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Picometer-precision atomic position tracking through electron microscopy

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TITLE:
Picometer-Precision Atomic Position Tracking through Electron Microscopy

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SUMMARY:
This work presents a workflow for atomic position tracking in atomic resolution transmission electron microscopy imaging. This workflow is performed using an open-source Matlab app (EASY-STEM).

ABSTRACT:
The modern aberration-corrected scanning transmission electron microscopes (AC-STEM) have successfully achieved direct visualization of atomic columns with sub-angstrom resolution. With this significant progress, advanced image quantification and analysis are still at the early stages. In this work, we present the complete pathway for the metrology of atomic resolution scanning transmission electron microscopy (STEM) images. This includes (1) tips for acquiring high-quality STEM images; (2) denoising and drift-correction for enhancing measurement accuracy; (3) obtaining initial atomic positions; (4) indexing the atoms based on unit cell vectors; (5) quantifying the atom column positions with either 2D-Gaussian single peak fitting or (6) multi-peak fitting routines for slightly overlapping atomic columns; (7) quantification of lattice distortion/strain within the crystal structures or at the defects/interfaces where the lattice periodicity is disrupted; and (8) some common methods to visualize and present the analysis.

Furthermore, a simple self-developed free MATLAB app (EASY-STEM) with a graphical user interface (GUI) will be presented. The latter can assist in the analysis of STEM images without the need for writing dedicated analysis code or software. The advanced data analysis methods presented here can be applied for the local quantification of defect relaxations, local structural distortions, local phase transformations, and non-centrosymmetry in a wide range of materials.

INTRODUCTION:

The development of spherical aberration correction in the modern scanning transmission electron microscope (STEM) has enabled microscopists to probe crystals with sub-angstrom sized electron beams[1,2]. This has allowed imaging of individual atomic columns in a wide variety of crystals with interpretable atomic resolution images for both heavy and light elements[3,4]. Recent developments in pixelated direct electron detectors and data analysis algorithms have enabled phase reconstruction imaging techniques, such as ptychography, with further improvements in spatial resolution to around 30 pm[5–7]. Additionally, the recent progress in STEM tomography has even enabled three-dimensional atomic resolution reconstruction of the single nano-particle[8]. The electron microscope has thus become an extraordinarily powerful tool for quantifying structural properties in materials with both high precision and site-specificity.

With the ultra-high resolution STEM images as the data input, direct measurements of structural distortions were performed to extract physical information from crystals at the atomic scale[9,10]. For example, the defect coupling between a Mo dopant in the WS_2 monolayer and a single S vacancy was directly visualized by measuring the atomic positions and then calculating projected bond lengths[11]. Furthermore, the measurement on crystal interfaces, such as the coalesced grain boundaries in monolayer WS_2, can exhibit the local atomic arrangement[12]. The interfacial analysis performed on the ferroelectric domain walls in $LiNbO_3$ revealed the domain wall to be a combination of Ising and Neel states[13]. Another example is the visualization of the polar vortex structures achieved in the $SrTiO_3$-$PbTiO_3$ superlattices, achieved through calculation of the titanium atomic column displacements with respect to the strontium and lead column positions[14]. Finally, the advances in computer vision algorithms, such as image denoising with non-local principle component analysis[15], Richardson and Lucy deconvolution[16], drift-correction with non-linear registration[17], and pattern recognition with deep learning, have significantly strengthened the accuracy of the measurement to sub-picometer precision[18]. One such example is the alignment and image registration of multiple fast-scan cryogenic-STEM images to enhance the signal-to-noise ratio. Subsequently, the Fourier-masking technique was applied to analyze the charge density waves in crystals by directly visualizing the periodic lattice distortion[19]. Even though the incredible aberration-corrected STEM instrumentation is increasingly accessible to researchers around the globe, the advanced data analysis procedures and methods remain uncommon and an enormous barrier for one without experience in data analysis.

In the present work, we showcase the complete pathway for the metrology of atomic resolution STEM images. This process includes firstly acquiring the STEM images with an aberration-corrected microscope followed by performing post-acquisition denoising/drift-correction for enhanced measurement accuracy. We will then further discuss the existing methods to clearly resolve and accurately quantify the atom column positions with either 2D-Gaussian single peak fitting or multi-peak fitting routines for slightly overlapping atomic columns[20,21]. Lastly, this tutorial will discuss methods for the quantification of lattice distortion/strain within the crystal structures or at the defects/interfaces where the lattice periodicity is disrupted. We will also introduce a simple self-developed free MATLAB app (EASY-STEM) with a graphical user interface (GUI) that can help with the analysis of STEM images without the need for writing dedicated analysis code or software. The advanced data analysis methods presented here can be applied for the local quantification of defect relaxations, local structural distortions, local phase

transformations, and non-centrosymmetry in a wide range of materials.

PROTOCOL:

NOTE: The flow chart in **Figure 1** shows the general procedure of the atomic position quantification.

[Place **Figure 1** here]

1. STEM image drift-correction and denoising

1.1. Acquire high-quality annular dark-field (ADF)/annular bright-field (ABF) STEM images.

NOTE: The quality of the input data is key to ensuring the accuracy of data analysis, so we start the protocol with a few tips for acquiring good image data.

1.1.1. Ensure a high-quality TEM sample. The sample quality is extremely crucial. Use thin and clean TEM samples with no beam damage for imaging. Avoid touching the sample during handling and loading as this can cause sample contamination.

1.1.2. Clean the sample before insertion (if possible). Clean the sample by using plasma cleaner, baking in a vacuum, or irradiating the region of interest in the sample at low magnifications by spreading the electron beam after sample insertion into the microscope ('beam shower'). Avoid damaged or contaminated areas when imaging.

1.1.3. Align the microscope and tune the aberration correctors to minimize the lens aberrations as much as possible. Test the resolution by acquiring a few STEM images on a standard sample to confirm that the spatial resolution can resolve the specific crystal structures and further fine-tune the aberrations in the image.

1.1.4. Tilt the sample until the optical axis is aligned with the specific zone axis of the crystal. For certain crystals, make observations from a required zone axis. For instance, align the viewing axis with the planes of the domain walls in ferroelectric crystals for the measurement.

1.1.5. Optimize the electron dose while limiting electron beam damage and the sample drift during imaging. If the sample is stable under the electron beam and does not show drift or damage during the acquisition, it may be possible to try a higher electron dose or acquire multiple images of the same region to boost the signal-to-noise ratio. The goal here is to have a higher signal-to-noise ratio without beam damage or image artifacts.

1.1.6. Acquire STEM images with different scanning directions to correct for potential drift during acquisition. First, acquire an image and then take the second one from the same region immediately after rotating the scan direction by 90°.

1.1.6.1. Take images using the same imaging condition except for the scan directions. The purpose of this step is to feed the rotated images to the drift correction algorithm developed recently[17].

NOTE: One can also input more than two images with more varying scanning directions (with arbitrary angles) into the algorithm. However, successive scanning of the same region may lead to lattice damage or drift in that area. Additionally, it is recommended that the scan direction and the low index lattice planes not maintain parallel or perpendicular directions with each other and instead maintain oblique angles. If the scan direction coincides with certain horizontal or vertical features (lattice planes, interfaces, etc.), the drift along the direction of the strong vertically/laterally varying features may cause artifacts during image registration.

1.2. Perform drift-correction with a non-linear correction algorithm.

NOTE: The non-linear drift correction algorithm was proposed and constructed by C. Ophus et al.[17], and the open-source Matlab code can be found in the paper. Two or more images with different scanning directions are fed into the correction algorithm, and the algorithm will output the drift corrected STEM images. The downloaded code package includes a detailed yet simple procedure for the implementation. A more detailed algorithm and description of the process can be found in the original paper.

