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Citation: The Journal of Chemical Physics 144, 080901 (2016); doi: 10.1063/1.4941375
View online: http://dx.doi.org/10.1063/1.4941375
View Table of Contents: http://scitation.aip.org/content/aip/journal/jcp/144/8?ver=pdfcov
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Perspective: 4D ultrafast electron microscopy—Evolutions and revolutions

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(Received 9 December 2015; accepted 13 January 2016; published online 23 February 2016)

In this Perspective, the evolutionary and revolutionary developments of ultrafast electron imaging are overviewed with focus on the “single-electron concept” for probing methodology. From the first electron microscope of Knoll and Ruska [Z. Phys. 78, 318 (1932)], constructed in the 1930s, to aberration-corrected instruments and on, to four-dimensional ultrafast electron microscopy (4D UEM), the developments over eight decades have transformed humans’ scope of visualization. The changes in the length and time scales involved are unimaginable, beginning with the micrometer and second domains, and now reaching the space and time dimensions of atoms in matter. With these advances, it has become possible to follow the elementary structural dynamics as it unfolds in real time and to provide the means for visualizing materials behavior and biological functions. The aim is to understand emergent phenomena in complex systems, and 4D UEM is now central for the visualization of elementary processes involved, as illustrated here with examples from past achievements and future outlook. © 2016 AIP Publishing LLC. [http://dx.doi.org/10.1063/1.4941375]

I. INTRODUCTION

In 1912, the twenty-two-year-old Bragg ushered in a major new idea, namely, that diffraction of X-rays by a crystal is best thought of as a process of reflection from atomic planes, similar to the reflection of light from mirrors. From the structure of sodium chloride to the myriad of complex structures in biological sciences, X-ray diffraction—in a century of developments—has proven essential for elucidating the atomic arrangements, especially in systems of crystalline order.1–3

Earlier in 1897, the electron was discovered by Thomson and thought to be only a particle of well-defined mass and charge. However, in a revolutionary contribution (a Ph.D. thesis) published in 1924, de Broglie provided a universal duality relationship, with the electron, for example, being a particle and at the same time having an associated wave characterized by \( \lambda = \frac{h}{p} \), where \( p \) is the particle momentum and \( h \) is Planck’s constant. Since then, the wave character of electrons has been exploited in diffraction and imaging (Fig. 1), providing spatial resolution in real space that can reach the sub-Ångström scale. With electrons, many complex biological structures have been determined including the first membrane protein structure and recently the ribosome6 and ion channels7—from single particles without crystals! As mentioned above, X-rays have been successful for decades in determination of crystal structures.

It is fair to say that there is no significant center of research in the world that does not include Electron Microscopy (EM) in its arsenal of studies of materials and biological structures using EM’s capabilities for real-space, Fourier-space, and energy-space imaging.8 The first electron microscope, using accelerated electrons, was invented by Knoll and Ruska in the 1930s.9 The images thus obtained were “static,” i.e., time-averaged, and initially the resolution was close to that of optical microscopy, but with design improvements, especially for the magnetic lenses, it has now reached the atomic scale! Many contributions10–12 to this field have laid the foundation for advances of the fundamentals of microscopy and for recent studies of electron interferometry.13,14 Today, with aberration-corrected microscopes, imaging has reached resolutions of less than an ångström.15 A comprehensive overview of these developments is given in Ref. 8. The applications in materials science are numerous.

Similarly, biological EM was transformed by several major advances, including electron crystallography, single-particle tomography, and cryo-microscopy, aided by high-performance computing (HPC). Beginning with the 1968 electron crystallography work of DeRosier and Klug,16 3D density maps became retrievable from EM images and diffraction patterns. Landmark experiments revealing high-resolution structures from 2D crystals, single-particle 3D cryo-EM images of different, though identical, particles (6 Å resolution), and 3D cryo-EM images of a single particle (tomography with 6-Å resolution) represent the impressive progress made. More recently, another milestone in EM structural determination was reported.6,17

Cryo-EM is revealing, for the first time, the structure of mitochondrial ribosome at near-atomic resolution, and, as importantly, without the need for protein crystallization or extensive protocols of purification. In the latter case, the biological structure is massive, being 3 MDa in content, and the subunit encompasses a 39 protein complex which is clearly critical for the energy-producing function of the organelle. This was achieved with a spatial resolution of 3.2 Å using direct electron detection. Some of our first ultrafast electron diffraction (UED) experiments involved direct electron detection but with clear difference to the technology of today.18,19 Comparisons with spatial resolutions possible with X-ray lasers are discussed in Ref. 8. Recently,
in a Correspondence to Nature, Henderson commented on the overzealous claims made by some in the X-ray laser community; he pointed out the unique advantages of electron microscopy and its cryo-techniques for biological imaging. The promise and challenges of X-ray free-electron lasers (XFELs) have been highlighted by several authors and are not the focus of this Perspective. With the sub-3 Å resolution achieved in a recent cryo-EM atomic structure measurement of anthrax protective antigen pore, it is clear that EM is leading the way in the determination of macromolecular (and non-crystalline!) structures; the highlight by Kühlbrandt and the book by one of us provide the perspective and relevant references for these developments.

All the (bio)materials’ structures thus determined are averaged over time, i.e., these are quasistatic 3D structures described by the spatial coordinates \((x, y, z)\). Incorporation of the fourth dimension—the dimension of time—into EM observations accomplished at Caltech is now providing first glimpses of spatiotemporal evolution of specimen (structural dynamics). Up to 15 years ago, we would not have dreamt of reaching the ultrastable, atomic-scale temporal resolution in EM. Improving the time resolution by nearly ten orders of magnitude—while maintaining the spatial atomic resolution characteristic of a state-of-the-art electron microscope—has given rise to the whole new area of ultrafast 4D \((x, y, z, t)\) imaging research that now encompasses all three microscopy domains: real-space, Fourier-space (diffraction), and energy-space, as discussed below.

