Ultramicroscopy (xxxx) xxxx–xxxx

Contents lists available at ScienceDirect

Ultramicroscopy

journal homepage: www.elsevier.com/locate/ultramic

Application of EELS and EFTEM to the life sciences enabled by the contributions of Ondrej Krivanek

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ARTICLE INFO

Keywords:
Electron energy loss spectroscopy
Energy-filtered transmission electron microscopy
Spectroscopic imaging
STEM-EELS
CCD cameras
Detective quantum efficiency
Detection limits

ABSTRACT

The pioneering contributions of Ondrej Krivanek to the development of electron energy loss spectrometers, energy filters, and detectors for transmission and scanning transmission electron microscopes have provided researchers with indispensible tools across a wide range of disciplines in the physical sciences, ranging from condensed matter physics, to chemistry, mineralogy, materials science, and nanotechnology. In addition, the same instrumentation has extended its reach into the life sciences, and it is this aspect of Ondrej Krivanek's influential contributions that will be surveyed here, together with some personal recollections. Traditionally, electron microscopy has given a purely morphological view of the biological structures that compose cells and tissues. However, the availability of high-performance electron energy loss spectrometers and energy filters offers complementary information about the elemental and chemical composition at the subcellular scale. Such information has proven to be valuable for applications in cell and structural biology, microbiology, histology, pathology, and more generally in the biomedical sciences.

1. Introduction

From the time of its origin, electron energy loss spectroscopy (EELS) was recognized as being a particularly sensitive technique for the detection of lighter elements because those atoms have favorable inelastic scattering cross sections for inner shell excitations [1–3]. It was therefore understood at the outset that EELS had potential for analyzing the composition of biological specimens, which are predominantly composed of lighter atoms. In addition, early studies in Albert Crewe's laboratory at the university of Chicago showed that EELS could distinguish between biological molecules, such as different amino acids, and different nucleic acid bases by analyzing details of the EELS fine structure caused by excitation of valence or core shell electrons [4,5]. However, in the 1970s electron spectrometers were not readily available commercially. Instead, the few existing EELS systems in laboratories around the world were home-built and often unsuitable for users who were non-physicists. For example, my own work on EELS began at the Department of Physics, Cambridge University, UK, where I used a 45° magnetic prism spectrometer on an AEI EM6G electron microscope operating at an accelerating voltage of 60 kV with the task of obtaining a better characterization of EELS core-edges and investigating the potential of EELS as a quantitative microanalytical tool. The spectrometer that I used had been built by David Wittry a few years earlier, when he was on sabbatical leave from the University of Southern California [6]. It was also in Cambridge where I met Ondrej Krivanek in 1975 since we were both graduate students at the Cavendish Laboratory. In fact, Ondrej's Ph.D. research did not involve EELS, but was concerned with analyzing high-resolution TEM images of amorphous materials to discriminate nanometer-sized ordered domains from random fluctuations in the structure [7]. I completed my studies at the Cavendish one year after Ondrej, and moved to Mike Whelan's group in the Department of Metallurgy and Materials in Oxford where I continued work on EELS with an in-column energy filter built by Egerton et al. [8] based on the original design by Castaing and Henry [9]. Then the following year, I moved to the Department of Applied and Engineering Physics at Cornell University in Ithaca, NY, where I worked in John Silcox's group using a third type of spectrometer, a Wien-Filter, which had been constructed by Curtis and Silcox [10], and which was attached to a somewhat ancient Hitachi HU11A TEM. Experience with these three instruments illustrates the point that in the 1970s most EELS and EFTEM systems were home-built and often quite difficult to use.

