The Electron Microscopy & Histology Research Core

The Electron Microscopy and Histology Research Core provides instrumentation and technical expertise for the preparation, acquisition and analysis of cell and tissue images obtained by light and electron microscopy. Services are provided for all rodent (mouse, rat, etc.) and human samples. The goal of the Core facility is to assist researchers in elucidating various phenotypes and in gaining mechanistic insights about the biological actions of specific molecules or the toxicity of exogenously administered substances.

Given the cost of such instrumentation and the high level of technical expertise required to perform these techniques, the Electron Microscopy and Histology Research Core was established to ensure the availability of these procedures, namely histopathology, ultrastructural morphology, cellular and subcellular localization, as well as tissue or cell isolation from slide preparations by laser microdissection. The Histology section of the core provides basic services, such as tissue preparation and embedding, paraffin block or frozen tissue sectioning and H&E staining. The Electron Microscopy Section provides transmission and scanning electron microscopy services, including specimen preparation, embedding, thin and ultrathin sectioning and staining (TEM), as well as critical point drying, sputter and/or carbon coating (SEM). The Core also provides additional services such as serial sectioning and special stains (Alcian Blue, Periodic Acid Schiff, Oil-Red-O, Masson's Trichrome). Localization of specific proteins with direct and indirect immuno-histochemistry or immunofluorescence, or with gold-labeled antibodies contributes to the next level of information after preliminary phenotyping. In addition, the Core laboratory provides training opportunities for histology and ultrastructural techniques as well as phenotyping analysis.

The Electron Microscopy and Histology Research Core is located on two sites. The Histology Service, located in the Pavillon de recherche appliquée sur le cancer (PRAC, Z8-2007), is equipped with a dissecting microscope, a Leica MZFLIII dissecting inverted brightfield and fluorescent microscope, a Leica CM3050 cryostat, a Leica DM LB2 brightfield and fluorescent microscope with digital image capture, a Shandon Histocentre 3 embedding center, a Shandon Finesse ME+ paraffin sectioning microtome, a Shandon Citadel 2000 automated tissue processor, a Shandon varistain 24-4 automated slide stainer with a manual staining station for special staining , a MMI Cellcut laser-dissecting scope coupled to a Nikon Eclipse TE2000-S inverted epifluorescent microscope and a Nanozoomer. The Electron Microscopy Service, located on the 9th floor of the Faculté de médecine et des sciences de la santé (FMSS), is equipped with a Cryo-microtome, an ultra-microtome, a critical point dryer, a transmission electron and scanning electron microscopes both provided with digital cameras.

The Electron Microscopy and Histology Core has established a fee for the service facility that is available to all researchers requiring histology and ultrastructural services. The Core provides services to more than 30 research teams at the Université de Sherbrooke and to research teams outside the University.

Services and Fees

- Fixation and Tissue Processing: The primary goal of fixation is to arrest tissue autolysis and to stabilize the morphological structure and the biochemical relationships between constituent proteins for subsequent analysis (e.g. H&E, immunohistochemistry). At the Core, we process tissues fixed in paraformaldehyde. Tissues fixed with other methods will be treated individually for an extra fee. Processing involves three major steps, namely dehydration with ethanol, clearing with xylenes, and infiltration with paraffin.

- Tissue Processing for Ultrastructural Analysis: Tissues are fixed in a 2 to 4% solution of glutaraldehyde buffered with 0.1 M sodium phosphate to preserve the fine structure of biological tissues. The material is then fixed in 1% osmic acid and thoroughly dehydrated in ethanol solutions. After dehydration, tissues are infiltrated with an epoxy-embedding medium.

- Paraffin Embedding: Paraffin embedding offers the best option for long-term preservation of tissue samples. Tissues must be fixed before being embedded in paraffin. Tissues are subsequently cut into ultra-thin slices using a microtome. Paraffin sections provide almost identical serial sections with high quality morphology preservation. However, some epitopes are denatured after fixation and embedding. In addition, paraffin-embedded tissues are not recommended for RNA recovery.

