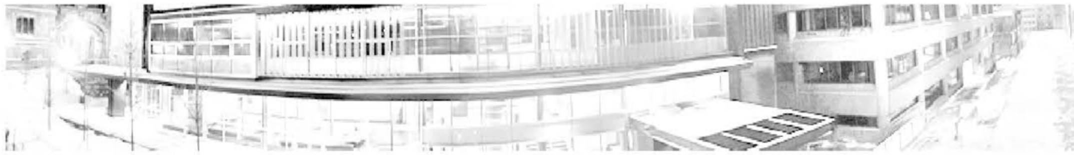
McGill
Faculty of Engineering



Course Outline
BIEN 535

Course Title:	**Electron Microscopy and 3D imaging for biological and soft materials**
Credits:	**3**
Contact Hours:	**3-3-3**
Course Prerequisite(s):	**Instructor's permission. Open to final year undergraduate bioengineering students and graduate students from all departments; capacity 16. Preference will be given to bioengineering students on a first-came-first-served basis. Graduate students from other departments must submit a 100-words statement of interest endorsed by their supervisor.**
Course Corequisite(s):	**none**
Course Description:	**Introduction to electron microscopy and 3D imaging. Dual-beam microscopy (FIB-SEM, or focused ion beam – scanning electron microscope); conventional and cryogenic preparation methods for biological materials. Complementary methods such as X-ray diffraction, X-ray tomography, atom probe tomography. 3D image processing and analysis, and the fundamentals of deep learning in imaging.**

Course Outline

This course is primarily dedicated to dual-beam microscopy (FIB-SEM, or focused ion beam – scanning electron microscope) and scanning electron microscopy (SEM). An emphasis will be placed on preparation methods for biological materials, such as cryo-preparation, or traditional fixation, staining and embedding. Transmission electron microscopy (TEM) will be covered briefly – for an in-depth course on TEM see ANAT 542 Transmission Electron Microscopy (Winter, 3 credits). As well, for in-depth analytical microscopy and materials characterization methods see MIME 317 Analytical and Characterization Techniques (Fall, 3 credits) and MIME 569 Electron Beam Analysis of Materials (Fall, 3 credits).
Several methods that are strictly speaking not electron microscopy methods (X-ray diffraction, X-ray tomography, atom probe tomography), but are valuable and pertinent to imaging and analysis of biological materials, will be presented as special topics throughout the course.

Motivation and Rationale

Biological materials – without exceptions – are characterized by tremendous structural complexity, as they form from the atomic scale up. Moreover, a nearly universal feature of biological materials is their hierarchical structure. This combination of hierarchy and complexity warrants a twopronged approach in their research, pursuing both high resolution and broad context. SEM and FIB-SEM, especially combined with other EM methods, occupy the central position on the landscape of ultrastructural imaging and analysis, as they enable to grasp both the resolution and the context to a sufficient extent. The specimen preparation module of this course primarily addresses the fact that the pristine state of biological and soft materials is the hydrated state. Therefore, various preparation techniques address the issue of compatibility of high vacuum and water.

Finally, one of the most fascinating and dynamic domains today is the domain of computer vision and artificial intelligence applied to imaging. We will cover contemporary approaches to 3D image processing and analysis, including applications of deep learning to imaging.

Short Rationale

This course focuses on 3D electron microscopy-based research methods capable of tackling the resolution/context conundrum of bioimaging. This course also covers fundamental and advanced image processing and deep learning-based 3D image segmentation.

Learning outcomes

The purpose of this course is to equip students with basic knowledge of electron- and ion-beam techniques, primarily scanning electron microscopy (SEM) and dual-beam focused-ion beam SEM (FIB-SEM). From this, students will be enabled to select and apply appropriate methods for research questions, to interpret results, and to understand limitations of the techniques. By the end of the course, students will acquire general literacy regarding the operation of artificial neural nets in the context of image processing, and will be enabled to produce educational videos, and surface meshes of 3D objects, suitable for 3D printing and modeling. Hands-on demo sessions on various microscopes and auxiliary equipment will be given throughout the course, although these sessions will not replace proper personal training.

