have scheduled an appointment.

#4
Your images will be backed up on the terabyte array. The terabyte array is available for accessing your images, but you will not be able to save to the terabyte. You should find all your images there. You need to find/search for the network computer, "NCI37B", using the search option under the "start" button of your computer (usually found at lower left hand corner of computer screen). Once you have found this computer, double click and then select "37_G9". Then double click on LECImage and you will see your user folder which contains all your images. You should map a drive for LECImage on your computer and then you will not have to do a search whenever you want to work with your images. Software for both the Zeiss (under software/fsm) and the BioRad (software/BioRad) confocals are stored on the terabyte for your use. For the Zeiss, under the fsm folder, just double click on the image browser and the tool bar will appear on your computer monitor. For the BioRad, you will have to double click on the setup program and instructions for setting up confocal assistant will appear on your computer monitor. Once you have installed Confocal Assistant on your computer, you will need to copy the red, green and blue "tut" files in the "tut" folder in the program. These will allow you to color the gray scale images. All software only works with PCs, not MACs. If you have any questions or problems, just let me know.

fig.2

Zeiss LSM 510 Calendar

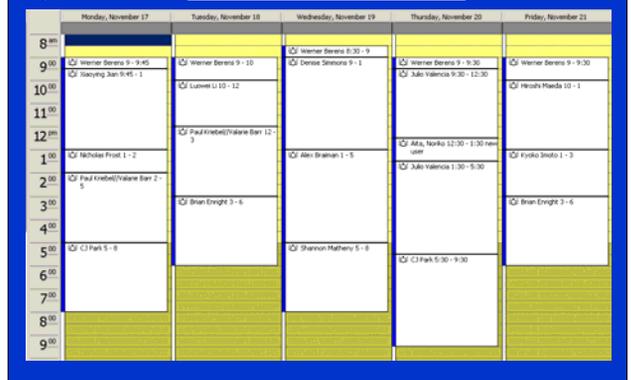


fig.3

Confocal Slide Submission Form

Click to proceed

Your network login name: [text box]
 Electronic Mail address: [text box]
 Date: 12/15/03 0:00:00

Fluorescence 1: [dropdown menu] (must be entered before submitting form)
 Fluorescence 2: none
 Fluorescence 3: none
 Instrument: [dropdown menu] (must be entered before submitting form)
 Line or Field: [dropdown menu] (must be entered before submitting form)
 Tissue or type of cell (must be entered before submitting form): [text box]

Brief description of experimental objectives (i.e. structures of interest): [text box]

User label 1: [text box] (must be entered before submitting form)
 User label 2: [text box]
 User label 3: [text box]
 User label 4: [text box]
 User label 5: [text box]
 User label 6: [text box]
 User label 7: [text box]

Completed Form

Order #: 2003982
 User ID: nles
 Date: 12/9/03 0:00:00
 Fluorescence: FITC
 Fluorescence 2: Rhodamine
 Fluorescence 3: none
 Instrument: Zeiss 510
 Type: Fixed
 Tissue or Type of Cell:
 Jurkat cells
 Description:
 Effect of FLAG-PZA of AGAP2 on cell s
 Slide count: 20
 Slide 20036931: FLAG-Smin
 Slide 20036932: eGFP-Smin
 Slide 20036933: pCIPZA-Sim
 Slide 20036934: pGIPZA-Smin
 Slide 20036935: FLAG-0min
 Slide 20036936: eGFP-10min

fig.4

Reminder Email

Don't forget your Confocal appointment today.

Submission form done? <http://elsie.nci.nih.gov/confocal>

Please, if you need to cancel your appointment, let us know.

Thank you,
 Susan & Stephen

Contacts:

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