Electron Tomography

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Electron Tomography: From 3D Statics to 4D Dynamics

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Human beings are used to seeing in three dimensions—we are born to live in a three-dimensional (3D) world. As we live, time is the fourth dimension that we do not feel, but experience. This four-dimensional (4D) observation and experience is so obvious in our daily life, yet it is not apparent in physics, chemistry, and biology, and especially not for details at the nanoscale at sub-millisecond time intervals. We need additional instruments for observation and recording. The transmission electron microscope (TEM), one of the two most powerful imaging instruments with resolution attainable to below 0.1 nm, produces only two-dimensional images of a nano-object (either biomolecules, viruses, or inorganic materials). The third-dimensional information of the object along the direction of the incident electron beam is lost by projection, along with any time-resolved information at a sub-millisecond timescale.

There have been several biologists, biophysicists, and biochemists who were pioneers in the "retrieval" of 3D information from two-dimensional (2D) TEM projections. Already in 1960s, Klug et al. reconstructed 3D biostructures of high symmetry from one or more projections,[1] and Hoppe et al. reconstructed asymmetric protein structure from a sufficient number of projections.[2] In 1968, Hart reconstructed the 3D structure of the tobacco mosaic virus using a recorded tilt series of images with a resolution of 0.3 nm,[3] and Gordon et al. introduced an algebraic reconstruction technique (ART) that could handle completely asymmetric objects.[4] Many theoretical refinements followed, for example, image reconstruction from projections by Zwick and Zeitler,[5] and reconstruction with orthogonal functions by Zeitler.[6] Although 3D electron tomography has remained a field of research in biology since that time,[7] its spread into other fields only started in this decade.[8] The driving force is the rapid development of nanoscience and catalysis,[8,9] where the 3D morphology of a nano-object and the spatial distribution of supported nanocatalysts become essentially important to understand certain physical properties of the nanodevices, or to develop catalysts with designed structure and performance. Advances in electron microscopy, and especially the availability of large-area charge-coupled device (CCD) cameras, microscope automation, and finally the advances in computational methods were required to bring the field into the state-of-the-art in high-resolution electron tomography with applications in various fields.[10] It is remarkable when we remember that Hart could only use 12 tilted images for the reconstruction.[3]

Due to their tubular morphology and high-aspect radio, carbon nanotubes (CNTs) induce peculiar properties for materials trapped inside (the confinement effect).[11] Although the selective deposition of metal particles only inside CNTs is already highly demanding,[12] determination whether the particles are really inside or simply outside the tubes remains even more challenging. A 2D electron micrograph does not distinguish particles inside or outside the tube; the third-dimension information along the electron beam is lost (Figure 1 A). However, a 3D tomogram can be reconstructed from a tilt series of 2D electron micrographs, thus revealing the spatial distribution of nickel nanoparticles inside or outside the CNT.[13]

So far so good, but the image and the tomogram in Figure 1 (and in all of these studies to date) is obtained from a static object that represents the time-averaged equilibrium state of the structure. Any dynamics, for instance the "breathing motion" of the supporting CNT or a transient process of the particles, if any, are lost in such an experiment. In a recent paper published in *Science*,[14] Zewail et al. realized 4D electron tomography for the first time. They integrated the time dimension into the electron tomogram, thus allowing real-space and real-time visualization of dynamics of nano-objects. This process requires the recording of various 2D projections of an object (the conventional 3D tomography) at a given time with a time resolution that is high enough to capture any transient process of the object. The reconstructed 3D tomogram obtained as a function of time gives then the 4D tomogram. The breakthrough was the ability to obtain the high spatial resolution of conventional electron microscopy but simultaneously enable the temporal resolution of atomic-scale motion.[15]

As conventional TEM could not provide any temporal information down to the submillisecond timescale, Zewail and co-workers have developed ultrafast electron microscopy (UEM) by combining ultrafast lasers to a modified electron microscope.[16] The technique is based on the fundamental concept of timed, coherent single-electron packets, or electron pulses, which are liberated with femtosecond dura-

