Ondrej Krivanek's early scientific research

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ABSTRACT

In 1806, Humphrey Davy said that “nothing promotes the advancement of science so much as a new instrument”. This paper reviews some of the lesser-known achievements of Ondrej's early career, and reminds us of the level of performance of instruments in those days, in order to appreciate how great has been the progress in instrumentation, much of it due to Ondrej and his leadership, since then. Some new results in the field of EELS are described, including extraction of the time-dependence of the dielectric response (with better time resolution than an X-ray free electron laser (XFEL)) from Nion EELS data. An approximation for atomic-resolution imaging which includes multiple scattering effects is given for biological samples, for use with aberration-corrected instruments when these become needed at the higher beam energies required to preserve the projection approximation, on which the 3D merging of single-particle cryo-EM images is based. We also discuss the requirements for out-running radiation damage using pulsed electron beams, a worthy final challenge for OLK.

1. Introduction

The early nineteen-eighties were an exciting time in the physics department at Arizona State University. Continuing the excellent Cavendish and CSIRO tradition of extended “morning tea” and coffee, John Cowley presided over lengthy discussions among the postdocs, faculty and students on their various projects, covering both theory and experiment. At various times, this group might have included Ondrej Krivanek, Sumio Iijima, David Smith, Ray Carpenter, Peter Rez, Johan Tafto, Neil Long, Mike O'Keefe, Gary Hembree and myself and our students and postdocs. These were the days of punched-card mainframe computer input with one-day turn-around, at a time when the Professors used the electron microscopes, working closely with the lab manager John Wheatley to keep the machines in top condition. Iijima, especially, was forever polishing gun components with John to extract more brightness from his etched pointed filaments, producing 0.34 nm resolution TEM images from the JEOL 100B (with special C₀=0.7 mm polepiece) always just a little crisper than anyone else’s. (In 1980 he had published the first HREM image of a buckyball, long before their fame on Ondrej’s early stage.

In 1980, Humphrey Davy said that “nothing promotes the advancement of science so much as a new instrument”. This paper reviews some of the lesser-known achievements of Ondrej's early career, and reminds us of the level of performance of instruments in those days, in order to appreciate how great has been the progress in instrumentation, much of it due to Ondrej and his leadership, since then. Some new results in the field of EELS are described, including extraction of the time-dependence of the dielectric response (with better time resolution than an X-ray free electron laser (XFEL)) from Nion EELS data. An approximation for atomic-resolution imaging which includes multiple scattering effects is given for biological samples, for use with aberration-corrected instruments when these become needed at the higher beam energies required to preserve the projection approximation, on which the 3D merging of single-particle cryo-EM images is based. We also discuss the requirements for out-running radiation damage using pulsed electron beams, a worthy final challenge for OLK.

Ondrej burst upon the scene at ASU (from 1981 to 1985), full of his remarkable drive and energy, committed to making EELS a user-friendly experience with higher performance than soft X-ray spectroscopy, and often working with Christian Colliex and the Cornell group from an early stage. The ASU winter schools and conferences were soon in full swing (Figs. 1–3), bringing international leaders in the field to a Hot Springs desert resort in Arizona every year, such as Albert Crewe, inventor of the modern STEM, and an inspiring figure for us all, with a lasting influence on Ondrej [28]. Ondrej had come to join the faculty from his Berkeley postdoc with Gareth Thomas, and a string of publications on interfaces in materials science, a theme he continued at ASU. His 1977 paper with Kobayashi [24] on grain-boundary structure in germanium had showed the power of the HREM method for semiconductor physics and attracted much attention, and he produced some of the first high-resolution images of the Si-SiO₂ interface, precursor to a most critical area of device physics under Moore’s relentless law. This followed his Ph.D. work with Archie Howie at the Cavendish (to which he came from Leeds in 1971, where he’d arrived from Czechoslovakia in 1968) on the structure of glasses, now mostly lost in this age of insistent email demands, deadlines and grant-writing, with funding agency rejection rates rising from about 75% then to around 90% now. John Cowley somehow managed to maintain this lifestyle to the end, and had worked on his VG HB5 on the day he died in 2005.