After the course the students will have learnt to:

• Comprehend and interpret the fundamental principles of interaction of an electron beam with matter (KB)
• Understand appropriate and adequate applications of various EM-based methods (KB, Inv.)
• Apply basic image processing tools to EM data (KB)
• Generate and implement a sound experimental plan combining several complementary methods of imaging and analysis (Inv., PA)
• Identify, understand and avoid inherent artifacts of EM-based imaging and analysis, as well as specimen preparation and image processing routines (KB, PA & LL)
• Optimize image acquisition and analysis parameters using appropriate hardware and software (PA & Inv.)
• Communicate results of an imaging-based study and conduct a scholarly dialogue (Comm., Prof.)

Canadian Engineering Accreditation Board Curriculum Content

CEAB curriculum category	Number of AUs	Description
Mathematics	0	Mathematics include appropriate elements of linear algebra, differential and integral calculus, differential equations,

		probability, statistics, numerical analysis, and discrete mathematics.
Natural science	58.5	Natural science includes elements of physics and chemistry, as well as life sciences and earth sciences. The subjects are intended to impart an understanding of natural phenomena and relationships through the use of analytical and/or experimental techniques.
Complementary studies	0	Complementary studies include the following areas of study to complement the technical content of the curriculum: engineering economics; the impact of technology on society; subject matter that deals with central issues, methodologies, and thought processes of the arts, humanities and social sciences; management; oral and written communications; healthy and safety; professional ethics, equity and law; and sustainable development and environmental stewardship.
Engineering science	0	Engineering science involves the application of mathematics and natural science to practical problems. They may involve the development of mathematical or numerical techniques, modeling, simulation, and experimental procedures. Such subjects include, among others, applied aspects of strength of materials, fluid mechanics, thermodynamics, electrical and electronic circuits, soil mechanics, automatic control, aerodynamics, transport phenomena, elements of materials science, geoscience, computer science, and environmental science.
Engineering design	0	Engineering design integrates mathematics, natural sciences, engineering sciences, and complementary studies in order to develop elements, systems, and processes to meet specific needs. It is a creative, iterative, and open-ended process, subject to constraints which may be governed by standards or legislation to varying degrees depending upon the discipline. These constraints may also relate to economic, health, safety, environmental, societal or other interdisciplinary factors.

Graduate attributes

This course contributes to the acquisition of graduate attributes as follows:

GA	KB	PA	IN	DE	ET	IT	CS	PR	IE	EE	EP	LL
level	A	A	D	n/a	n/a	n/a	D	n/a	n/a	n/a	n/a	I

n/a = not applicable; I = introduced; D = developed; A = applied

Evaluation and grading

Quizzes (KB, LL): After each of four major modules (EM Basics, Digital Imaging, Sample Preparation, FIB-SEM) students will receive an in-class quiz of 5 multiple choice question. Quiz solving will be timed (7 minutes). Maximal score per quiz is 5 (one point per question), and the maximal total score is 20 points.

Creative project – 3D image processing (KB, PA, IN, CS): Students will be provided access to cloud-based virtual graphic computers with the Dragonfly software for multiscale image analysis. Each student will be allocated 20 hours of cloud instance access time and a Dragonfly license for the duration of the course (January-March) for their autonomous work. A library of 3D images will be provided for the imaging project, in which students will characterize an object of their choice and compose a graphic and/or multimedia-based report. The library will include 3D images of the following objects for students' selection: snail shell, sea urchin spine, mouse mandible, mouse skeleton, human tooth, bay leaf, chick in an egg, dog-day cicada, ant, and others. These free-form projects will be assessed by the instructor and TA based on logic, wealth of analytical approaches, and aesthetics (10 points on each axis). Maximal score is 30, and selected authors will be invited to present.

