THE MICRO TOME

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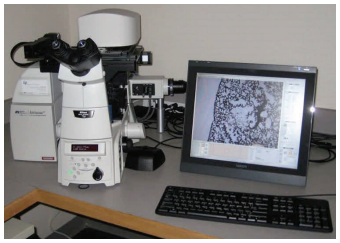
MICROSCOPY IMAGING CENTER

http://www.uvm.edu/medicine/MIC

https://www.facebook.com/The-Microscopy-Imaging-Center-786501598145292/

Fall 2015

The Captivating Laser Capture Microdissector



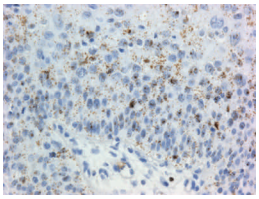
The Arcturus XT-Ti Laser Capture Microdissector (LCM), housed in HSRF 207, is the latest generation of microdissection instruments from Life Technologies (Arcturus). The software allows the user to select, either morphologically or by using fluorescently-labeled molecules, single cells or small groups of cells from a heterogeneous population on a glass or PEN-membrane slide. The LCM is equipped with an infrared laser which melts a polymer onto the cells of interest and a UV laser which allows for the cutting of a larger group of cells from membrane slides. After microdissection, RNA, DNA, and proteins can be extracted for downstream molecular analysis. High resolution images can be taken to document the tissue before and after LCM, and also the isolated cells from which the biomolecules will be purified.

Past studies using the Arcturus LCM system in the MIC allowed gene expression analysis of asbestos-induced changes in bronchiolar epithelial cells, pinpointed the genetic profiles of normal and malignant stromal and ductal cells in breast carcinoma, and investigated the differential expression in stretched versus relaxed fibroblasts from mouse skin.

To set up a demonstration of the Arcturus-XT Laser Capture Microdissection system, contact Jan Schwarz at 656-0813, janet.schwarz@uvm.edu.

Itching for ISH

The MIC is now offering RNA *in situ* hybridization analyses using the ***RNAscope*** technology from Advanced Cell Diagnostics. A unique probe design strategy provides signal amplification together with background suppression, resulting in single-molecule localization on a setting of well-preserved tissue morphology. The technique employs formalin-fixed, paraffin-embedded tissue sections, with either a chromogenic dye for bright-field microscopy, or a fluorescent dye for wide-field or confocal microscopy analysis. The unique nucleic acid probe design overcomes the questions of specificity and cross-reactivity often accompanying antibody-based assays.



Please see the other side of this newsletter for a description of recent research results obtained with ***RNAscope*** by Dr. Mark Evans from the Department of Pathology & Laboratory Medicine. If you are interested in learning more about this novel biomarker technique, please contact Nicole Bishop, Nicole Bouffard, or Doug Taatjes.

Microscopy Imaging Center Outreach Activities

During this past academic year, the Microscopy Imaging Center's Outreach efforts encompassed all ages, from preschool to adult, and covered the entire state of Vermont from Brattleboro to Barnet to Grand Isle. Now in our 15th year facilitating Project MICRO, we delivered programs to 5 middle schools, 11 libraries, and the College of Medicine Reunion 2014, reaching over 500 students and more than 80 adults. Jan Schwarz "womanned" the Microscopy Outreach booth at the annual Microscopy & Microanalysis meeting in Hartford, CT and presented a Project MICRO "festival" with conference participants and local teachers.

In addition to working with UVM medical students and FAHC Pathology residents, we were involved in tours and demos for UVM Engineering, Botany, and Medical Laboratory and Radiation Sciences. We also hosted FAHC School of Cytotechnology, Marlboro College, and Johnson State College students, and gave tours to prospective UVM graduate students in the CMB & Neuroscience programs. Students from Champlain Valley Union and Colchester High Schools spent many hours job-shadowing in the lab, and we gave hands-on demos to A.P. Biology classes from Stowe, Mount Mansfield, Colchester, and Harwood Union High Schools.

HAPPY TRAILS TONY!

Our expert AFM technician and all around good guy Tony Quinn retired earlier this year. He is dearly missed. Nicole Bouffard has taken on the duties of AFM analysis, training and troubleshooting.

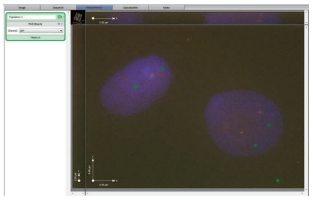
LIKE US ON FACEBOOK!

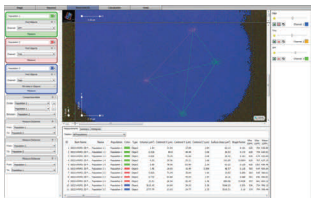
The Microscopy Imaging Center has created a Facebook page. https://www.facebook.com/The-Microscopy-Imaging-Center-786501598145292/ **We invite you to like our page for a chance to win 2 free hours on the scope of your choice.** We will be drawing the lucky winner on December 1st.

Volocity – Analysis of FISH probe distribution by creating a 'Measurement Protocol'

Volocity allows the user to perform 2D and 3D analysis by designing measurement protocols that can be applied to future images. In this example the user is interested in information about the distribution relationships between FISH probes and the shape factor of the nucleus.