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Our machine-shop at ASU was kept busy building serial-ELS systems to his designs using photomultipliers, with their excellent dynamic range, and the performance of these rapidly improved due to his skill in optimizing experimental conditions, and in instrument design. This led to the publication of the famous "EELS Atlas" with Channing Ahn, an astonishing achievement aimed at publishing the inner shell edges of practically every atom in the periodic table, which was done (eg by leaking hydrogen into the JEOL 200CX) over many long nights at ASU. Parallel-detection EELS systems were in development by several groups at the time since the appearance of the first linear diode arrays and one-dimensional charge-coupled diode arrays (CCD) (Jones, Johnson, Shuman, Kruit, Egerton - see [9] for a review) and all this work led eventually to Ondrej’s design of the hugely successful Gatan parallel-detection EELS (PEELS) system, which has been in continuous development ever since, and is in use today. The electron-optical design, and subsequent design of the imaging filter, based on quadrupoles, allowed many of the ideas of later STEM aberration-correction systems to be developed. Ondrej’s first PEELS paper with Ahn and Keeney [22] is one of the most highly cited papers in Ultramicroscopy. But several other lesser-known projects were ongoing in parallel at that time which turned out to influence his future scientific career, and a few other unreported failures which I’ll mention to save somebody time repeating them.

By good fortune, I had hired as my first postdoc Johann Tafto, from the powerful group of Jon Gjonnes in Oslo. Johann arrived full of his Ph.D. work on the theory of electron channeling, which I’d been simulating in the hope of reproducing on EELS spectra some of the standing-wave effects on X-ray fluorescence which Batterman had observed using X-rays at Cornell. But the EELS case involved double-channeling, difficult to interpret, and instead Johann conducted a brilliant series of experiments on channeling effects on EDX spectra from polyatomic crystals containing dopants. (Since multiple scattering affects host and dopant atoms equally, the effect cancels). This lead to our Alchemi technique for locating foreign atoms in crystals, used recently in the study of turbine-blade alloys [20] and ceramics [10]. But in a brilliant collaboration with Ondrej and his new spectrometer, they were able to combine the standing-wave effect with the sensitivity of near-edge structure (ELNES) to chemical valence for site-specific valence determination in iron oxide [47]. Johann went on to use the channeling effect to study localization in EELS by a clever experimental
arrangement [43], solved the double-channelling problem (through reciprocity and a large beam divergence) and established the usefulness of ELNES with a famous series comparing spectra from octahedral and tetrahedrally coordinated Mg and K in oxides, showing how these could be distinguished [46]. All this provided an important market for future applications of Ondrej’s wonderful spectrometers.

