4D-STEM of Beam-Sensitive Materials

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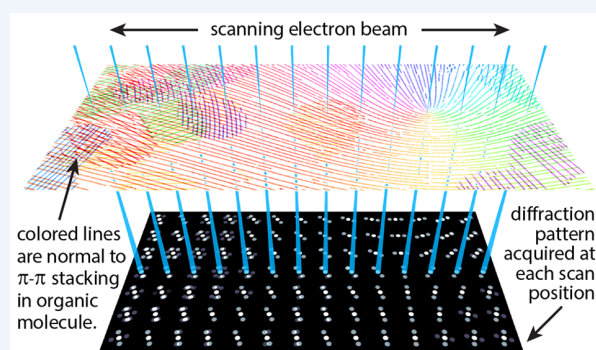Cite This: https://doi.org/10.1021/acs.accounts.1c00073

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CONSPECTUS: Scanning electron nanobeam diffraction, or 4D-STEM (four-dimensional scanning transmission electron microscopy), is a flexible and powerful approach to elucidate structure from "soft" materials that are challenging to image in the transmission electron microscope because their structure is easily damaged by the electron beam. In a 4D-STEM experiment, a converged electron beam is scanned across the sample, and a pixelated camera records a diffraction pattern at each scan position. This four-dimensional data set can be mined for various analyses, producing maps of local crystal orientation, structural distortions, crystallinity, or different structural classes. Holding the sample at cryogenic temperatures minimizes diffusion of radicals and the resulting damage and disorder caused by the electron beam. The total fluence of incident electrons can easily be controlled during 4D-STEM experiments by careful use of the beam blanker, steering of the localized electron dose, and by minimizing the fluence in the convergent beam thus minimizing beam damage. This technique can be applied to both organic and inorganic materials that are known to be beam-sensitive; they can be highly crystalline, semicrystalline, mixed phase, or amorphous. One common example is the case for many organic materials that have a $\pi-\pi$ stacking of polymer chains or rings on the order of 3.4−4.2 Å separation. If these chains or rings are aligned in some regions, they will produce distinct diffraction spots (as would other crystalline spacings in this range), though they may be weak or diffuse for disordered or weakly scattering materials. We can reconstruct the orientation of the $\pi-\pi$ stacking, the degree of $\pi-\pi$ stacking in the sample, and the domain size of the aligned regions. This Account summarizes illumination conditions and experimental parameters for 4D-STEM experiments with the goal of producing images of structural features for materials that are beam-sensitive. We will discuss experimental parameters including sample cooling, probe size and shape, fluence, and cameras. 4D-STEM has been applied to a variety of materials, not only as an advanced technique for model systems, but as a technique for the beginning microscopist to answer materials science questions. It is noteworthy that the experimental data acquisition does not require an aberration-corrected TEM but can be produced on a variety of instruments with the right attention to experimental parameters.

KEY REFERENCES

- Panova, O.; Ophus, C.; Takacs, C. J.; Bustillo, K. C.; Balhorn, L.; Salleo, A.; Balsara, N.; Minor, A. M. Diffraction imaging of nanocrystalline structures in organic semiconductor molecular thin films. *Nat. Mater.* **2019**, *18*, 860−866.[1] Using 4D-STEM, the orientation of $\pi-\pi$ stacking from two different molecular film systems was visualized as flow-lines providing an orientation map that shows overlapping grains. The resulting structure maps show grains with sharp grain boundaries and overlapping grains, as well as liquid-crystal like structures that contain disclinations representing singularities in the director field.
- Gallagher-Jones, M.; Bustillo, K. C.; Ophus, C.; Richards, L. S.; Ciston, J.; Lee, S.; Minor, A. M.; Rodriguez, J. A.