1.3. Apply various image denoising techniques.

NOTE: After the drift correction, perform image denoising to enhance the accuracy of future analysis. Some of the common denoising techniques are listed here. Furthermore, we introduce a free interactive Matlab app named EASY-STEM with a graphical user interface to help with the analysis. The interface is shown in **Figure 2**, with all the steps labeled on the corresponding buttons.

[Place **Figure 2** here]

1.3.1. Apply the Gaussian filtering. In the EASY-STEM app, find a tab called **Gaussian** on the bottom left. Use the slider to select how many nearby pixels to average. Move the slider to apply the Gaussian filter to the image.

[Place **Figure 3** here].

NOTE: This technique uses a filter that averages the intensity of the nearby pixels in the images. The effect of the Gaussian filtering is presented in **Figure 3a-d.**

1.3.2. Apply Fourier filtering. In the EASY-STEM app, find a tab called **FFT** on the bottom left. There is a slider to restrict the spatial frequency to reduce high-frequency noise. Move the slider to apply the Fourier filter to the image.

NOTE: This technique limits the spatial frequency of the image to remove the high-frequency noise in the image.

1.3.3. Apply the Richardson-Lucy deconvolution. In the EASY-STEM app, find a tab called **Deconvolution** on the bottom left, where there are two input boxes for the iterations of blind deconvolution and Richardson-Lucy deconvolution, respectively. Change the value and apply this denoising algorithm by clicking the button.

NOTE: This technique is a deconvolution algorithm for effectively removing the noise in the image by calculating the point spread function.

2. Finding and refining the atom position

2.1. Find the initial atomic positions.

NOTE: After the post-acquisition image processing, the initial atomic positions can be simply extracted as the local intensity maximum or minimum for the ADF or ABF STEM images respectively. A minimum distance between the neighboring atomic columns needs to be defined to remove the extra positions.

2.1.1. Define the minimum distance (in pixels) by changing the value in the input box that determines the distance between the neighboring peaks.

2.1.2. Click the **Find Initial Positions** button in the EASY-STEM app. The result is shown in **Figure 3e**.

NOTE: Frequently, extra positions or missing positions are observed with a simple local max/min finding algorithm. Thus, a manual correction mode is created in the EASY-STEM app to further refine the atomic positions (**Add Missing/Remove Extra Points** buttons). This feature enables the addition and removal of the initial positions by using the mouse cursor.

2.2. Index the initial atomic positions with a unit-cell vector-based system.

2.2.1. Define an origin point in the image. In the EASY-STEM app, click on the **Find Origin** button. After clicking the button, drag the pointer to one of the initial atomic positions to define it as the origin.

2.2.2. Define the 2D unit cell u and v vectors and the unit cell fractions.

2.2.2.1. Click the **Find U/V** button and drag the pointer to the end of the unit cells.

2.2.2.2. Define the lattice fraction value by changing the value in the **Lat Frac U** and **Lat Frac V** input boxes.

NOTE: This value determines the lattice fraction value along the unit cell vector. For example, in the ABO_3 perovskite unit cell, the unit cell can be divided equally into two halves along the two perpendicular unit cell vector directions. Consequently, there are two fractions along each unit cell vector direction, so the unit cell fraction values are 2 and 2 for u and v directions, respectively. The example result of the indexing and the corresponding u and v unit cell vectors are

demonstrated in **Figure 3f**. For example, in **Figure 3f**, we will index the atoms on the corners as (0, 0), (1, 0), (0, 1), (1, 1); and we will index the atom in the center as (1/2, 1/2). This indexing system helps with information extraction in the following steps.

2.2.2.3. Click on the **Calculate Lattice** button to index all the atoms.

2.3. Click on the **Refine Positions** button in the EASY-STEM app to refine atomic positions with 2D-Gaussian fitting.

NOTE: After obtaining the initial atomic positions and indexing the atoms in the image, a 2D-Gaussian fitting around each atomic column needs to be applied to achieve the sub-pixel level precision in the analysis. Using this algorithm, it is possible to first crop an area in the image around each initial atomic position in the image and then fit a 2D-Gaussian peak in the cropped image. We then use the centers of the fitted 2D-Gaussian peaks as the refined atomic positions. This algorithm fits the 2D-Gaussian function to each atomic column in the image and the center of the fitted peak will be plotted after fitting. The result of the 2D-Gaussian fitting is shown in **Figure 3g,h**.

2.4. (Optional) Click the **mpfit Overlaps** button in EASY-STEM to refine atomic positions with 2D-Gaussian multi-peak fitting (mp-fit).

NOTE: Refine the atomic positions using the mp-fit algorithm when the intensities from adjacent atomic columns are overlapping with each other. The mp-fit algorithm and its effectiveness are discussed in detail by D. Mukherjee et al.[21]. The EASY-STEM app has incorporated this algorithm and can be used to separate neighboring atoms with overlapping intensities. The result of mp-fit is shown in **Figure 3i**.

2.5. Save the results by clicking the **Save Atomic positions** button.

NOTE: The app will prompt the user for location saving and file name. All saved results are included in the variable called "atom_pos".

3. Physical information extraction

3.1. Measure the atomic displacements based on the unit cell vector indexing and atomic positions.

3.1.1. Define a unit cell center.

NOTE: For example, for an ABO_3 perovskite unit cell looking from its [100] axis, the unit cell centers can be defined as the average position of the four A-site atoms. In the first unit cell, those A-site atoms have been previously indexed as (0, 0), (1, 0), (0, 1), (1, 1).

3.1.2. Find the position of the displaced atoms.

NOTE: In the case of the ABO_3 perovskite unit cell, the displaced atom is the B-site atom, which was previously labeled as (1/2, 1/2).

3.1.3. Iteratively find the position of the reference unit cell centers and displacement atoms for all the complete unit cells in the image.

NOTE: Unit cells may be incomplete near the edge of the TEM image. The atomic positions in those unit cells are discarded.

3.1.4. Measure the displacement vector by entering the following command:
d = pos(B) – mean(pos(A))

3.2. Quantify the lattice strain.

3.2.1. Extract the unit cell vectors from each unit cell based on the atomic positions.

NOTE: Extract vector matrix "C", which is a 2x2 matrix consisting of u-vector and v-vector for each unit cell in x and y directions.

3.2.2. Define a reference vector, "C_0".

NOTE: C_0 can be defined as the average unit cell vectors from the part of the image (recommended) or the theoretically calculated unit cell vector value.

3.2.3. Calculate the 2x2 transformation matrix "T" using the following equation:
$C_0 \cdot T = C$ or $T = C_0^{-1}C$ (1)

3.2.4. Calculate the distortion matrix "D":
D = T – I (2)
where the "I" is the identity matrix.

3.2.5. Decompose the distortion "D" to symmetric strain matrix "ε" and anti-symmetric rotation matrix "ω":
$$D = \begin{bmatrix} \varepsilon_{xx} & \varepsilon_{xy} \\ \varepsilon_{yx} & \varepsilon_{yy} \end{bmatrix} + \begin{bmatrix} 0 & \omega_{xy} \\ -\omega_{xy} & 0 \end{bmatrix} \quad (3)$$

NOTE: Strain matrix "ε" and rotation matrix "ω" can be extracted by using the equations:
$\varepsilon = \frac{D+D^T}{2}$ (4) And $\omega = \frac{D-D^T}{2}$ (5).

3.2.6. Iteratively calculate strains for all unit cells.

3.2.7. In the EASY-STEM app, click on the **Calculate Strain based on the atomic positions** button under the **Quantify** tab on the top-left of the interface.

