# bulletin

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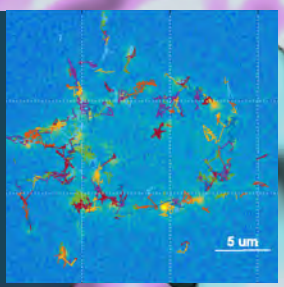


MSC SMC

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The Society welcomes to membership any individual or organization interested in the Microscopical Sciences. Memberships (Ordinary, Student, Corporate) are initiated and renewed as of January 1st annually. Applications received prior to November 1st will be credited to the current year, and back issues of the current year will be supplied if available. Correspondence concerning the Society should be directed to: Line Mongeon, McGill University, 3775 University Street, Montréal, QC (Canada), H3B 2B4; Tel: 514 398-7354; line.mongeon@mcgill.ca.

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**About the cover:** *Helicobacter pylori* are bacteria that colonize the stomach of approximately 50% of human beings. The vacuolating cytotoxin A (VacA) is a major virulence factor secreted by the bacteria that is associated with the development gastric cancer. The VacA structure consists of a dodecamer of individual subunits called p88. Each map seen here illustrates a different conformational state observed by cryoEM, thus providing the ability to visualize the heterogeneity observed in the toxin's structure. The underlying heterogeneity may play an important role in governing the many functions of VacA. Scale bar represents 50 Å. The micrograph represents an image taken of VacA that has been isolated from H. pylori cultures, purified, and frozen in a thin layer of vitreous ice. Individual VacA oligomer, with a total diameter of 280 Å, can be observed in the ice in orientations ranging from side, top, and oblique views.

**Cover insert:** S. Makaremi et al. Tracks found for TLR2 receptors on the membrane of a macrophage in "Investigating diffusion receptors on macrophage membranes using single molecule tracking" *60th Annual meeting of the Biophysical Society*. Los Angeles 2016.


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## NOTE DE L'ÉDITEUR

*Chers lecteurs,*

*Après pratiquement un an de mutisme, je suis fier de vous présenter la plus récente édition du Bulletin de la Société de microscopie du Canada (SMC) qui a repris de plus belle avec plusieurs nouvelles contributions. Nous comptons tous que cette recrudescence dans la participation à la rédaction d'articles puisse se maintenir pour permettre au Bulletin de combler le rôle pour lequel il a été créé, c'est-à-dire celui de diffusion de nouvelles, d'activités et de travaux de recherche de la communauté de la SMC.*

*Dans ce numéro du Bulletin, nous publions des résumés des lauréats des prix de la Fondation canadienne pour le développement de la microscopie. Ces bourses de 1000$ sont offertes pour soutenir les étudiants qui présenteront leur projet de recherche lors d'une conférence nationale ou internationale. Cette année, quatre étudiants ont reçu des bourses de déplacement. Il s'agit de Dheva Setiaputra de l'Université de la Colombie Britannique ainsi que de Sagar Prabhudev, Sara Makaremi et Steffi Woo, de l'Université McMaster. Le résumé de la conférence des quatre gagnants est reproduit dans les pages du Bulletin et témoigne de la qualité du travail effectué par les étudiants du MSC.*

*Par ailleurs, nous présentons le premier d'une série de quatre articles sur la cathodoluminescence de Sandra Gibson, étudiante qui a travaillé avec Micheal Robertson (Université Acadia) et qui est boursière post-doctorale à l'Université de Waterloo. Aussi dans cette édition, Harry Leung (Children's Hospital Boston, Harvard) partage ses trucs et astuces nouvellement acquis et des conseils sur la façon d'effectuer de la microscopie « super-résolution » avec des équipements de base.*

**Nadi Braidy**
Éditeur du Bulletin de la SMC

Dear readers,

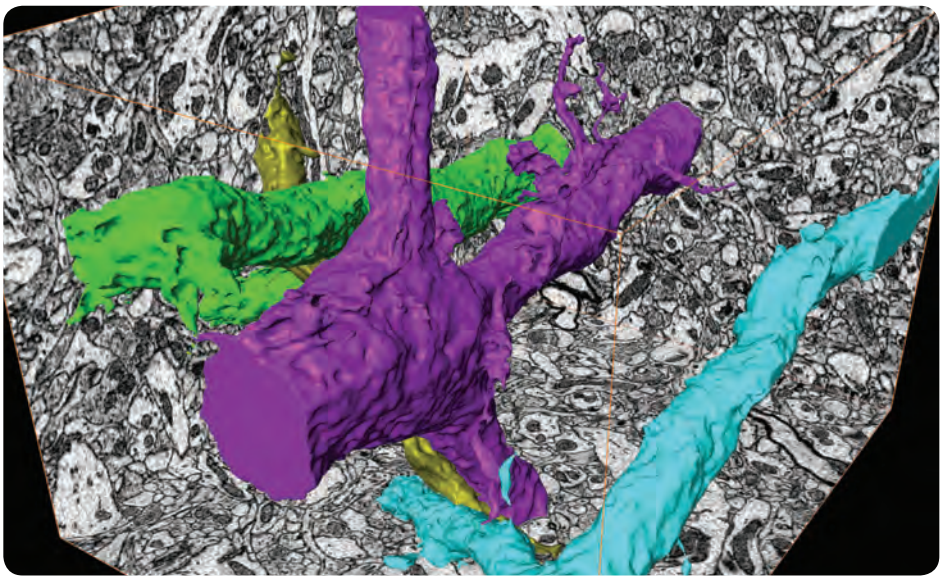
After almost a year, I am proud to present the most recent edition of the Bulletin of the Microscopical Society of Canada (MSC) which has resumed from its slumber with many new contributions. We are all expecting that this recent increase in involvement will continue to allow the Bulletin to fill the role for which it was created, that is to say, the diffusion of news, activities and research activities of the MSC community.

In this issue of the Bulletin, we publish summaries of the winners of the Canadian Foundation for the Development of Microscopy awards. This $1000 bursary is offered to support students who will present their research project at a national or international conference. This year, four students were awarded travel bursaries. These were Dheva Setiaputra from the University of British Columbia, and Sagar Prabhudev, Sara Makaremi and Steffi Woo from McMaster University. The conference summary of the four winners are reproduced in the pages of the Bulletin and testifies to the quality of the work carried out by the students of the MSC.

In addition, we present the first in a series of four articles on cathodoluminescence by Sandra Gibson, a student who has worked with Micheal Robertson (Acadia University) and is now at the University of Waterloo on a post-doctoral fellowship. Also in this edition, Harry Leung (Children's Hospital Boston, Harvard) shares his newly acquired tricks and tips on how to perform "super-resolution" microscopy with basic equipment.

**Nadi Braidy**
MSC Bulletin Editor

Part of the MSC Council present at our Edmonton annual meeting, left to right / *une partie du Conseil de la SMC présent lors de notre rencontre annuelle à Edmonton, gauche à droite*: Zygmunt Jakubek, Isabelle Rouiller, Nadi Braidy, Jeff Fraser, Sara Makaremi, Michael Robertson, Pierre-Mathieu Charest, Youssef Chebli, Joaquin Ortega.