Atomic structures determined from digitally defined nanocrystalline regions. *IUCrJ* **2020**, *7*, 490−499.[2] The atomic structure of this protein crystallite was confirmed with high spatial resolution using a 4D-STEM tomography series, a 4D-STEM data set acquired at each 1° tilt step in a ±30° tilt range. The 4D-STEM technique was compared to similar results from MicroED and selected area diffraction.
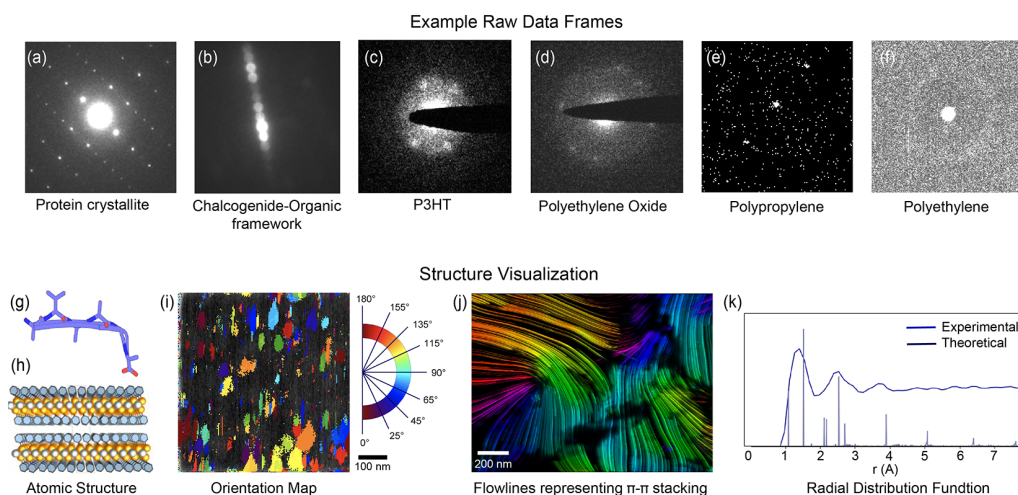
Figure 1. Example diffraction patterns and structure maps: (a) protein crystallite;[2] (b) Ag−Se−organic layered material;[8] (c−f) semicrystalline organic materials; (g) atomic structure of protein crystallite;[2] (h) layered structure of Ag−Se−organic framework;[8] (i) orientation map of $\pi-\pi$ stacking in acid-polyethylene;[9] (j) flowlines representing overlapping grains from organic small molecule;[1] (k) radial distribution function from polyethylene. Panels a and g adapted with permission from ref 2. Copyright 2020 International Union of Crystallography. Panel h reproduced with permission ref 8. Copyright 2019 American Chemical Society.

• Panova, O.; Chen, X. C.; Bustillo, K. C.; Ophus, C.; Bhatt, M. P.; Balsara, N.; Minor, A. M. Orientation mapping of semicrystalline polymers using scanning electron nanobeam diffraction. *Micron* **2016**, *88*, 30−36.[3] 4D-STEM was applied to a photovoltaic polymer blend to map the size and orientation of crystallites, as well as the degree of crystallinity.

■ INTRODUCTION

As a technique, scanning electron nanobeam diffraction, or 4D-STEM,[4] can be applied to electron beam-sensitive materials to produce structural maps from a series of diffraction patterns acquired during a scanning transmission electron microscopy (STEM) image collection. Cooling the sample with LN_2 to slow diffusion of ionizing species,[5] in combination with 4D-STEM acquisition, allows one to collect data from some of the most electron beam-sensitive materials. Samples can be transferred into the TEM in the frozen vitreous state allowing for imaging in a hydrated state as is commonly described with the term "Cryo-EM", or they can be transferred as dry samples at room temperature and then cooled only during electron beam irradiation. This Account will focus on the latter, although 4D-STEM could be applied to vitreous samples.[6] For samples that are easily damaged by the electron beam, the luxury of finding the right location, doing careful focus, and perhaps spectroscopy as a function of position is seldom possible. Instead, making every incident electron count and keeping track of the numbers of electrons used is critical. Ultimately, the goal is to optimize the signal-to-noise ratio (SNR) in the raw data and enable dose control, both of which can be facilitated using 4D-STEM.

The kinds of material systems that are appropriate for cryogenic 4D-STEM include organic materials, such as polymers,[3] small molecules,[1] and proteins,[7] inorganic materials, such as perovskites, oxides, 2D materials, and organic frameworks, or composite materials, such as nanoparticle assemblies in contact with an organic component. Typical diffraction patterns showing the wide distribution of raw data types are shown in Figure 1a−f.

In addition to being easily altered by the electron beam, many of the materials of interest are weakly scattering. They may be comprised of light elements that have a weak electrostatic potential, or the long-range order may be compromised, causing the signal to be spread in reciprocal space or to be mixed with signal from disordered regions. These kinds of materials often produce little image contrast or images that are difficult to interpret in a conventional bright-field or dark-field TEM image. In general, we seek structural information in the form of image contrast; Figure 1g−k shows several examples of maps of local structure and orientation.

The 4D-STEM technique uses a focused electron beam that scans across the TEM sample (Figure 2a). At each X and Y probe position, a 2D diffraction pattern is acquired with a pixelated camera. The two reciprocal space dimensions of the diffraction pattern, k_x and k_y, together with the real space probe positions of x and y, are the four dimensions referred to in the term "4D-STEM". A typical 4-dimensional data set might contain 100 × 100 probe positions or 10 000 diffraction patterns.