This Perspective is not a review article, but rather an overview of the evolutions and revolutions that led to the development of the field of “4D Ultrafast Electron Microscopy” (4D UEM), and with focus on its potential and future impact on other fields, such as materials and biological sciences. The 4D UEM developed at Caltech over the past 15 years began with theory and culminated in its experimental implementation, followed by further refinements. Williamson and Zewail theoretically showed in 1991 that the replacement of optical pulses by electron ones would yield structural bond dynamics of a chemical system through diffraction of its constituent atoms. It took almost a decade to achieve this goal in 2001 and to establish UED as the experimental method of choice for the study of isolated, transient molecular structures. For condensed-phase studies, we advanced, in this laboratory, ultrafast electron crystallography (UEC) as the diffraction method best suited for the determination of structural dynamics. Both UED and UEC were employed in various studies of molecular and phase transitions, and they were the precursors that led ultimately to the development of UEM. It is important to consider conceptual foundations of these precursors prior to describing UEM in greater detail.

II. UED

The determination of isolated intermediate structures formed during the course of chemical reactions was impossible because of their fleeting nature—on the time scale of a picosecond or less (see Ref. 29 for a historical perspective). In addition, these transient structures are often optically "dark" in that they undergo radiationless transitions into reactive or nonreactive channels, and in most cases, they do not emit light. With a properly chosen frame referencing, i.e., with an adequate selection of the points in time that correspond to the different states of the system under study, typically before \((t < 0)\) and after \((t > 0)\) the arrival of the exciting laser pulse, or a number of distinct points
on the positive time axis, the spatiotemporal evolution of transient structures was established. A textbook example is the transient behavior observed upon consecutive elimination of iodine atoms from 1,2-diodotetrafluoroethane (C$_2$F$_4$I$_2$) to form tetrafluoroethylene (C$_2$F$_4$) on the ultrafast time scale. Freezing the resulting intermediate in time enabled us to determine the picosecond-lived C$_2$F$_4$I$^\ddagger$ structure (Fig. 2).

Upon excitation, the diffraction pattern of the anti–gauche conformation mixture characteristic of the ground state of C$_2$F$_4$I$_2$ at $t = -95$ ps (Fig. 2(b)) was referenced to those obtained for a variety of time points corresponding to $t > 0$, and the diffraction differences were analyzed. The significance of this referencing is evident in the results of Figs. 2(e) and 2(f); a reference at positive time shows the absence of I···I nonbonded distance, as the first C–I bond breaks on the sub-picosecond time scale. The results are indicative of the nonconcerted nature of the reaction: the first step (C$_2$F$_4$I$_2$ → C$_2$F$_4$I$^\ddagger$ + I) is essentially complete within 5 ps (see evolution in Fig. 2), whereas the second step (C$_2$F$_4$I$^\ddagger$ → C$_2$F$_4$ + I) takes place with a time constant of 26 ± 7 ps. Importantly, the former step is dissociative and occurs within ~200 fs, whereas the latter step is a barrier-crossing process involving energy redistribution.

Knowing the time scales involved, two structures were considered for the intermediate C$_2$F$_4$I$^\ddagger$: a classical structure, in which primary halide (I) resides predominantly on one –CF$_2$ moiety, and a bridged structure, in which the primary halide is shared equally between the two –CF$_2$ moieties (Figs. 2(c) and 2(d)). Theoretical curves obtained for the classical structures provided an almost perfect fit to the experimental data, whereas the fit to the bridged structure was poor (Figs. 2(c) and 2(d)), thereby elucidating the nature of the intermediate: the structure of the C$_2$F$_4$I$^\ddagger$ intermediate is classical.

![UED: Elimination Reaction](image)

**FIG. 2.** Structural dynamics of the elimination reaction of C$_2$F$_4$I$_2$. (a) Reactant, intermediate, and product structures were determined under collisionless condition. (b) Shown are UED patterns of the anti–gauche conformation mixture characteristic of the reactant as obtained for the ground state; internuclear distances for the anti (black) and gauche (green) isomers are indicated by vertical bars at the bottom of the panel. (c) and (d) The structure of the intermediate is determined to be classical (c), not bridged, as evidenced by the agreement between diffraction theory and experiment; the discrepancy between theory and experiment is indicated in white. (e) and (f) Frame-referencing reveals temporal changes in diffraction with respect to the two different reference points, before (e) and after (f) the arrival of the exciting laser pulse. Note the absence of the I···I peak in panel (f). Adapted with permission from D. Shorokhov and A. H. Zewail, J. Am. Chem. Soc. 131, 17998 (2009). Copyright 2009 American Chemical Society.
Evolutions of bond distances and angles are indicative of the chemical transformation underway. The C–I and C–C distances of the intermediate [2.153(13) and 1.48(5) Å] are, respectively, longer and shorter than those of the reactant [2.136(7) and 1.534(13) Å]. These results elucidate the increased C–C and decreased C–I bond order resulting from the formation of the transient C$_2$F$_4$I$_2$ structure. Moreover, the C–C–F$^\prime$ and F$^\prime$–C–F$^\prime$ angles (Fig. 2(a)) become larger than the corresponding angles of the reactant (by $\sim 9^\circ$ and $\sim 12^\circ$, respectively), suggesting that the intermediate relaxes following the loss of the first I atom. One important conclusion is that the retention of stereochemistry is dynamical in origin (i.e., it is not caused by the electronic structural changes); bonds are broken before rotations scramble the orientation. See Ref. 27 for further details.