Final oral presentation of a selected paper (PA, CS, LL)
During two last weeks, each student will deliver a short oral presentation and analysis of any paper from the course's reading list, or any paper of their own choice that utilizes electron microscopy, or any related 3D imaging technique. Each presentation will be graded along 3 axes, i) content; ii) flow and logic; iii) quality of delivery and layout. Maximal score along each axis is 10 points, maximal total score is 30. Class will evaluate their peers and will submit to TA evaluation matrices for each presenter, including mandatory verbal comments.

Critique (PA, CS, LL)
Each student will also critique peer presentations. This is to demonstrate understanding of the paper, professionalism and courtesy. Presenters and referees will be paired up by the instructor. Each referee will receive score along the axes i) substance; ii) logic; iii) delivery. Maximal grade along each axis is 6, maximal total score is 18 (peer grading using evaluation matrices with mandatory verbal comments).

Attendance
Two extra points will be given to those who attend 100% of lectures and tutorials either in person or virtually, so that the total attainable score is 100 for the entire course.

Course Outline (zoom link for lectures)

Date	Module	Lecture topic	Instructor	Microscopy tutorial 2 hours	Imaging tutorial 1 hour	Notes
05.01	I. EM Basics	Introduction. History and purpose of microscopy	Reznikov	Housekeeping	-	Start reading from the list
10.01		Optics. Intro to TEM	Reznikov	Optics tutorial	Intro to Dragonfly (cloud)	Select object for imaging project
12.01		Intro to SEM	Reznikov			
17.01		SEM (continued)	Reznikov	Facility visit 1	Image transforms (cloud)	
19.01		Low voltage SEM. Scanning transmission EM (STEM)	Guest Prof Gauvin, McGill			
24.01	II. Digital Imaging	Introduction to digital imaging	Reznikov	Facility visit 2	Image filters	EM basics quiz on 24.01

Date	Module	Lecture topic	Instructor	Microscopy tutorial 2 hours	Imaging tutorial 1 hour	Notes
26.01		Deep learning	Reznikov		(cloud)	
31.01		Advanced image processing 1	Reznikov	Facility visit 3	ROI (cloud)	
02.02		Advanced image processing 2	Reznikov			
07.02	III. Sample Prep	Classic specimen preparation	Reznikov	Facility visit 4	Multi-ROI (cloud)	Digital Imaging quiz on 07.02
09.02		Water	Reznikov			
14.02		Cryogenic specimen preparation	Reznikov	Q&A with TA, spillover topics	Linear classifier (cloud)	Presenters and opponents paired
16.02		Cryo-EM tomo, SPA, CLEM	Guest Prof Huy Bui, McGill			
21.02	IV. FIB-SEM	FIB-SEM 1. Intro	Reznikov	Facility visit 5	Destructive tomography of red cabbage	Sample prep quiz on 21.02
23.02		FIB-SEM 2. Lamella lift out	Reznikov			
Spring break						
07.03		FIB-SEM 3. Slice & View tomography	Reznikov	Facility visit 6	Deep learning 1 (cloud)	
09.03	V. Guest lectures and other methods	X-ray tomography	Reznikov			FIB quiz on 09.03
14.03		Introduction to crystallography	Reznikov	Facility visit 7	Miller indices	Submit selected presentation topic
16.03		Protein purification, crystallization, and structure analysis	Guest Prof Zeytuni, McGill			
21.03		Ultrastructural methods for protein localization	Guest Prof McKee, McGill	Facility visit 8	Deep learning 2 (cloud)	Quiz results released
23.03		In situ imaging: liquid cell TEM, atmospheric SEM	Guest Prof Kröger, U of York (UK)			
28.03		Analytical electron microscopy, EELS, EDX	Guest Prof Lagos, McMaster U (Canada)	Facility visit 9	Video making (cloud)	Deadline for creative project submission
30.03		Atom probe tomography	Guest Prof Joester,			

Date	Module	Lecture topic	Instructor	Microscopy tutorial 2 hours	Imaging tutorial 1 hour	Notes
			Northwestern U (USA)			
04.04	VI. Students present	Presentations and critique			3D mapping (cloud)	Evaluation matrices due
06.04						
11.04		Creative project show				

For the core facility-based tutorials, the class will be divided into 4 permanent groups

