

LIGHT MICROSCOPY CORE FACILITY (LMCF)

Standard Operating Procedure (SOP) for transporting live human and animal-derived cell lines to the LMCF, handling them in the LMCF and transporting them from the LMCF when finished.

General description:

Experimental procedures will be the microscopic imaging of fixed and live cells of animal origin (human-derived, non-primate human-derived, and other animal-derived, e.g. mice) using microscope instruments located in the Light Microscopy Core Facility in B047A and B049 Porter. In the case of live cells, cells are grown in glass-bottom or suitable culture dishes in the laboratory of the investigator. The cells are transported to the LMCF (see procedure below) where the culture dish is removed from the transport container and placed immediately into the imaging chamber – no cells are removed, or transferred during this procedure, the culture dish is simply placed into the imaging chamber and the chamber is sealed and placed onto/into the microscope where it remains for the duration of the experiment without disruption. Two of the microscopes use named “Dante” and “Thor” utilize plexiglass boxes that surround the microscope and have small doors to provide access; the third microscope named “Black Widow” does not have a plexiglass box, but is in an isolatable space; each microscope space is isolatable by either drawing heavy curtains or closing doors. While an experiment with live cells is being performed a sign that reads “Microscopy of Live Animal Cell in Progress – Do Not Disturb” will be placed on the exterior doors and at the instrument.

Signage indicating the potential use of live cells will be placed on each LMCF access door. Contact information will (is) also on each door. The online scheduler will indicate which if any instrument is being used or live cell work. A sign will be placed at the instrument or closed curtains indicating when live cells are being imaged. Printed copies of this SOP will be made available at each instrument and as a downloadable pdf from the LMCF website.

Scheduling:

All users who wish to perform live cell microscopy must first receive training from Dr. Jolien Tyler, Dr. James Orth, or both and demonstrate proficiency in using the microscope and the incubation chamber that will hold their sample prior to work. Each such user must have passed appropriate training for handling of the live cells through their laboratory’s (PI’s) approved IBC protocol.

Approved users schedule their imaging session through the web-based sign-up system (<https://mcdbcal.colorado.edu/phpScheduleIt/index.php>) that must be logged into using their IdentiKey. Dr. Tyler is notified when instrument scheduling occurs.

The scheduling system and status of each instrument can be viewed by any user – when the imaging session involves live cells, we require that the user indicates for example, “Orth – live cells” so that other users will know.

Transport from the lab to the facility and the facility back to the laboratory:

The live cells in the respective cell culture room are transported to the LMCF by placing the culture dish into a suitable secondary container, e.g., crush-resistant styrofoam or plastic “Igloo” lunch container that has been wiped with 70% ethanol. The secondary container must be securely closed, either by zipper or velcro (lunch bag) or secured strap or tape to contain any spilling of the culture medium that may occur if the container is dropped during transport to the LMCF. In the event of a spill into the transport contained it will be decontaminated with 70% ethanol wash and 10% diluted bleach. In the event of contamination with an agent (viral or bacterial) that does not respond to traditional decontamination approaches, additional decontamination steps may be required. Note – currently no such agents are being used and we will check this when new agents are introduced through investigator’s work. In this case, decontamination procedures recommended for those agents will be used, e.g. Wescodyne for bacteria. The EHS website contains important information for disinfecting with different agents: <http://ehs.colorado.edu/resources/common-chemicals-used-for-cleaning-and-decontamination-guideline/>.

After the experiment is finished, the sample is placed into the sealed secondary container, transported to the laboratory’s certified cell culture room and treated as cell culture waste and appropriately discarded.

Transfer sample plate into imaging chamber:

The cells are growing in imaging dishes that function as a standard cell culture dish and are covered with well-fitted lids. Using at least one gloved hand, the imaging dish is transferred into the imaging chamber, the chamber is sealed, as is required for correct functioning of the chamber, and the chamber is placed onto the microscope stage. No aerosols are generated during the sample transfer or during the imaging procedure. Lids on the imaging dishes prevent nearly all spills from occurring. In the event of a medium spill it will be cleaned (see below). The glove(s) used to transfer the dish is/are will be placed into the transport container and discarded appropriately back in the laboratory. Gloved hands will not be used when operating the equipment. After the experiment, the imaging dish is removed, transported back to the laboratory in the transport container for proper disposal. The inside and surface of the imaging chamber is wiped with 70% ethanol after each use.

For handling live samples that contain live bacteria (e.g. *Salmonella enterica*). Imaging dishes with tight-fitting and/or closable lids will be used, e.g. ibidi or In Vitro Scientific 35 mm dishes. The lids will be parafilm to the dish, and transported to the LMCF in a crush-proof hard-sided container. At the microscope, the imaging dish will remain parafilm at all times. After imaging, surfaces will be wiped down with 70% EtOH and sample goes back into the transport container for transport back to laboratory for proper disposal.

Clean up in case of a spill:

In the event of a spill during transport to the LMCF, the area of the spill (<15 ml) will be wiped clean with 10% bleach and disposable paper towels that are discarded into the hazardous waste trash (e.g. red bag) that is autoclaved before disposing into the certified collection bins in the autoclave rooms. The area will then be doused with 70% ethanol for total contact time of 15 minutes (min. 10 minutes) and wiped clean; used paper towels will be discarded as

those used for the bleach solution. The EHS w