With the same methodology, numerous other reactions and intermediate structures have now been studied, and a number of structural dynamics determination techniques such as laser desorption, ensemble-wide orientation mapping, or resonant-scattering fingerprinting have been developed. A comprehensive UED reference list for the body of work from this laboratory is provided in the Appendix of Ref. 25. Recently, the groups of Centurion and Krausz reported on the temporal evolution of structures representative of aligned molecular ensembles, a subject that has been of intense interest at Caltech for use in optical and diffraction mapping.

III. UEC

The experimental techniques employed in the studies of isolated molecules were further developed to encompass the solid state. Due to its characteristic atomic-scale spatio-temporal resolution, UEC provides unprecedented insights into the areas of phase transitions, quantum wells, and novel materials of nanometer-length scale. Two illustrative examples of such studies are given here: the microscopic observation of temperature-induced structural (metal–insulator) transition in vanadium dioxide and the determination of transient structures of superconducting cuprates.

The UEC study of VO$_2$ was performed both on films and on single crystals. The phase transition exhibits a well-defined hysteresis between the two thermodynamically stable structures (Fig. 3(a)). In order to map pathways of motion, the Bragg diffraction spots representative of different planes and zone axes were examined on the femtosecond-to-nanosecond time scale. Because the transformation under study takes place in a strongly correlated system, the dependence on excitation fluence is evident in a threshold behavior, and such dependence has been explored at short—and long—times to elucidate the nonequilibrium transition from local atomic motions to shear at sound wave (and carrier) velocity.

Similar to many chemical reactions, the concept of concerted (or concurrent) vs. consecutive nuclear motions, which deal with reorganization of the lattice and microscopic restructuring within unit cells, is pertinent to understanding the elementary steps of the mechanism involved. The 3D sampling of diffraction—and long-range order—allows for the separation of different nuclear motions, which are mirrored in the temporal change of the structure factor for various Miller indices. For two different kinds of investigated Bragg spots (hkl), characterized by $h \neq 0$ and $h = 0$, two different types of dynamics were observed: a femtosecond process ($\sim 300$ fs) and another process with a time constant reaching 10 ps. This distinct behavior in dynamics is indicative of stepwise atomic motions along different directions. Because an atomic movement along a certain direction can only affect the Bragg spots that have nonzero contributions in the corresponding Miller indices, it has been concluded that the initial femtosecond motion is along the $a$-axis (Fig. 3(a)), which is the direction of the V–V bond in the spatial structure characteristic of the monoclinic phase.

From a chemical perspective, the excitation is to an antibonding state, which instantly results in a repulsive force exerted on the atoms as they separate along the bond direction. In sequence, and on a slower time scale, the unit cell transforms toward the configuration of the metallic, rutile phase. On the nanosecond time scale, the system attains equilibration nearly at sound wave shear motion. The observed stepwise atomic motions indicate that the phase transition follows a non-direct pathway on the multidimensional potential energy surface—i.e., it does not occur through a direct structural conversion—thus defining a transition-state intermediate for the metal-to-insulator transformation (Fig. 3(a)). Further studies of the above system were reported recently.

The second example representative of nonequilibrium structural phase transitions is that of superconducting cuprates. The specific material studied is oxygen-doped La$_2$CuO$_{4+\delta}$ (LCO); the undoped material is an antiferromagnetic Mott insulator, whereas the doping confers superconductivity below 32 K and metallic properties at room temperature. From the Bragg spots observed in UEC, the unit-cell parameters were determined using the patterns associated with different zone axes. Structural dynamics were then obtained by recording the diffraction frames at different time delays, before and after the arrival of the optical excitation pulse. What was expected, in line with earlier UEC studies from this laboratory, was that the peak would shift continuously and the intensity would decrease with time. Instead, all the profiles obtained at different times appear to cross at a single value of $s$-coordinate, which parameterizes the momentum-transfer space. This intensity sharing with a common crossing point, a structural isosbestic point, is indicative of a transformation from the initial phase to a new (transient) phase.

The structural interconversion associated with isobestic point observation is schematized in Fig. 3(b). The diffraction difference profile, as a function of time, reveals the depletion of initial structure and the accumulation of the transient-phase structure. The population of the initial (transient) phase decays (builds up) with a time constant of 27 ps, but the newly formed phase restructures on a much longer time scale (307 ps). Because the linear expansion coefficient is $\alpha_{1} \leq 1.0 \times 10^{-5}$ K$^{-1}$, the observed 2.5% increase in the lattice constant would correspond to an unphysical 2500 K rise in the lattice temperature at equilibrium. Another striking feature of the structural phase transition is its dependence on the fluence of the initiating laser pulse. A threshold was observed, above
which the lattice constant of the transient-phase structure was changing linearly with the fluence (Fig. 3(b)).

At 1.55 eV, the transformation\(^{44,45}\) is the result of a charge transfer from oxygen (O\(^2\)\(^-\)) to copper (Cu\(^{2+}\)) that occurs in the \(a-b\) copper–oxygen planes, as stated in the literature. With the lattice relaxation being involved, the excitation is shared microscopically (exciton type), and subsequently a transition to a transient phase is accomplished (macroscopic domain). In the transient phase, the net charge distribution leads to weakening of the interplanar Coulomb attractions, resulting in a pronounced expansion along the \(c\)-axis. The behavior is nonlinear in that, when the number of transformed sites is below a critical value, the macroscopic transition is not sustainable. The crystal domain is greater than 20 nm\(^2\), and symmetry breaking is not evident because charge transfer can only occur in a plane perpendicular to the \(c\)-axis expansion. By consideration of Madelung energy and charge distributions, both linear fluence dependence and pronounced anisotropic lattice expansion were accounted for.

