Vol .04 - March 2023

Research Core Facilities

Newsletter

Tissue Culture

Check your cultures!

Continuous cell lines are ideal model systems- (i) repeatedly passaged (ii) reliably recover from cryopreservation and (iii) retain properties of its cell type or tissue.

Several continuous cell lines are reported to be cross-contaminated, which may occur due to:

- Poor technique (spread via aerosols or accidental contact)
- Use of unplugged pipets
- Sharing media and reagents among cell lines
- Use of mitotically inactivated feeder layers or conditioned medium etc.

Cross-contamination is a serious issue, check each cell line before it is used, search for previous references and obtain cell lines from repositories that authenticate them.

A 2010 study reports testing 598 leukemia–lymphoma cell lines for cross-contamination. 187 were contaminated with Mycoplasma and/or a second cell line and 38 cell lines contaminated with both. The commonest contaminaned cell lines are Hela, T-24 and HT-29.

Read article https://pubmed.ncbi.nlm.nih.gov/20143388/ for further information.

Flow Cytometry

Clogs happen... but you can prevent them!

Causing a clog in the system can create substantial system downtime!

Clumps and debris can clog the instrument fluidics and either distort the measurements or obstruct them completely. If the flow rate is perturbed, illumination time can be affected which can lead to inaccurate data.

Common causes and fixes:

- **Cations -** calcium and magnesium can promote cell clumping, use Ca++ and Mg++ free PBS, chelators such as EDTA (1-2 mM) can be used to remove calcium, 1% BSA in Flow Buffer instead of FBS (contains calcium)
- **Clump removal -** filtration is the fastest and easiest approach, always sieve the cells prior to acquisition through a 35-50 µm nylon mesh
- **Dead cells -** dead cells release DNA which in turn produces a viscous sticky consistency that binds cells together, the easiest remedy is to add 10 units of DNAase per ml of sample (best for tissue digests)

A. Suspecting a clog due to a poor event rate - what now?

Run water on the highest flow rate – see fluid displacement?
No - Go to B, Yes - try the steps below:

- Re-vortex the sample and inspect it. Does it need to be re-filtered?
- Did you run the last sample dry, introducing an air bubble? Prime and run water for 3-5 min
- Did you perform a cell count? Does the sample need to be concentrated?
- Check tube integrity - chipped flow tubes can prevent proper pressurization (BD Fortessa and Sony SP) – change tubes

B. Still no events? Try clearing the flow cytometer injection point.

10-20% Contrad for 5-10 min (can be found by the Fortessa station), followed by water for 10-15 min. If this fails, report the issue to your flow specialist.

What's New

Recycling in the labs

We are happy to announce that **recycling is now available on the 4th, 5th and 6th floor lab spaces.** A large recycling bin has been set up in the north hallways to recycle paper, cardboard and packing boxes. A large landfill container has also been set up for packaging material such as bubble wrap and ice packs. Please ensure that you flatten the cardboard boxes before putting them in the recycling container.

Save your Styrofoam

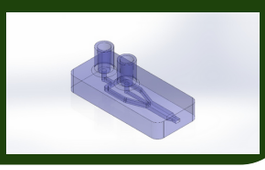
In partnership with the University of Toronto, styrofoam boxes smaller than 20" x 20" x 20" can be returned to MedStore to be reused. We have set up a small bin in the LKSKI receiving dock area as a designated collection point. Please place any styrofoam boxes in this bin for MedStore to pick up.

Microfabrication

We have a new workstation with CAD software in the Rapid Device Prototyping room on the 7th floor (Rm 772) available to researchers in the institute.

With the software installed, you can create fast and accurate designs, including 3D models and 2D drawings of complex parts and assembly designs. You can design your 3D models for printed pieces, mask layouts for the photolithography processes when making devices in the Microfabrication Cleanroom, and 3D models and animations for your presentations.

The current list of software includes SolidWorks, Blender and KLayout. If you want to use the workstation or need help with your models, please get in touch with Dario.Bogojevic@unityhealth.to



Histology

Redeveloping the Histology Core

Starting March 2023, the new Histology Core will have two labs. The **Histo lab 1, room 666** will be used for tissue processing, embedding, staining, antigen retrieval, and cytospinning. The **Histo lab 2, room 643** will be dedicated for section, which includes paraffin sectioning, cryosectioning, vibratome sectioning.

Equipment Updates

- The Leica ST 5010 Autostainer XF has replaced the existing one and will continue providing reliable, reproducible, consistent high-quality staining. Please contact Xiaofeng Lu (xiaofeng.lu@unityhealth.to) if you require refresher training.
- The new Leica tissue processor – PEGASUS has been installed and will be ready for use soon. Training is required to use the new processor. Please wait for further announcements regarding training sessions.
- E-manuals for all the new equipment have been uploaded on our Research Facilities website. This includes the PEGASUS Tissue Processor, ST 5010 Autostainer, PrintMate™ AS 450 Cassette Printer and SlideMate™ AS Slide Printer et al. For more information, please visit here.

Pre Clinical Imaging

New Equipment in House

U-CT

MILabs' U-CT is extremely fast and offers ultra-high resolution at low X-ray doses enabling demanding multispecies 3D/4D in vivo applications, including dynamic contrast-enhanced (DCE) CT imaging. The system allows for dual-gated cardiac and respiratory imaging for freeze-frame acquisitions and features sensor-free respiratory gating for imaging up to:

- Available for four mice simultaneously and medium-sized animals, such as rabbits
- Dynamic contrast-enhanced (DCE) CT imaging
- Dual-energy CT imaging
- Ultra-high resolution 2.4 µm
- High speed and low dose, down to < 2 mGy whole-body mouse

Light Microscopy

Microscope Monday

This Spring, stay tuned for Microscope Monday. Each week will have a new theme to improve your imaging.

Equipment Updates

- The last day to use the LSM700 as a confocal scanner is March 6, 2023.
- The Cell Discoverer 7 will be available for use starting in April. Stay tuned for more details.

Genomics

What is spatial biology and what is all the fuss about?

It is the study of molecules in a two-dimensional or three-dimensional context. Using spatial biology techniques, researchers can visualize molecules in their unique contexts within individual cells of tissues. Biological systems exist in three-dimensional space and for many biological processes, spatial orientation and positioning to each other is critical for that process to work. For example, during development, patterning of the body, such as the orientation and number of fingers, is driven by gradients of signaling molecules. Aberrations to this spatially important cell signaling can lead to polydactyl or extra digits.

Spatial biology typically refers to a specific technique for looking at spatially resolved transcriptional dynamics: it may also be called "spatial profiling, spatial genomics or spatial transcriptomics".

Regardless of the name, the technique relies on two well-established techniques, immunofluorescence and molecular barcoding/next-generation sequencing (depending on the system). Using both technologies together, the user can ascertain how transcriptional dynamics vary within a spatial context. Spatial information can be obtained at various scales, including at the tissue, single cell, and subcellular levels.

Spatial biology is a promising new field, consequently, Nature Methods chose spatially resolved transcriptomics as the method of the year in 2020. Hopefully, this technology will be coming soon to Toronto – for more information please see Genomics Specialist, Pamela Plant or www.nanostring.com.