- Frozen Embedding: One of the benefits of frozen tissue samples is that the initial fixation step required for paraffin-embedded tissues is not needed. Fresh tissues are frozen in cryoprotectant embedding media (O.C.T.). Frozen sections more often retain enzyme and antigen functions but morphology preservation is low. Fresh frozen tissues are recommended for optimal RNA recovery. However, manipulation of fresh frozen tissues can be extremely challenging, and RNA purity and yield depend on optimal tissue preparation.

- Microtomy: Thin sections (between 3 um and 4 um) are cut with a microtome on paraffin tissue blocks. These sections are best suitable for H&E staining and other staining procedures, as well as for immunohistochemistry and immunofluorescence.

- Cryo-Microtomy: Sections (between 4 um and 60 um) are cut with a microtome on frozen tissue blocks. Frozen tissue microtomy is performed for a variety of applications, either H&E staining, RNA recovery, immunohistochemistry or immunofluorescence, or confocal microscopy.

- Ultramicrotomy and Cryo-Ultramicrotomy: Ultra-thin sections (between 40 nm and 200 nm) are used for electron microscopy analyses.

- Hematoxylin and Eosin (H&E) Staining and other staining procedures: These first procedures for the phenotypic analysis of cellular components are performed on paraffin sections as well as on frozen sections. We use Shandon Varistain (24-4) Automatic Stainer for H&E and Periodic Acid Schiff staining. Other staining procedures performed manually at the Core are the Alcian Blue, Masson's trichrome, Sirius Red and Oil-Red-O staining and many more. Uranyl-acetate and lead citrate staining is used for electron microscopy analyses.

- **Immuno-Cytolocalization:** This method detects specific cellular or intracellular proteins by an antigen-antibody reaction. Immunohistochemistry and immunofluorescence are the methods of choice in histology. The immunogold technique is used for ultrastructural analysis.

- **Tissue Microdissection**: Microdissection is a method used to isolate individual cells or group of cells from tissue sections, in order to prepare homogeneous cell samples for sensitive and accurate molecular assays. The investigators must bring their own Capsure™ and special eppendorf tubes to obtain and secure microdissected samples for further analyses.

- **Digital whole slide scanning and virtual pathology**: Scan entire slides containing one or multiple tissue sections at high-resolution magnification in brightfield imaging modes with the Nanozoomer. Slide scanning can replace hours of photographing regions using a microscope. Slide scanning is an excellent means to create publication quality snap-shots. The digital images are high-resolution and preserve information from the original glass slide.