A second parallel development related to Ondrej’s future involved my first Ph.D. student, Mark Disko, who took on a summer project with John Cowley in 1980 on Rochigrams, which eventually became crucial to the success of the Nion STEM. I’d become interested in the possibilities for Ptychography using STEM, and how this related to the theory of lattice imaging in STEM, as a result of a visit to Joachim Frank in Hoppe’s lab in Munich while I was a postdoc at Oxford around 1974. I wanted to connect Hoppe’s proposal for Ptychography [14] with the experimental observation of the coherent interference effects between overlapping Bragg orders published by [8], and it dawned on me in Oxford in 1975 that even in a dynamical treatment, scanning the beam with a detector in the overlapping region would produce a lattice image, and that there was an “achromatic circle” (on which Ondrej had found a point [21]). So I showed the relevant dynamical theory on arrival at ASU to Cowley and we wrote it up [44]. It did not dawn on me till much later that the far-out-of-focus Ronchigram was a coherent atomic-resolution shadow image (although the effect is there in our dependence of fringe period on defocus), or how this related to in-line holography and large-angle convergent-beam electron diffraction (LaCBED). Cowley later elucidated all this in a series of papers on new imaging modes for STEM, which led to him hiring Mark Disko in summer 1980 to simulate Ronchigrams [5]. At the ASU library in 1979, I’d found Malacara’s “Optical Shop Techniques” text beside the book I was actually looking for, with its fascinating chapter on Ronchigrams for testing astronomical mirrors and lenses. This I took back to show John Cowley at our morning tea. John immediately understood the usefulness of this for measuring aberrations in his Vacuum-Generators HB5 STEM (and for electron holography) as described in his papers in the early nineteen eighties. Modern autotuning methods, such as used by Nion, are based mainly on discrete components, with, for example, a complete removable printed circuit board for one binary half-adder, and an associated paperback book for the manual of each board. The retardation-field electrostatic energy filter had a bias voltage applied to it (across the high voltage) using a set of nylon strings running down into the oil tank from relays (driven by the PDP8) which operated binary switches floating at high voltage. Writing instrument-control machine code and constantly rebuilding this system with others, occasionally getting results (mainly plasmon spectra and elastically filtered Bragg scattering for quantification), constituted the four years of my Ph.D., all of us working very long hours against the background of the Vietnam war protests and the music of the Beatles. We were surprised to see the paper [34,35] reporting energy gain in the beam from interaction with previously beam-excited surface plasmons, and immediately reversed the polarity of the bias batteries in the high-voltage tank to seek this effect, without success. Now, 43 years later, the sad recent death of Ahmed Zewail reminds of the energy-gain possibility in his fascinating PINEM observations, discussed elsewhere in this volume. Later at ASU, starting around 1978, with my first student Mark Disko’s Ph.D. we developed a tight-binding theory of near-edge structure [7] and spent fruitless hours in a search for optical pumping effects from a laser on both high-resolution lattice images and inner-shell EELS spectra during Ondrej’s time at ASU. In the first case, Naoki Yamamoto and I had built a cathodoluminescence apparatus for STEM [48] for study of luminescence from individual dislocation in diamond at 25 K (this part was very successful), and this could be run backwards to illuminate the sample through a spectrometer tuned to the optical absorption edge of strongly absorbing dopants in a crystal. We expected to see the dopant atoms change appearance in the HREM lattice image when they were optically illuminated (because the low-angle electron scattering, well within the resolution of our TEM, is so sensitive to changes in valence due to ionization) but didn’t. Later calculations showed we’d have needed to melt the sample to get an effect, and a superlattice of dopants would be needed. Similar attempts to observe optical pumping effects on ELS spectra with this apparatus failed, as did our attempts to detect co-incidence spectra between EDX or CL and EELS (to reduce background in EELS), where it finally dawned on us that we could only expect the statistics of the channel with the poorest counting statistics in such experiments. All this work was done on the superb Philips EM400 TEM/STEM which we shared with Ondrej’s development of his serial EELS system amid intense discussion at morning teas.

About fifteen years after Ondrej left ASU, Dr Nan Jiang joined us from John Silcox’s group at Cornell to take up the EELS mantle. He decided to focus on time-resolved EELS in oxides, and on direct-write inorganic lithography by STEM [17]. His achievements, using the vastly improved spectrometers then resulting from Ondrej’s leadership at Gatan with Peter Swann [27] have been remarkable and too numerous to review here, but include striking maps in doped silicate glasses of the spatial variation in local atomic coordination. In these glasses, obtained from fast, low dose, pre-damage near-edge structure analysis, as the nanometer beam is scanned [16]. Most recently he has written an extensive review of radiation damage in STEM, using his time-resolved low-dose ELNES method to identify the sequence of

