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 core facility's goal is to assist researchers in elucidating various phenotypes and in gaining mechanistic insights about the biologic 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 and 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) and 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 at "Faculté de Médecine et des Sciences de la Santé" (FMSS). 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 Leica MZFLIII dissecting inverted brightfield and fluorescent microscope, 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 and a MMI Cellcut laser-dissecting scope coupled to a Nikon Eclipse TE2000-S inverted epifluorescent microscope. The Electron Microscopy service, located on the 9th floor of the FMSS, is equipped with a Cryo-microtome, an ultra-microtome, a critical point dryer, a transmission electron and a scanning electron microscopes both provided with digital cameras.

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.

- **Tissue Preparation for Ultrastructural Immunolocalization:** Light fixation of the tissues followed by fast freezing of embeding in Aradilte or Lowicryl K4M for the preservation of immunoreactivity.

- **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 tissue is not needed. Fresh tissue is 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 will be 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, as well as 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. Uranyl-acetate and lead citrate staining is used for electron microscopy analyses.

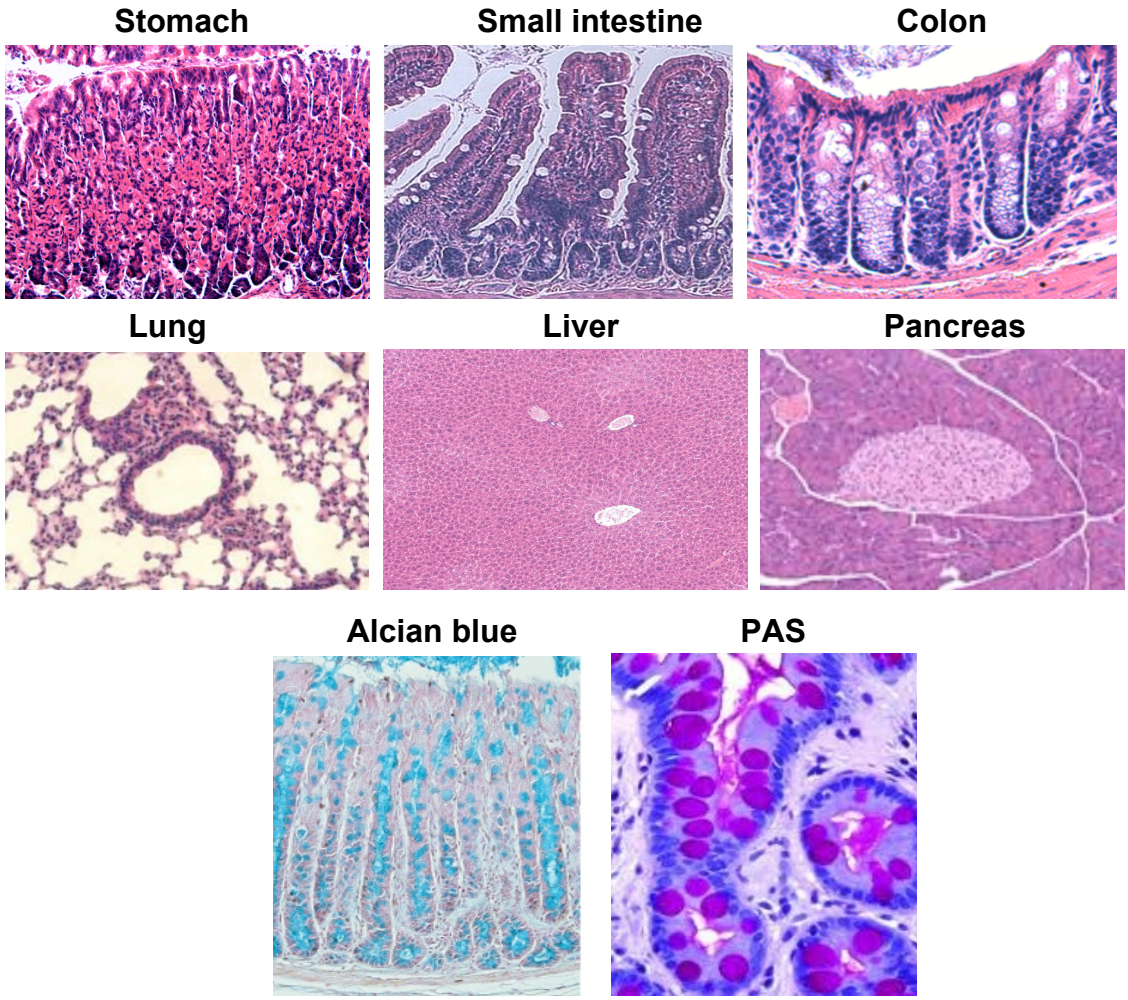
- **Immuno-Cytolocalization:** This method detects specific cellular or intracellular proteins by an antigen-antibody reaction. Immunohistochemistry and immunofluorescence are the methods of choice in histology. The immunogold technique is used for ultrastructural analysis.

- **Tissue Microdissection**: Microdissection is a method 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.

