

Zeiss LSM 510 Confocal Microscope

Introduction and Instrument Specifications

Laser scanning confocal microscopy provides researchers with the ability to optically section whole specimens of cells and small organisms, such as developing embryos, or very thick sections from bone, brain, and other organ tissues that have been tagged with fluorescent or reflective probes. This technology provides us with the highest light microscope resolution obtainable, giving the scientist a clearer picture of sub-cellular structures, function, and cellular or organism architecture. This can be performed on single or multiply labeled specimens. The advantage of confocal microscopy is the ability to collect light from a single plane. This is achieved in laser scanning microscopy. A laser light beam is expanded to make optimal use of the optics in the objective. Through an x-y deflection mechanism this beam is turned into a scanning beam, focused to a small spot by an objective lens onto a fluorescent specimen. The mixture of reflected light and emitted fluorescent light is captured by the same objective and is focused onto a photodetector (photomultiplier) via a dichroic mirror (beam splitter). The reflected light is deviated by the dichroic mirror while the emitted fluorescent light passes through in the direction of the photomultiplier. A confocal aperture (pinhole) is placed in front of the photodetector, so that the fluorescent light (not the reflected light!) from points on the specimen that are not within the focal plane (the out-of-focus light) where the laser beam was focused will be largely obstructed by the pinhole. In this way, out-of-focus information (both above and below the focal plane) is reduced. This becomes especially important when dealing with thick specimens. The spot that is focused on the center of the pinhole is often referred to as the "confocal spot". Ultimately, displaying the scanned specimen with high resolution. Optical sectioning can be performed in the xy plane (perpendicular to the optical axis of the microscope) and also in the vertical plane (parallel to the optical axis of the microscope) in the xz or yz plane. Scanning in the z-axis, as well as the x and y-axis, gives the effect of viewing the focal plane from the side. True, three-dimensional data sets can be recorded. Stacks of optical sections obtained from successive focal planes (a z series) can be reconstructed to produce the 3-D view of the specimen. The IBB Flow Cytometer and Confocal Microscopy Laboratory is equipped with the Zeiss LSM510 Confocal Microscope and has the software package for image analysis, processing and 3D image reconstruction of these confocal images.

LSM 510 Confocal Microscope (Carl Zeiss Inc.)

The LSM 510 UV confocal microscope is the latest generation of laser scanning microscopes from Carl Zeiss Inc. The system is equipped with 3 lasers:

LASER TYPE	Type of cooling	Excitation lines (nm)	Maximum Power
Argon	Water cooled	351-364	80.0mW
Argon	Air cooled	458,488	15.0mW
HeNe	None	543	0.5mW

LSM 510 OBJECTIVE SPECIFICATIONS

<u>MAG</u>	<u>NA</u>	<u>WD(mm)</u>
10X	0.25	6.5
20X	0.30	2.2
40X (OIL)	1.3	0.1
63X (OIL)	1.25	0.1

The microscope stand is an Axiovert (inverted configuration). The stage is equipped with slide holder that can accommodate 12X75 mm slides and 35 and 65 mm dishes.

Zeiss LSM 510 Confocal Microscope Operation and Guidelines

Policy

Projects:

All new users should meet with Johnafel Crowe (Office: 404.894-2212, johnafel.crowe@ibb.gatech.edu) to discuss their project **BEFORE** they use the instrument. An abstract or brief description of each user's project involving microscopy should be discussed in person or forwarded to johnafel.crowe@ibb.gatech.edu. At that time objectives and goals of the user can be discussed as well as the best means of achieving these objectives. **Important note:** If projects involve potential biohazards, the user must inform Johnafel and supply protocols for decontamination at this time.

Instrument Use:

Prior to confocal microscope use it is imperative that every person that intends to use this instrument be properly trained. This is done after project consultation described above. Visit ibb.gatech.edu/~avesper/confocal/ to schedule a training session. Basic and advanced training sessions will be administered at least once a semester or as needed for new and current users. These sessions usually last about 2 hrs. The training session topics include but are not limited to operation of the system, laser safety, how to acquire, save, export, and transfer an image as well as proper microscope operation.

User Certification:

The confocal is a very expensive and sensitive piece of equipment. We expect all confocal users to be certified before they use this instrument. Only certified users may operate the instrument. Certification is attained after a person has attended a Basic or Advanced Training session. Basic Training sessions will be held at least once a semester or as needed. Advanced training sessions are available but are not required for certification.

Who is a certified user?

A certified user is defined as a person who has attended a basic training session on the operation and proper use of the confocal microscope. This person has also agreed to accept and follow all of the guidelines and related safety measures discussed in this document.

Confocal Sessions:

All users will read and have available the condensed equipment instructions for the confocal microscope. Equipment will be left as clean or cleaner than the user originally encountered it. Each user is required to sign a log before and after each session. Users are required to report any instrument problems encountered during each session to Johnafel Crowe. This action will help to keep the confocal microscope up and running properly. Each user will receive a username, password and a secured folder to temporarily store their images. The hard drive connected to the confocal has a limited amount of disk space. **Therefore, users are required to bring blank storage media** (e.g. blank Zip disks, blank CDs, MO disks, or hard drives via the GT network) **to each**

session and transfer their images to some form of storage media **BEFORE** each session is complete. **The core laboratory is not responsible for the backup of user folders and files.**

Instrument Modifications:

No modifications are to be made to the confocal microscope stand, scan head, laser sources, or the attached PC. Only certified Zeiss representatives may make hardware and LSM 510 software modifications to the system

Reserving Time for Confocal Sessions:

Equipment reservations can be made by visiting <http://www.ibb.gatech.edu/~avesper/confocal/> and following the instructions provided. **IF YOU REQUIRE OPERATOR ASSISTANCE, YOU SHOULD NOTIFY JOHNAFEL AND RESERVE A TIME NO LATER THAN THE DAY BEFORE YOUR APPOINTMENT.** In the event of a cancellation or an error when scheduling a time, please notify Johnafel at 404-894-2212; johnafel.crowe@ibb.gatech.edu as soon as possible and he will make the necessary corrections to the schedule. If the core laboratory has to cancel an appointment, then the user will be notified in a timely manner.