NOTE: The users can customize the displayed range of the strain map by changing the value within the **Strain Upper/Lower limit** input box.

4. Data Visualization

4.1. Create colored line maps.

NOTE: Colored line mapping of the atomic bonds is a straightforward way to present the distance between nearby atoms. In Matlab, the command to draw a line between two points is: *Line([x1 x2],[y1 y2],'Color',[r g b]).* The inputs [x1 x2] and [y1 y2] are the coordinate values of the first and the second position. The distance variation can be presented with varying colors in the line map, which is defined by the [r g b] value. The [r g b] values stand for the red, green, and blue color values, each ranging from 0 to 1. Then iteratively connect all nearby atoms with colored lines.

4.1.1. Generate colored line maps in the EASY-STEM app.

NOTE: In the EASY-STEM app, line maps can be generated by a simple button clicking, which is under the **Quantity** tab on the top right of the interface.

4.1.1.1. Adjust the value (in pm) in the **Mean Distance** input box and **Measurement Range** input box in EASY-STEM. These two values define the average distance of the projected atom distance and the distance range of the measurement.

4.1.1.2. In the EASY-STEM app, click on the **Calculate Bond Length Based on Near Neighbor** button.

NOTE: The line maps will be generated automatically. The users can adjust the colormap, line style, and line width for better visualization.

4.2. Create vector maps.

NOTE: Vector maps can present atomic displacements in an area of the crystal. Since the displacement analysis is unique to individual systems, we have not integrated the code into the EASY-STEM app, but instead, here, we will introduce the Matlab commands for such analysis based on the standard ABO_3 perovskite unit cells.

4.2.1. Calculate the reference position for displacement measurement.

NOTE: In the example of ABO_3 perovskite, we have indexed the atoms on the corners (A-site) as (0, 0), (1, 0), (0, 1), (1, 1), and the atom in the center (B-site) as (1/2, 1/2). To compute the displacement with respect to the unit cell center, we first calculate the reference position as the averaged position of the corner (A-site) atoms. The Matlab command for this calculation is:
ref_center=(positionA1+positionA2+positionA3+PostionA4)/4

4.2.2. Calculate the displacement by entering the command:

[displace_x displace_y] = PositionB – ref_center

4.2.3. Implement the vector map:
quiver(x,y,displace_x,displace_y)

NOTE: The input `x` and `y` are the positions of the displaced atom. The variables *displace_x* and *displace_y* are the displacement magnitudes in x and y directions. The vector maps can be uniformly colored (e.g., yellow, white, red…) or shaded based on the displacement magnitude.

4.3. Create false-colored maps.

4.3.1. Generate the false-colored maps by upsampling to estimate the measured value (displacement, strain, etc.) for each pixel in the image:
ImageSize = Size(Image);
[xi,yi] = meshgrid(1:1:ImageSize(1),1:1:ImageSize(2));
Upsampled_Data = griddata(x,y,YourData,xi,yi,'v4');

NOTE: The "griddata" function upsamples the data at position (x,y) to estimate the value for each pixel in the entire image. The inputs xi and yi are the grid coordinates, and the 'v4' is the bicubic upsampling method.

4.3.2. Plot the upsampled data using user-defined color scale.

REPRESENTATIVE RESULTS:
Figure 3 shows the example results of atomic position tracking by following steps 1 and 2 in the protocol. A raw ADF-STEM image of a unit cell of the ABO_3 perovskite is shown in **Figure 3a**, and its intensity profile is plotted in 3-D in **Figure 3b**. **Figure 3c** shows the result after Gaussian filtering is applied to the STEM image in **Figure 3a**, and the intensity profile is plotted in **Figure 3d**. The initial positions are determined by finding the local maxima in the image and the positions are indicated by yellow circles in **Figure 3e**. The atomic positions are indexed based on the unit cell vector and shown in **Figure 3f**. After the initial position is found and indexed, 2D-Gaussian fitting is applied to further refine the measurement. In **Figure 3g** and **Figure 3h**, the fitted positions are indicated as red circles, the measurement precision is improved as the refined positions are closer to the center compared to the initial positions (yellow circles). Lastly, the advantage of applying the mpfit algorithm on the overlapping intensities is showcased in an ADF-STEM image of $BaMnSb_2$ crystal (**Figure 3i**). The regular 2D-Gaussian fitting (red circles) fails severely on the Mn columns as highlighted by yellow arrows, while the mpfit algorithm can determine the positions much more accurately (green circles).

[Place **Figure 4** here].

The HAADF-STEM image of the $Ca_3Ru_2O_7$ (CRO) is shown in **Figure 4a** and **Figure 4b** (with the overlaid crystal structure). CRO is a Ruddlesden-Popper phase perovskite crystal with the polar space group $A2_1am$. ADF-STEM imaging shows the contrast from the heavier elements well (Ca and Ru), but the O atoms are not visualized as lighter atoms do not scatter the beam strong enough to become visible with HAADF detectors. The non-centrosymmetry of the crystal structure is

caused by the tilting of O octahedra and can be visualized in ADF-STEM images by analyzing the displacement of the Ca atom in the center of the double perovskite layer. By following the steps listed in the Protocol section, all atomic positions in this image can be located by finding the centers of the fitted 2D-Gaussian peaks, as shown in **Figure 4c**. Furthermore, using the indexing system, in step 3.2, each type of atom in the unit cell can be identified and used for further processing. For example, the Ca atoms at the upper, center, and lower side of the perovskite double layer can be easily identified and their positions are presented with circles filled with different colors, as shown in **Figure 4d**.

[Place **Figure 5** here].

After positioning and indexing the atoms in the STEM images, the physical information can be extracted and visualized via various types of plots, as shown in **Figure 5**. The vector map that shows the polarization direction is shown in **Figure 5a**. The arrows point towards the projected polarization direction, and by coloring the arrows based on their orientation, a vertical head-to-head 90° domain wall (labeled with blue arrows), and a horizontal 180° domain wall (labeled with red arrows) are shown at the top of the image. By constructing the false-colored map as shown in **Figure 5b**, a decreasing polar displacement magnitude can be observed via the fading color in the center, and thus the head-to-tail domain wall can be visualized. By combining the vector map and false-color map, the T-junction formed by three domain walls is shown in the ADF-STEM image. Additionally, with the dimension of each unit cell in the image measured, an ε_{xx} strain map can be constructed, as shown in **Figure 5c**.

FIGURE AND TABLE LEGENDS:

Figure 1: The workflow of the atomic position quantification and structural measurement.

Figure 2: The graphical user interface (GUI) of the Matlab app EASY-STEM. All steps described in the protocol section are labeled accordingly.

Figure 3: Example results of atomic position tracking. (**a**) The drift-corrected ADF-STEM image showing a typical unit cell of the ABO_3 Perovskite. (**b**) The 3D plot of the intensity in (**a**). (**c**) The same image denoised with a Gaussian filter. (**d**) The 3D plot of the intensity in (**c**). (**e**) The contour plot of the intensity in (**c**) with the initial atomic positions (yellow circles) overlaid. (**f**) An example of the unit cell vector indexing system showing the index of the atomic positions in the image. (**g**) The contour plot of the intensity in (**c**) with the initial atomic positions (yellow circles) and refined atomic positions (red circles) overlaid, and (**h**) the 3D plot of the intensity with initial and refined atomic positions shown with yellow and red circles.