Week of	Jan 16	Jan 23	Jan 30	Feb 6	Feb 20	March 6	March13	March20	March27
Group 1	µCT	TEM Spirit	XRD	FIB-SEM Ethos	FS or embed	µ-tome	FIB-SEM Helios	HPF	EDX SU8000
		CPD + sputter	SEM Quanta		HRTEM Titan	STEM Talos		FLM-CLEM	VP-SEM SU3500
Group 2	µ-tome	µCT	FS or embed	XRD	FIB-SEM Ethos	STEM Talos	VP-SEM SU3500	FIB-SEM Helios	HPF
	TEM Spirit		CPD + sputter	SEM Quanta		HRTEM Titan	EDX SU8000		FLM-CLEM
Group 3	FS or embed	µ-tome	µCT	HRTEM Titan	XRD	FIB-SEM Ethos	EDX SU8000	FLM-CLEM	FIB-SEM Helios
	STEM Talos	TEM Spirit		CPD + sputter	SEM Quanta		VP-SEM SU3500	HPF	
Group 4	TEM Spirit	HRTEM Titan	FIB-SEM Helios	µCT	µ-tome	XRD	FIB-SEM Ethos	FS or embed	VP-SEM SU3500
	CPD + sputter	HPF			FLM-CLEM	SEM Quanta		STEM Talos	EDX SU8000

Tutorials may be shifted based on the availabilities of equipment and operators (e.g., due to maintenance). Tutorials missed for any reason will not be compensated or rescheduled. Students are encouraged to enter the sequence of the tutorials into calendar. Wearing indoor shoes during facility visits is compulsory. Storage space for bags and coats will be organized. It is responsibility of the group to arrive on time for tutorials (5 minutes earlier than scheduled time), to the locations indicated below:

Facility for Electron Microscopy Research (FEMR) https://www.mcgill.ca/femr/

TEM Spirit:
Transmission Electron Microscope FEI Tecnai G2 Spirit Twin, 120 kV
Room B29, Strathcona Anatomy & Dentistry Building
Bright- and dark-field imaging of thin plastic-embedded sections of cells, selected-area electron diffraction

STEM Talos:
Scanning Transmission Electron microscope Thermo Scientific Talos F200X G2, 200kV
Room B30, Strathcona Anatomy & Dentistry Building
Bright- and dark-field imaging of thin plastic-embedded sections of incipient mineralization in bone, selected-area electron diffraction, EDX elemental mapping, STEM tomography

HRTEM Titan:

Aberration-corrected high-resolution cryo-(scanning) transmission electron microscope FEI Titan Krios 300 kV
Room B32, Strathcona Anatomy & Dentistry Building
Cryo-electron tomography, single protein analysis – watching of ongoing operation. Tutorial is preceded by plunge-freezing of protein suspension using FEI Vitrobot grid plunging system

FIB-SEM Helios:
Focused Ion Beam Scanning Electron Microscope FEI Helios Nanolab 660 DualBeam
Room B27, Strathcona Anatomy & Dentistry Building
FIB milling, FIB nanofabrication, FIB-SEM Slice & View 3D tomographic imaging

FIB-SEM Ethos:
Focused Ion Beam Scanning Electron Microscope Hitachi High-Technologies Ethos NX5000
Room1280, WH Wong Building
FIB milling, FIB nanofabrication, FIB-SEM Slice & View 3D tomographic imaging

SEM Quanta:
FEI Quanta 450 Environmental Scanning Electron Microscope
Room 0360, WH Wong Building
Secondary electron imaging of a critical point dried insect. Tutorial is paired with (preceded by) critical point drying, stub mounting and sputter-coating with platinum of the specimen

FLM-CLEM:
FEI CorrSight Fully Automated Correlative Fluorescence Light Microscope System
Strathcona Anatomy & Dentistry Building
Identification of rare and transient events in a wet specimen for subsequent vitrification and electron microscopy, correlative microscopy

FEMR specimen preparation suite (meeting point: Eastern entrance to Strathcona, basement)