↑ **Volume reconstruction of a mouse kidney.** 8 × 8 nm pixels (x,y) 554 slices @ 50 nm, volume 28.7 × 25.9 × 27.7 µm

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# Cover Artist SPOTLIGHT

Dr. Martin Smith joined the SickKids Research Institute after finishing his PhD at the University of Waterloo, where he studied protein folding. At SickKids, Dr. Smith joined the labs of Dr. John Rubinstein and Dr. Nicola Jones as a postdoctoral fellow to continue his research and develop his skills in electron microscopy. As part of this exciting collaboration, Dr. Smith studied the structure of the vacuolating cytotoxin A, from the bacteria Helicobacter pylori, using electron cryomicroscopy. Successfully finishing his postdoctoral fellowship, Dr. Smith joined The Ontario Brain Institute where he works with researchers to study brain disease.

ABSTACT PRESENTED AT THE 4TH INTERNATIONAL SYMPOSIUM
ON ADVANCED ELECTRON MICROSCOPY FOR CATALYSIS (EMCAT 2016), IN BERLIN

# ATOMIC RESOLUTION IMAGING AND SPECTROSCOPY OF FUEL CELL NANOCATALYSTS

Sagar Prabhudev[a,*], Matthieu Bugnet[a], Guozhen Zhu[b], David Rossouw[a] and Gianluigi A. Botton[a]

Catalytic nanoparticles play a crucial role in accelerating chemical reactions by offering their active sites and surfaces. Further fine-tuning of the surface structure and bulk composition can enhance their reactivity by manifolds, thanks to the electronic structure modification that is achievable through alloying. In the case of Polymer Electrolyte Membrane (PEM) fuel cells, it is also necessary to reduce the catalyst cost for these devices to be commercialized. Here, Pt nanoparticles supported on carbon (Pt/C) are traditionally being used to accelerate the sluggish kinetics of oxygen reduction reaction (ORR). Owing to high cost of Pt, there is a major ongoing effort to replace Pt/C with suitable Pt-alloy/C nanoparticles[1]. Consequently, it is imperative to obtain feedback on the synthesis/phase transformation studies and this demands employing characterization techniques that can provide structural and compositional information on the atomic level. Electron microscopy has always played an important role in the development and the understanding of new materials. In the last ten years, there has been a renaissance in the role of microscopy due to the improved capabilities of its instrumentation (aberration correctors, ultra-fast electron energy loss spectrometers, monochromators) and specimen holders (*in situ* liquid cell, *in situ* heating, tomography). Here we summarize recent examples of work related to the *in situ* study of Pt-alloy nanocatalysts, illustrating the wealth of structural and compositional information that is obtainable with the electron microscopic techniques.

An FEI Titan (80-300 Cubed) microscope fitted with an electron energy loss spectroscopy (EELS) capability (Quantum 966) was used for this work. With high-angle annular dark-field scanning transmission electron microscopy (HAADF-STEM) and EELS, we have studied the evolution of alloy catalysts following annealing in-situ and ex-situ procedures. Starting with a disordered PtFe alloy nanoparticle, we captured the ordering transformation *in situ*, showing evidence of formation of ordered Pt and Fe-rich planes, and evidence of both Pt and Fe-rich shells over a Pt-Fe ordered core (**Figure 1**)[2]. Owing to instrumental limitations, this evolution process had so far been approached ex situ, wherein the particles were characterized before and after annealing. Furthermore, we also show that the Pt surface segregation induces local strain and atomic displacements[2] (**Figure 2**) that can be further correlated to the enhanced catalytic activity of the material, in addition to the enhanced durability shown previously[3,4]. Using in-situ heating, and taking advantage of fast acquisition capabilities with EELS, it has also been possible to study the alloying phenomena of AuPt nanoparticles showing evidence of full miscibility starting at 200 °C (**Figure 3**), well below the thermodynamically expected temperature, due the reduced size-scale of the system[5]. At high-temperature, we have also detected the formation of unexpected ordered structures. Furthermore, we find that annealing under reducing atmospheres led to mostly phase separation and monolayer surface segregation.

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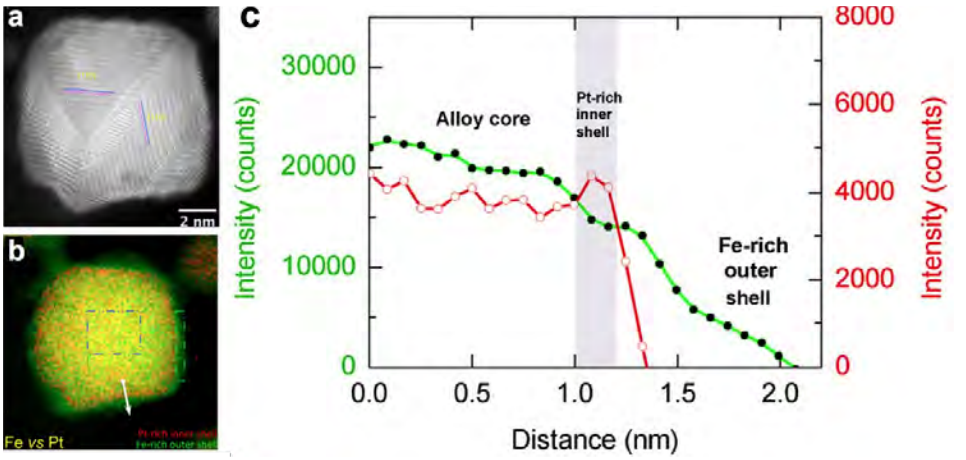


**Figure 1.** (a) and (b), STEM–HAADF and Pt versus Fe STEM–EELS composite map of a heat-treated Pt-Fe nanoparticle, respectively. (c) EELS line profiles of Fe L-edge (green) and Pt M-edge (red) taken along the white arrow highlighted in (b).

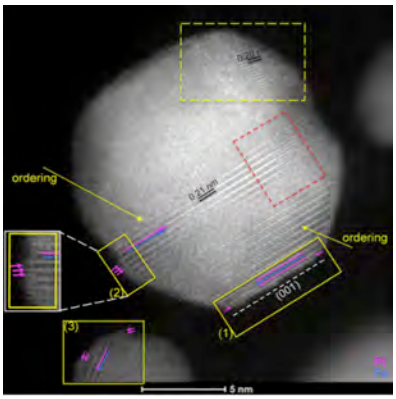


**Figure 2.** , STEM-HAADF image of an ordered PtFe particle illustrating Pt atoms wedged at the surface (highlighted in the yellow boxes).

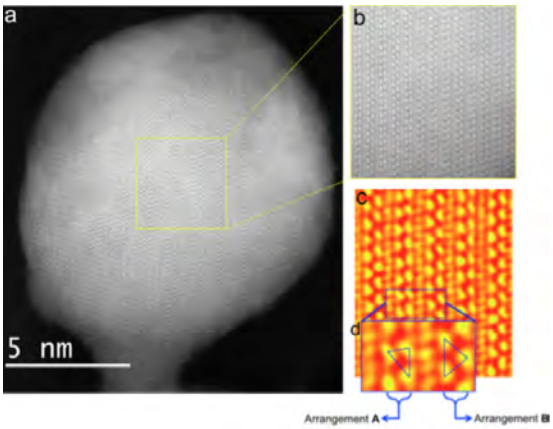


**Figure 3.** , STEM-HAADF image of the ordered AuPt particle.
(a) STEM-HAADF image. (b) and (c) Enlarged images of the selected region in (a).

