

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 website contains important information for disinfecting with different agents: <http://ehs.colorado.edu/resources/common-chemicals-used-for-cleaning-and-decontamination-guideline/>.

In the event of a spill in the LMCF (<15ml) but not on the equipment, e.g. on the floor or bench-top, the spill will be treated as above. The LMCF will keep a spill kit under the fume hood within the facility that contains 70% ethanol, 10% bleach solution, latex gloves, paper towels, and clean red bags to discard the waste into. The red bag with all waste shall be taken back to the user's laboratory for proper tagging, autoclaving and disposal. There is no source of flame in the LMCF.

In the event of a spill onto the equipment, it will be treated with 70% ethanol while maintaining a total contact time of 15 minutes (min. 10 minutes) and wiped with paper towels that are then discarded into a red bag from the spill kit.

What to do if the equipment is contaminated and you need to bring in a vendor to fix the equipment:

If there has been a spill on the equipment in an area that a service technician needs to contact, we will make them aware that there was a spill and that the spill was cleaned per IBC approved SOP and that the risk is essentially none.

Security for the facility:

The LMCF is a secured facility with either hard key entry or coded key card entry from 4 pm until 9 am except Saturday, Sunday, and holidays are keycard required 24 hours. All entry from keycards is logged. Between the hours of 9 am and 4 pm during the week the access doors are open. Dr. Tyler is often in the core and has a sense of who is there. Instruments themselves are secured in that they cannot be operated unless logged into (see below). Hard keys exist only for Dr. Tyler, Dr. Orth, trained EHS personnel and Jen Ryan, the building manager. Spare hard keys are stored in the MCDB Front Office in case of emergency.

Each microscope can only be operated by computer login which uses IdentiKey and can be tracked. Further, a log-in sheet must be completed each time a microscope is used, which keeps track of usage and purpose of use.

Each microscope area can be isolated from the main space by heavy, overlapping curtains or doors that act as a barrier to light and disruption by foot traffic, etc. During live cell imaging, the samples are secured from access by being inside of a sealed chamber (Dante, Thor, Black Widow) that is inside of an additional plexiglass box (Dante and Thor).

How the facility is managed, contact information, who to call if something goes wrong etc.):

The facility is managed by Dr. Jolien Tyler (contact info listed on the LMCF entry doors) and Dr. James Orth is the faculty Proctor (contact info listed on the LMCF entry doors). If something occurs that needs attention Dr. Tyler and/or Orth can be contacted.