The sensitivity of the sample to the electron beam varies greatly for different materials.[10,11] In the simplest observation, one experiences a decrease in the diffracted signal over time or perhaps no diffracted signal in the parallel TEM diffraction condition because the crystalline fraction is a small part of the total. The critical dose, a fluence that causes the diffracted spots to decrease to $1/e$ in intensity, is reported for different materials (see recent review by Egerton).[10] The beam sensitivity depends not only on the material but also on the synthesis and morphology. As a practical example, we have observed solution phase crystallites of polyethylene with order that lasts seconds at room temperature, while the order in a less-crystalline polyethylene phase is undetectable without cooling and a low-noise counting detector. Many samples are damaged with a few picoamp probe current and 50 ms dwell time (time at each scan position) to the point of forming a hole; others can be scanned multiple times allowing for a tomographic series of 4D-STEM data sets.[2] In addition to damage due to radiolysis, it is possible that electrostatic charging effects enhance the damage.[10,12] With the exception of graphene, we have found no advantage to
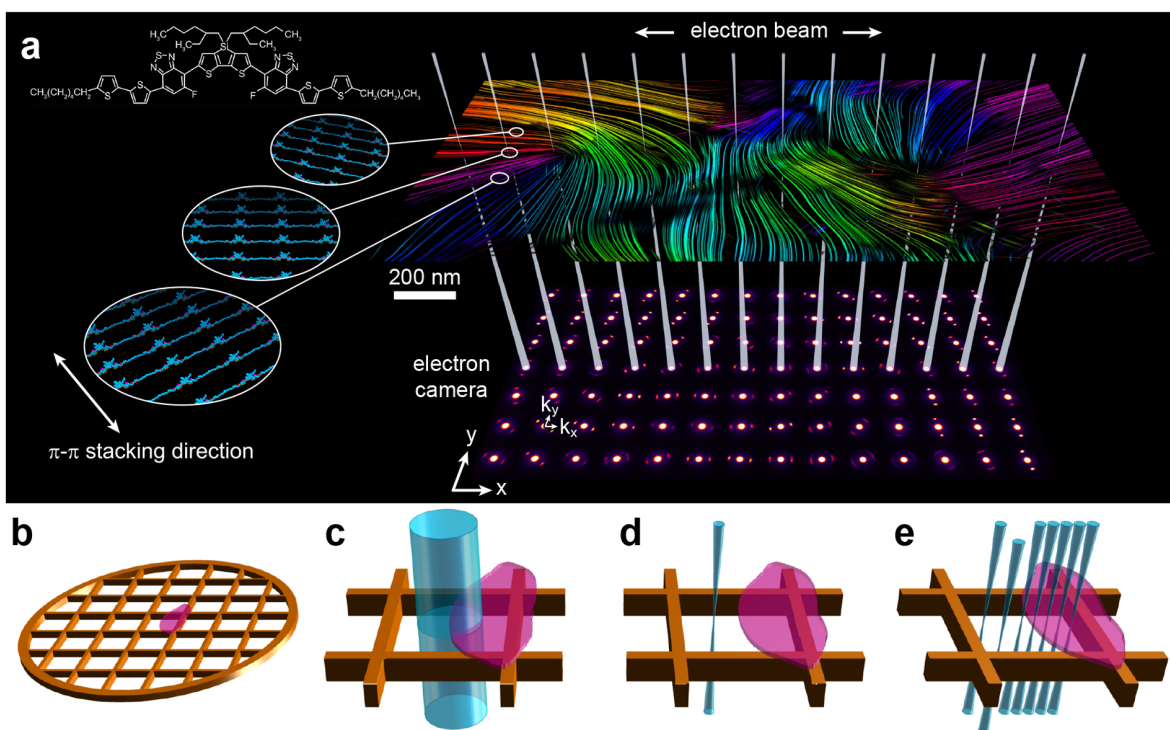
Figure 2. 4D-STEM experiment. (a) Schematic of focused beam stepping across organic small molecule shown in upper left. The conjugated backbone is represented by colored lines. $\pi-\pi$ stacking generates diffraction patterns; some frames show multiple pairs of reflections indicating overlapping grains, and other frames contain only a weak amorphous halo indicating that region is either noncrystalline or does not have $\pi-\pi$ stacking aligned in a diffracting condition. (b) TEM sample grid with microtome slice. (c) TEM parallel beam exposes large area of sample. (d) Convergent beam limits area exposed. (e) Once sample is found, probe is moved to unexposed sample for each new data set.

performing 4D-STEM at accelerating voltages lower than 300 kV; radiolysis is the presumed damage mechanism, and this type of damage increases with decreasing accelerating voltage.[13]