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Microscopy was carried out at the Canadian Centre for Electron Microscopy, a National facility supported by The Canada Foundation for Innovation, under the MSI program, NSERC and McMaster.

The authors are grateful to CFDM for providing travel assistance to present this work at the Electron Microscopy for Catalysis Meeting (EMCAT-2016) held in Berlin. The abstract was awarded the "Best Poster" at the meeting.

THIS ABSTRACT WAS PRESENTED
AT THE 2016 EM GORDON CONFERENCE IN HONG KONG, CHINA

## MOLECULAR ARCHITECTURE OF THE YEAST ELONGATOR COMPLEX REVEALS AN UNEXPECTED ASYMMETRIC SUBUNIT ARRANGEMENT AND POTENTIAL COOPERATIVITY BETWEEN CATALYTIC ACTIVITIES

D. Setiaputra, D.T.H. Cheng, S. Lu, C.H.Y Lam, J.L. To, M.Q. Dong, C.K. Yip

The yeast Elongator complex is involved in multiple cellular process such as mRNA transcription and tRNA modification. It is a conserved 850 kDa complex consisting of two copies of six subunits (Elp1-Elp6) organized into two subcomplexes (Elp123 and Elp456). The structure of the full complex has not been characterized. Here we present the first three-dimensional reconstruction of the complex generated by electron microscopy. Elongator adopts a bilobal configuration with two different-sized lobes connected by a slender density. The Elp456 RecA-like ATPase ring associates with only one lobe, causing the size asymmetry. Using cross-linking mass spectrometry, we identified the subunit architecture of Elongator. By integrating the available Elongator structural data, we generated a model of the subunit configuration within the complex and show that the catalytic Elp3 subunit is positioned near the Elp456 ATPase ring, suggesting potential cooperativity. Our model also revealed that a series of loop regions facing the major subunit interface of Elongator is important for its function. Our results provide the first view of the asymmetric molecular architecture of Elongator.



THIS ABSTRACT WAS PRESENTED AT THE 2016 BIOPHYSICAL SOCIETY
60TH ANNUAL MEETING IN LOS ANGELES, USA

## INVESTIGATING DIFFUSION OF RECEPTORS ON MACROPHAGE MEMBRANES USING SINGLE MOLECULE TRACKING

Sara Makaremi[a], Kyle Novakowski[b], Markus Rose[c], Dawn M.E. Bowdish[d,e], Jose Moran-Mirabal[a,f*]

Among the central constituents of the innate immune system are macrophages, which are known for phagocytosis or "eating" foreign particles or pathogens. Macrophages express several cell-surface molecules including Toll-like receptors (TLR), and scavenger receptors which play a vital role in their response to pathogenic stimuli. Studies suggest that macrophages exhibit age-related deficiencies in TLR function, and as a result elderly are more susceptible to infections. Previous investigations in aged populations have revealed an increase in the number of bone marrow macrophages that have an impaired ability to produce or release cytokines. The increase in macrophage numbers may reflect a compensation for their reduced function. Our hypothesis is that the reduced function of the receptors is due to the effect of aging on fluidity

and rigidity of the membrane. Therefore, we aim to measure the mobility of macrophage receptors in young and old individuals to investigate whether there are significant differences in membrane fluidity, and if that impacts receptor function. Our experimental methodology includes imaging fluorescently labeled membrane receptors on macrophages isolated from mice peritoneum to track their displacement. Single-particle tracking has been successfully utilized in previous studies to track the trajectories of molecules in the cell membrane, and total internal reflection fluorescence microscopy (TIRF) has enabled visualization of single molecules inside living cells. In this study, we have captured a sequence of images for each cell using TIRF, and have analyzed the images with a custom routine algorithm to detect and localize the receptors to calculate their diffusion coefficient using mean square displacement (MSD) as the most common measure of motion. To our knowledge, this is the first study that has used this technique to compare receptors' diffusion through the membrane of macrophages for young and old organisms.

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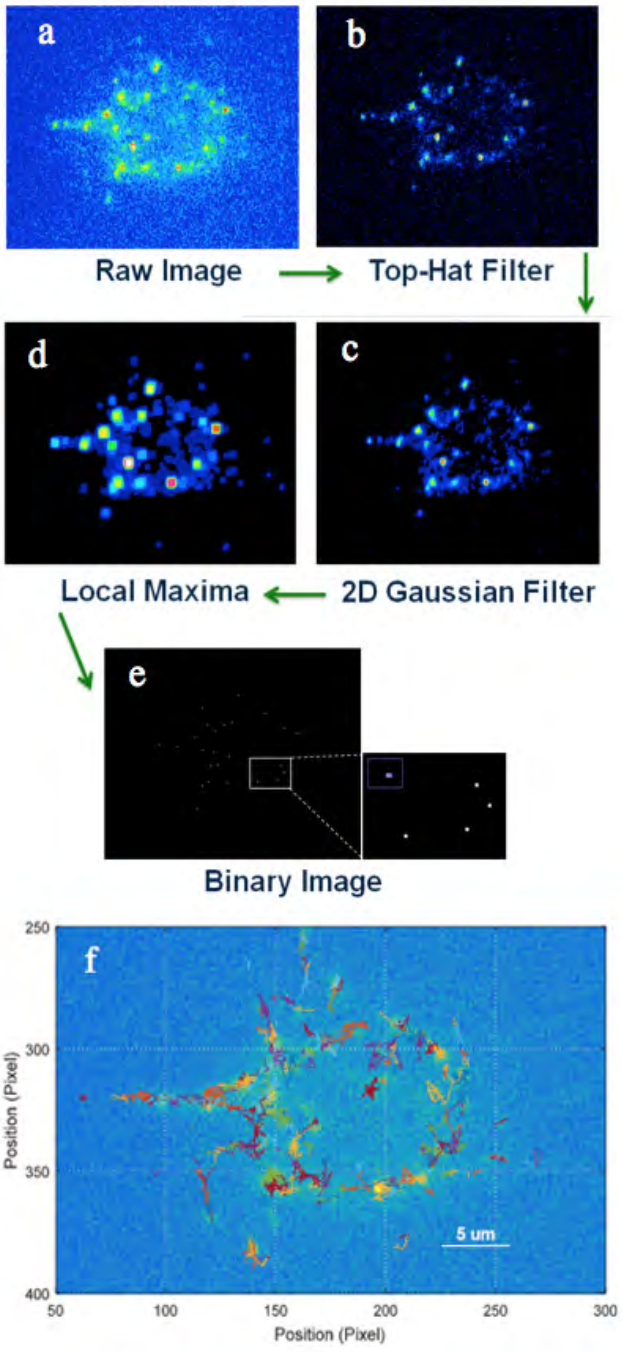


**Figure 1.** Particle detection and tracking: (a-e) Image processing; (f) Tracks found for TLR2 receptors on the membrane of a macrophage.