2. EELS

My own education in energy-loss spectroscopy (EELS) began with my Ph.D. in Melbourne, and was continued by Ray Egerton at Oxford when Ondrej was working in Cambridge on his Ph.D. My Ph.D. was devoted to detection of a second-order “double-plasmon” process [40], and, with David Johnson, to developing the logarithmic deconvolution method of removing multiple-scattering effects from EELS spectra [18], now described in detail in Ray Egerton’s book [9]. Cowley, at that time in the Physics department in Melbourne, had purchased one of the first DEC PDP8 computers in order to automate EELS data collection from our JEOL JEM 7 TEM [3]. These early “mini” computers were based mainly on discrete components, with, for example, a complete removable printed circuit board for one binary half-adder, and an associated paperback book for the manual of each board. The retardation-field electrostatic energy filter had a bias voltage applied to it (across the high voltage) using a set of nylon strings running down into the oil tank from relays (driven by the PDP8) which operated binary switches floating at high voltage. Writing instrument-control machine code and constantly rebuilding this system with others, occasionally getting results (mainly plasmon spectra and elastically filtered Bragg scattering for quantification), constituted the four years of my Ph.D., all of us working very long hours against the background of the Vietnam war protests and the music of the Beatles. We were surprised to see the paper [34,35] reporting energy gain in the beam from interaction with previously beam-excited surface plasmons, and immediately reversed the polarity of the bias batteries in the high-voltage tank to seek this effect, without success. Now, 43 years later, the sad recent death of Ahmed Zewail reminds of the energy-gain possibility in his fascinating PINEM observations, discussed elsewhere in this volume. Later at ASU, starting around 1978, with my first student Mark Disko’s Ph.D. we developed a tight-binding theory of near-edge structure [7] and spent fruitless hours in a search for optical pumping effects from a laser on both high-resolution lattice images and inner-shell EELS spectra during Ondrej’s time at ASU. In the first case, Naoki Yamamoto and I had built a cathodoluminescence apparatus for STEM [48] for study of luminescence from individual dislocation in diamond at 25 K (this part was very successful), and this could be run backwards to illuminate the sample through a spectrometer tuned to the optical absorption edge of strongly absorbing dopants in a crystal. We expected to see the dopant atoms change appearance in the HREM lattice image when they were optically illuminated (because the low-angle electron scattering, well within the resolution of our TEM, is so sensitive to changes in valence due to ionization) but didn’t. Later calculations showed we’d have needed to melt the sample to get an effect, and a superlattice of dopants would be needed. Similar attempts to observe optical pumping effects on ELS spectra with this apparatus failed, as did our attempts to detect co-incidence spectra between EDX or CL and EELS (to reduce background in EELS), where it finally dawned on us that we could only expect the statistics of the channel with the poorest counting statistics in such experiments. All this work was done on the superb Philips EM400 TEM/STEM which we shared with Ondrej’s development of his serial EELS system amid intense discussion at morning teas.

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atomic processes [15]. This exhaustive treatment concludes that a new mechanism arises at the high doses normally used, due to the intense electric field established by the tube of positive charge running through a dielectric sample, created by the ionizing beam. The resulting force on ions causes migration. Two types of insulating materials are found – those which respond with increased resistivity causing phase separation, and those in which resistivity decreases, causing phase transformation. Below a certain threshold, the conventional mechanisms of knock-on and radiolysis dominate.

Radiation damage is certainly a serious limiting factor in STEM EELS studies. In view of recent work showing that damage can be "outrun" using sufficiently brief pulses of X-ray radiation (see [42,1]), it is natural to ask if it might be out-run using a pulsed electron beam [41]. X-ray lasers have provided 0.2 nm resolution scattering from biological samples (protein nanocrystals) using 50 fs pulses, which are nevertheless destroyed following collection of the elastic scattering. The idea would be to collect the EELS data before secondary electrons cause additional ionization, or before knock-on damage occurs (despite drilling a hole in the sample with each shot!). Equally important is the goal of damage-free imaging, where sufficient elastic scattering might be collected to form an image before the onset of damage, or at least that which consists of irreversible nuclear displacements. Since a cold field-emitter emits only a few hundred electrons per picosecond, whereas the exposure time needed to out-run damage is about equal to the Debye period of 100 fs or less, it is not clear that this can be done in a single shot, despite the larger cross section for electron scattering compared to X-rays, unless by summing data from identical particles. [32] has considered the use of STEM for high-resolution imaging in biology, where he concludes that less than one electron is scattered from a pixel-sized STEM probe whose intensity lies below the damage dose normally used in cryo-EM (about 5 electrons per square cm). For a sample thin enough to avoid multiple scattering, density 5.3×10^8 electrons per square cm and 0.05 Mrad beam divergence (providing adequate spatial coherence for di