One advantage of using STEM instead of parallel beam TEM is the spatially resolved control of dose that can be a more straightforward approach for limiting exposure, especially for the nonexpert microscopist. As shown in Figure 2c, conventional TEM exposes an area of the sample that is 100 nm to a few micrometers, and there is a habit of spreading and condensing the beam as the magnification is changed without paying attention to the size of the illuminated area and the position of the stage. In contrast, a STEM image only illuminates the square area scanned (Figure 2d), and the scan position is saved in the image tags. By unblanking the beam only when acquiring an image, the regions nearby will remain unexposed (Figure 2e). In many cases, it is necessary to work "without seeing", meaning the beam is blanked while moving around the sample and unblanked only when acquiring a data set. Working "without seeing" in this manner means that there is no opportunity to focus the sample. In conventional TEM, focusing takes time and electron dose, and while it can be performed adjacent to the region of interest, parallel beam imaging is not as robust to defocus as acquiring diffraction patterns. Working "without seeing" also means that there should be large areas of sample on every grid so that time is not wasted looking for the sample. In practice, it is sometimes possible to acquire a low-magnification (~140×) STEM image quickly at the beginning of the session to confirm the location of the region of interest. Microtomed sections can often be seen and photographed in the optical microscope, in order to orient the section with respect to the center of the grid. Acquiring movies of the diffraction while moving the sample quickly while

scanning can also help locate the signal. Constant use of the prespecimen beam blanker is essential. The first goal in any attempt to image these materials is to acquire some detectable signal in reciprocal space; this is usually detected by the eye as the frames flash on the computer monitor. In many cases, cooling the sample makes the difference between capturing some signal or none at all, and the final optimization of parameters can be done empirically.

■ SAMPLE PREPARATION AND COOLING

Typically, samples are supported on a TEM grid with an amorphous support that is several to 20 nm thick. The "lacey" structures in some TEM grids provide features on which to focus, as well as to track sample drift. The samples can be spin-cast resulting in a thin film, drop-cast from a solution, thinned with a focused ion beam, or embedded in a resin and then sliced with a microtome knife, often at cryogenic temperatures, to produce thin sections. 4D-STEM does not require ultrathin sections; <200 nm thick samples are acceptable at 300 kV accelerating voltage for weakly scattering samples. However, if the thickness of the sample starts to include many domains through the thickness, the diffraction patterns can be challenging to analyze.

The sample is placed in a TEM holder and the foot-long copper rod that holds the sample is cooled by an external liquid nitrogen dewar after the sample holder is inserted into the TEM (Figure 3a). The sample is cooled usually to the lowest temperature possible, although one can control the temperature with a local heater over the range of −10 °C to −170 °C. Because the cooling of the copper rod causes contraction and
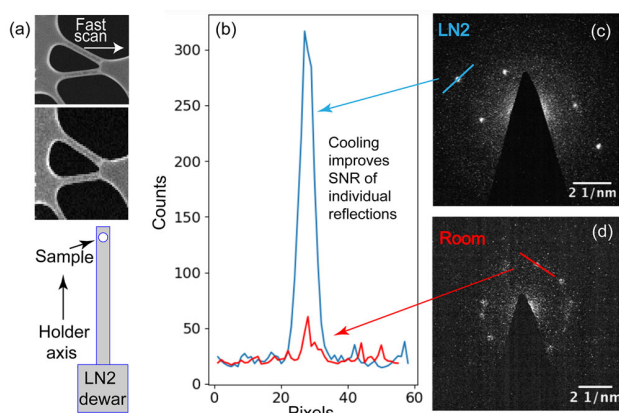
Figure 3. Cryogenic 4D-STEM: (a) drift along holder axis causes the reconstructed image to be stretched compared with the actual region; (b) line scan from acid-polyethylene sample[9,14] shows advantage in SNR when cooling; (c) LN$_2$; (d) 20 °C. The best diffraction frame from each data set was chosen to make the comparison. Panels c and d adapted with permission from ref 14. Copyright 2020 Cambridge University Press.

expansion, the sample drifts severely during the first ~60 min of cooling. Eventually the sample drift slows to ~0.5 nm/s, but its direction is predictably along the holder axis. It is necessary to take into account the number of pixels in the fast scan direction, dwell time, step size, and drift rate in order to prevent sampling the same region twice. If the magnitude and direction of the drift

is known, one can place the fast scan direction perpendicular to the holder axis and scan opposite to the drift, and consequently the reconstructed map shows more of a stretch and less of a skew.

Figure 3b highlights the SNR improvement when keeping the sample cold. Here we compare a line scan through the strongest reflections from data sets acquired near −170 °C (Figure 3c) and 20 °C (Figure 3d). The improvement is material dependent, but we observe a factor of ~2−6 improvement in SNR at the lower temperatures with others reporting similar numbers.[10]