The transient-phase structures, which are inaccessible by means of equilibrium methods, cannot be detected using optical probes with wavelengths longer than typical lattice spacings. Moreover, the time scales of optical response on the one hand and structural changes on the other are very different. For the cuprate studied, the observed phase transition is the result of electron density redistribution and collective lattice interactions to form domains. The apparent similarity of thresholds observed for the “photon doping” of \(\sim 0.12\) photons per copper site and for the “chemical doping” corresponding to fractional charge of 0.16 per copper site—required for superconductivity—may have its origin in the nature of the photoinduced inverse Mott transition. If general, the implications are significant.

FIG. 3. Ultrafast dynamics of structural phase transitions. (a) Bragg diffractons of different directions and zone axes, when temporally resolved, indicate that the insulator-to-metal transition between the monoclinic and tetragonal structures of vanadium dioxide (upper left) occurs stepwise on the femtosecond and picosecond time scales, as depicted in the two-coordinate energy landscape (upper right). The full temporal behavior is illustrated at the bottom. (b) Shown is the structural phase transition in oxygen-doped cuprate, La\(_2\)CuO\(_4\)+\(\delta\). Displayed are schematic phase diagram (upper right) and the fluence dependence of the structural change (lower right) for La\(_2\)CuO\(_4\)+\(\delta\). The temporal evolution of the referenced diffraction profile (left) indicates the depletion of the initial structure and the buildup of the transient-phase structure. Note the presence of the isosbestic point at \(\sim 4.76\) Å\(^{-1}\) and the threshold at \(\sim 5\) mJ/cm\(^2\), which corresponds to a critical value for the number of photons (0.12) per copper site. Adapted with permission from D. Shorokhov and A. H. Zewail, J. Am. Chem. Soc. 131, 17998 (2009). Copyright 2009 American Chemical Society.
More recently, the above studies were extended to other cuprates of different structures and chemical composition, and to various doping levels. In these studies, variable polarization of the exciting laser pulse was used to elucidate the physics. It was concluded, at least for these systems, that the electron–phonon coupling was a significant player in structural changes and in electron correlation. Such condition is at the heart of the transformation mechanism characteristic of high $T_c$ materials—phonons vs. magnetic interactions.\textsuperscript{46,47}

IV. UEM

Building on the leaps forward made in the areas of UED and UEC, an effort was taken to extend the methodology to UEM—but with no success. In order to attain the atomic-scale temporal resolution in UEM, a new way of thinking was required!\textsuperscript{48} First, we had to overcome the limitations associated with the slow response of detection-system electronics of electron microscopes (the millisecond regime)—or the nanosecond regime characteristic of using intense optical pulses (see Ref. 48 for a historical perspective). Second, the probe pulses encompassing millions of electrons, as in UED and UEC, would not produce images of desirable spatial resolution because electrons would repel each other on their way through the microscope column, resulting in substantial “defocusing” in the image plane of the objective lens. Third, the spatiotemporal responses had to be characterized \textit{in situ} on the scales of ångströms and femtoseconds. These hurdles could only be overcome by the development of \textit{single-electron imaging},\textsuperscript{48} and by exploiting “ultrashort optical pulses” in simultaneous generation of electron packets for imaging and electronic excitation (or temperature jump) initiating the dynamical change in the specimen. Gating of electron pulses\textsuperscript{49,50} or compression with RF\textsuperscript{50} can make them accommodate large numbers of electrons.

In UEM, we are not limited by the microscope’s CCD detector response! This is because the temporal resolution is determined by the duration of the pulses and the delay between them. The first ultrafast pulse, called the pump pulse, initializes the structural change and thus sets the experimental clock “at zero.” The second laser pulse, called the probe pulse, generates an electron beam through the photoelectric effect; the electrons arrive at the specimen after a time delay and record a snapshot of the process under study at that particular point in time. In order to gather information about different stages of the change, the probe records successive images at different time delays. To control, and manipulate, the time delay between the pump and the probe pulses, we initially tune the optical system so that both pulses reach the specimen at the same time. We then divert the probe pulse so that it travels a longer distance than does the pump pulse before it reaches the specimen (Fig. 4). If the electron-generating optical pulse travels $1 \mu$m farther than the pump pulse, it is delayed by 3.33 fs. Probe electron pulses can picture structural dynamics through the change in real-space image contrast, Bragg or Kikuchi diffraction, or the electron energy loss (or gain), as discussed below.

We note that unlike in the case of single-object photography, e.g., that of a galloping horse, here we have to synchronize the motions of numerous independent atoms—or molecules—so that all of them arrive at a similar point during the course of their structural evolution when a snapshot of the entire ensemble is taken; to achieve such synchronization for millions—or billions—of objects under study, the relative timing of the clocking (pump) and probe pulses must be of ultrashort temporal precision, and the launch configuration must be defined to a sub-ångström resolution. Unlike with photons, in imaging with electrons, we must also consider the consequences of the Pauli exclusion principle. The maximum number of electrons that can be packed into a state (or a cell of phase space) is two, one for each spin; in contrast, billions of photons can be condensed in a state of the laser radiation. This characteristic of electrons represents a fundamental difference in what is termed the \textit{degeneracy}, or the mean number of electrons per cell in phase space. Typically, it ranges from $10^{-4}$ to $10^{-6}$ but in UEM, it is possible to increase the degeneracy by orders of magnitude, a feature that could be exploited in quantum electron optics investigations.\textsuperscript{8}