THIS ABSTRACT WAS PRESENTED AT THE 2016 EUROPEAN MICROSCOPY CONGRESS (EMC) MEETING IN AUGUST IN LYON, FRANCE

# ATOMIC-SCALE COMPOSITIONAL FLUCTUATIONS IN TERNARY III-NITRIDE NANOWIRES

S. Y. Woo[a], M. Bugnet[a], H. P. T. Nguyen[b,c], S. Zhao[b], Z. Mi[b], G. A. Botton[a]

Ternary InGaN and AlGaN alloys have been sought after for the application of various optoelectronic devices spanning a large spectral range between the deep ultraviolet (DUV) and infrared (IR), including light-emitting diodes, and laser diodes. Conventional planar devices suffer from a high density of dislocations due to the large lattice mismatch, which together with the non-ideal alloy mixing, are established as the cause for various phase separation, surface segregation, and chemical ordering processes commonly observed in nitride alloys. Growth in a nanowire (NW) geometry can overcome these processes by providing enhanced strain relaxation at the free surfaces. In both InGaN and AlGaN, their superior operational characteristics can be attributed to enhanced charge carrier localization at alloy inhomogeneities down to the atomic-scale. Atomic-level chemical ordering in wurtzite InGaN and AlGaN epilayers, describing preferential site occupancy of the cation sublattice by the group III atoms, has been reported mostly with a 1:1 periodicity along the [0001] growth direction[1]. Reports of atomic ordering in cubic ternary III-V alloys (including III-As and III-P) have remained limited to planar thin films; its prevalence within NWs had not been explored.

InGaN/GaN dot-in-a-wire nanostructures grown on Si(111) by molecular beam epitaxy (MBE) were recently developed to achieve more controlled light emission across the entire visible spectrum[2], and characterized using aberration-corrected scanning transmission electron microscopy (STEM)[3]. High-angleannular dark-field (HAADF) Z-contrast imaging shows the InGaN quantum dots (QDs) with atypical oscillating HAADF image intensity at the atomic-level along the c-axis growth direction, exhibiting alternating bright/dark atomic-planes within the QDs[3]. Electron diffraction patterns obtained from the QDs show the presence of otherwise forbidden superlattice reflections, unambiguously confirming the presence of 1:1 bilayer atomic ordering[1]. In addition, atomic-resolution elemental mapping using electron energy-loss spectroscopy (EELS) shows significant In-enrichment in alternating c-planes matching the maxima in the ADF signal collected concurrently, with a deviation from the local mean composition by >25%. Corresponding annular bright field imaging (ABF) enables the visualization of light elements like N, and was used to directly deduce the NWs as N-face polarity. It also indicates that the In-atoms have a preferential occupation at the lower-coordination site along a pyramidal surface facet, which is the first experimental evidence[3] validating the existing theoretical structure model for ordered InGaN layers[4].

Compositional inhomogeneities were also investigated in MBE-grown self-catalyzed AlGaN NWs, which exhibit high luminescence efficiency in the DUV range[5]. With increasing Al concentration, atomic-scale compositional modulations can be induced due to differences in Ga- and Al-adatom migration and incorporation at the growth front. The modulating HAADF intensities were confirmed as Ga-rich/Al-rich regions using EELS elemental mapping at atomic-resolution. Furthermore, their QD/quantum dash-like nature was determined based on multi-orientation views of the same atomic-scale Ga-rich regions. Such atomic-scale compositional modulations in AlGaN can provide energy band fluctuations leading to strong three-dimensional confinement of charge carriers[6].

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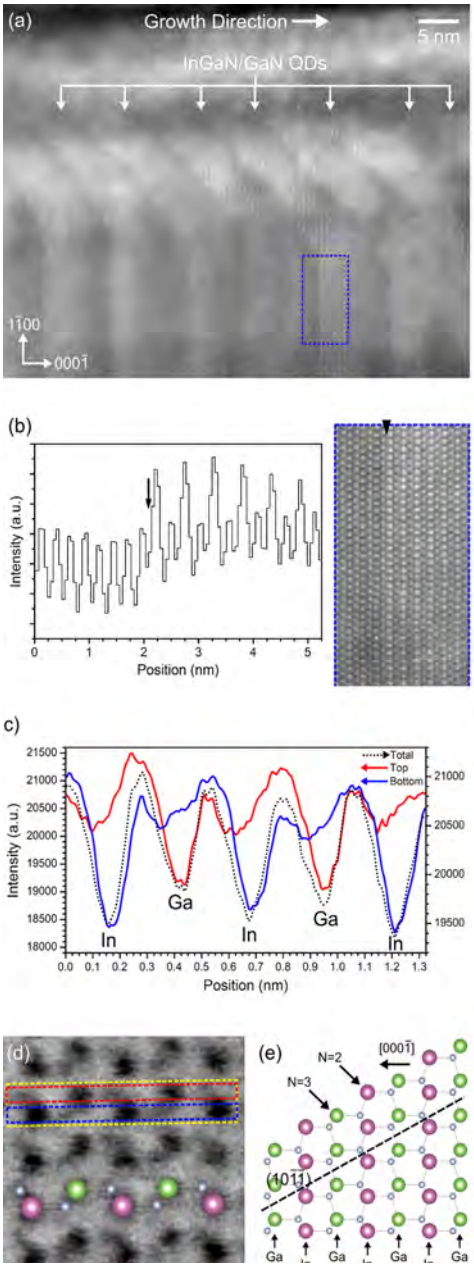


**Figure 1**: (a) HAADF image showing the oscillatory intensity at the atomic-level within the InGaN QDs, (b) integrated intensity line profile from boxed region. (c) Line profiles of the ABF image in (d), including both projected columns (Total, black line from yellow box), and separating into Top (red box) and Bottom (blue box) columns. (e) Model of ordered InGaN from the preferential incorporation of In at the reduced N-coordination site (N=2).

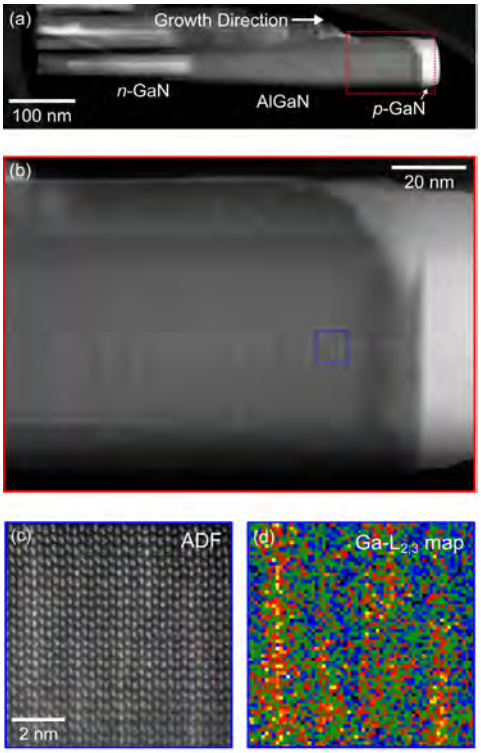


**Figure 2**: (a) STEM-HAADF image of an AlGaN nanowire, highlighting the n- and p-GaN segments in bright contrast. (b) High-magnification view of the p-AlGaN region (red box in (a)), showing intensity modulations along the c-axis, indicative alternating Ga-rich/Al-rich planes at the atomic-scale. (d) The Ga-map (displayed in temperature-scale) and concurrently acquired ADF signal from EELS spectrum image at atomic-resolution (green box in (b)), showing a direct correspondence between the local increases in Ga-signal with the ADF signal within single atomic-planes. Weighted-PCA was applied for noise reduction.