In order to avoid space-charge (Coulomb interaction) effects, which introduce unwanted beam divergence and energy spread as more electrons are packed into a sub-picosecond pulse, higher beam energies have been used, since the electric and magnetic field interactions in charged-particle beams cancel as the relativistic factor γ goes to infinity. In recent experimental work [31], the smallest beam (limited perhaps by these space charge effects) which could be formed at 3 MeV was 500 μm in diameter, with a pulse duration of 100 fs and current density 5.3×10^8 electrons per square cm and 0.05 Mrad beam divergence (providing adequate spatial coherence for diffractive imaging). Using the appropriate cross sections for elastic scattering this arrangement would scatter a total of 0.002 electrons per shot from the virus. The most favorable case, however, would match this beam diameter to the bioparticle size. Assuming this same beam (same number of electrons per shot) could be focused down to 100 nm, the number of elastically scattered electrons increases to about 50,000 a useful number. We can conclude that out-running damage using electron beams is new a problem in electron optics and brighter electron source development, a suitable final challenge for Ondrej, with the aim of minimizing Coulomb interactions sufficiently to allow formation of sub-micron beams at high energy. For fast imaging, rather than fast diffraction, we have pointed out that the hollow-cone geometry has advantages, since it allows the use of a large, extended incoherent source (packing more photons into a briefier pulse) while, as simply shown by reciprocity, also capable of atomic-resolution imaging [41].

These estimates assume that the allowed pulse duration to avoid damage is the same for X-rays and electrons. In fact electrons have an advantage by also being scattered by the more slowly moving nuclei, unlike X-rays. And if the electron dose is increased to the level at which 1% atomic displacements occur by knock-on per atom, then the above estimate can be increased by an order of magnitude. But we should note that the X-ray pulse duration limit was obtained from nanocrystals, which benefit from the coherent amplification of scattering by the Bragg process. The XFEL resolution for "single particles" is currently around 10 nm, probably limited by other factors. Simulations, however, all agree that single-particle damage is negligible at atomic resolution when using 10 fs pulses, which are currently provided by XFELs. (Again this is a destructive-readout process, in which the sample is destroyed after detecting the elastic scattering).