Besides these requirements, we must also achieve atomic-scale resolutions for structural dynamics in both space and time. Single-electron imaging\textsuperscript{48,51} (Fig. 4) is the key concept behind the success of 4D UEM as the repulsion between electrons is negligible; thus, the atomic-scale spatiotemporal resolution can be achieved. Attaining the atomic scale resolution in both the spatial and temporal domains is of pivotal significance in the observations of structural dynamics. Atomic motions, phase transitions, nanomechanical movements, and the nature of fields at interfaces are examples of phenomena that have now been charted in unprecedented structural detail, and at a rate that is ten orders of magnitude faster than hitherto (for a recent review article, see also Ref. 52). Furthermore, as stated above, UEM yields information in three distinct ways: in real space, in reciprocal space, and in energy space, all with the structural changes being followed on the femtosecond time scale.

The concept of single-electron imaging is based on the premise that coherent and timed single-electron packets (Fig. 4) can provide an image equivalent to that obtained using continuous electron beams in conventional microscopes. Unlike the randomly distributed—in space and time—in UEM the packets are timed with an ultrashort temporal precision, and each electron has a unique coherence volume. Putting it in Dirac’s famous dictum, \textit{each electron interferes only with itself}. As such, each electron of finite de Broglie wavelength is (transversely) coherent over the object length scale to be imaged, with a longitudinal coherence length that depends on its velocity. On the detector, the electron produces a “click” behaving as a classical particle, and—when a sufficient number of such clicks are accumulated stroboscopically—the whole image of the specimen emerges, and movies representing its structural dynamics can be made. This was the idea realized in electron microscopy for the first time at Caltech. By contrast, in the single-pulse mode of operation, with its Coulomb repulsion broadening, each frame is recorded with a pulse consisting of $10^{4}$–$10^{6}$ electrons.
One has the freedom to operate the apparatus in either single-electron or single-pulse mode. When an electron is accelerated in a 100 kV microscope, its wavelength approaches $4 \times 10^{-2}$ Å (the picometer scale), a distance far shorter than that separating individual atoms in crystals or in isolated molecules. The spatial resolution of state-of-the-art electron microscopes now extends all the way to the sub-Ångström domain, and new approaches for imaging studies of catalysis and environmental EM have been pioneered by Gai and Boyes. Liquid-cell EM has also been successful in the studies of nanomaterials by Alivisatos and colleagues, and of biological cells by de Jonge and others.

Essentially, all the variant techniques of 4D EM have now been reported (Fig. 5); currently, these include tomography, stereography, convergent-beam (CB) imaging, electron energy loss spectroscopy, scanning microscopy, conical...
scanning, environmental microscopy, Lorentz microscopy, cryo-microscopy, and single-particle imaging. Also, a multitude of ultrafast phenomena have been uncovered, which led to the realization of novel experimental techniques. Thus, e.g., the newly discovered photon induced near-field electron microscopy (PINEM) approach is well suited for imaging nanostructures and their near electric fields in both space and time. 4D EM applications are wide-ranging; to date, these include direct visualization of atomic motions in nanomaterials, mechanical motions of nanostructures, phase transitions, melting and crystallization, and macromolecular assemblies of polymers and biological structures (e.g., proteins and DNA). In what follows, we select from the studies summarized in Fig. 5 a few experimental techniques—and their respective applications—to highlight the impact of 4D EM development on a number of key areas of research, such as materials science and biological imaging (Fig. 6).

**A. Single-particle imaging**

In order to enable the studies of single particles of nanoscale, we developed 4D nanodiffraction imaging technique utilizing convergent (pulsed) electron beams. Instead of using a parallel electron-beam illumination with a single-electron wave vector, a CB with a span of incident wave vectors is tightly focused on the specimen. This method of CB UEM affords determination of 3D structures with high precision for local areas, reaching below one unit cell. The left panel of Fig. 6(a) displays the concepts pertinent to CB-UEM, together with a typical zero-order Laue zone disk and a sample nanodiffraction frame. By following the individual spots in the ring as a function of time, the dynamics of the probed nanoarea of the specimen are obtained. For silicon, the structural dynamics (rate of 0.14 ps\(^{-1}\)), the temperatures (rate of 10\(^{14}\) K/s), and the amplitudes of atomic vibrations (up to 0.084 Å) are all obtained for the local site probed (10–300 nm), and not for the bulk material. Importantly, CB-UEM and its variant techniques are equally suited for the studies of structural dynamics of single particles and their heterogeneous assemblies.

More recently, we applied parallel-beam electron pulses to realize combined real- and reciprocal-space probing of single, isolated nanoparticles on the intrinsic length- and time scale of chemical phase transitions. To avoid inflicting damage on radiation-sensitive samples, the electron beam was spread over the entire specimen. Attaining the single-particle selectivity is required to obtain well-defined diffraction patterns. Introducing a small aperture in the image plane of the objective lens (Fig. 6(a), center) enables one to select a single particle to observe. The proof of principle was first demonstrated in the spatiotemporal visualization of single-spin crossover in nanoparticles of Fe(pyrazine)Pt(CN)\(_4\), for which most optical and X-ray studies have dealt with the dynamics of either molecules in solution or nanoparticle ensembles (or bulk crystals).