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6. The authors are grateful to NSERC for supporting this research. The microscopy was carried out at the Canadian Centre for Electron Microscopy, a National facility supported by NSERC, CFI under the MSI Program, and McMaster.

*This article is the first of a four-part series detailing some of the equipment and operational considerations of a cathodoluminescence (CL) system. Each article will focus on a different aspect of a CL experiment: (1) components, (2) calibration, (3) optimization and (4) artefacts. This work was originally published in the Proceedings of the Mineralogical Association of Canada Short Course held in Fredericton NB, May 2014 (In Coulson, I.M., (ed.) Cathodoluminescence and its Application to Geoscience, Mineral. Assoc. Can. Short Course 45, 29–45 (2014)) and permission to reprint the material has been graciously granted by the Mineralogical Association of Canada.*

Sandra Gibson is a Postdoctoral Fellow with the Quantum Photonics Lab at the Institute for Quantum Computing. Her current research focuses on using semiconductor nanowires to fabricate scalable, chip integrated devices for electron-to-photon quantum state conversion, single photon emission and detection. She became interested in nanotechnology while doing CL studies as an undergraduate student at Acadia University and went on to complete a PhD in nanostructure growth at McMaster University.

# THE COMPONENTS, CALIBRATION, OPTIMIZATION AND AVOIDANCE OF ARTIFACTS OF A CATHODOLUMINESCENCE SYSTEM

## PART 1 – COMPONENTS

Michael D. Robertson[a] and Sandra J. Gibson[b]

The electronic and optical properties of many non-metallic materials can be characterized by studying the luminescence emitted by the material when excited by non-thermal energy sources, including: (i) photons (photoluminescence); (ii) electrons (cathodoluminescence); (iii) chemical reactions (chemiluminescence); and (iv) electric fields (electroluminescence). Although each of these methods can provide unique insights into the nature of optical transitions within the material, cathodoluminescence (CL) imaging and spectroscopy using a scanning electron microscope (SEM) offers the advantages of high spatial resolution and the simultaneous excitation of all of the luminescent pathways. There has been much written in the literature on the physical mechanisms of CL emission (e.g., Mason 2014; Yacobi & Holt 1990) and instrumentation (Edwards & Lee 2014; Yacobi & Holt 1990) and the reader is directed to these resources, and the references contained therein, for a more detailed treatment of these topics.

### COMPONENTS OF A CL SYSTEM

Generally speaking, an optically based CL system is useful for studying relatively large sample areas on the order of square millimetres, while SEM-based systems are mainly used for higher resolution work ranging from hundreds of square micrometres down to square nanometre length scales. However, all CL systems, whether optically or SEM-based, have certain elements in common, as illustrated in **Figure 1**. In both methods a beam of electrons generated by cold- (field emission) or hot-cathode (thermionic) emission is directed towards the surface of the sample. The impinging electrons interact with the material and light is emitted by bandgap or activator mechanisms. In an optical CL system, the sample surface is illuminated and the light generated is collected over the entire viewable area, as governed by the magnification and numerical aperture (NA) of the objective lens, and an image or spectra can be obtained. In an SEM-based CL system, the surface of the sample is scanned with an electron beam and the luminescence is acquired point by point. In each case, the light is collected using either a mirror or an optical lens and then directed either immediately onto a photodetector or into a spectrometer prior to measurement. Mirrors are the preferred method of light collection, especially in SEM–CL, since they can collect light over a larger solid angle than an optical lens. The collected light intensity must then be converted to an electrical signal by means of a photodetecting device. Typical photodetectors include: (i) photodiodes; (ii) avalanche photodiodes; (iii) charge-coupled devices (CCDs); and (iv) photo-multiplier tubes (PMTs). PMTs are the most sensitive photodetectors and are suited to the measurement of low light signals with typical gains on the order of 106–108 (Hamamatsu 2007). In addition, PMTs have high response speed for imaging applications. CCDs, however, have the advantage of parallel detection of a continuous spectrum of light from a large number of pixels aligned in

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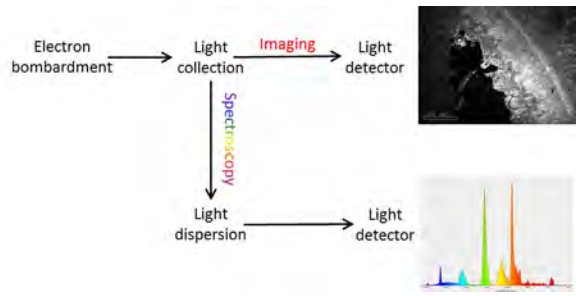


**Figure 1.** A simplified schematic diagram of the typical CL system flow from electron bombardment to light detection and spectroscopy. The panchromatic CL image is from a brachiopod sample and the spectrum was collected from the light emitted from a fluorescent bulb.

a linear array. Photodiodes and avalanche photodiodes are relatively inexpensive options, with the disadvantage of lower gain (~102–103, similar to CCDs), as compared to PMTs.

The distinction between imaging and spectroscopy paths is somewhat artificial. A window can be placed over a selected range of wavelengths and an image can be acquired using a limited spectral range. This imaging mode is useful for determining which regions of the sample are emitting at a particular wavelength. In addition, if a parallel CCD detector is used to acquire a spectrum at every pixel, then a full color image, similar to that obtained in an optical microscope, can be constructed. A full color image is not acquired all at once as in the case of an optical camera, but rather is based on a mathematical transformation relating the CL spectrum and response of the human eye to a perceived color at each pixel (Sun *et al.* 2000, 1998).

A photograph of a typical optical–CL system is presented in **Figure 2**. In this case the system has been built around a conventional optical microscope, with a vacuum chamber and electron gun added to the stage. The microscope can be operated as either a conventional optical microscope using white or cross-polarized light, or as a CL microscope by evacuating the chamber using a rotary pump and irradiating the sample with an electron beam. The electron beam potential and current, as well as the vacuum level, are set by a separate controller. The light is collected by the objective lens, magnified, and can be imaged with either the binoculars or a CCD camera mounted at the top of the microscope. In this image, the microscope has been configured with a fibre optics adaptor for spectroscopy work.

The principle of operation of an SEM–CL system is very similar to an optical system. Shown in **Figure 3** are the collection optics of a Gatan MonoCL3 system mounted onto a JEOL LV5900 SEM as viewed into the specimen chamber. The electron beam is focused onto the sample by the objective lens, passing through a small hole located in the top of a parabolic CL collection mirror. The sample is located at the focal point of this mirror. The diameter of the electron beam is typically on the order of nanometres and so the CL emission from the surface can be approximated as a point source. The outwardly emitted light is collected by the parabolic mirror and reflected down a light pipe as a parallel beam into the spectrometer or directly onto the PMT.