Figure 4: HAADF-STEM image of the $Ca_3Ru_2O_7$ (CRO). (**a**) The magnified image of the ADF-STEM image of the $Ca_3Ru_2O_7$ (CRO) crystal with the crystal structure schematic superimposed. The relative displacement of the Ca atom in the perovskite layer is highlighted with the yellow arrow. (**b**) Drift-corrected and denoised ADF-STEM image of CRO and (**c**) with overlaying refined atomic positions (red dots). (**d**) An example of using an indexing system to identify the upper (red), center (blue) and lower (yellow) Ca atoms in the perovskite layer.

Figure 5: Physical information. (**a**) An example of the implementation of the vector map showing

the polarization obtained from the center Ca displacement pattern. The arrows are colored based on the orientation (red to the right, blue to the left). The vertical 90° head-to-head and head-to-tail domain walls are indicated with blue arrows and a horizontal 180° domain wall is indicated with a red arrow. (**b**) An example of the implementation of the false-colored map showing the polarization. The color indicates the magnitude in left (yellow) and right (purple) directions. Reduced magnitude results in faded color. (**c**) An example of implementation of the false-colored map showing the ε_{xx} strain in the image. The color indicates the value of tensile (red) and compressive (blue) strain.

Figure 6: Statistical quantification of the atomic position finding. (**a**) The distribution of the perovskite A-site to A-site distance presented in a histogram. The normal distribution fitting is plotted and overlaid as the red dashed line showing the mean of 300.5 pm and the standard deviation of 4.8 pm. (**b**) The statistical quantification of the perovskite unit cell vector angle measurement is presented as a histogram. The normal distribution fitting is plotted and overlaid as the red dashed line showing the mean of 90.0° and the standard deviation of 1.3°. (**c**) The statistical quantification of the polar displacement measurement in $Ca_3Ru_2O_7$ (CRO) is presented as histogram. The normal distribution fitting is plotted and overlaid as the red dashed line showing the mean of 25.6 pm and the standard deviation of 7.7 pm.

DISCUSSION:

When working on the post-acquisition processing, some caution needs to be taken as well. To start with, during the image drift-correction, the algorithm assumes that the 0° image has the horizontal fast scanning direction, so double-check the direction before the calculation. If the scanning direction is not correctly set, the drift-correction algorithm will fail and may even introduce artifacts in the output[17]. Then during the imaging denoising, certain methods may introduce an artifact; for example, the Fourier filtering may create atom column contrast at the vacancy sites or remove fine features in the images, if the spatial resolution is not limited properly. As a result, it is vitally important to verify if the denoised images closely resemble the original raw input images.

Next, when determining the initial atomic positions based on local maximum/minimum, try to adjust the restriction minimum distance between peaks to avoid creating redundant positions between atomic columns. These redundant positions are artifacts generated due to the algorithm mistakenly recognizing the local maxima/minima in the image as atomic columns. Additionally, one can adjust the threshold value to find most of the positions if there are large contrast differences between various atomic species in the image (e.g., in ADF-STEM images of WS_2). After obtaining most of the initial atomic positions in the image, try to manually add missing ones or remove extra ones with best effort. Moreover, the method for the indexing of the atoms is the most effective when there are not large interruptions in the periodicities within the image. When there are interruptions such as grain boundaries or phase boundaries presented in the image, the indexing may fail. The solution to this problem is to define the areas of interest in the image (by clicking the **Define Area of Interest** button in EASY-STEM app), and then indexing and refining the positions within each area separately. Afterward, one can easily combine datasets of different areas in the same image into one set of data and work on the analysis.

Finally, after applying 2D-Gaussian peak fittings, scatter the refined positions points onto the input image to verify the fitting results to see if the refined positions deviate from the atomic columns.

The accuracy provided by the single Gaussian fitting algorithm is sufficient in most of the STEM experiments; however, if the position deviates due to the intensity from a neighboring atom, use the multi-peak fitting (mpfit) algorithm instead to isolate the intensity from adjacent atomic columns[21]. Otherwise, if the position deviates due to the image quality issue or the low intensity from the specific atom columns, it is suggested to discard the fitted position at that location.

There are several existing and specialized algorithms for the atomic position measurement, for example, the oxygen octahedra picker software[22], Atomap python package[23], and StatSTEM Matlab package[24]. However, these algorithms have some limitations in certain aspects. For instance, the oxygen octahedra picker requires the input of STEM images to contain only clearly resolved atomic columns and thus failed to address the issue in the images with atomic columns overlapping intensities[21]. On the other hand, although Atomap can calculate the positions of "dumbbell-like" atomic columns, the process is not very straightforward. Additionally, the StatSTEM is a great algorithm for quantifying the overlapping intensities, but its iterative model-based fitting process is computationally expensive[21]. In contrast, our approach, introduced in this work along with the Matlab app EASY-STEM, which is integrated with the advanced mpfit algorithm, can address the problem of the overlapping intensity and is less computationally expensive than StatSTEM, while offering competitive measurement precision. Furthermore, the analysis from Atomap and the oxygen octahedra picker software packages are designed and specialized for analyzing the data from ABO_3 perovskite crystals, while the indexing system shown in this work is much more flexible about different materials systems. With the method in this work, users can fully design and customize the data analysis for their unique material systems based on the output results that contain both refined atomic positions and the unit cell vector indexing.

[Place **Figure 6** here].

The method introduced here provides picometer-level precision and simplicity for implementation. To demonstrate the measurement precision, the statistical quantification of the atomic position finding is presented in **Figure 6**. The measurements of cubic ABO_3 perovskite A-site distance distribution and unit cell vector angle distribution are plotted using histogram in **Figure 6a** and **Figure 6b**, respectively. By fitting the normal distribution curve to the distributions, the A-site distance distribution shows a mean of 300.5 pm and standard deviation of 4.8 pm and unit cell vector angle distribution shows a mean of 90.0° and standard deviation of 1.3°. The statistical quantification indicates the method proposed here enables picometer-level precision and can greatly alleviate the distortion due to drift during imaging. This result suggests that this measurement is trustworthy when the physical information to be measured is greater or equal to roughly 10 pm. For example, in the case of aforementioned CRO crystals, the measurement of the magnitude of the polar displacement is presented in **Figure 6c**. The measurement shows a mean of 25.6 pm, a standard deviation of 7.7 pm, and it shows that the polar displacement measurement in CRO STEM images is solid. Additionally, more caution needs to be taken in case of experimental limitations such as low signal-to-noise ratio when imaging beam-sensitive samples. In those cases, the measured atomic positions need to be closely examined against the raw images to ensure the validity of the measurement. Consequently, the analysis method introduced here has limitations to the measurement precision when compared to more recent and advanced algorithms. Our method is insufficient when the precision is required at the sub-picometer level, so a more advanced analysis routine is necessary if the feature to be extracted in the image is below a certain

threshold. For example, the non-rigid registration algorithm has shown sub-picometer precision measurement on silicon and it enables accurate measurement of bond length variation on a single Pt nanoparticle[25]. Most recently, the deep learning algorithm was employed to identify various types of point defects in 2-D transition metal dichalcogenides monolayers from a huge amount of STEM image data. Later, the measurement was conducted on the averaged image of different types of defects and this method also demonstrated sub-picometer level precision on the distortion around those defects[18]. Consequently, as a future plan for increasing the analysis capacity, we are in the progress of developing and implementing more advanced algorithms such as deep learning. We will also try to integrate them into the future data analysis tool updates.

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The authors have nothing to disclose.

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TITLE:
Picometer-Precision Atomic Position Tracking through Electron Microscopy

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SUMMARY:
This work presents the workflow for atomic position tracking in the atomic resolution transmission electron microscopy imaging. This workflow is performed using an open-source Matlab app (EASY-STEM).