Another UEM technique which provides visualization of single-particle dynamics of a polycrystalline heterogeneous ensemble undergoing phase transitions is that of conical-scanning dark-field imaging. The approach was first applied in a study of ultrafast metal-insulator transition in VO\(_2\), which was induced using laser excitation and followed in time by recording electron-pulsed images and diffraction patterns. The single-particle selectivity was achieved by identifying the origin of all the constituent Bragg spots on Debye-Scherrer rings representative of the ensemble (Fig. 6(a), right). Orientation mapping and dynamic scattering simulations of electron diffraction patterns of the monoclinic
and tetragonal structures of VO$_2$ were in good agreement with the temporal change observed for Bragg spots during the transition. It was found that the threshold temperature for recovery increases with increasing particle sizes, and the observation was quantified through a theoretical model developed for single-particle phase transitions. The above methodology of conical-scanning, orientation mapping in 4D imaging is best tailored for the studies of heterogeneous ensembles, as it enables imaging and diffraction measurements at a given time with a full archive of structural information for each particle—for example, size, morphology, and orientation—while minimizing radiation damage to the specimen. Single crystals are not required and a large number of particles can be studied simultaneously.

B. PINEM discovery

The phenomenon and the nanostructure imaging method associated with it have been discovered serendipitously! Photon-electron coupling is the basic building block of the PINEM effect which takes place in the presence of nanostructures, provided that the energy-momentum conservation condition is satisfied. This coupling mechanism leads to inelastic gain—or loss—of photon quanta by electrons in the electron packet, which can be resolved in the electron energy spectrum. The spectrum then displays discrete peaks separated by multiples of the photon energy $\pm n\hbar\omega$, where $\hbar\omega$ is the energy of the laser photon; $n = 0, 1, 2, \ldots$ is an integer reflecting the number of photons, and $\pm$ denotes the energy gain (absorption of photons) or loss (emission of photons) by the electron, on the higher and lower energy sides of the zero loss peak (ZLP), respectively.$^{56}$ In PINEM, a photon of wavelength $\lambda$ excites a nanoobject of a characteristic dimension $d \ll \lambda$, giving rise to the evanescent near-field that turns into electromagnetic waves in the far-field. In our experiments, an ultrashort optical pulse induces the near-field and—simultaneously—an ultrashort electron pulse images the field through inelastic scattering of ultrafast electrons.

FIG. 6. Selected experimental techniques and their applications illustrating the power of 4D UEM. (a) The selection made here is for the convergent beam, single particle (aperture) imaging, and the conical scanning techniques. (b) Essential physics of the PINEM phenomenon and materials imaging with PINEM. (c) Examples of biological imaging. See text.
the field’s longitudinal component (z), that is, the component parallel to the trajectory of incident electrons (Fig. 6(b)).

It is impossible for the electron and photon to interact in the absence of the nanostructure, which provides spatial localization sufficient for momentum conservation. The extent of the spatial localization in the longitudinal direction needed for momentum conservation can be estimated by considering the uncertainty relationship between $\Delta z$ and $\Delta p$ ($\Delta z \Delta p \sim h$). Assuming that both $\Delta z$ and $\Delta p$ are normally distributed, for the condition of $\Delta p = \hbar \omega / v$ to hold, a spatial confinement with a full-width-at-half-maximum of $\Delta z = 2\pi / \omega$ is required. This gives $\Delta z < 350$ nm for photons with $\hbar \omega = 2.4$ eV and electrons traveling with $v = 0.7c$. It follows that a nanoobject having a dimension smaller than this value generates a scattering potential that has a significant $\omega / v$ and makes the incident electrons inelastically couple to the field. A semiclassical representation of the PINEM effect was demonstrated in solving the Schrödinger equation for an ultrafast electron in the presence of the field.60,61

Fig. 6(b) illustrates perspective applications of PINEM imaging in materials science. We observed that when two nanoparticles were brought in close proximity to one another, as shown in the right panel of Fig. 6(b), “entanglement” took place through their fields (the total electric field at any point in space and at a given time $t$ is the coherent vector sum of the particles’ fields). At separations larger than the field decay length for a single particle, the electric fields do not interact significantly, and the observed images are those of two separate dipoles. On the other hand, when particle separation becomes comparable to, or smaller than, the field decay length, the near-fields interfere and channels open up in the space between the particles. Because the shape and width of such channels depend on the spatial separation and polarization, for an assembly of particles, one can manipulate the entanglement channel directions and presence.62 Overall, the development of PINEM enables visualization of the spatiotemporal dielectric response of nanostructures, visualization of plasmonic fields and their spatial interferences, imaging of low-atomic-number, nanoscale materials, characterization of ultrashort electron packets, and imaging of various biological assemblies.63–66

C. Biological 4D-EM

Determination of time-averaged biomolecular structures is important and has led to an impressive list of achievements, for which more than ten Nobel Prizes have been awarded, but the macromolecular structures relevant to biological function are those that exist in the non-equilibrium state. Understanding their behavior requires an incorporation of the trilogy: structure, dynamics, and function. In Fig. 6(c) displayed are a micrograph of a catalase protein crystal (with the lattice-plane separations of 9.3 nm), image of Caulobacter crescentus (CC; the image was obtained by cryo-microscopy), and a 3D tomographic reconstruction—and vibrational motion analysis—of an isolated amyloid beam (relevant to Alzheimer’s).