Illustrated in **Figures 4a to 4c** are ray diagrams of the light paths for a) panchromatic imaging mode; b) monochromatic spectroscopy mode; and c) from the back of the spectrometer

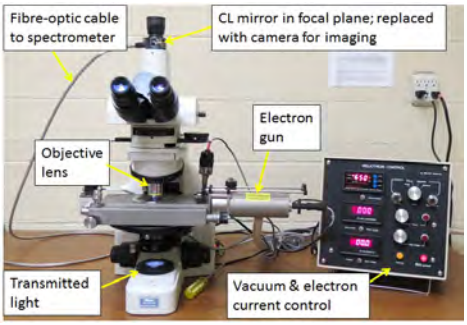


**Figure 2.** A photograph of the optical CL system at Acadia University equipped with a vacuum stage, electron gun and controller. It is presently configured for CL spectroscopy and the fibre-optic adaptor at the top can be replaced with a CCD camera for CL imaging. The instrument is a Reliotron from Relion Industries, LLC.

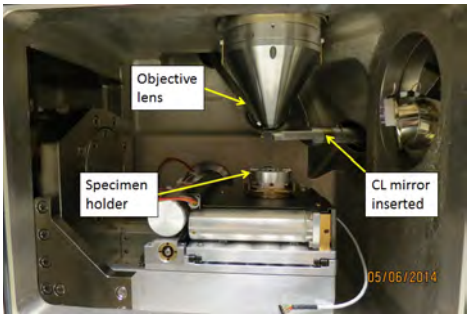


**Figure 3.** A photograph of the inside of the specimen chamber of a JEOL LV5900 SEM displaying the location of the light collection mirror of a Gatan MonoCL3 CL system.

in monochromatic spectroscopy mode. Note that the vertical line down the SEM column in **Figures 4a and b** represents the path of the electron beam from the electron gun to the sample location. In the panchromatic imaging mode (**Figure 4a**), the entire collected CL signal is directed towards the PMT and there is no distinction between the wavelengths of the emitted light. This mode has the highest signal-to-noise ratio and is useful for alignment and survey work. For spectroscopy work (**Figures 4b and c**), the light is directed upwards into a Czerny-Turner spectrometer where it passes through the entrance slit and diffracts to fill the first concave mirror. It is then reflected as a parallel beam onto the diffraction grating, which in turn reflects wavelength dispersed light onto a second concave mirror. The dispersed light is focused at different points as a function of wavelength, such that a specific wavelength band may be directed onto the exit slit. Lastly, the selected light leaving the exit slit is reflected by a plane mirror onto the PMT.

The wavelength resolution of the spectrometer is controlled by the width of the slits and by the density of grooves of the diffraction grating, specifically, narrower slits and higher groove density increase resolution. However, as the slits are narrowed to increase resolution the intensity of the signal decreases, and these two factors will need to be balanced according to the needs of the experiment, as discussed in Part 3 of the series focusing on CL

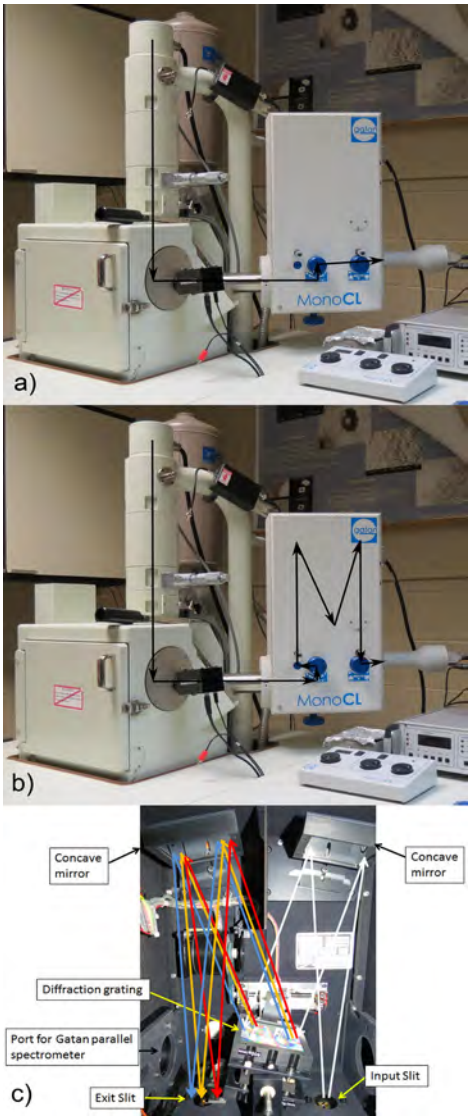


**Figure 4.** Light paths in a Gatan MonoCL3 CL system for: a) panchromatic imaging; ) monochromatic imaging and spectroscopy; and c) from the rear of the spectrometer where the components are displayed.

optimization. Furthermore, although increasing the groove density on the diffraction grating can increase spectrometer resolution, it limits the spectral range that can be investigated. The dispersion of a spectrometer is defined as its ability to spread out light into separate

wavelengths and is usually expressed as a range of wavelengths of light in nanometres as a function of distance in millimetres in the focal plane of the second mirror where the second slit is located (units of nm/mm). Dispersion is inversely proportional to groove density of the diffraction grating and a 1200 g/mm (grooves per mm) diffraction grating would have twice the dispersion of a 600 g/mm grating. Note that the number of grooves per mm of a diffraction grating is also commonly expressed as lines/mm. Again, a balance needs to be struck between the desired resolution and range of wavelengths to be investigated, and in the case of the particular spectrometer discussed herein two gratings are fastened onto the same mount, which can each be used depending on the application.

Another useful feature of this CL system is the option to insert an optical filter, often referred to as an "order-blocking filter", into the light path to prevent unwanted wavelengths of light from interfering with the desired signal. Potential benefits of using an order-blocking filter are to remove a long-lived CL emission which can lead to streaking within an image (Reed & Milliken 2003), or to remove light from a higher diffraction order, preventing interference with the first order diffracted light, as will be discussed in greater detail in Part 4 which focuses on CL artifacts. Images of an in-house designed filter jig are shown in **Figure 5**, which allows a filter to be inserted into the light path just before the PMT.

Other options that may be included in a CL system are optical polarizers to study the polarization properties of emitted CL radiation, a parallel spectrometer for rapid collection of spectra, and a low-temperature specimen stage for increasing CL emission and the study of fine structure in the CL spectra such as phonon and exciton effects. These topics are beyond the scope of these series of articles.

This concludes Part 1 of 4 and the calibration of a CL system will be discussed in Part 2 to be published in the next edition of the MSC-SMC Bulletin.

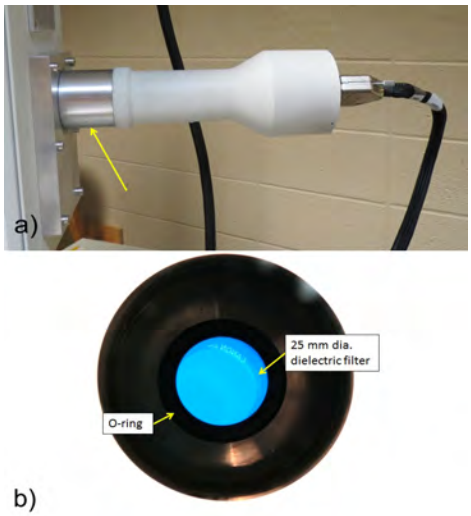


**Figure 5.** a) A side view of the optical filter holder showing its mounting location between the spectrometer casing and the PMT; b) A view of the top of the filter holder highlighting the red-block dielectric filter and supporting o-ring.