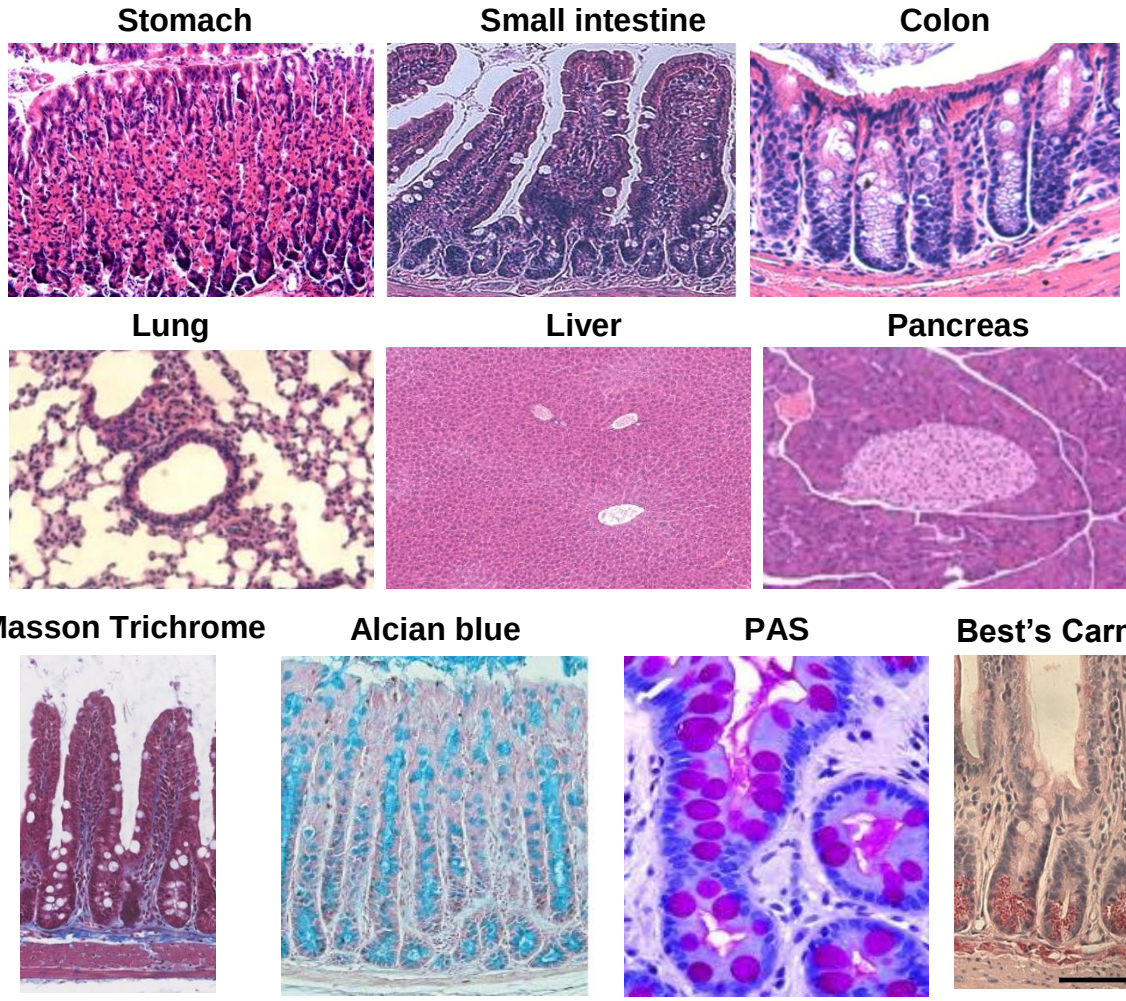
The Platform provides services to members of the Université de Sherbrooke as well as to domestic and foreign academic research institutions. For more information on our corporate rates or to obtain a quote, please contact Mrs. Marilène Paquette (marilene.paquette@usherbrooke.ca)

For information on histology and phenotyping, please contact Prof. Nathalie Perreault (Nathalie.Perreault@USherbrooke.ca).
For information on electron microscopy analysis, please contact Prof. Jean-François Beaulieu (Jean-François.Beaulieu@USherbrooke.ca).
You can also visit our web site at http://www.usherbrooke.ca/dep-anatomie-biologie-cellulaire/recherche/plateformes-facilities/the-electron-microscopy-histology-research-core/