In the latter case of amyloids, having defined the shapes of three distinct fibers, we then recorded the evolution of beam images with time.67 The space–time evolution of each beam was revealed by a time series of referenced difference images of the oscillating beams, in particular, for motions of the beam tips. It is important to note that the dynamics were obtained for a “single beam” and not for an ensemble of beams, which would provide an average; this may be the cause of the dispersion of literature results.68 Importantly, in the above study of amyloids by imaging and diffraction we succeeded in determining both the elasticity of individual fibers (Young’s modulus, $Y$) and anisotropy of the bonding strength. Given that cell membranes are highly flexible ($Y \sim 1$ MPa) and that amyloid beams were found to be exceptionally stiff ($Y \sim 1$ GPa), the extracellular deposition of intractable amyloid plaques is likely to stiffen normally elastic or contractile tissues. In addition, it has recently been shown that amyloid fibrils distort cell membranes, which may lead to leakage and ultimately cell death, and this may be a pathological consequence of the inflexibility of amyloid’s cross-$\beta$ structure.68

In other studies involving image contrast enhancement, the PINEM effect was invoked to visualize the membrane of an Escherichia coli bacterium.63 An ultrashort laser pulse generated an evanescent electromagnetic field in the cell membrane at time zero. By collecting only the imaging electrons that gained energy from the field, the technique produced high-contrast, relatively high-spatial resolution snapshots of the membrane. Importantly, the method can capture events occurring on very short time scales, as is evidenced by the field’s significant decay after 200 fs (the field vanishes by 2000 fs). More recently, time-resolved cryo-EM has been successfully introduced in the 4D EM studies of amyloids.67–69 In parallel, theoretical efforts have been launched at Caltech8,35,70–74 to explore the areas of research pertaining to biological structures, dynamics, and the energy landscapes, with focus on the elemental processes involved. Other studies have addressed the nanomechanical properties of DNA networks.75

V. OUTLOOK

Over the past 15 years, developments and discoveries involving 4D EM have taken the field of imaging way beyond the known temporal and spatial resolutions of conventional electron microscopes—the speed has increased by ten orders of magnitude and both resolutions are at the atomic scale. The discovery of the PINEM phenomenon has opened up the field to applications in both plasmonics and photonics.76 Future directions of exploration will involve three major areas of research: variant techniques (such as electron holography), materials science (such as structural and nanomechanical dynamics), and biological imaging.

In materials, the capability to examine structural dynamics at various temperatures and pressures, and for individual (single) particles, rather than for large-scale heterogeneous ensembles of nanostructures, makes it possible to visualize the elementary stages of microscopic transformations—and hence identify origins of the associated macroscopic (bulk) properties of matter. Moreover, for a variety of nanoscale devices, such as nano-electromechanical systems (NEMS) and micro-electromechanical systems (MEMS), it is important to verify
FIG. 7. Ultrafast “optical gating” of electrons using 3-pulse sequence in PINEM. (a) PINEM spectrum at $\tau_1 = 0$ fs, which consists of discrete peaks on the higher and lower energy sides of the zero loss peak (ZLP) separated by multiple photon-energy quanta (2.4 eV). The shaded curve presents the normalized ZLP measured at $\tau_1 = 1000$ fs. (b) PINEM spectrogram of photon-electron coupling of the first optical and electron pulse as a function of the first optical pulse delay ($\tau_1$). The ZLP area between $-1.5$ eV and $1.5$ eV has been reduced for visualization of the adjacent discrete peaks. Optical gating is clearly manifested in the narrow strip corresponding to the width of the optical pulse ($210 \pm 35$ fs) shown in red in the vertical plane at right, which is superimposed on the ultrafast electron pulse (1000 fs) in blue. The material studied is vanadium dioxide nanoparticles which undergo insulator-to-metal phase transition when appropriately excited.\textsuperscript{49} Adapted with permission from M. T. Hassan et al., Proc. Natl. Acad. Sci. U. S. A. 112, 12944 (2015). Copyright 2015 National Academy of Sciences.\textsuperscript{49}

FIG. 8. Infrared PINEM. (a) and (b) Electron energy spectra obtained at $t = 0$ in the diffraction mode at the interface between vacuum and copper grid with two different wavelengths of the optical pulse (note the identical beam size (130 nm) and two-orders-of-magnitude fluence difference used in visible (a) and infrared (b) optical pulse based experiments). (c) Shown is the zero-loss energy spectrum obtained in the diffraction mode at $t = -3$ ps, demonstrating the energy resolution of 0.63 eV as determined by the Gaussian fit (solid line). (d) Time delay dependence of the first and second PINEM peak amplitudes. The solid lines are Gaussian fits (FWHM: 910 fs, 640 fs).\textsuperscript{88} Adapted with permission from H. Liu, J. S. Baskin, and A. H. Zewail, Proc. Natl. Acad. Sci. U. S. A. (in press). Copyright 2016 National Academy of Sciences.\textsuperscript{88}
their efficiency and functionality, and 4D EM studies of such devices are invaluable in doing so.

On the biological front, one problem that can now be tackled using the already developed cryo-4D EM is folding/unfolding in proteins. A glassy (noncrystalline) ice holds the protein. For each shot of the movie, a laser pulse melts the ice around the sample, causing the protein to unfold in the warm water. The movie records the protein refolding before the water cools and refreezes. The protein could be anchored to the substrate to keep it in the same position for each shot.

In this laboratory, we are also exploring a variety of aspects pertinent to radiation damage inflicted by the incident electrons, including its possible attenuation through usage of probing electron pulses of given shape and delay time.

The overwhelming complexity associated with the ensemble-wide macromolecular (un)folding behavior renders theoretical simulation a partner tool for understanding of the full nature of the energy landscape. Model systems representing the influence of hydrophobicity are also essential. Novel coarse-graining approaches must be employed to elucidate the essential ensemble-wide aspects of structural change, while preserving the mechanistic nature of the dynamics (e.g., cooperative motion or resonance phenomena). Our "4D computational microscopy" approach, which is based on the adequate degree of coarse-graining and the ensemble-convergent numerical simulations, enables studies of structural dynamics of complex systems in real time with atomic-scale spatial resolution, and with adequate statistical certainty and signal-to-noise ratio that are comparable to those of experimental observations.