To begin designing a measurement protocol for this application first look at a function called '**Find Objects**.' This is a powerful tool which allows the user to indentify 2D or 3D areas of interest and quantify them based on staining intensity, size and volume. To start a protocol for finding objects simply drag a finding task such as '**Find Objects'** to the protocol pane and select the channel of interest. A histogram of intensity values are shown within the dialog box and can be used to threshold for significant intensities and avoid background. The '**Find Objects'** task allows the exclusion of objects below a size threshold by selecting the gear symbol in the upper left corner of the measurement item. The default value in this field will exclude most noise in biological images, but can be adjusted. The result of a **'Find Objects'** task is then referred to as a '**Population**.' The user can now create a new '**Population**' to identify each of the fish probes and the DAPI stain. After the three '**Populations**' (GFP probe, Tritc probe and DAPI stain) are identified by threshold the user can begin to make measurements between them. Next the user needs to consider that only measurements between probes within one nucleus make sense. So, the '**Compartmentalize**' function is used. In '**Compartmentalize**' we will '**Divide**' '**Population 1**' (GFP probe) and '**Population 2**' (Tritc probe) '**Between**' '**Population 3**' (DAPI stain). Now the '**Measure Distances**' function can be used to automatically measure distances between each '**Object**' identified within each '**Population**' and across populations. Simply add a '**Measure Distances**' item and select from '**Population 1**' to '**Population 2**', then add another '**Measure Distances**' item and select from '**Population 1**' to '**Population 1**' and a third item selecting from '**Population 2**' to '**Population 2.'**





A table will appear in the display window with '**All Populations**' selected that gives all of the distance measurements as well as surface area, shape factor and volume information for each '**Object**' within each '**Population**'. To save this protocol to be applied to future images simply go to '**Measurements**', '**Save Protocol**' and assign the protocol a name.

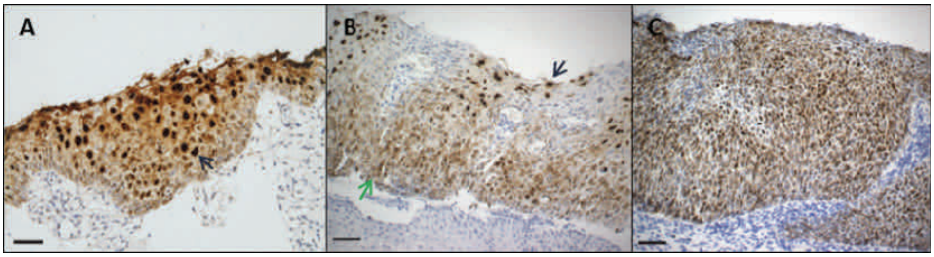
Visualizing RNA Expression by *in situ* Hybridization by Mark Evans PhD

We have been using the RNAscope CISH in two translational applications using formalin-fixed, paraffin-embedded (FFPE) clinical specimens: 1) to investigate human papillomavirus (HPV) *E6/E7* mRNA oncogene expression as a marker of pre-invasive cervical lesion grade (http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0091142) (discussed below) and 2) to examine long noncoding RNA (lncRNA) expression in the progression from breast ductal carcinoma *in situ* (DCIS) to invasive breast carcinoma (IBC).

HPV *E6/E7* mRNA showed qualitatively distinct staining patterns along with lesion grade (Figure). CIN 1 (cervical intraepithelial neoplasia grade 1) demonstrated HPV 'productive' phase staining in which cell nuclei stain diffusely indicative of active proliferation of HPV virions; CIN 2 showed a diminution of productive pattern staining and a shift towards 'transformative' phase staining indicated by fine punctate signals in cell nuclei and cytoplasm; CIN 3 tended to 'transformative' pattern staining only with some lesions also showing sporadic 'productive' pattern staining.

These data highlight the potential of RNA ISH to reveal RNA expression in the histopathologic context as standalone tests or that may be used to complement data from *in vitro* tests. Demonstration of the cellular compartment expressing a target RNA is a particular strength of ISH.

We are now expanding upon our primary findings using the RNAscope CISH expertise available through the MIC. The assay is a one day test and requires use of a 'HybEZ™' hybridization oven system located in the MIC. Custom designed probes and detection kits are purchasable from ACD via the MIC or directly from the company (http://www.acdbio.com/products). The more than 300 publications that have utilized RNAscope ISH (http://www.acdbio.com/science/scientific-resources/publications) illustrates the scope of potential research applications for the assay.



Equipment Available:

- JEOL 1400 TEM
- JEOL JSM 6060 SEM with Oxford INCA EDS system
- Nikon STORM Super Resolution
- Zeiss LSM 510 META Confocal
- Applied BioPhysics ECIS Zθ
- AR MFP-3D BIO™ Atomic Force Microscope
- Arcturus XT-Ti Laser Microdissector
- CompuCyte Laser Scanning Cytometer
- IVIS Whole Animal Imager
- Olympus BX50 Microscope
- Olympus IX70 Inverted Microscope
- Olympus SZX12 Dissecting Microscope
- Leica MZ16F Fluorescence Dissecting Microscope
- Universal Imaging MetaMorph Workstation
- Volocity 3D Software
- MBF Biosciences Stereo Investigator
- RNAscope—HybEZ Oven for ISH

MIC Services Provided:

- Morphologic services and consultation at the light and electron microscopy level
- Morphometry
- Light and electron microscopic immunocytochemistry
- Confocal scanning laser microscopy
- Laser scanning cytometry
- Atomic force microscopy
- Scanning and transmission electron microscopy
- Laser capture microdissection
- Super resolution microscopy
- Preparation of paraffin and frozen sections
- Whole animal imaging
- Electric Cell Substrate Impedance Sensing
- Image analysis and processing
- Training for use of the above equipment
- Special histological staining
- Testing of new antibodies and developing new staining techniques