ABSTRACT:
The modern aberration-corrected scanning transmission electron microscopes (AC-STEM) have successfully achieved direct visualization of atomic columns with sub-angstrom resolution. With this significant progress, advanced image quantification and analysis are still at the early stages. In this work, we present the complete pathway for the metrology of atomic resolution scanning transmission electron microscopy (STEM) images. This includes (1) tips for acquiring high-quality STEM images; (2) denoising and drift-correction for enhancing measurement accuracy; (3) obtaining initial atomic positions; (4) indexing the atoms based on unit cell vectors; (5) quantifying the atom column positions with either 2D-gaussian single peak fitting or (6) multi-peak fitting routines for slightly overlapping atomic columns; (7) quantification of lattice distortion/strain within the crystal structures or at the defects/interfaces where the lattice periodicity is disrupted; and (8) some common methods to visualize and present the analysis.

Furthermore, a simple self-developed free MATLAB app (EASY-STEM) with a graphical user interface (GUI) will be presented. The latter can assist in the analysis of STEM images without the need for writing dedicated analysis code or software. The advanced data analysis methods presented here can be applied for the local quantification of defect relaxations, local structural distortions, local phase transformations, and non-centrosymmetry in a wide range of materials.

INTRODUCTION:

The development of spherical aberration correction in the modern scanning transmission electron microscope (STEM) has enabled microscopists to probe crystals with sub-angstrom sized electron beams[1,2]. This has allowed imaging of individual atomic columns in a wide variety of crystals with interpretable atomic resolution images for both heavy and light elements[3,4]. Recent developments in pixelated direct electron detectors and data analysis algorithms have enabled phase reconstruction imaging techniques, such as ptychography, with further improvements in spatial resolution to around 30 pm[5–7]. Additionally, the recent progress in STEM tomography has even enabled three-dimensional atomic resolution reconstruction of the single nano-particle[8]. The electron microscope thus has become an extraordinarily powerful tool for quantifying structural properties in materials with both high precision and site-specificity.

With the ultra-high resolution STEM images as the data input, direct measurements of structural distortions were performed to extract the physical information from crystals at the atomic scale[9,10]. For example, the defect coupling between a Mo dopant in WS_2 monolayer and a single S vacancy was directly visualized via measuring the atomic positions and then calculating projected bond lengths[11]. Furthermore, the measurement done on crystal interfaces, such as the coalesced grain boundaries in monolayer WS_2, can exhibit the local atomic arrangement[12]. The interfacial analysis performed on the ferroelectric domain walls in $LiNbO_3$ revealed the domain wall to be a combination of Ising and Neel states[13]. Another example is the visualization of the polar vortex structures achieved in the $SrTiO_3$-$PbTiO_3$ superlattices, achieved through calculation of the titanium atomic column displacements with respect to the strontium and lead column positions[14]. Finally, the advances in computer vision algorithms, such as image denoising with non-local principle component analysis[15], Richardson and Lucy deconvolution[16], drift-correction with non-linear registration[17], and pattern recognition with deep learning, have significantly strengthened the accuracy of the measurement to sub-picometer precision[18]. One such example is the alignment and image registration of multiple fast-scan cryogenic-STEM images to enhance the signal-to-noise ratio. Subsequently, the Fourier-masking technique was applied to analyze the charge density waves in crystals by directly visualizing the periodic lattice distortion[19]. Even though the incredible aberration-corrected STEM instrumentation is increasingly accessible to researchers around the globe, the advanced data analysis procedures and methods remain uncommon and an enormous barrier for one without experience in data analysis.

In the present work, we will showcase the complete pathway for the metrology of atomic resolution STEM images. This process includes firstly acquiring the STEM images with an aberration-corrected microscope followed by performing post-acquisition denoising/drift-correction for enhanced measurement accuracy. We will then further discuss the existing methods to clearly resolve and accurately quantify the atom column positions with either 2D-gaussian single peak fitting or multi-peak fitting routines for slightly overlapping atomic columns[20,21]. Lastly, this tutorial will discuss methods for the quantification of lattice distortion/strain within the crystal structures or at the defects/interfaces where the lattice periodicity is disrupted. We will also introduce a simple self-developed free MATLAB app (EASY-STEM) with a graphical user interface (GUI) that can help with the analysis of STEM images without the need for writing dedicated analysis code or software. The advanced data analysis methods presented here can be applied for the local quantification of defect relaxations, local structural distortions, local phase

transformations, and non-centrosymmetry in a wide range of materials.

PROTOCOL:

NOTE: The flow chart in **Figure 1** shows the general procedure of the atomic position quantification.

[Place **Figure 1** here]

1. STEM image drift-correction and denoising

1.1. Acquire high-quality annular dark-field (ADF)/annular bright-field (ABF) STEM images.

NOTE: The quality of the input data is key to ensuring the accuracy of data analysis, so here we start the protocol with a few tips for acquiring good image data.

1.1.1. Ensure a high-quality TEM sample. The sample quality is extremely crucial. Use thin and clean TEM samples with no beam damage for imaging. Avoid touching the sample during handling and loading as this can cause sample contamination.

1.1.2. Clean the sample before insertion (if possible). Clean the sample using plasma cleaner, baking in a vacuum, or irradiating the region of interest in the sample at low magnifications by spreading the electron beam after sample insertion into the microscope ('beam shower'). Avoid damaged or contaminated areas when imaging.

1.1.3. Align the microscope and tune the aberration correctors to minimize the lens aberrations as much as possible. Test the resolution by acquiring a few STEM images on a standard sample to confirm that the spatial resolution can resolve the specific crystal structures and further fine-tune the aberrations in the image.

1.1.4. Tilt the sample until the optical axis is aligned with the specific zone axis of the crystal. For certain crystals, make observations from a required zone axis. For instance, the viewing axis needs to align with the planes of the domain walls in ferroelectric crystals for the measurement.

1.1.5. Optimize the electron dose while limiting electron beam damage and the sample drift during imaging. If the sample is stable under the electron beam and does not show drift or damage during the acquisition, it may be possible to try a higher electron dose or acquire multiple images of the same region to boost the signal-to-noise ratio. The goal here is to have a higher signal-to-noise ratio without beam damage or image artifacts.

1.1.6. Acquire STEM images with different scanning directions to correct for potential drift during acquisition. First, acquire an image and then take the second one from the same region immediately after rotating the scan direction by 90°.

1.1.6.1. Take images using the same imaging condition except for the scan directions. The purpose of this step is to feed the rotated images to the drift correction algorithm developed recently[17].

NOTE: One can also input more than two images with more varying scanning directions (with arbitrary angles) into the algorithm. However, successive scanning of the same region may lead to lattice damage or drift in that area. Additionally, it is recommended that the scan direction and the low index lattice planes not maintain parallel or perpendicular directions with each other and instead maintain oblique angles. If the scan direction coincides with certain horizontal or vertical features (lattice planes, interfaces, etc..), the drift along the direction of the strong vertically/laterally varying features may cause artifacts during image registration.

1.2. Perform drift-correction with a non-linear correction algorithm.

NOTE: The non-linear drift correction algorithm was proposed and constructed by C. Ophus et al.[17], and the open-source Matlab code can be found in the paper. Two or more images with different scanning directions are fed into the correction algorithm, and the algorithm will output the drift corrected STEM images. The downloaded code package includes a detailed yet simple procedure for the implementation. A more detailed algorithm and description of the process can be found in the original paper.