From the beginning, the connection between EELS data and optical spectroscopy was clear, and this was emphasized by the pioneers who based their analysis on the dielectric formulation of the energy-loss problem [6]. The Melbourne EELS group in the nineteen sixties contributed to this with an efficient algorithm for removing multiple scattering and applying the Kramers-Kronig analysis to EELS data to provide the real and imaginary parts of the dielectric function as a function of frequency ω (corresponding to energy loss hω) [19]. The analysis of Drude and Lorentz derives this function using a classical model of electrons bound to atoms, or vibrating ions. A Fourier transform from frequency to time gives, for linear materials, the time-dependent impulse response of the system. In simple cases, therefore, it is possible to extract a plot of atomic displacement against time by Fourier Transform of the dielectric function [2]. This is consistent with the relationship between EELS peak widths and the lifetime of excitations. From the uncertainty principle, the energy range of the EELS spectrum ΔE is related to the time resolution Δt by Δt = 4.14/ΔE (eV) while the time range Δt is related to the energy resolution ΔE by Δt = 4.14/ΔE (fs, eV). Millivolt energy resolution in EELS spectra was first obtained using broad electron beams [11], Ondrej's leadership has since made this possible using the sub-nm electron probe of the STEM. 10 meV energy resolution on the Nion STEM thus corresponds to a time range of 0.4 ps (several vibrational periods of an atom with Debye period of 100 fs), and a range of 30 eV to a time resolution of 0.2 fs, better than current XFELs. Using this approach, we have compared the time-dependent dielectric response of hexagonal ice with that of protein, finding that molecular oscillations are more rapidly damped in protein than in ice. A similar analysis for BaF2 (Fig. 4) using data collected on the ASU Nion STEM at 100 meV resolution with 0.15 nm diameter STEM probe showed many identifiable spectral features below 30 eV above the bandgap, including F 2p transitions, Ba 5p core transitions, band-gap excitons, color centers (created by the beam) and plasmons. The time-dependent dielectric function, plotted as a function of a single configuration coordinate (Fig. 5), shows damped oscillations corresponding to these peaks. With the improvement of energy resolution on the Nion to the level of phonon energies [26], it will be possible to apply this analysis to the localized modes associated with defects and foreign atoms using the sub-nm probe of the Nion. When extended to the thermal energy range using millivolt EELS, this may be helpful in understanding the way in which biomolecules are able to take up energy through their various excitations. The results can also provide us with an estimate of the lifetime of excitations in biological specimens. Excitation by a pulse much briefer than this lifetime is not expected to show effects of radiation damage in the resulting elastic scattering, however, irreversible damage may occur at longer times.

3. Aberration correction

The story of Ondrej’s pioneering role, first in Cambridge and later working with Nicholas Delhoy and the staff at Nion, in making aberration corrected electron microscopy in STEM a reality has been told several times [4,13]. I’ll just add one point here not previously mentioned in connection with this remarkable achievement.

The projected charged density (PCD) approximation has been almost entirely ignored since it was first proposed in 1975 [30]. It cleverly includes all multiple scattering effects within the projection approximation (flat Ewald sphere) unlike the weak phase approxima-
tion, and holds for small focus defects in the absence of spherical aberration, as can now be well approximated on modern aberration-corrected HREM machines. It provides an exact summation of the Born approximation to all orders in the approximation of a flat Ewald sphere.

With the usual symbols, the diffracted amplitude from a thin HREM sample (not STEM mode) is written (without making the weak phase approximation)

\[ F_{uv} = \Psi(u,v) = \{\exp(-i\sigma(x,y)\varphi(x,y))\exp(i\Delta f(u^2 + v^2))\} \approx \Phi(u,v) \{1 + \frac{\Delta f}{2}\} \]

for a defocus \( \Delta f \). If \( \Phi(u,v) \) and \( f(x,y) \) are a Fourier transform pair, it is well established that

\[ \delta^{-1}(u^2 + v^2)\Phi(u,v) = \frac{1}{4\pi^2}V^2f(x,y) \]

The image intensity \( I(x, y) = FT(\Psi^*(u,v)\Psi(u,v)) \) is therefore

\[ I(x, y) \approx 1 + (\Delta f\sigma(2\pi v_0))p_r(x, y) \]

where Poisson's equation

\[ \nabla^2\varphi(x,y) = -\rho_c/\varepsilon \]

has been used and \( \rho_c(x,y) \) is the projected charged density in the sample, including the nuclear contribution, not seen in X-ray charge density maps. Here \( \varepsilon \) is the dielectric constant.

This results shows that a high-resolution bright-field image in a modern aberration-corrected TEM, if slightly out-of-focus, shows a faithful map of the charge-density (not the projected electrostatic potential), projected in the beam direction, including the nuclear contribution. The contrast is proportional to the focus.