After Hours Use:

Please see Johnafel if you are a certified user and require access. Certified users who wish to operate the confocal microscope after normal operating hours may do so after they have logged at least 3 successful sessions with Johnafel Crowe present. To gain access to the confocal microscope after hours when you come to your session, remember to bring your buzz card in order to gain access to the lab.

Supplies

The core laboratory is primarily equipped for image acquisition, and on a limited basis, for image analysis. We will provide Zeiss immersion oil, Kimwipes, cotton tip applicators, ethanol, and lens paper. The staff will be available for technical support Monday –Friday between the hours of 9 A.M. and 5 P.M. EST. The core laboratory will provide three types of storage devices: a 650 MB MO drive, a 250MB Zip drive and a CD-RW drive. Users whose computers have ethernet connections may transfer images to their computers via the GT network. We do not supply MO disks, Zip disks or blank CDs. We have an offline workstation where image analysis can be performed. If you need to access files on the acquisition workstation, you can do so by using the offline workstation.

What users should consider purchasing:

Users may consider purchasing the following items prior to their scheduled session:

Mounting agent (e.g. Fluoromount G)	Glass Coverslips (# 1.5)	Glass slides
Blank CDs	ZIP disks	Clear fingernail polish
35mm stainless steel 25mm coverslip holder (used for viewing live cells/living tissue in a fluid)		

Guidelines for viewing dry specimens:

Users must prepare their samples prior to entering the core laboratory. The samples must be stable and free of any material that might come in contact with the objectives. Samples must lie parallel to the stage. Glass cover slips (# 1.5) must be used. If a mounting or sealing agent is used (e.g. fingernail polish, anti-fading mounting reagents), it must be completely dry and fixed to a slide before using the confocal microscope. If the sample is not dry, the coverslip may move and the wet sealing agent may damage the objectives.

Guidelines for viewing wet specimens:

Wet specimens must be self-contained. A glass coverslip must come between the specimen and the objectives. Users may consider purchasing a coverslip holder from Zeiss or making their own. Homemade coverslip holders must be leakproof. This will prevent fluid spills on the objectives and nosepiece.

Safety

Emergency procedures:

In case of an emergency, please contact:

Johnafel Crowe Office: 404-894-2212, Home: 770-210-9145

Steven Woodard Office: 404-894-5891, Cell: 404-725-0023, Home: 770-322-0187

Kay Kinard Office: 404-894-8896, Home: 770-944-0519

In the event we cannot be reached and the matter requires immediate attention, call Georgia Tech Police 404-894-2500. These numbers will be posted near the instrument and on the outside of Room 1328.

Laser Safety

We are operating a type IV laser and there are some precautions. Do not look directly into the beam and do not disable any of the safety features on the microscope. Knock before entering a room with this type of laser. Do not use any type of reflective surfaces when operating this instrument.

Biohazardous Materials:

Please notify Johnafel of any potentially biohazardous samples that are used with the confocal microscope. Any specimen that is either human or primate in nature is considered a potential biohazard. Therefore it is the user's responsibility to perform their image acquisition in such a way as to not contaminate the instrument. Furthermore, it is the user's responsibility to provide the facility with decontamination protocols. In the event that the instrument becomes contaminated by the specimen (e.g. blood, sputum or waste products), it is the user's responsibility to notify Johnafel Crowe immediately. All biohazardous waste will be disposed of in red biohazardous bags and placed in a designated waste area. Regular waste pick-ups will be scheduled with the Department of Environmental Health and Safety.

FLUORESCENT DYES FOR CONFOCAL MICROSCOPY

BEST DYES FOR THE ZEISS LSM 510

<i>Laser Line Excitation</i>	<i>Dyes</i>	<i>Emission Color</i>
364 nm. UV	<ul style="list-style-type: none">• Hoechst 33342, for DNA• Dapi, for DNA• AlexaFluor-350• Cascade Blue• AMCA	<ul style="list-style-type: none">• Violet / Blue
488 nm. Argon	<ul style="list-style-type: none">• AlexaFluor-488• Oregon Green• YOYO-1, for DNA• Cy-2• FITC• PE	<ul style="list-style-type: none">• Green• Green• Green• Green• Green• Red
543 nm. HeNe	<ul style="list-style-type: none">• AlexaFluor-546• AlexaFluor-568• Tetramethyl Rhodamine• Lisamine Rhodamine• Propidium Iodide, for DNA• BOBO-1 for DNA	<ul style="list-style-type: none">• Red

General References

Cheng, P. C., Lin, T.H., Wu, W. L., and Wu, J. L., eds. (1994). "Multidimensional Microscopy." Springer Verlag, New York.

Cogswell, C. J., and Carlsson, K. (1994). "Three-dimensional microscopy : image acquisition and processing." SPIE. Bellingham, Washington, USA.

Matsumoto, B., ed. (1993). "Cell biological applications of confocal microscopy." Academic Press. San Diego, California.

Pawley, J. B., ed. (1990). "Handbook of Biological Confocal Microscopy." Plenum, New York.

Stevens, J. K., Mills, L. R. and Trogadis, J. E., eds. (1994). "Three- Dimensional Confocal Microscopy: Volume Investigation of Biological Systems." Academic Press, London.