1.3. Apply various image denoising techniques.

NOTE: After the drift correction, perform image denoising to enhance the accuracy of future analysis. Here some of the common denoising techniques are listed. Furthermore, here we introduce a free interactive Matlab app named EASY-STEM with a graphical user interface to help with the analysis. The interface is shown in **Figure 2**, with all the steps labeled on the corresponding buttons.

[Place **Figure 2** here]

1.3.1. Apply the Gaussian filtering. In the EASY-STEM app, find a tab called **Gaussian** on the bottom left. There is a slider to select how many nearby pixels to average. Move the slider to apply Gaussian filter to the image.

[Place **Figure 3** here].

NOTE: This technique uses a filter that averages the intensity of the nearby pixels in the images. The effect of the Gaussian filtering is presented in **Figure 3a-d.**

1.3.2. Apply Fourier filtering. In the EASY-STEM app, find a tab called **FFT** on the bottom left. There is a slider to restrict the spatial frequency to reduce high-frequency noise. Move the slider to apply the Fourier filter to the image.

NOTE: This technique limits the spatial frequency of the image to remove the high-frequency noise in the image.

1.3.3. Apply the Richardson-Lucy deconvolution. In the EASY-STEM app, find a tab called **Deconvolution** on the bottom left, where there are two input boxes for the iterations of blind deconvolution and Richardson-Lucy deconvolution respectively. Change the value and apply this denoising algorithm by clicking the button.

NOTE: This technique is a deconvolution algorithm for effectively removing the noise in the image by calculating the point spread function.

2. Atom position finding and refining

2.1. Find the initial atomic positions.

NOTE: After the post-acquisition image processing, the initial atomic positions can be simply extracted as the local intensity maximum or minimum for the ADF or ABF STEM images respectively. A minimum distance between the neighboring atomic columns needs to be defined to remove the extra positions.

2.1.1. Define the minimum distance (in pixels) by changing the value in the input box that determines the distance between the neighboring peaks.

2.1.2. Click the **Find Initial Positions** button in the EASY-STEM app. The result is shown in **Figure 3e**.

NOTE: Frequently, extra positions or missing positions are observed with a simple local max/min finding algorithm. Thus, a manual correction mode is created in the EASY-STEM app to further refine the atomic positions (**Add Missing/Remove Extra Points** buttons). This feature enables the addition and removal of the initial positions by using the mouse cursor.

2.2. Index the initial atomic positions with a unit-cell vector-based system.

2.2.1. Define an origin point in the image. In the EASY-STEM app, click on the **Find Origin** button. After clicking the button, drag the pointer to one of the initial atomic positions to define it as the origin.

2.2.2. Define the 2D unit cell u and v vectors and the unit cell fractions.

2.2.2.1. Click the **Find U/V** button and drag the pointer to the end of the unit cells.

2.2.2.2. Define the lattice fraction value by changing the value in the **Lat Frac U** and **Lat Frac V** input boxes.

NOTE: This value determines the lattice fraction value along the unit cell vector. For example, in the ABO_3 perovskite unit cell, the unit cell can be divided equally into two halves along the two perpendicular unit cell vector directions. Consequently, there are two fractions along each unit cell vector direction, so the unit cell fraction values are 2 and 2 for u and v directions, respectively.

The example result of the indexing and the corresponding u and v unit cell vectors are demonstrated in **Figure 3f**. For example, in **Figure 3f**, we will index the atoms on the corners as (0, 0), (1, 0), (0, 1), (1, 1); and we will index the atom in the center as (1/2, 1/2). This indexing system helps with information extraction in the following steps.

2.2.2.3. Click on the **Calculate Lattice** button to index all the atoms.

2.3. Click on the **Refine Positions** button in the EASY-STEM app to refine atomic positions with 2D-Gaussian fitting.

NOTE: After obtaining the initial atomic positions and indexing the atoms in the image, a 2D-Gaussian fitting around each atomic column needs to be applied to achieve the sub-pixel level precision in the analysis. Using this algorithm, it is possible to first crop an area in the image around each initial atomic position in the image and then fit a 2D-Gaussian peak in the cropped image. We then use the centers of the fitted 2D-Gaussian peaks as the refined atomic positions. This algorithm fits the 2D-Gaussian function to each atomic column in the image and the center of the fitted peak will be plotted after fitting. The result of the 2D-Gaussian fitting is shown in **Figure 3g,h**.

2.4. (Optional) Click the **mpfit Overlaps** button in EASY-STEM to refine atomic positions with 2D-Gaussian multi-peak fitting (mp-fit).

NOTE: Refine the atomic positions using the mp-fit algorithm when the intensities from adjacent atomic columns are overlapping with each other. The mp-fit algorithm and its effectiveness are discussed in detail by D. Mukherjee et al.[21]. The EASY-STEM app has incorporated this algorithm and can be used to separate neighboring atoms with overlapping intensities. The result of mp-fit is shown in **Figure 3i**.

2.5. Save the results by clicking the **Save Atomic positions** button.

NOTE: The app will prompt the user for location saving and file name. All saved results are included in the variable called "atom_pos".

3. Physical information extraction

3.1. Measure the atomic displacements based on the unit cell vector indexing and atomic positions.

3.1.1. Define a unit cell center.

NOTE: For example, for an ABO_3 perovskite unit cell looking from its [100] axis, the unit cell centers can be defined as the average position of the four A-site atoms. In the first unit cell, those A-site atoms have been previously indexed as (0, 0), (1, 0), (0, 1), (1, 1).

3.1.2. Find the position of the displaced atoms.

NOTE: In the case of the ABO_3 perovskite unit cell, the displaced atom is the B-site atom, which was previously labeled as (1/2, 1/2).

3.1.3. Iteratively find the position of the reference unit cell centers and displacement atoms for all the complete unit cells in the image.

NOTE: Unit cells may be incomplete near the edge of the TEM image. The atomic positions in those unit cells are discarded.

3.1.4. Measure the displacement vector by entering the following command:
d = pos(B) – mean(pos(A))

3.2. Quantify the lattice strain.

3.2.1. Extract the unit cell vectors from each unit cell based on the atomic positions.

NOTE: Extract vector matrix "C", which is a two by two matrix consisting of u-vector and v-vector for each unit cell in x and y directions.

3.2.2. Define a reference vector, "C_0".

NOTE: C_0 can be defined as the average unit cell vectors from the part of the image (recommended) or the theoretically calculated unit cell vector value.

3.2.3. Calculate the two by two transformation matrix "T" using the following equation:
$C_0 \cdot T = C$ or $T = C_0^{-1}C$ (1)

3.2.4. Calculate the distortion matrix "D":
D = T – I (2)
where the "I" is the identity matrix.

3.2.5. Decompose the distortion "D" to symmetric strain matrix "ε" and anti-symmetric rotation matrix "ω":

$$D = \begin{bmatrix} \varepsilon_{xx} & \varepsilon_{xy} \\ \varepsilon_{yx} & \varepsilon_{yy} \end{bmatrix} + \begin{bmatrix} 0 & \omega_{xy} \\ -\omega_{xy} & 0 \end{bmatrix} \quad (3)$$

NOTE: strain matrix "ε" and rotation matrix "ω" can be extracted by using the equations:

$\varepsilon = \frac{D+D^T}{2}$ (4) And $\omega = \frac{D-D^T}{2}$ (5).

3.2.6. Iteratively calculate strains for all unit cells.

3.2.7. In the EASY-STEM app, click on the **Calculate Strain based on the atomic positions** button under the **Quantify** tab on the top-left of the interface.

NOTE: The users can customize the displayed range of the strain map by changing the value within the **Strain Upper/Lower limit** input box.