## ACKNOWLEDGWEMENTS

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# EXPANSION MICROSCOPY ENABLES SUPER-RESOLUTION WITHOUT SPECIALIZED INSTRUMENTS OR SOFTWARE

Harry Leung[a]

In a recent microscopy symposium, I learnt of an innovative technique that can produce super-resolution results without the usual approach in highly specialized instruments. The key to its success centers entirely on how the specimen is being prepared. I feel motivated once again to get on my computer and share the story with our readers.

## DEFINITION OF SUPER-RESOLUTION

Any optical imaging technique capable of achieving a resolution below the roughly 200nm limit imposed by the "Diffraction Barrier" is known as super-resolution. There have been several of such techniques emerging during the past decade. They all require expensive instrumentation and/or highly sophisticated computation algorithms though.

## THE B I G IDEA

A recent innovation from MIT's Synthetic Neurobiology Research Group offers a low cost alternative to enable super-resolution through an ingenious way of preparing their samples. The technique is called Expansion Microscopy (ExM) and was first revealed in the Journal Science early 2015[1]. The idea was to blow the sample up, making it bigger, without distorting its overall gross anatomy. Unlike those images seen in sci-fi movies, depicting 50-foot killer ants, ExM only blows up the sample four to five times. That is enough to render molecules normally too small to be resolved resolvable. For example, blowing up the sample four times is equivalent to achieving a 70nm resolution on a conventional microscope capable of 280nm resolution. Instead of exploiting further into more instrument resolution, the ExM's approach is to simply make the sample bigger.

## DOING THE TRICK

The basic idea of ExM is to infiltrate samples with swellable gel polymers (commonly found in diaper materials) that expand when exposed to water, to swell up the samples. The procedure involves:

1. Chemical fixation,
2. Cross link fluorescent labels to gel monomers and then anchor them to cell structures,
3. Polymerize the gel in situ,
4. Digest the cell content away for uniform expansion in all XYZ dimensions,
5. Expand the gel in water.

The protocol was described in detail in the Science paper[1].

## RESULTS

An enlarged sample with structures delineated with fluorescent labels. The sample was highly transparent because its content was mainly water. This gives the advantage of low optical aberrations with improved imaging depth.

## QUALITATIVE AND QUANTITATIVE ASSESSMENT

Side by side comparison of Confocal images obtained on pre and post expansion microtubules were "indistinguishable" after rescaling (**Figure 1**). Microtubule lengths calculated from these samples were within 1% discrepancy. As for nanoscale molecules, measurement accuracy was found to be "within the digitization error of measurements". In an ExM versus SIM* comparison, image resolution was said to be better in ExM.

* Structured Illumination Microscopy (SIM) is a commonly used super-resolution technique.)

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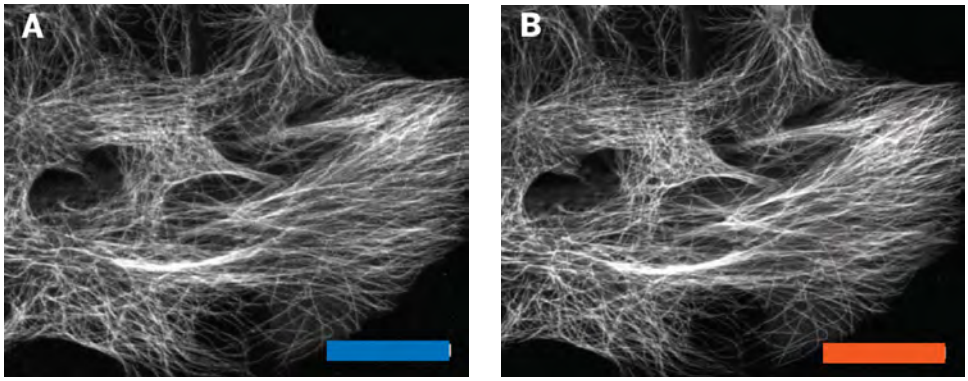


**Figure 1.** Microtubules in HEK293 cells from pre expansion (A) and post expansion (B) samples; scale bars=20 µm, reprinted from (1): Chen, Tillberg and Boyden *Science* 347 (2015) 543. Reprinted with permission from AAAS.

## THE GOOD AND THE BAD

GOOD:

- Uses readily available "off-the-shelf" ingredients.
- Converts any standard widefield or confocal microscope (including spinning disc) to super-resolution. (Much faster image acquisition compared to other super-resolution techniques that rely on heavy computation).
- Expands equally in XYZ (ie. resolution increases equally in XYZ). Unlike other super-resolution methods where Z resolution is always about two to three times worse than that of XY.
- Many super-resolution techniques suffer from signal/noise issues and can be a challenge for thick and opaque biological samples. ExM renders the sample transparent, with improve signal/noise ratio and imaging depth.

BAD:

- since the sample expands in the Z dimension, its usefulness is limited by the working distance of available lenses and becomes a challenge to study large (pre-expansion) samples.
- not a live cell technique.

## WHAT'S NEXT

ExM was developed using cultured cells and brain slices. However, in theory, any molecular structure capable of cross linking both to the gel and to a fluorescent tag can be used. These include most proteins, antibodies, and molecules as small as RNAs[2]. Since the ExM preparation renders the sample "totally transparent", minimizing many optical aberrations, it naturally is a prime candidate for Light Sheet Microscopy (Light Sheet has been discussed previously in this column[3]). Expect to see more applications using both techniques together in complimenting studies. ExM offers a neat idea for those interested in studying nanoscale molecular mechanisms who have been discouraged by the high cost of pursuing super-resolution. Enlarging a micro structure in a high density cellular micro-environment, to "un-crowd" target sites, is also an ingenious approach to better single molecule detection. The possibility of doing this without investing in expensive instruments is a very attractive proposition indeed. Since ExM works with most standard antibodies and florescent agents, minimizing the need to redesign experimental protocols, a quick adaptation to this technique can be expected.

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# ADVANCED EELS WORKSHOP AT CANADIAN CENTRE FOR ELECTRON MICROSCOPY

Alison F. Mark[a], Canadian Centre for Electron Microscopy

The Canadian Centre for Electron Microscopy held a workshop on Advanced Electron Energy Loss Spectroscopy (EELS) on Nov. 3rd 2016. The workshop came together quite quickly so we were happy to see a lot of interest in the topic, so much interest that we had to close the registration early. It was very well attended; 41 people came, with about half of those from outside McMaster University. We were particularly pleased to have four industry researchers registered.

Two speakers, Paolo Longo from Gatan and Gianluigi Botton from the Centre, discussed advanced EELS from a users' perspective. The advantages of EELS in combination with Energy Dispersive X-ray Spectroscopy (EDXS) for obtaining specific composition and chemistry data were described by Dr. Longo, with many examples. His experience in teaching the technique was evident in the many practical tips he gave about how to get the best EELS data. Dr. Botton spoke of the power of EELS to reveal fine details of atomic bonding and nearest neighbour positions. Despite the advanced topics, the informal atmosphere encouraged questions, and even students felt comfortable engaging with the speakers.