■ PROBE SIZE AND SHAPE

The semiconvergence angle, α, is defined by the probe forming aperture (usually just below the second condenser lens, C2) and the angle of the beam when it hits the aperture (Figure 4a). One can tailor the angle by using different sized apertures and, in a 3-condenser TEM, by adjusting the relative strength of the second and third condenser lenses. The semiconvergence angle has an inverse effect on the size of the probe: the larger the α, the smaller the probe. Typical semiconvergence angles for beam-sensitive materials that require cryogenic conditions are 0.5−0.01 mrad, in contrast to HRSTEM, which uses α = 10−30 mrad, or 4D-STEM strain mapping, which uses α = 1−4 mrad. In reciprocal space, the size of the Bragg disks in the diffraction patterns are proportional to α. For many 4D-STEM applications, one wants to separate the reflections so that they can be individually located by template matching. For beam-sensitive semicrystalline materials, it is advantageous to make the reflections even smaller or sharper.[14] Reducing the
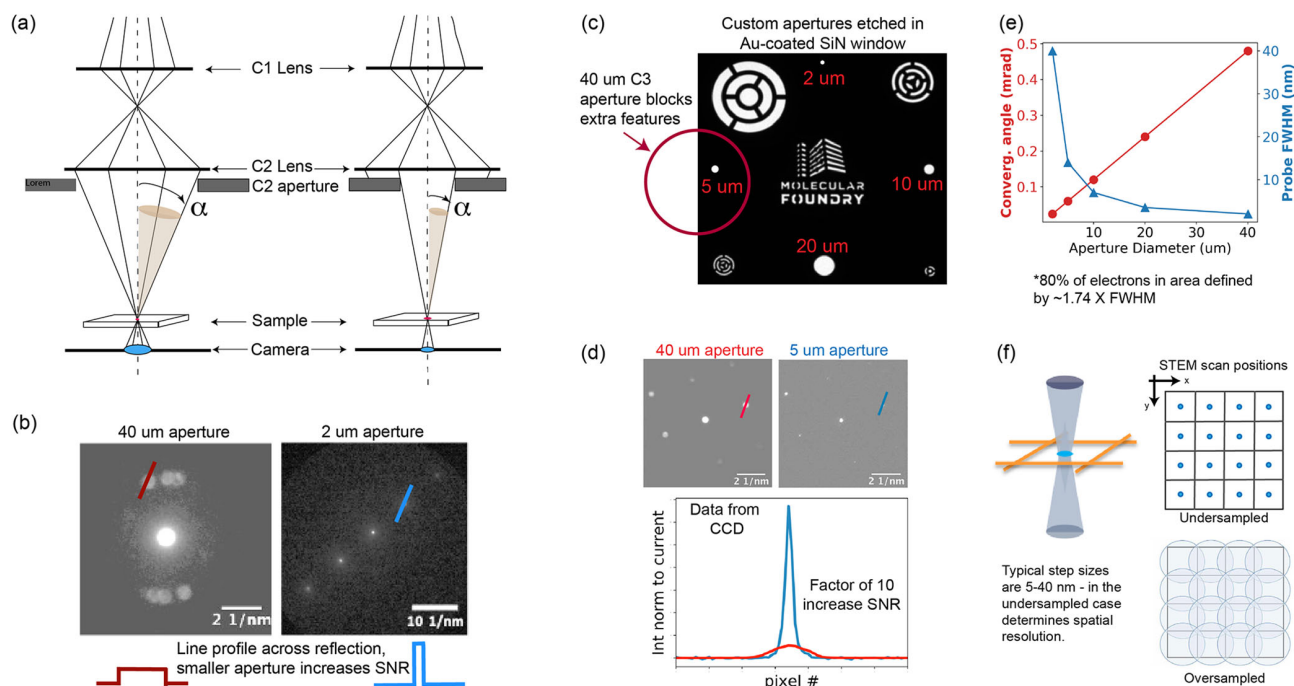
Figure 4. Small probe forming apertures: (a) Ray diagrams for two different aperture sizes showing semiconvergence angle (α) and corresponding size of diffraction spots; (b) squeezing electrons into fewer pixels on the camera increases SNR, comparison of 40 μm aperture (organic small molecule) and 2 μm aperture (rubrene); (c) custom apertures on Au-coated SiN window, red circle is additional aperture that selects the 5 μm;[15] (d) experimental comparison of two apertures; Au foil measured on CCD shows 10× improvement in SNR with smaller aperture for the same fluence; (e) real-space probe size (blue) is inversely proportional to α (red); (f) probe intersecting sample determines exposure area, typical step sizes of 5−40 nm can result in either undersampled or oversampled region of interest. Panel b reproduced with permission ref 14. Copyright 2020 Cambridge University Press.

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convergence angle while maintaining the same probe current localizes the signal into fewer pixels on the detector, helping to boost the signal above the noise floor of the detector as shown in Figure 4b. The <0.5 mrad beam is quite parallel with its crossover point suitably aligned for positional control by the scanning deflectors.