For electrons in 4D UEM, the challenge is to push the limit of the temporal resolution into the sub-femtosecond domain, the attosecond regime. For light (photon) pulses, this has already been achieved and for electron pulses, several schemes—including temporal optical-grating, tilted-pulses, and temporal-lens methodology—have already been proposed and discussed in the review article by Baum and Zewail.

One well-known technique is that of microwave compression of electron pulses, which has recently been applied in a femtosecond electron diffraction setup. A novel “all-optical” method for compression directly to the attosecond domain involves the creation of “temporal lenses” made by ultrashort laser pulses. The technique relies on the ponderomotive

![Spatial & Temporal Domains of UEM](image_url)

**FIG. 9.** Resolutions in space and time achieved in electron microscopy. The focus here is on the comparison of ultrafast electron microscopy (UEM) and transmission electron microscopy (TEM), but other variants of the techniques (scanning EM, tomography, as well as electron spectroscopy) can similarly be considered. The horizontal dimension represents the spatial resolution achieved from the early years of EM to the era of aberration-corrected instruments. The vertical axis depicts the temporal resolution scale achieved up to the present time and the projected extensions into the near future. The domains of “fast” and “ultrafast” temporal resolutions are indicated by the areas of high-speed microscopy (HSM) and ultrafast electron microscopy (UEM). Care should be taken in not naming the HSM “ultrafast electron microscopy.” See text. Adapted with permission from A. H. Zewail, Science 328, 187 (2010). Copyright 2010 AAAS.
force—or ponderomotive potential—that influences electrons when they encounter an intense electromagnetic field.

Very recently, at Caltech, a new variant of PINEM has been developed, which constitutes a breakthrough in electron-pulse slicing and imaging. In all the previous experiments conducted in 4D UEM, a single optical pulse had been used to initiate the change in the nanostructure. In a recent report,\textsuperscript{49} based on the conceptual framework given in Ref. 64, we utilized two optical pulses to generate excitation and one electron pulse to monitor the structural change. The implementation of this pulse sequence led to the concept of “photon gating” of the electron pulses as shown in Fig. 7. We note that the sequence gives rise to an electron pulse width limited only by the optical-gate pulse width. A picosecond electron pulse was shown to compress into the femtosecond width of the exciting optical pulse. This is a very important leap forward with the potential for attaining the attosecond time domain and with numerous perspective applications in the field of 4D materials visualization.

Another method that was developed recently is that of infrared PINEM (Fig. 8), with diffraction detection and unprecedented UEM energy resolution (0.63 eV, Fig. 8(c)).\textsuperscript{88} Because most biological systems do not absorb in the visible, this methodology is ideal for such studies with the two orders of magnitude enhancement in the IR PINEM signal intensity (Figs. 8(a) and 8(b)). With the optical gating of electron pulses and IR capabilities in UEM, it is now possible to examine electron-sensitive materials, and with at least an order-of-magnitude improved time resolution; both directions of research are currently being explored in this laboratory.

With this in mind, there exist clear-cut limits separating “fast” and “ultrafast” imaging capabilities, and these boundaries are sometimes overlooked in the literature (see references in Ref. 76 and also Ref. 89). Depicted in Fig. 9 are the spatial and temporal dimensions of 4D UEM, and—for comparison—those of TEM. The time-resolution boundaries are representative of the transition from the millisecond (video) temporal resolution typical of TEM, to the fast—or “high-speed”—nanosecond-to-microsecond microscopy, and on to the ultrafast, femtosecond-to-attosecond imaging regime, which currently represents the state of the art. As stated above, the spatial resolution in the nanosecond imaging domain indicated in the figure is limited by the electron-electron (space-charge) repulsion characteristic of the nanosecond pulses of electrons. We note that the UEM landscape is that of single-electron imaging, which, owing to the absence of inter-electron repulsion, attains the spatial resolution of the TEM, but with the temporal resolution extending all the way to the ultrafast time domain.

The field is now well established with laboratories around the world, including those in Minnesota, Purdue, Harbin, Osaka, Giza, Lausanne, Lansing, Göttingen, Ulsan, and Stockholm, among others, using UEM as the method of choice for imaging in space and time. Furthermore, electron microscopy is arguably among the most powerful imaging techniques\textsuperscript{90–93} for the study of physical and biological structures, and including complex molecular machines. In his visionary highlight published back in 1991, Sir Thomas stated “If the experiment does indeed prove successful . . . it will mark the dawn of an important new era in structural chemistry.”\textsuperscript{94} Following the accomplishments made at Caltech, he wrote a piece with the title “A Revolution in Electron Microscopy.”\textsuperscript{90} On the X-ray side, we have already mentioned the potential and challenges of future XFELs.\textsuperscript{4,20–23} Chergui, in a recent Perspective, discusses the dynamical X-ray research direction with a thoughtful overview, which includes comparison with developments in electron imaging—UEM and related areas.\textsuperscript{95} We hope this Perspective will inspire the community, especially the young scientists, to join this burgeoning field of 4D visualization of matter and to take part in the race—not only against time but also for the highest resolution in space!

**ACKNOWLEDGMENTS**

The research summarized in this contribution had been carried out with support from the National Science Foundation (No. DMR-0964886) and the Air Force Office of Scientific Research (No. FA9550-11-1-0055) in the Physical Biology Center for Ultrafast Science and Technology (UST), which is supported by the Gordon and Betty Moore Foundation at Caltech. The contributions made by colleagues in this laboratory are highlighted in the references given, and without them this Perspective would not have been possible.

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Chem. Phys. 144, 080901 (2016)