We had so much interest that we wondered if we would be able to accommodate everyone for the practical demonstrations in the afternoon. Thanks to a generous demonstrator and everyone's willingness to make space, everyone who wanted to had chance to see the demonstrations in one of two afternoon sessions. Dr. Longo demonstrated advanced applications of EELS and particularly focused on energy-filtered imaging (EFTEM), which is often an underused analysis technique. He also highlighted the speed and relative ease of use of the Quantum spectrometer system installed in the Centre's Titan 80-300 HB TEM.

Our intention was to introduce the EELS technique to new users, to show researchers and industry members what data it can provide, specifically at the highest levels of its capability. The excellent turn-out shows that the interest is there; it is our hope that many of the participants will return to the centre with their own materials to push the limits of EELS even further.

The Canadian Centre for Electron Microscopy would like to announce its 2017 Summer School on Electron Microscopy.

Advanced EELS workshop organized by the CCEM.

a. McMaster University, Hamilton, ON, Canada

Each year the Centre welcomes participants from around the world to the McMaster University campus in Hamilton, Ontario, Canada for an advanced school on electron microscopy.

The course runs over five days and aims to provide students with advice in solving characterization problems using advanced electron microscopy techniques. With the help of experts, users with experience in electron microscopy learn about the fundamentals of aberration-corrected imaging, electron energy loss spectroscopy, electron tomography, ultimate physical limits (beam damage and resolution) and the use of aberration-corrected electron microscopes.

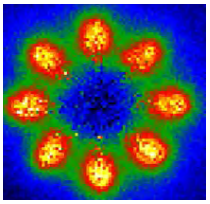
There are many opportunities for hands-on training on the alignment and operation of the electron microscopes with experts from the microscope and spectrometer companies. Several hands-on data processing sessions are also organised.

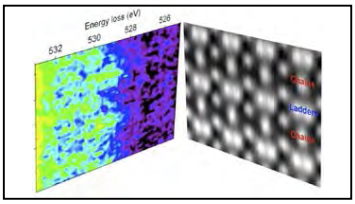
For more information please go to: ccem.mcmaster.ca/outreach-courses

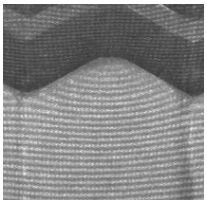

Gianluigi Botton lecturing during the advanced EELS workshop organized by the CCEM.

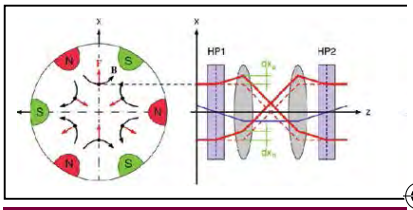
# CCEM Summer School on
# Electron Microscopy

**A 5-DAY COURSE** for users with experience in electron microscopy, on the fundamentals of aberration-corrected imaging, electron energy loss spectroscopy, electron tomography, ultimate physical limits (beam damage and resolution) and the use of aberration-corrected electron microscopes. The aim is to provide students advice in solving characterization problems with the help of experts. The course will include lectures given by experts in the use of the technique and experts in electron optics, alignment and optimization of electron microscopes and EELS spectrometers. Students will have plenty of opportunities for hands-on training on the alignment and operation of the electron microscopes with the experts from the microscope and spectrometer companies. Two FEI Titan microscopes with correctors and monochromators (Quantum and Tridiem spectrometers) will be used for training. Several hands-on data processing sessions are also organised.








# June 5-9, 2017

**McMaster University,** Hamilton ON Canada. On-campus accommodation is available for confirmed registered students.

## COST

All meals and course notes are included in the registration fee ranging from **$500.CDN**/full-time students to **$1500.CDN**/industry researchers. Accommodation will be separate and the responsibility of attendees *(see full details on registration form)*.

## REGISTRATION

**Register online** by **January 15, 2017** at the web address below.
For inquiries, email: **ccem@mcmaster.ca.**
Payment details are given on the registration form.

*Places are limited to 15 registrants. Please contact us if you are interested to reserve a place.

## TOPICS:

- Aberration-corrected TEM, STEM
- Alignment of microscopes with correctors
- EELS data processing and MMLS fitting
- Independent component analysis
- Fundamental limits from beam damage
- Inelastic scattering and ultimate resolution
- Geometric Phase Analysis
- Monochromated EELS, EELS mapping
- Operation of monochromators
- Optimization of spectroscopy data acquisition
- Simulations of images and diffraction patterns
- STEM quantitative image analysis

LECTURERS: Various instructors from academic institutions and technical experts from manufacturers of microscopes, spectrometers and aberration correctors.

CONFIRMED SPEAKERS:
R. Egerton (U. Alberta); P. Hartel (CEOS); M. Hÿtch (CEMES, Toulouse) ; L. Jones (Oxford); S. Lazar (FEI); P. Longo (Gatan); Q. Ramasse (SuperSTEM, UK); D. Rossouw (McMaster); E. Sourty (FEI); P. Stadelmann (EPFL); P. Tiemeijer (FEI); R. Twesten (Gatan)

# http://ccem.mcmaster.ca/outreach-courses

Brockhouse Institute for Materials Research · ccem Canadian Centre for Electron Microscopy · INNOVATION.CA Canada Foundation for Innovation / Fondation canadienne pour l'innovation · CEOS Corrected Electron Optical Systems GmbH · FEI · GATAN · McMaster University

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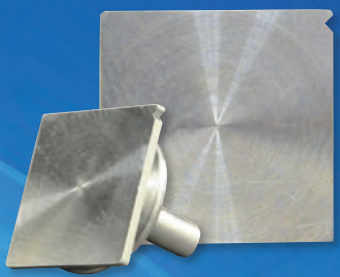

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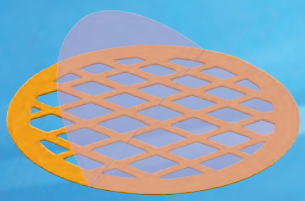

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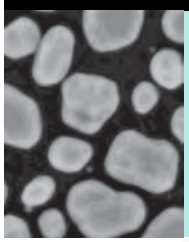

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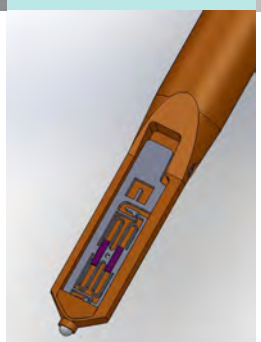
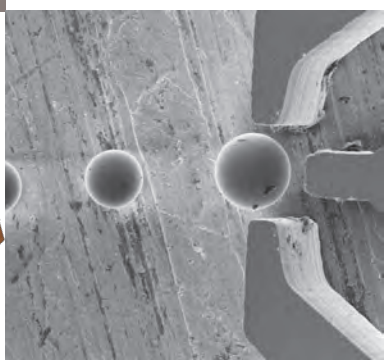
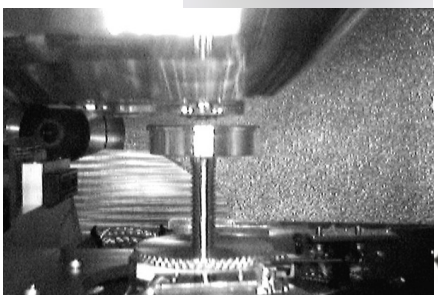


**Chamberscope**





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