A typical probe forming aperture is 40−150 μm in diameter, but condenser apertures exist that are 20, 10, 5, or 2 μm in diameter.[15,16] The aperture plate shown in Figure 4c has 8 different apertures; the bullseye design helps with strain mapping precision.[16,17] The 3 mm aperture plate fits in many TEM aperture rods; it requires an aperture 40−70 μm downstream (usually below C3) to select the aperture of interest. These smaller apertures allow for control of the convergence angle to smaller angles than is typical. The small apertures have the added advantage that they can reduce the fluence of electrons, which is especially helpful for TEMs that do not have a monochromator with continuously adjustable gun lens to control the fluence. Data from an oriented Au foil showing the measured difference of SNR between 40 and 5 μm apertures is shown in Figure 4d. For a given electron fluence, the smaller aperture realizes a factor of 10 improvement in SNR. Theoretically, the peak height should depend on the square of the aperture diameter,[18] but because these images were acquired on a scintillator-coupled camera, the point spread function causes "blooming" over neighboring pixels and only a factor ∼10 is realized. What is most significant for the beam-sensitive materials is that one can reduce the electron fluence by a factor of 10 without compromising SNR by using a smaller aperture.

The disadvantage of using a smaller aperture and corresponding small convergence angle is that it increases the size of the probe in real space. The plot in Figure 4e shows how the fwhm of the probe, measured in real-space on the camera, increases with decreasing aperture diameter. Similar numbers have been reported by those who perform fluctuation microscopy and intentionally want a few nanometer sized probe.[18] Because the probe-forming aperture is sharp in Fourier space, the shape of a STEM probe in real space is an Airy disk pattern, which in its most focused state can be approximated by a Gaussian, although the true Airy disk does possess tails.[19] The probe size for a 0.5−0.01 mrad semiconvergence angle has a fwhm of typically 1−15 nm. In actuality, this fwhm represents only ∼50% of the electron fluence; ∼80% of the fluence is contained in 1.74 × fwhm, with long tails that extend nanometers from its center. However, measurement of the fwhm from a real-space image of the probe is easy to make as a check of the illumination conditions. Depending on the step size of the scan, the size of the probe in real space can limit the spatial resolution of the final structure map.

A critical experimental parameter for 4DSTEM experiments is the step size, or the distance between probe positions. For many beam-sensitive materials that lose their order from damage due to radiolysis, there is a damage ring (sometimes called "collateral damage") that extends well beyond the first minimum of the Airy disk.[10,20] This damage ring then defines the closest one can separate the probe positions without illuminating a damaged region that provides less or no useful signal. Often this step size is what defines the spatial resolution in real space; the scan positions are commonly spaced 10−40 nm apart, often significantly larger than the probe size. Two extremes of sampling are shown in Figure 4f. In one case the material is oversampled with a step size that is smaller than the area illuminated by the probe, and in this case the area of the probe is

what defines the spatial resolution. In the other extreme, the material is undersampled to avoid the damage ring or perhaps to cover a large region of interest, and in this case the spatial resolution is defined by the step size.[10] During the experiment, the loss of diffraction information is a clear indicator that the damage radius exceeds the step size: the first scan position will have signal and subsequent scan positions will produce no signal.

In real space, the sample is "in focus" when it is placed at the crossover point of the beam (Figure 5a,b). If the sample is out of
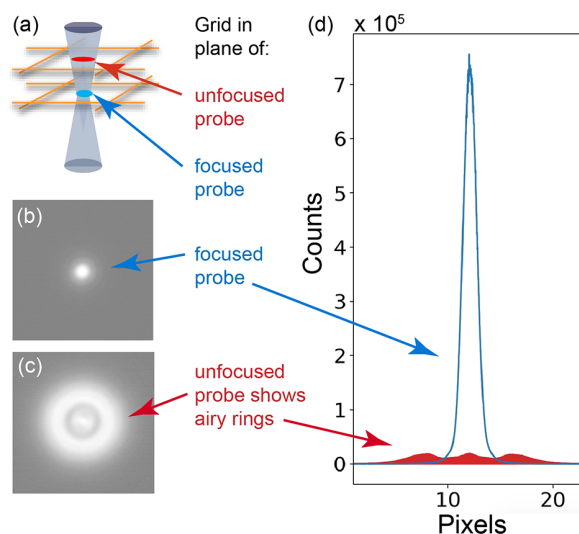
Figure 5. Probe shape: (a) focus of STEM image defined by placing sample at crossover height, sample not in this plane intersects the probe in a defocused state; (b) real-space image of focused probe; (c) real-space image of defocused probe; (d) line scan through focused (blue) and defocused (red).

focus and not at this crossover point (Figure 5c), then the intersection area is larger as shown in Figure 5a. If the beam is strongly defocused, then the shape of the Airy rings becomes more dominant, and the tails start to contain more of the electrons as shown in Figure 5d. Therefore, another advantage of using a smaller semiconvergence angle is that it increases the depth of focus. This means that the sample does not need to be at the perfect crossover point, and yet it will be close enough to not significantly increase the intersected area of the probe with the sample, nor change the sharpness of the reflections. A large depth of focus means that it is hard to focus in STEM mode, but one can get around this by temporarily inserting a large aperture without changing the C2/C3 conditions, finding the eucentric height of the sample, and then going back to the smaller aperture. For the example of a 0.12 mrad semiconvergence angle, the sample needs to have a z-height that is ±30 μm in order to be "in focus"; in this measurement, "in focus" was defined by a change in maximum intensity of the center peak by <15%. This allows the sample to change height and bend across the grid and not compromise the imaging conditions.