CPD:
Leica Microsystems EM CPD030 Critical Point Dryer

Microtome:
Reichert Ultracut E Ultramicrotome

Sputter coater:
Leica Microsystems EM ACE600 High-Resolution Sputter Coater with room temperature stage

HPF:
Leica Microsystems EMPACT2 High-Pressure Freezer System, or Leica Microsystems ICE High-Pressure Freezer System (coming soon, this summer)

FS or embed:
Freeze substitution Leica Microsystems EM AFS2 Automatic Freeze Substitution System, or infiltration and resin embedding of a dehydrated sample depending on availability and ongoing work

Materials Characterization https://www.mcgill.ca/materials/research/shared-facilities

XRD:
Powder X-ray diffraction, Bruker D8 Discovery X-Ray Diffractometer (Cobalt source)
Room 1280, WH Wong Building

Crystallographic analysis of calcite $CaCO_3$ using sea urchin spine (single crystal) and avian eggshell (polycrystalline), texture analysis, identification of impurities

VP-SEM SU3500:
Variable Pressure – Scanning Electron Microscope Hitachi SU-3500
Room 0230, WH Wong Building
Secondary and backscattered electron imaging of a sea urchin spine, EDX analysis of elemental composition

EDX SU8000:
Cold Field Emission Gun Scanning Electron Microscope Hitachi SU-8000
Room 0320 WH Wong Building
Secondary and backscattered electron imaging of paper and elemental analysis of typographic inks

Integrated Quantitative Biology Initiative IQBI https://www.mcgill.ca/iqbi/

µCT:
Micro-computed Tomography Zeiss Xradia Versa
Room N3-15, Stewart Biology Building
Non-destructive X-ray tomographic imaging

Classroom-based tutorials (2hr) will take place in the allocated classroom during the weeks of Jan 2, Jan 9, Feb 13 and Apr 3, with no peculiar requirements. For the **1-hr image analysis tutorials**, except for those during the week February 20 and March 13, students must bring laptops with input devices (mouse or digital pad). It is possible to attend **cloud-based image analysis tutorials** remotely and access virtual instance from a desk-top computer. However, attending in person is encouraged. For the **creative imaging project**, virtual instance log in credentials will be provided for access at any time, up to 20 hours during the course. The format of the creative project is unrestricted. Example can be found here and here.

Detailed syllabus

Module I: EM basics

Week 1, January 5th

Lecture 1. Scale of objects, and human perception. History of microscopy. Questions for microscopy. Interpretation of data.

Papers from the list (Bader, Kolb et al. 2018, Crameri, Shephard et al. 2020)
Following this lecture, students are encouraged to start studying the reading list.

https://www.fei.com/introduction-to-electron-microscopy/types/#gsc.tab=0

Instead of tutorials, we will hold a housekeeping session where the principles of evaluation and assignments will be explained, as well as the rules of facility visits, facility visits calendar, facility locations, digital image library contents, creative project assessment criteria, rules for using virtual instances, and other subjects.

Week 2

Lecture 2, January 10th. Basics of ray optics. Cardinal points. Aperture. Wavelength. Focus. Intro to wave optics. Light versus electrons. Lenses. Electron source. Electron properties. Types of electron microscopes. Aberrations.
Papers from the list (Carlson and Evans 2012).
Refresher on ray optics: https://courses.lumenlearning.com/physics/chapter/introduction-10/
Refresher on wave optics: https://courses.lumenlearning.com/physics/chapter/introduction-8/

Lecture 3, January 12th. Column. Electron sources. Magnetic lenses. Detectors. Vacuum systems. Transmission electron microscopy at a glance. Contrast transfer function. Intro to scanning electron microscopy. Electron microscopy in the Life Sciences.
Papers from the list (Schatten 2011)

Webinars to watch
Grant Jensen's online EM course vids 4-13 (~130 min. total, great stuff), starting from this one: https://www.youtube.com/watch?v=Z2U0pcNk1jo&list=PL8_xPU5epJdctoHdQjpfHmd_z9WvGxK8-&index=4
Tonya Coffey SEM intro: https://www.youtube.com/watch?v=d7ch1XSmOgI
How to make your own SEM: https://www.youtube.com/watch?v=VdjYVF4a6iU

Instrument tutorial 1, January Xth. Recap of ray and wave optics, observation of light diffraction pattern using a laser and a banknote, assembly of camera obscura.