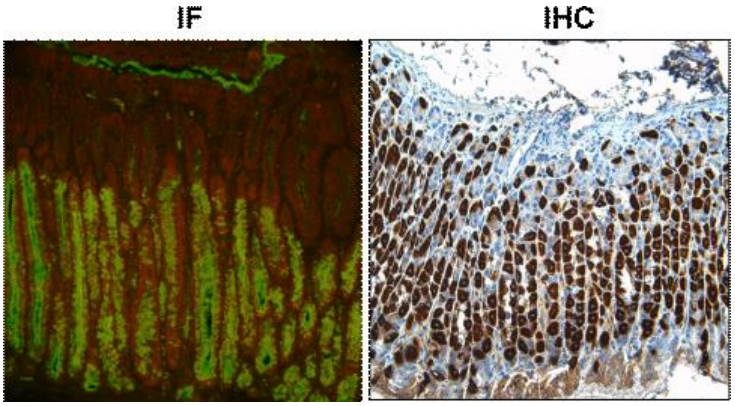
Related Publications

1. Roy SAB, Langlois MJ, Carrier JC, Boudreau F, Rivard N and Perreault N. 2012. Dual regulatory role for Pten in specification of intestinal endocrine cell subtypes. World J of Gastro 18(14): 1579-1589.
2. Maloum F, Allaire JM, Gagné-Sansfaçon J, Roy E, Belleville K, Sarret P, Morisset J, Carrier JC, Mishina Y, Kaestner KH and Perreault N. 2011. Epithelial Bmp signaling is required for proper specification of epithelial cell lineages and gastric endocrine cells. Am J Physiol 300(6): G1065-79.
3. Allaire J, Darsigny M, Marcoux SS, Roy SAB, Schmouth JF, Umans L, Zwijsen A, Boudreau F and Perreault N. 2011. Loss of Smad5 leads to disassembly of the apical junctional complex and increasing susceptibility to experimental colitis. Am J Physiol 300 (4):G586-97.
4. Lussier CR, Brial F, Roy SAB, Langlois MJ, Verdu EF, Rivard N, Perreault N and Boudreau F. 2010. Loss of Hepatocyte-Nuclear-Factor-1α Impacts on adult mouse intestinal epithelial cell growth and cell lineages differentiation. PloS One 24 (8):12 378.
5. Benoit YD, Paré F, Francoeur C, Jean D, Tremblay E, Boudreau F, Escaffit E and Beaulieu JF. 2010. Cooperation between HNF-1alpha, Cdx2, and GATA-4 in initiating an enterocytic differentiation program in a normal human intestinal epithelial progenitor cell line. Am J Physiol 298 (4):G504-17.
6. Lussier CR, Babeu JP, Auclair BA, Perreault N and Boudreau F. 2008. Hepatocyte nuclear factor-4 alpha (HNF-4α) promotes differentiation of intestinal epithelial cells in co-culture system. Am J Physiol 294(2):G418-28.
7. Auclair BA, Benoit YD, Rivard N, Mishina Y and Perreault N. 2007. Bone morphogenetic protein signaling is essential for terminal differentiation of the intestinal secretory cell lineage. Gastroenterology 133: 887-896.
8. Boudreau F, Lussier CR, Mongrain S, Darsigny M, Drouin JL, Doyon G, Ran Suh E, Beaulieu JF, Rivard N and Perreault N. 2007. Loss of cathepsin L activity promotes claudin-1 overexpression and intestinal neoplasia. FASEB J 21(14): 3853-65.
9. Groulx JF, Gagné D, Benoit YD, Martel D, Basora N, Beaulieu JF. 2011. Collagen VI is a basement membrane component that regulates epithelial cell-fibronectin interactions. Matrix Biol. 30(3):195-206.

- Histological staining procedures performed routinely at the Core: H&E, Alcian Blue, Periodic Acid Schiff (PAS) and more.



- Detection of specific cellular or intracellular proteins in tissues or cells by immunohistochemistry (IHC) and immunofluorescence (IF).



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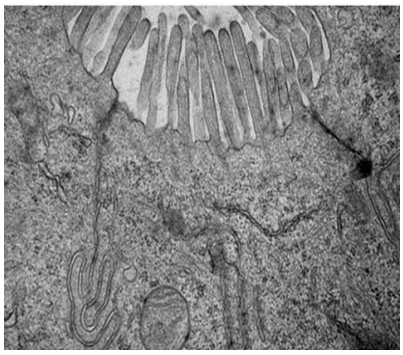
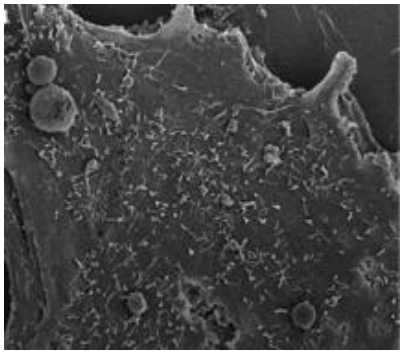
- Ultrastructural analyses of cell components by transmission or scanning electron microscopy.

Trasmission electronic microscopy



Scanning electronic microscopy



Immunogold