In analogy to refractive lenses, we consider the probe-forming aperture as the numerical aperture (NA) of the illumination system. Table 1 lists how parameters of interest should depend on the NA.[18,21] The first row lists semiconvergence angle as indicated in the TEM software. The second row shows improvement in peak height of the signal from a CCD camera for a given electron fluence; this value is less than theoretically

Table 1. Probe Parameters As a Function of Probe-Forming Aperture or Numerical Aperture (NA)

	dependence on numerical aperture (NA)	measured with 40 μm aperture	measured with 5 μm aperture
semiconvergence angle	NA	0.48 mrad ind.	60 μrad ind.
SNR or peak height	$1/(NA)^2$	1	10
beam current	$(NA)^2$	6 pA	94 fA
real space fwhm	$1/(NA)$	2 nm	14 nm
depth of focus (theoretical)	$1/(NA)^2$	~10 μm	≫10 μm

expected as the point spread function limits accurate measurement on the CCD. The third row is measured screen current, which scales appropriately with the indicated semiconvergence angle. The fourth row is the real-space fwhm measured at high magnification. The fifth row is the estimated depth of focus.[21]

Because of the need for small convergence angles, the 3-condenser TEMs are set in "Microprobe" STEM mode; the mini-condenser lens is active with a large angle range. There is no advantage to using a probe-corrected TEM for these experiments as the probe size is limited by diffraction from the probe-forming aperture, rather than by aberrations in the lenses.[18]

■ **ELECTRON FLUENCE**

The fluence, or dose, in e Å^{-2} describes the total sum of electrons incident in a region of the sample. To calculate the fluence, the following steps are used: (1) measure the screen current on the calibrated fluorescent screen in picoamps, (2) convert to electrons (1 pA = 6.24×10^6 e/s), (3) multiply by the exposure time, and (4) divide by the relevant area. Sometimes the flux, or dose rate, in e Å^{-2} s^{-1} is important.[22] However, for many materials, it is believed that the damage is independent of dose-rate and instead is dependent on total dose.[10] In STEM imaging where oversampling is typical, the relevant area is the scanned area and the exposure time is the frame time; this is appropriate in 4D-STEM in cases where we are not undersampling. However, in the example of Figure 4f where the material is undersampled, such a measurement would under-report the fluence from the area that generates the signal. Instead, we take the relevant area to be the area defined by the fwhm as an easy measurement with the time being the dwell time. Experiments with beam-sensitive materials can range from 0.5 to 10 000 e Å^{-2}. Table 2 shows comparisons of fluence for different conditions. A common benchmark for low dose imaging is a fluence <20 e Å^{-2}.[23] As can be seen by the italic entries in Table 2, reducing the fluence by using a small aperture can achieve these low-dose conditions; the spatial resolution of the structure map is now limited by the large size of the real-space probe. The point to recognize is that the signal arises from the area that is

Table 2. Fluence (e Å^{-2}) Measured Two Ways: Total Image Area and Probe Area

	40 μm aperture	5 μm aperture
beam current	6 pA	94 fA
real space fwhm	2 nm	14 nm
step size	10 nm	10 nm
dwell time	50 ms	50 ms
fluence 1 μm × 1 μm image	200 e Å^{-2}	*~3 e Å^{-2}*
fluence using fwhm	6000 e Å^{-2}	*~2 e Å^{-2}*

illuminated, and it is that area that should be used to calculate the fluence. The small beam current is measured on the fluorescent screen at a weak C1 excitation (small spot number) and then extrapolated to the spot size used. A dwell time of 2−50 ms is typical, but this parameter can also be used to tune the fluence.

■ **DETECTORS**

During a 4D-STEM experiment, a pixelated camera is used to acquire diffraction patterns. Either a scintillator-transduced (CCD or CMOS) or a direct-electron (hybrid pixel array detector or monolithic active pixel, counted or integrated) camera[24] can be used, although the subtleties of their operation and performance will dictate the experiment. Acquiring diffraction patterns from a beam-sensitive material usually does not require high reciprocal-space sampling, so 512 × 512 pixels is often enough. There is a need for dynamic range because the unscattered beam can be strong compared to the scattered beam, and the reflections can be of variable intensity as the probe scans over regions of varying crystallinity, although if one is not using the intensity of the disks in the analysis, it is acceptable to saturate some reflections intentionally. Because there exists sample drift (especially when cooling), the frame rate (frames per second, fps) is important. The figure of merit that addresses both sensitivity and noise of the sensor is the detector quantum efficiency (DQE);[25] the DQE is important because of the need to maximize the SNR of the diffracted spots. For most applications, a CCD running at 20 fps is a good compromise of speed and dynamic range.