Imaging tutorial 1, January Xth. Cloud-based. Introduction to the Dragonfly software. Image formats, import and export, orthogonal and 3D views, regions of interest, histogram, look-up-tables, shapes and structural grids

Week 3

Lecture 4, January 17th. Scanning electron microscopy SEM, continued. Interaction of electrons with matter. SEM column. Focusing. Image formation. Spot size. Aberrations. Astigmatism. Image quality. Working distance. Detectors. Acquisition parameters.

Papers from the list (Joy and Joy 1996)

Lecture 5, January 19th. *With Raynald Gauvin, special topic*. Low voltage SEM. Scanning Transmission Electron Microscopy (STEM). Microanalysis of materials. Simulation of interaction of electrons with matter.

Papers from the list (Brodush, Trudeau et al. 2012, Golozar, Hovington et al. 2018)

Instrument tutorial 2, January Xth. Facility visit 1 in rotating groups

Imaging tutorial 2, January Xth. *Cloud-based*. Image alignment, stitching, superimposition, transformation

Module II: Digital Imaging

Week 4

Lecture 6, January 24th Introduction to digital image processing and analysis. Pixel. Histogram. Channels. Look-up tables. Filters. Fast Fourier transform. Image calculus. Syntax and semantics in image analysis. Image patterns. Image segmentation.

Papers from the list (Prum, Torres et al. 1999, Reznikov, Almany-Magal et al. 2013)

Lecture 7, January 26th. Deep learning for image segmentation. Convolutional neural networks. Convolution and pooling. Encoding and decoding. Loss functions. Back-propagation. Hyperparameters. Transfer of learning.

Webinars to watch
Stanford lecture 5 Conv Nets: https://www.youtube.com/watch?v=bNb2fEVKeEo&t=2471s
Stanford lecture 6 training Conv Nets: https://www.youtube.com/watch?v=wEoyxE0GP2M

Instrument tutorial 3, January Xth. Facility visit 2 in rotating groups

Imaging tutorial 3, January Xth. *Cloud-based*. Image filters, Fourier transform

Week 5

Lecture 8, January 31st. Advanced image processing and analysis 1. 3D mapping approaches. Anisotropy mapping. Skeletonization and graph analysis.

Papers from the list (Zeiler and Fergus 2014, LeCun, Bengio et al. 2015, Cheng, Cardone et al. 2019, Pashaei, Kamangir et al. 2020, Reznikov, Buss et al. 2020)

Lecture 9, February 1st. Advanced image processing and analysis 2. Meshing. Watershed transform, digital object separation. Hyperdimensional data. Case studies.

Instrument tutorial 4, January Xth. Facility visit 3 in rotating groups

Imaging tutorial 4, January Xth. *Cloud-based*. Image segmentation, regions of interest, morphological operators, Boolean operations, meshing

Module III: Specimen Preparation

Week 6

Lecture 10, February 7th. Classic specimen preparation for electron microscopy. Peculiarities of biological samples. Fixation. Staining. Dehydration. Embedding. Sectioning. Critical point drying. Freeze-drying. Mounting. Sputter-coating. Microwave-assisted preparation.

Papers from the list (Wischnitzer , Chissoe, Vezey et al. 1995, Chicoine and Webster 1998)

Lecture 11, February 9th. Water. Hydrogen bonds. Anomalous properties of water. Surface water. Bulk water. Phase diagram of water. Ice. Hydration of ions. Hydration of polysaccharides. Hydration of proteins. Folding and denaturation. Hydration of DNA. Colloids. Hydration of membranes. Hydrophobic hydration. Intracellular water. Exclusion zone water. Water as a prestress agent in biological materials.