In the early 1980s this situation changed radically due to two events: first, Ondrej Krivanek designed a compact electron energy loss spectrometer offering relatively high performance in terms of collection efficiency and energy resolution; and second, Peter Swann, an ex-Professor of Materials Science from the University of London, who was an accomplished electron microscopist and inventor, and co-founder of Gatan Inc., entered into a partnership with Ondrej, who oversaw the launch of the first commercial post-column EELS system. Initially, the

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http://dx.doi.org/10.1016/j.ultramic.2017.01.002
Received 8 November 2016; Received in revised form 29 December 2016; Accepted 10 January 2017
0304-3991/ Published by Elsevier B.V.

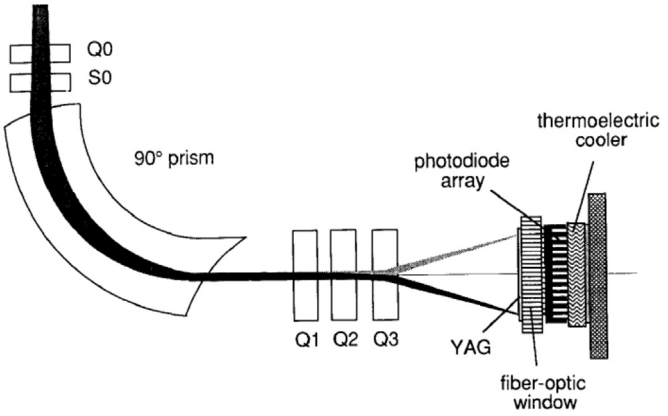

Fig. 1. Schematic diagram of Gatan parallel EELS showing the magnetic sector, the pre-sector focusing coils, post-spectrometer lens assembly, and the photodiode detection system. From O.L. Krivanek et al. [12].

spectrometer collected spectra serially using a photomultiplier tube coupled to a scintillator [11], but soon afterwards Ondrej and his team developed an electron energy loss spectrometer with a parallel detection based on a photodiode array coupled to a YAG scintillator, as indicated in Fig. 1 which is taken from Ondrej's *Ultramicroscopy* paper from 1987 [12]. Shuman working on muscle physiology at the University of Pennsylvania with a home-built system had also demonstrated in 1981 that electron energy loss spectra could be collected in parallel [13]. The Gatan detector provided an enormous improvement in sensitivity, expanded the scientific community pursuing EELS, and enabled near single atom detectability in STEM with a sufficiently small probe. In particular, exciting results were obtained from a wide range of specimens, often using what was then the state-of-the-art STEM, the VG Microscope HB501 [14–17].

With the advent of CCD detectors, Ondrej realized that it was possible to modify the PEELS system by incorporating a slit at the dispersion plane and a series of other lenses (quadrupoles and sextupoles) after the slit, thus converting the TEM/PEELS into an energy filtering transmission electron microscope (EFTEM). The system could also easily revert to PEELS operation by retracting the slit and changing the lens strengths to image the dispersion plane onto the CCD detector. This provided high-end TEMs with great analytical flexibility since these instruments were equipped with field-emission guns that produced a small probe in STEM. For the first time TEMs could be operated in EFTEM or STEM-EELS mode, which attracted considerable interest from researchers in the life sciences, as indicated in Fig. 2, which is taken from Ondrej's 1995 *Ultramicroscopy* paper [18]. Over the past two decades there have been many further refinements in energy loss spectrometers and filters, including increased sophistication in the software used to acquire STEM-EELS hyperspectral images, as well as correction of higher order spectrometer aberrations, which give improved collection efficiency with larger spectrometer entrance apertures. However, Ondrej and his colleagues at Gatan had established the essential principles of today's post-column imaging filters some twenty years ago, i.e., before Ondrej's embarked upon the next stage of his career: the quest and ultimate realization of aberration-corrected STEMs, and the founding, together with Niklas Dellby, of Nion Inc.