4. Data Visualization

4.1. Create colored line maps.

NOTE: Colored line mapping of the atomic bonds is a straightforward way to present the distance between nearby atoms. In Matlab, the command to draw a line between two points is: *Line([x1 x2],[y1 y2],'Color',[r g b]).* The inputs [x1 x2] and [y1 y2] are the coordinate values of the first and the second position. The distance variation can be presented with varying colors in the line map, which is defined by the [r g b] value. The [r g b] values stand for the red, green, and blue color values, each ranging from 0 to 1. Then iteratively connect all nearby atoms with colored lines.

4.1.1. Generate colored line maps in the EASY-STEM app.

NOTE: In the EASY-STEM app, line maps can be generated by a simple button clicking, which is under the **Quantity** tab on the top right of the interface.

4.1.1.1. Adjust the value (in pm) in the **Mean Distance** input box and **Measurement Range** input box in EASY-STEM. These two values define the average distance of the projected atom distance and the distance range of the measurement.

4.1.1.2. In the EASY-STEM app, click on the **Calculate Bond Length Based on Near Neighbor** button.

NOTE: the line maps will be generated automatically. The users can adjust the colormap, line style, and line width for better visualization.

4.2. Create vector maps.

NOTE: Vector maps can present atomic displacements in an area of the crystal. Since the displacement analysis is unique to individual systems, we have not integrated the code into the EASY-STEM app, but instead, here, we will introduce the Matlab commands for such analysis based on the standard ABO_3 perovskite unit cells.

4.2.1. Calculate the reference position for displacement measurement.

NOTE: In the example of ABO_3 perovskite, we have indexed the atoms on the corners (A-site) as (0, 0), (1, 0), (0, 1), (1, 1), and the atom in the center (B-site) as (1/2,1/2). To compute the displacement with respect to the unit cell center, we first calculate the reference position as the averaged position of the corner (A-site) atoms. The Matlab command for this calculation is:
ref_center=(positionA1+positionA2+positionA3+PostionA4)/4

4.2.2. Calculate the displacement by entering the command:

[displace_x displace_y] = PositionB – ref_center

4.2.3. Implement the vector map:
quiver(x,y,displace_x,displace_y)

NOTE: The input `x` and `y` are the positions of the displaced atom. The variables *displace_x* and *displace_y* are the displacement magnitudes in x and y directions. The vector maps can be uniformly colored (e.g., yellow, white, red…) or shaded based on the displacement magnitude.

4.3. Create false-colored maps.

4.3.1. Generate the false-colored maps by upsampling to estimate the measured value (displacement, strain, etc.) for each pixel in the image:
ImageSize = Size(Image);
[xi,yi] = meshgrid(1:1:ImageSize(1),1:1:ImageSize(2));
Upsampled_Data = griddata(x,y,YourData,xi,yi,'v4');

NOTE: The "griddata" function upsamples your data at position (x,y) to estimate the value for each pixel in the entire image. The inputs xi and yi are the grid coordinates, and the 'v4' is the bicubic upsampling method.

4.3.2. Plot the upsampled data using user-defined color scale.

REPRESENTATIVE RESULTS:
Figure 3 shows the example results of atomic position tracking by following steps 1 and 2 in the protocol. A raw ADF-STEM image of a unit cell of the ABO_3 perovskite is shown in **Figure 3a**, and its intensity profile is plotted in 3-D in **Figure 3b**. **Figure 3c** shows the result after Gaussian filtering is applied to the STEM image in **Figure 3a**, and the intensity profile is plotted in **Figure 3d**. The initial positions are determined by finding the local maxima in the image and the positions are indicated by yellow circles in **Figure 3e**. The atomic positions are indexed based on the unit cell vector and shown in **Figure 3f**. After the initial position is found and indexed, 2D-gaussian fitting is applied to further refine the measurement. In **Figure 3g** and **Figure 3h**, the fitted positions are indicated as red circles, the measurement precision is improved as the refined positions are closer to the center compared to the initial positions (yellow circles). Lastly, the advantage of applying the mpfit algorithm on the overlapping intensities is showcased in an ADF-STEM image of $BaMnSb_2$ crystal (**Figure 3i**). The regular 2D-gaussian fitting (red circles) fails severely on the Mn columns as highlighted by yellow arrows, while the mpfit algorithm can determine the positions much more accurately (green circles).

[Place **Figure 4** here].

The HAADF-STEM image of the $Ca_3Ru_2O_7$ (CRO) is shown in **Figure 4a** and **Figure 4b** (with the overlaid crystal structure). CRO is a Ruddlesden-Popper phase perovskite crystal with the polar space group $A2_1am$. ADF-STEM imaging shows the contrast from the heavier elements well (Ca and Ru), but the O atoms are not visualized as lighter atoms do not scatter the beam strong enough to become visible with HAADF detectors. The non-centrosymmetry of the crystal structure is

caused by the tilting of O octahedra and can be visualized in ADF-STEM images by analyzing the displacement of the Ca atom in the center of the double perovskite layer. By following the steps listed in the Protocol section, all atomic positions in this image can be located by finding the centers of the fitted 2D-gaussian peaks, as shown in **Figure 4c**. Furthermore, using the indexing system, in step 3.2, each type of atom in the unit cell can be identified and used for further processing. For example, the Ca atoms at the upper, center, and lower side of the perovskite double layer can be easily identified and their positions are presented with circles filled with different colors, as shown in **Figure 4d**.

[Place **Figure 5** here].

After positioning and indexing the atoms in the STEM images, the physical information can be extracted and visualized via various types of plots, as shown in **Figure 5**. The vector map that shows the polarization direction is shown in **Figure 5a**. The arrows point towards the projected polarization direction, and by coloring the arrows based on their orientation, a vertical head-to-head 90-degree domain wall (labeled with blue arrows), and a horizontal 180° domain wall (labeled with red arrows) are shown at the top of the image. By constructing the false-colored map as shown in **Figure 5b**, a decreasing polar displacement magnitude can be observed via the fading color in the center, and thus the head-to-tail domain wall can be visualized. By combining the vector map and false-color map, the T-junction formed by three domain walls is shown in the ADF-STEM image. Additionally, with the dimension of each unit cell in the image measured, an ε_{xx} strain map can be constructed, as shown in **Figure 5c**.

FIGURE AND TABLE LEGENDS:

Figure 1: The workflow of the atomic position quantification and structural measurement.

Figure 2: The graphical user interface (GUI) of the Matlab app EASY-STEM. All steps described in the protocol section are labeled accordingly.

Figure 3: Example results of atomic position tracking. (**a**) The drift-corrected ADF-STEM image showing a typical unit cell of the ABO_3 Perovskite. (**b**) The 3D plot of the intensity in (**a**). (**c**) The same image denoised with a Gaussian filter. (**d**) The 3D plot of the intensity in (**c**). (**e**) The contour plot of the intensity in (**c**) with the initial atomic positions (yellow circles) overlaid. (**f**) An example of the unit cell vector indexing system showing the index of the atomic positions in the image. (**g**) The contour plot of the intensity in (**c**) with the initial atomic positions (yellow circles) and refined atomic positions (red circles) overlaid, and (**h**) the 3D plot of the intensity with initial and refined atomic positions shown with yellow and red circles.

Figure 4: HAADF-STEM image of the $Ca_3Ru_2O_7$ (CRO). (**a**) The magnified image of the ADF-STEM image of the $Ca_3Ru_2O_7$ (CRO) crystal with the crystal structure schematic superimposed. The relative displacement of the Ca atom in the perovskite layer is highlighted with the yellow arrow. (**b**) Drift-corrected and denoised ADF-STEM image of CRO and (**c**) with overlaying refined atomic positions (red dots). (**d**) An example of using an indexing system to identify the upper (red), center (blue) and lower (yellow) Ca atoms in the perovskite layer.