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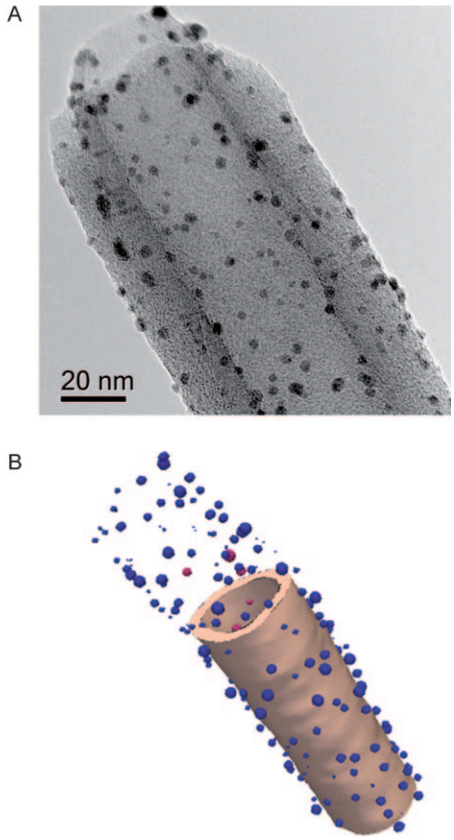

Figure 1. A) A typical 2D TEM image of a carbon nanotube (CNT) with nickel nanoparticles. The information as to whether the nickel particles are outside or inside the tube, is lost because of the projection. B) Reconstructed tomogram from a titled series. Pink: CNT, red: Ni particles inside the tube; blue: Ni particles on the external surface. Reproduced from Ref. [13] with permission.

tions.[17] This new UEM has been successfully applied to study time-resolved image and diffraction,[18] and time-resolved electron-energy-loss (or gain) spectroscopy.[19]

For 4D electron tomography, the time dimension is then integrated into any electron tomogram that spans a whole tilt series. These simultaneous real–space and real-time-resolved images are obtained stroboscopically with single-electron coherent packets. As is shown in Figure 2 A, a specimen tilt arrangement is configured in an UEM to enable the recording of various 2D projections of an object at a given time. The frames are taken for each degree of tilt with time intervals of femtoseconds or nanoseconds, as dictated by the timescale of the motions involved. The concept is illustrated in Figure 2 B, which depicts the construction of tomograms from the 2D projections at different angles and times. Because of the various dimensions involved, at a given time, each 2D projection represents a 3D frame (including time), whereas a 3D tomogram when constructed from all the 2D projections represents a 4D frame.

4D electron tomography allows the constitution of movies of objects in motion, thus enabling studies of nonequilibrium structures and transient processes. The method was demonstrated using carbon nanotubes of a bracelet-like ring structure for which 4D tomograms display different modes