The big advantages to using a direct electron camera for beam-sensitive materials are higher DQE and faster frame rate.[26,27] The camera frame rate dictates the speed of data acquisition: at 20 fps (dwell time = exposure time = 50 ms), a 100 × 100 position scan will take 8 min; at 400 fps, it will take 25 s. In addition to the sensor having a higher DQE, many direct electron cameras can be operated in "counting mode", resulting in lower noise. In order to operate in counting mode, a pixel fill factor of ~1% is recommended such that the probability of acquiring >1 electron per pixel is low.[24] The relevant pixel fill factor is for the regions within the reflections. Such a low fill factor is achieved by a combination of reducing the flux of electrons and a fast frame rate. Because each frame has little data, many frames need to be summed. Consequently, although the camera has a high frame rate, the number of summed frames may approach the same 50 ms exposure that is common on a CCD camera, and so the number of pixels in the STEM scan is still limited by the speed. Newer direct electron cameras, like the 4D camera at NCEM[28,29] running at 87 000 fps, make higher fluxes possible while maintaining a ~1% fill factor.

Because many of the soft materials are weakly scattering, the unscattered beam is much stronger than the reflections of interest. Consequently, either the unscattered beam must be blocked by a beam stop or the camera must be robust enough to withstand a large flux of electrons in the central spot. The shape and size of the beamstop may limit the camera length, and it certainly adds to the trouble of aligning all the diffraction patterns in postprocessing. With large area (>100 nm) scans, there can be sway in the beam due to misaligned descan, which can move the beam out from under the beam stop. An active pixel direct electron camera can be damaged by a direct beam and so these cameras usually retract automatically when a dangerous level is reached limiting the dynamic range. The hybrid pixel array direct electron cameras, like the EMPAD[30] or

Medipix,[26] were designed as high dynamic range cameras that are able to withstand the full flux of the unscattered electron beam.

Some pixelated cameras are placed at the end of an energy filter, a prism that spreads the electrons in energy allowing for a slit to be placed such that the diffraction image is constructed of electrons that have lost only a certain amount of energy. We have used this feature to bracket the zero-loss peak with a 15 eV slit, which removes some electrons that are inelastically scattered to improve SNR of the diffraction spots; this is especially useful for thick samples. While the pixelated camera is acquiring the diffraction patterns, it is common, although not necessary, to acquire a conventional STEM image, albeit pixelated, with a monolithic annular dark field detector.[27] A similar STEM image could be reconstructed from the 4D-data set.

DATA ANALYSIS

Once a data set of ~10 000 frames is acquired, computer algorithms process the data to reconstruct a real-space map.[31,32] One could imagine choosing one reflection (a disk of pixels, k_x–k_y, in reciprocal space) and reconstructing a real-space image of 100×100 pixels where the pixel intensity is the strength of that reflection.[31] This real-space reconstruction is analogous to a conventional dark-field image obtained by placing an objective aperture in the TEM around a diffracted spot and acquiring a dark-field TEM image. The advantage of 4D-STEM is that one can construct unlimited virtual apertures or combinations of apertures to explore contrast mechanisms, all in postprocessing. Similarly, one can sum diffraction signals from an arbitrary collection of scan spots and then display the resulting diffraction pattern, analogous to selected area diffraction in conventional TEM. Thus, nanobeam diffraction, enables low-dose determination of crystal orientation over large, real-space features. This feature of 4D-STEM has been applied to beam-sensitive battery materials,[33] catalytic nanoparticles,[34] and framework materials, and it has potential to identify orientation in perovskite[35] and oxide materials that are often beam-sensitive.

Several open-source packages have been developed to analyze data sets,[27,36] such as py4DSTEM[32] and pyXem.[37] First steps in the analysis include aligning all diffraction frames, subtracting a background, and correcting for ellipticity introduced by stigmation in objective or projector lenses. Then, a cross-correlation is performed against a template of the probe-forming aperture to identify the positions of Bragg reflections. If the material exhibits short- or medium-range order, a radial distribution function (rdf) can be generated that tracks correlations, which correspond to bonding arrangements. If distinct rdf fingerprints exist, one can create a phase map distinguishing the different regions.[38] Even for semicrystalline, mixed phase, or amorphous samples, measurements using 4D-STEM can allow for comparison of different synthesis methods or processing conditions.[1]

CONCLUSION

4D-STEM is an emerging technique that can visualize structural contrast in materials that are conventionally challenging to image. In this Account, we have discussed experimental parameters that make it possible for even a beginning microscopist to take advantage of this rich technique. In the future, the technique will be improved by faster cameras and more comprehensive data analysis pipelines.

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