Following the "resolution revolution" in cryo-EM due to direct electron detector systems, it is natural to ask what now limits resolution in the single-particle mode, where many different projections of different copies of similar particles must be merged (and possibly also sorted by conformational changes). Factors include the ability of the software to distinguish orientational changes from conformational changes (the internal structure of a protein may change with conformation, but not with orientation, allowing these to be distinguished), beam-induced motion, further detector improvements, and curvature of the Ewald sphere. Once these are addressed, aberration-correction may also be needed for biologists, who are currently limited by the above factors to about 0.2 nm resolution. The Ewald sphere curvature is limiting, because in order to merge real-space projections in the correct orientations we rely on the projection approximation, which fails with significant curvature of the Ewald sphere (for details and conditions, see [36]). Unlike kinematic diffraction patterns, these real-space images do not show Freidel symmetry, a symmetry which makes diffraction patterns more difficult to orient. For cryo-EM tomography, rather than single-particle imaging, where many different projections are recorded from the same particle (and for sub-tomogram averaging), radiation damage imposes a more severe limit on resolution.

It has therefore been suggested, that, unlike the materials scientists studying graphene who have moved to lower energies, the biology community should move to much higher beam energies, such as 1 MeV, in order to allow merging of 3D images at higher resolution, in the assurance that each image is a true projection of the structure. Knock-on damage may not be important at the very low doses used, and this cross section is proportional to the square of atomic number, while the ratio of elastic to inelastic scattering cross sections, which controls background, is approximately independent of beam energy, so

![Fig. 4. Low-loss electron energy-loss spectra (EELS) data from BaF2 acquired using the monochromated Nion UltraSTEM 100 at Arizona State University. Convergence semi-angle 30 mrad, EELS collection angle 15 mrad. Probe size 0.15 nm and full-width at half-maximum of the zero-loss peak was 100 meV. The pre band gap, Fluorine 2p and Ba 5p electronic transition features are highlighted as peaks (a–i), notably (a) the color center peak and (b) the band-gap exciton peak.](image)

![Fig. 5. Time-dependent dielectric function of BaF2 obtained over an energy-loss range of 9 eV using only peaks (a) and (b) from Fig. 4.](image)
that this may be a good strategy. When comparing imaging of proteins in amorphous ice with high-resolution bright-field TEM imaging of graphene, the most important difference is the resistance of the covalent bonding in graphene to damage, relative to proteins. The PCD approximation given above may therefore prove valuable for single particle imaging in biology at high beam energies with aberration correction.

Recently, we have seen many protein crystal structures solved by transmission electron diffraction using cryo-EM. In view of the early pioneering work solving monolayer protein crystals, in which the onset of multiple scattering artifacts could already been seen (but was not a show-stopper), it may seem surprising that 3D protein nanocrystals can be solved, with thicknesses (or “size”) up to a fraction of a micron. Since they contain helices and are all non-centrosymmetric, the presence of Freidel symmetry in these electron diffraction patterns provides a fortunate test for single-scattering conditions, which tends to fail first in high-symmetry zone-axis beam directions (see [45] for a review). The occurrence of reflections of negligible intensity which are forbidden by space-group symmetry elements is also often used to confirm single-scattering conditions – for inorganic samples these would remain forbidden due to dynamical cancellation effects [12], however for broadly illuminated regions of bent protein crystals (which take the sample out of the dynamical cancellation window) this should remain a good test. However the use of direct methods for phasing this data will fail as the number of atoms in the protein becomes large, as the width of the Cochran distribution for phases increases. Spectacular results have recently been obtained by micro-electron diffraction for amyloids [33], whose small molecules form excellent microcrystals. The size of these is limited by the build-up of strain due to the twist in the beta-sheets. This important work has clear implications in therapy for Alzheimer’s disease.

4. Summary

In a long career, Ondrej’s remarkable energy, focus and drive have produced a string of exceptional achievements, reflecting his unusual commitment, efficient organization and use of time, together with his rare gifts as an experimental physicist and instrument designer. Worldwide, we all have reason to be grateful to him for the advances he has made possible in instrumentation, and only by being reminded of the limited performance and challenges we all confronted when starting out, some of which I have tried to indicate here, can users of modern electron microscope understand how great this progress has been. As Humphrey Davey once said in 1806 - “nothing promotes the advancement of science so much as a new instrument”.

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