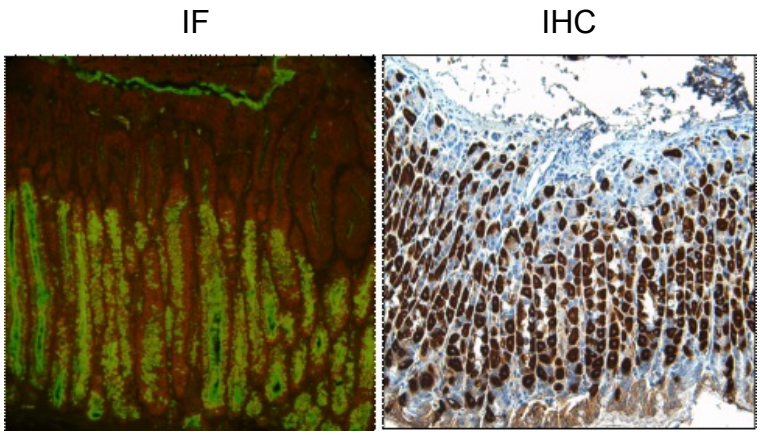
For informations on histology and phenotyping, contact Pr Nathalie Perreault (Nathalie.Perreault@USherbrooke.ca). For informations on electron microscopy analysis, contact Pr Jean-François Beaulieu (Jean-François.Beaulieu@USherbrooke.ca), or our technician Anne Vézina (Anne.Vezina@USherbrooke.ca). You can also visit our web site at www.USherbrooke.ca/dep-anatomie-biologie-cellulaire/recherche/plateforme-danalyses/

Category	Services	Description	Units	Price FMSS (CAD $)	Academic price U de S (CAD $)
Paraffin	Processing one block	Paraffin embedding (less than 5 slides comission per block on a 3 months average)	Tissue block	4,25	4,25
		Paraffin embedding (5 slides or more comission per block on a 3 months average)	Tissue block	2,25	2,25
	Serial sectionning	Paraffin (4-6um) (1 to 150 slides commission on a 3 months average)	Per slide	1,50	1,50
		Paraffin (4-6um) (151 to 375 slides commisison on a 3 months average)	Per slide	1,25	1,25
		Paraffin (4-6um) (over 375 slides commision on a 3 months average)	Per slide	1,00	1,00
	Non-serial sectionning	Paraffin (4-6um) First slide	First slide	5,25	5,25
		Paraffin (4-6um) Additonal slide from same block above	Addtional slide	3,00	3,00
Frozen	Sectioning	1st slide (5 µm)	First slide	4,00	4,00
		Additonal slide from same block above 5 µm	Addtional slide	2,00	2,00
Staining	Periodic Acid Schiff	From pre-existing slide	Per slide	3,75	3,75
	H&E	From pre-existing slide	Per slide	1,50	1,50
	Masson Trichrom	From pre-existing slide	Per slide	5,00	5,00
	Alcian blue	From pre-existing slide	Per slide	2,75	2,75
	Development of new staining	From pre-existing slide	Per slide	TBD	TBD
Electron microscopy	Processing sample	Embedding for EM + thin sectioning + staining	Per sample/ one slide	Supplies cost only	80,00
	Cryo section	Cryo-thin sectioning	3 hours min.	Supplies cost only	40,00/h
	Immunogold	gold-labeled antibodies	Per sample	Supplies cost only	200,00
	Use of microscope		Hour	40,00/h	40,00/h
LCM	Use of LCM	Supplies provide by investigator	Hour	40,00/h	100,00/h

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

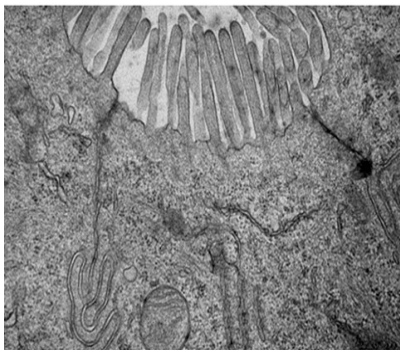


- **Detection of specific cellular or intracellular proteins in tissues or cells by immunohistochemistry (IHC) and immunofluorescence (IF).**

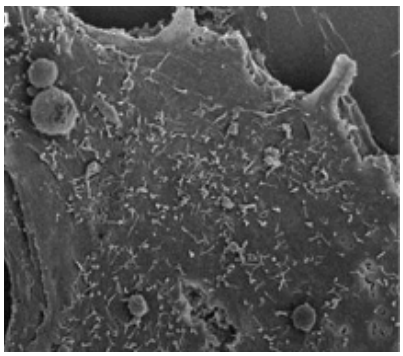


- **Ultrastructural analyses of cell components by transmission or scanning electron microscopy.**

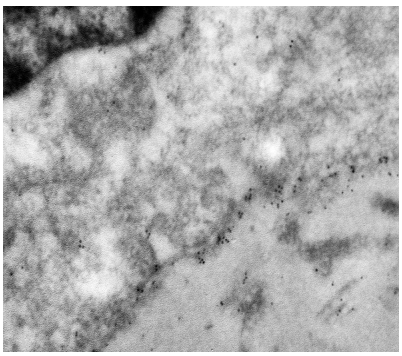
Microscopie électronique à transmission



Microscopie électronique à balayage



Immunolocalisation ultrasctructurale



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.