Papers from the list (Pollack 2001, Wang, Von Euw et al. 2013, Huss, Fratzl et al. 2019)

Martin Chaplin's water portal:
https://water.lsbu.ac.uk/water/?_ga=2.76435029.1588563357.1640042296-863327536.1640042296

Webinars to watch
Nobel Prize Jacques Dubochet:
https://www.nobelprize.org/prizes/chemistry/2017/dubochet/lecture/
RMS Joaquim Frank: https://www.rms.org.uk/rms-event-calendar/2021-events/imfls-professor-joachim-frank.html
Lars Pettersson on water and life:
https://www.youtube.com/watch?app=desktop&v=1ijHgccuhLE&list=PLnqQJI0EhuwwdoH18CnKcOC6j4qaU_yXI
Martin Chaplin on oddities and anomalies of water, short video:
https://www.youtube.com/watch?v=XnT6SZd1toE

Instrument tutorial 5, February X^th^. Facility visit 4 in rotating groups

Imaging tutorial 5, February X^th^. *Cloud-based*. Multiple regions of interest, object connectivity, morphometrics, watershed transform

Week 7

Lecture 12, February 14^th^. Cryogenic specimen preparation. Vacuum and biology. Dehydration. Freezing. Glass transition. Cryoprotectants. Vitrification. Heat transfer. No-man's land. High-pressure freezing. Cryo-sectioning. Freeze-substitution.

Lecture 13, February 16^th^. *With Khanh Huy Bui, special topic.* Cryo-TEM, cryo-electron tomography, subtomogram averaging and single particle analysis. Correlative light-electron microscopy

Papers from the list (Callaway 2015, Cianfrocco and Kellogg 2020)

Webinars to watch:
Eva Nogales on single particles: https://www.ibiology.org/techniques/transmission-electron-microscopy/
MRC Richard Henderson:
https://www2.mrc-lmb.cam.ac.uk/research/scientific-training/electron-microscopy/

Instrument tutorial 6, February X^th^. Q&A with TA, spillover topics

Imaging tutorial 6, February X^th^. *Cloud-based*. "Shallow learning" and linear classifier, random forest and segmentation wizard

Module IV: FIB-SEM

Week 8

Lecture 14, February 21^st^. FIB-SEM part 1. Components of the dual beam microscope. Ion source. Interaction of ions with matter. Ion column. FIB aberrations. Sputtering. Milling. Patterning. Sectioning.

Lecture 15, February 23rd. FIB-SEM part 2. Specimen preparation for (S)TEM and Atom Probe Tomography (APT). In situ and lift-out. Room temperature and cryogenic temperature operation

Webinars to watch:

FIB intro Honghui Zhao: https://www.youtube.com/watch?v=X8aw6k4bdhg
FIB-SEM for ToF-SIMS application: https://www.youtube.com/watch?v=TsUH4uHOhAg

Instrument tutorial 7, February Xth. Facility visit 5 in rotating groups

Imaging tutorial 7, February Xth. In class. Destructive tomography of red cabbage. Deep learning for image segmentation I

Spring break

Week 9

Lecture 16, March 7th. FIB-SEM part 3. Slice&View tomography. Acquisition parameters. Slice geometry. Correlative imaging.

Papers from the list (McIntosh 2005, Li 2006, Drobne, Milani et al. 2007, Desbois, Urai et al. 2008, Leser, Drobne et al. 2009, Hurbain and Sachse 2011, Rigort 2012, Young 2014, Narayan and Subramaniam 2015, Vidavsky, Akiva et al. 2016, Rykaczewski, Howell et al. 2017, Xu, Hayworth et al. 2017, Suzuki, Kubota et al. 2019, Weiner and Enninga 2019)

Module V: Complementary methods and guest lectures

Lecture 17, March 9th. X-ray tomography. Sources of X-rays. Principles of image formation and reconstruction. Attenuation contrast and phase contrast.