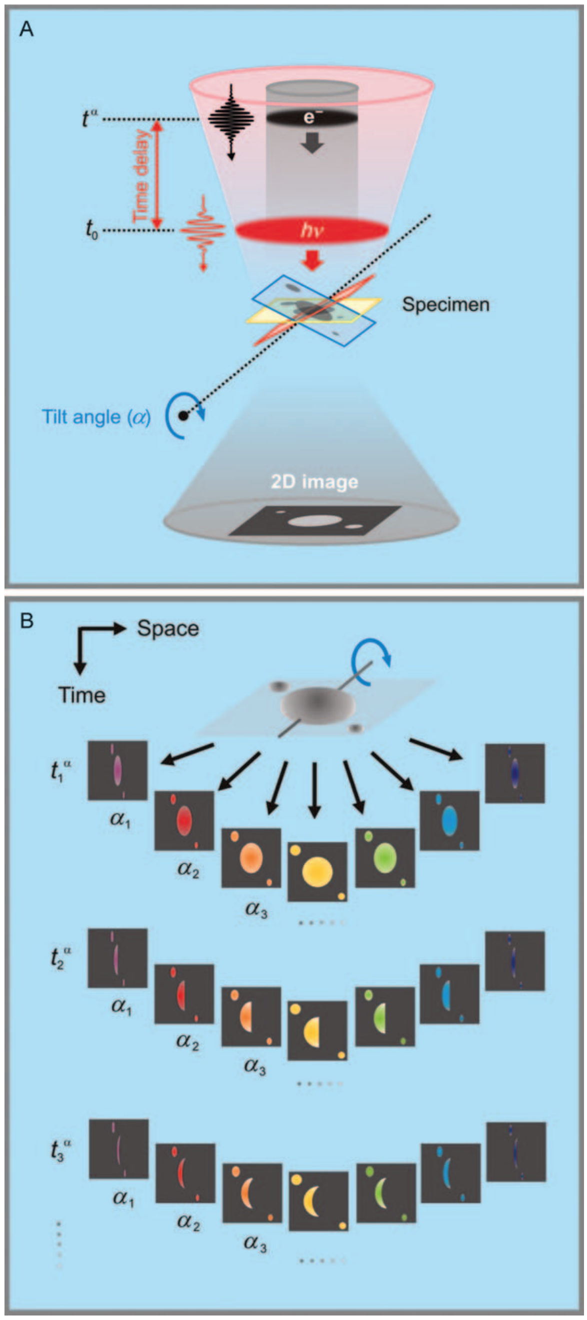

Figure 2. A) Representation of time-resolved 4D electron tomography. The heating pulse (at t_0) initiates the structural change and acts as a clocking pulse, whereas the time-delayed electron packet (at t^{α}) with respect to the clocking pulse images the structure at a given tilt angle α. B) A series of 2D images at various projection angles and time steps are taken to construct the tomograms. In this case, increments of 1° and scans from −58° to +58° were used to define α and its range; the time scale ranged from femtoseconds to microseconds. The number of total spatiotemporal projections made was near 4000, and these were used to construct the tomographic movies of the object in motion. Reprinted from Ref. [14] with permission. Copyright 2010, AAAS.

of motion, such as breathing and wiggling, with resonance frequencies of up to 30 MHz (Figure 3).[14] The mechanical and morphological dynamics of a MWCNT can be obtained in

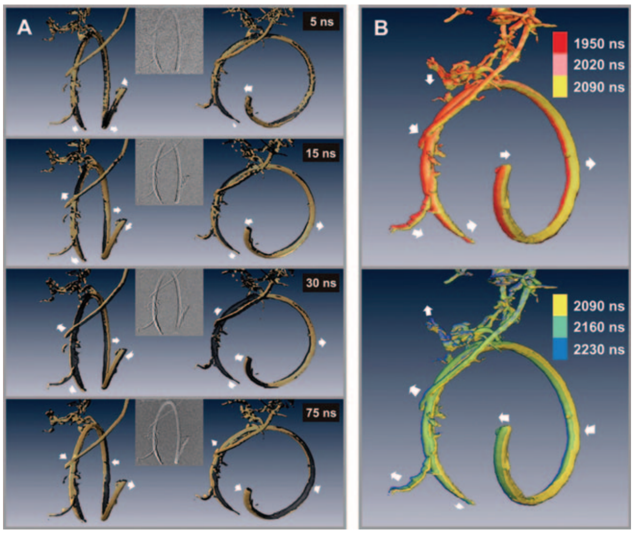

Figure 3. 4D tomographic visualization of motion. A) Representative 3D volume snapshots of the nanotubes at relatively early times. Each 3D rendered structure at a different time delay (beige) is shown at two view angles. A reference volume model taken at $t=0$ ns (black) is merged in each panel to indicate the resolved nanometer displacements. Arrows in each panel indicate the direction of motion. B) The time-dependent structures visualized at later times and with various colors to indicate different temporal evolution. The wiggling motion of the whole bracelet is indicated with arrows. Reprinted from Ref. [14] with permission. Copyright 2010, AAAS.

the 4D tomograms by investigating the frequency change in various resonances induced by laser-impulse heating.

With the new development of 4D electron tomography, we should be able to view a "movie" of the dynamics of various nano-objects. The method enables the study of transient states of materials, structural dynamics of large molecular objects, and biological systems under controlled conditions. Performing the 4D-electron tomography under environmental conditions, that is, in the presence of atmospheric gases suitable for chemical reactions, would allow the recording of the response and dynamics of nanocatalytic particles and the four-dimensional viewing of their behavior during the reaction. A truly new understanding of processes and discoveries for catalysis, chemistry, and nanoscience are now on the horizon.

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