It seems clear that Ondrej's deep understanding of the optics of electron spectrometers and energy filters, and how to correct spectrometer aberrations based on multipole magnetic lenses, helped Ondrej's path towards correcting the spherical aberration of the probe-forming lens in the STEM. Also, Ondrej's hands-on experience in interfacing spectrometers to the VG Microscopes HB501 instrument provided him with useful experience on how design features of dedicated STEMs could be improved. Therefore much of Ondrej's and his colleagues' experience with energy loss spectrometers helped lead to Nion's

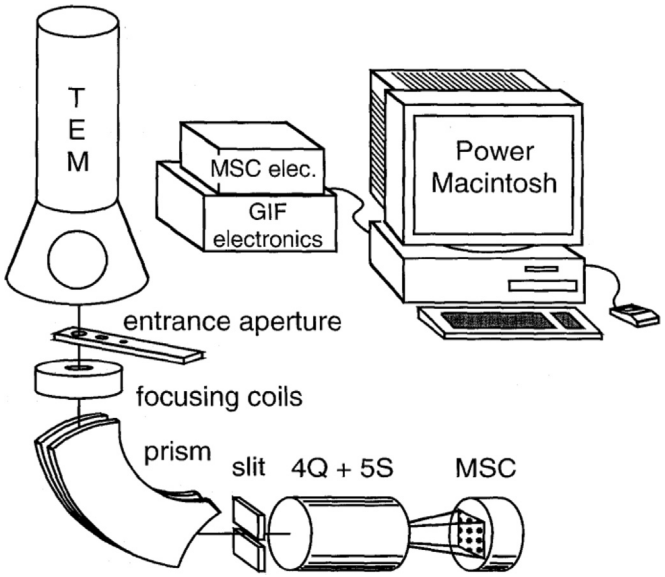

Fig. 2. Principal components of the Gatan imaging filter system: (Q) quadrupole lens, (S) sextupole lens, (MSC) multi-scan CCD camera, (GIF) Gatan imaging filter.From O.L. Krivanek et al. [18].

development of the aberration-corrected UltraSTEM [19,20].

It is should be pointed out that development of the energy filters with high-performance cooled CCD detectors, in turn, led to improvements in detectors for dedicated EELS systems, first the Enfina spectrometer, and more recently the Enfinium spectrometer which incorporates features of the Quantum GIF. In particular, the new generation of EELS enables 1000 spectra to be read out in 1 s, and the Dual-EELS capability making it possible to acquire two regions of the energy loss spectrum almost simultaneously.

The account below includes examples of the application of electron energy loss spectrometers and post-column imaging filters, which have been developed by Ondrej and his colleagues, and which we have used in our laboratory to study biological specimens. Many other laboratories have been working in this area too, and we do not attempt to present a comprehensive review of EELS and EFTEM in the life sciences. For example, researchers have been using various in-column imaging filters to address important biological questions [21–24], and this is also outside the scope of this article.

2. Applications of the GIF

One method for obtaining quantitative elemental distributions from large regions of cells and tissues is to acquire a series of energy-selected images successively over a range of energy losses containing core-edges of interest, i.e., EFTEM spectroscopic imaging. Although this is inefficient in terms of the incident electron dose required to obtain a signal of a specific size, the acquisition is fast since the signal from ~10^6 pixels is read out in parallel. In this case, the signal is determined by the width of the energy-selecting slit, which is typically set to the required spectral energy channel width. For example, this approach has been applied to map the composition of hormone-containing secretory granules in mouse pancreatic islets of Langerhans, which are aggregates of endocrine cells that secrete hormones to control blood glucose levels [25,26]. Fig. 3 shows the phosphorus $L_{2,3}$ edge, sulfur $L_{2,3}$ edge, and nitrogen K edge maps from a sectioned region of an islet containing portions of an insulin-secreting β-cell (right), and a glucagon-secreting α-cell (left). Nitrogen serves as a general marker for protein, nucleic acid and other biological molecules, and is observed at high concentrations in both α-cells and β-cells, as well as in the nucleus. Phosphorus is concentrated in the DNA-containing chromatin of the β-cell nucleus as well as lesser amounts in membranes and ribosomes,