Kennesaw State University Microscopy Core Facility Users Guide

Before using any of the equipment in KSU's Microscopy Core Facility, you must read and sign this document. DocuSign is required for KSU faculty and staff.

I have read the following users guide and confirm that I will adhere to facility policies.

Signed: _____ Name: _____ Date: _____

Principle investigator: _____ Dept. affiliation: _____

To schedule training or discuss capabilities and/or projects with Dr. Nowak, please email him at snowak@kennesaw.edu to get on his training. calendar.

Facility instrumentation: Multiple instrument upgrades have happened recently so even experienced users should consult this guide and familiarize yourself with new features.

Zeiss LSM 900 Confocal – This is an inverted confocal microscope equipped with four solid-state lasers (405, 488, 555, and 639nm). The LSM 900 confocal is equipped with two GaSP PMT detection modules, enabling simultaneous four-beam acquisition. The microscope is equipped with an incubation chamber with controlled temperature and humidity for prolonged live cell imaging. A full range of objectives (e.g., 10x, 20x, 40x, 63x (oil immersion), 100x (oil immersion)) is installed on the microscope. This system is suitable for routine fixed and live imaging of either prepared specimens or live cells in conventional tissue culture vessels. Flow-through capabilities for long-term live imaging, including feeding kinetics, can be accommodated with this system.

Leica TCS SP8 Tau-STED 3X Confocal – This is an inverted confocal microscope equipped with all three depletion lasers (592 nm, 660 nm, and 775nm), capable of multi-channel STED, as well as a spectrally tunable excitation with a pulsed White-Light Laser suitable for most conventional fluorophores (effective range 470 nm - 670 nm). The setup includes five spectrally tunable detectors (2 PMT detectors, as well as 3 HyD detectors). Additionally, this microscope is equipped with a 405 nm laser suitable for DAPI imaging, as well as FLIM image capabilities. A full range of objectives (e.g. 10x, 20x (aqueous immersion), 63x (oil), 100x (oil)) is installed on the microscope. This microscope is suitable for routine and STED imaging of fixed prepared specimens. Ambient live cell imaging (i.e., incubator-free) is possible with this system, but not recommended.

Zeiss AxioImager M2 Upright Compound – This is an upright compound microscope equipped with 4-color epifluorescence and an Apotome2 slider module. The AxioImager is capable of conventional epifluorescence as well as phase illumination. Two cameras for high-resolution (4K compatibile) imaging (mRM mono, mRM color) are mounted on the microscope for use. A full range of objectives (e.g., 5x, 10x, 20x, 40x, 63x (oil immersion) are installed on the microscope. Deconvolution capabilities are possible using the Apotome2 slider.

Training: All users of the KSU Microscopy Core Facility must be trained for appropriate microscopy use. This training includes fluorophore and illumination selection, experimental design, and proper

image acquisition and processing. For users already familiar with confocal microscopy, this will likely be a quick refresher. For users new to the facility, Dr. Nowak offers *ad hoc* training, preferably in groups of up to 5 users at a time. For scheduling training time, please email Dr. Nowak at snowak@kennesaw.edu.

Sample preparation: Users are required to provide their own microscope slides and cover glasses. No reagents (e.g., mounting medium, antibodies, or tissue culture chambers) will be provided by the MCF. The Microscopy Core Facility is not a wet lab- all experimental manipulations must be carried out prior to entering the Facility. Advance arrangements for limited wet bench space can be made if necessary.

Scheduling: Imaging time can be booked at https://csm-resources.kennesaw.edu/reservation Users are limited to advance scheduling of one 2-hour block of time during core hours (defined as 9AM-5PM, Monday-Friday). Longer imaging runs can be accommodated after 5PM and/or on weekends. Users may schedule extra time on the day of use if available. Laboratories are asked to refrain from scheduling more than two 2-hour blocks in a single day on the same instrument.

Tidiness: It is expected that users will clean up after themselves. Microscope samples left unattended will be discarded after 24 hours. Please do not leave paper waste (e.g., lens paper) in the facility. Users will be trained in the proper method for cleaning the microscopes during their training session.

Data storage and removal: Please make every effort to organize datasets into folders following a regular scheme. Data hygiene is very important. Flash drives must be free of malware or viruses or we will implement a flash drive ban. Imaging data on the microscope computers is not intended for long-term storage; drives fail so it is strongly recommended that you transfer your data offsite for processing. Data processing of images must be handled off-site.

Publications: In the acknowledgement section for publications resulting from work performed on confocal microscopes in the KSU Microscopy Core Facility, please provide the following statement: "*The authors would like to acknowledge Kennesaw State University Academic Affairs for support of the Microscopy Core Facility, which made possible the research necessary for the completion of in this project.*" If the work was performed on the Zeiss AxioImagerM2 microscope, please provide the following statement: "*The authors would like to acknowledge Kennesaw State University Academic Affairs and Georgia BIO for support of the Microscopy Core Facility, which made possible the research necessary for the completion of in this project.*"

Special pandemic-related instructions: Masks must be worn by unvaccinated users while in public areas of the KSU campus, including the Microscopy Core Facility. In addition, please make every effort to social distance and to wipe down keyboards and work surfaces with 70% Ethanol and Kimwipes provided. ***Do not use the sanitizing wipes on MCF equipment!***