Papers from the list (Guldberg, Lin et al. 2004, Friedman, Nguyen et al. 2012, Liebi 2015, Beliaev, Zoellner et al. 2020)

Webinar to watch
https://www.nobelprize.org/prizes/medicine/1979/hounsfield/lecture/

Instrument tutorial 8, March Xth. Facility visit 6 in rotating groups

Imaging tutorial 8, March Xth. Cloud-based. Deep learning for image segmentation I

Week 10

Lecture 18, March 14th. Diffraction. Crystals and minerals. Symmetry. Unit cell. Miller indices. X-ray diffraction. Electron diffraction.

Papers from the list (Bacon 1990, Britton, Jiang et al. 2016, Combes, Cazalbou et al. 2016)
https://www.nobelprize.org/uploads/2018/06/wl-bragg-lecture.pdf
https://www.nobelprize.org/uploads/2018/06/klug-lecture.pdf
Lecture 19, March 16th. *With Natalie Zeytuni, special topic*. Protein purification and crystallization. Applications of X-ray crystallography in structural biology.

Webinar to watch

Sir Peter Hirsch (RMS):
https://www.rms.org.uk/rms-event-calendar/2021-events/imfls-professor-sir-peter-hirsch.html
Dan Shechtman: https://www.nobelprize.org/prizes/chemistry/2011/shechtman/lecture/

Instrument tutorial 9, March Xth. Facility visit 7 in rotating groups

Imaging tutorial 9, March Xth. Class-based. Miller indices and crystallographic orientations

Week 11

Lecture 20, March 21st. *With Marc McKee, special topic.* The history of electron microscopy at McGill, and ultrastructural methods for protein localization (radioautography and immunogold labeling).

Papers from the list:(Sodek, Ganss et al. 2000, Hoffmann, Shtengel et al. 2020, Heinrich, Bennett et al. 2021, Xu, Pang et al. 2021)

Lecture 21, March 23rd. *With Roland Kroger, special topic on zoom.* In situ electron microscopy. Liquid cell TEM, atmospheric SEM. Observation of dynamic processes. Crystal nucleation

Papers from the list (Verch, Morrison et al. 2013, Ross 2015, Kroeger and Verch 2018)

Webinar to watch
UPenn doctoral defense, liquid cell: https://www.youtube.com/watch?v=ZWhWy0xpmjs

Instrument tutorial 10, March Xth. Facility visit 8 in rotating groups

Imaging tutorial 10, March Xth. Cloud-based. Deep learning for image segmentation II

Week 12

Lecture 22, March 28th. *With Maureen Lagos, special topic on zoom.* Analytical electron microscopy. EELS, EDX

Papers from the list (Gordon 2011, Krivanek, Lovejoy et al. 2014, Lagos, Truegler et al. 2017, Dubosq, Gault et al. 2020)

Lecture 23, March 30th. *With Derk Joester, special topic on zoom*. Atom probe tomography (APT). Ion flight. Reconstruction. System layout. Specimen preparation. Case studies.

Papers from the list (Gordon 2012, DeRocher, Smeets et al. 2020)
Webinars to watch:
RMS Ray Egerton: https://www.rms.org.uk/rms-event-calendar/2021-events/imfls-professor-ray-egerton.html
Cameca collection of videos: https://www.atomprobe.com/keyaptlinks/videos
McMaster CCEM intro to APT:
https://www.youtube.com/watch?app=desktop&v=eR2323Evz3U

Instrument tutorial 11, March Xth. Facility visit 9 in rotating groups

Imaging tutorial 11, March Xth. Cloud-based. Video making

Module VI: Student presentations

Week 13

Seminar 24 April 4th . Student presentations

Seminar 25 April 6th . Student presentations

Instead of Instrument tutorial 12, April Xth. Student presentations

Imaging tutorial 12, April Xth. Cloud-based. Image analysis using 3D mapping methods, thickness and anisotropy, skeletonization and graph analysis

Week 14

Seminar 26 April 11th. Creative project selected presentations

Instead of Imaging tutorial 12, April Xth Creative project selected presentations

Reading list

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Drobne, D., M. Milani, V. Leser and F. Tatti (2007). "Surface damage induced by FIB milling and imaging of biological samples is controllable." Micros Res Tech **70**: 895-903.

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