Figure 5: Physical information. (**a**) An example of the implementation of the vector map showing

the polarization obtained from the center Ca displacement pattern. The arrows are colored based on the orientation (red to the right, blue to the left). The vertical 90° head-to-head and head-to-tail domain walls are indicated with blue arrows and a horizontal 180° domain wall is indicated with a red arrow. (**b**) An example of the implementation of the false-colored map showing the polarization. The color indicates the magnitude in left (yellow) and right (purple) directions. Reduced magnitude results in faded color. (**c**) An example of implementation of the false-colored map showing the ε_{xx} strain in the image. The color indicates the value of tensile (red) and compressive (blue) strain.

Figure 6: Statistical quantification of the atomic position finding. (**a**) The distribution of the perovskite A-site to A-site distance presented in a histogram. The normal distribution fitting is plotted and overlaid as the red dashed line showing the mean of 300.5 pm and the standard deviation of 4.8 pm. (**b**) The statistical quantification of the perovskite unit cell vector angle measurement is presented as a histogram. The normal distribution fitting is plotted and overlaid as the red dashed line showing the mean of 90.0° and the standard deviation of 1.3°. (**c**) The statistical quantification of the polar displacement measurement in $Ca_3Ru_2O_7$ (CRO) is presented as histogram. The normal distribution fitting is plotted and overlaid as the red dashed line showing the mean of 25.6 pm and the standard deviation of 7.7 pm.

DISCUSSION:

When working on the post-acquisition processing, some caution needs to be taken as well. To start with, during the image drift-correction, the algorithm assumes that the 0° image has the horizontal fast scanning direction, so double-check the direction before the calculation. If the scanning direction is not correctly set, the drift-correction algorithm will fail and may even introduce artifact in the output[17]. Then during the imaging denoising, certain methods may introduce an artifact; for example, the Fourier filtering may create atom column contrast at the vacancy sites or remove fine features in the images, if the spatial resolution is not limited properly. As a result, it is vitally important to verify if the denoised images closely resemble the original raw input images.

Next, when determining the initial atomic positions based on local maximum/minimum, try to adjust the restriction minimum distance between peaks to avoid creating redundant positions between atomic columns. These redundant positions are artifacts generated due to the algorithm mistakenly recognizing the local maxima/minima in the image as atomic columns. Additionally, one can adjust the threshold value to find most of the positions if there are large contrast differences between various atomic species in the image (e.g., in ADF-STEM images of WS_2). After obtaining most of the initial atomic positions in the image, try to manually add missing ones or remove extra ones with the best effort. Moreover, the method for the indexing of the atoms is the most effective when there are not large interruptions in the periodicities within the image. When there are interruptions such as grain boundaries or phase boundaries presented in the image, the indexing may fail. The solution to this problem is to define the areas of interest in the image (by clicking the **Define Area of Interest** button in EASY-STEM app), and then indexing and refining the positions within each area separately. Afterward, one can easily combine datasets of different areas in the same image into one set of data and work on the analysis.

Finally, after applying 2D-Gaussian peak fittings, scatter the refined positions points onto the input image to verify the fitting results to see if the refined positions deviate from the atomic columns.

The accuracy provided by the single Gaussian fitting algorithm is sufficient in most of the STEM experiments; however, if the position deviates due to the intensity from a neighboring atom, use the multi-peak fitting (mpfit) algorithm instead to isolate the intensity from adjacent atomic columns[21]. Otherwise, if the position deviates due to the image quality issue or the low intensity from the specific atom columns, it is suggested to discard the fitted position at that location.

There are several existing and specialized algorithms for the atomic position measurement, for example, the oxygen octahedra picker software[22], Atomap python package[23], and StatSTEM Matlab package[24]. However, these algorithms show some limitations in certain aspects. For instance, the oxygen octahedra picker requires the input of STEM images to contain only clearly resolved atomic columns and thus failed to address the issue in the images with atomic columns overlapping intensities[21]. On the other hand, although Atomap is capable of calculating the positions of "dumbbell-like" atomic columns, the process is not very straightforward. Additionally, the StatSTEM is a great algorithm for quantifying the overlapping intensities, but its iterative model-based fitting process is computationally expensive[21]. In contrast, our approach, introduced in this work along with the Matlab app EASY-STEM, which is integrated with the advanced mpfit algorithm, can address the problem of the overlapping intensity and is less computationally expensive than StatSTEM, while offering competitive measurement precision. Furthermore, the analysis from Atomap and the oxygen octahedra picker software packages are designed and specialized for analyzing the data from ABO_3 perovskite crystals, while the indexing system shown in this work is much more flexible about different materials systems. With the method in this work, users can fully design and customize the data analysis for their unique material systems based on the output results that contain both refined atomic positions and the unit cell vector indexing.

[Place **Figure 6** here].

The method introduced here provides picometer-level precision and simplicity for implementation. To demonstrate the measurement precision, the statistical quantification of the atomic position finding is presented in **Figure 6**. The measurement of cubic ABO_3 perovskite A-site distance distribution and unit cell vector angle distribution is plotted using histogram in **Figure 6a** and **Figure 6b**, respectively. By fitting the normal distribution curve to the distributions, the A-site distance distribution shows a mean of 300.5 pm and standard deviation of 4.8 pm and unit cell vector angle distribution shows a mean of 90.0° and standard deviation of 1.3°. The statistical quantification indicates the method proposed here enables picometer-level precision and can greatly alleviate the distortion due to drift during imaging. This result suggests that this measurement is trustworthy when the physical information to be measured is greater or equal to roughly 10 pm. For example, in the case of aforementioned CRO crystals, the measurement of the magnitude of the polar displacement is presented in **Figure 6c**. The measurement shows a mean of 25.6 pm, a standard deviation of 7.7 pm, and it shows that the polar displacement measurement in CRO STEM images is solid. Additionally, more caution needs to be taken in case of experimental limitations such as low signal-to-noise ratio when imaging beam-sensitive samples. In those cases, the measured atomic positions need to be closely examined against the raw images to ensure the validity of the measurement. Consequently, the analysis method introduced here has limitations to the measurement precision when comparing to more recent and advanced algorithms. Our method is insufficient when the precision is required at the sub-picometer level, so a more advanced analysis routine is necessary if the feature to be extracted in the image is below a certain

threshold. For example, the non-rigid registration algorithm has shown sub-picometer precision measurement on silicon and it enabled accurate measurement of bond length variation on a single Pt nanoparticle[25]. Most recently, the deep learning algorithm was employed to identify various types of point defects in 2-D transition metal dichalcogenides monolayers from a huge amount of STEM image data. Later, the measurement was conducted on the averaged image of different types of defects and this method also demonstrated sub-picometer level precision on the distortion around those defects[18]. Consequently, as a future plan for increasing our analysis capacity, we are in the progress of developing and implementing more advanced algorithms such as deep learning. We will also try to integrate them into our future data analysis tool updates.

ACKNOWLEDGMENTS:

L.M. and N.A.'s work supported by the Penn State Center for Nanoscale Sciences, an NSF MRSEC under the grant number DMR-140620. D.M. was supported by ORNL's Laboratory Directed Research and Development (LDRD) Program, which is managed by UT-Battelle, LLC, for the U.S. Department of Energy (DOE). A.C. and N.A. acknowledge the Air Force Office of Scientific Research (AFOSR) program FA9550-18-1-0277 as well as GAME MURI, 10059059-PENNfor support.

DISCLOSURES:

The authors have nothing to disclose.

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Figure 1

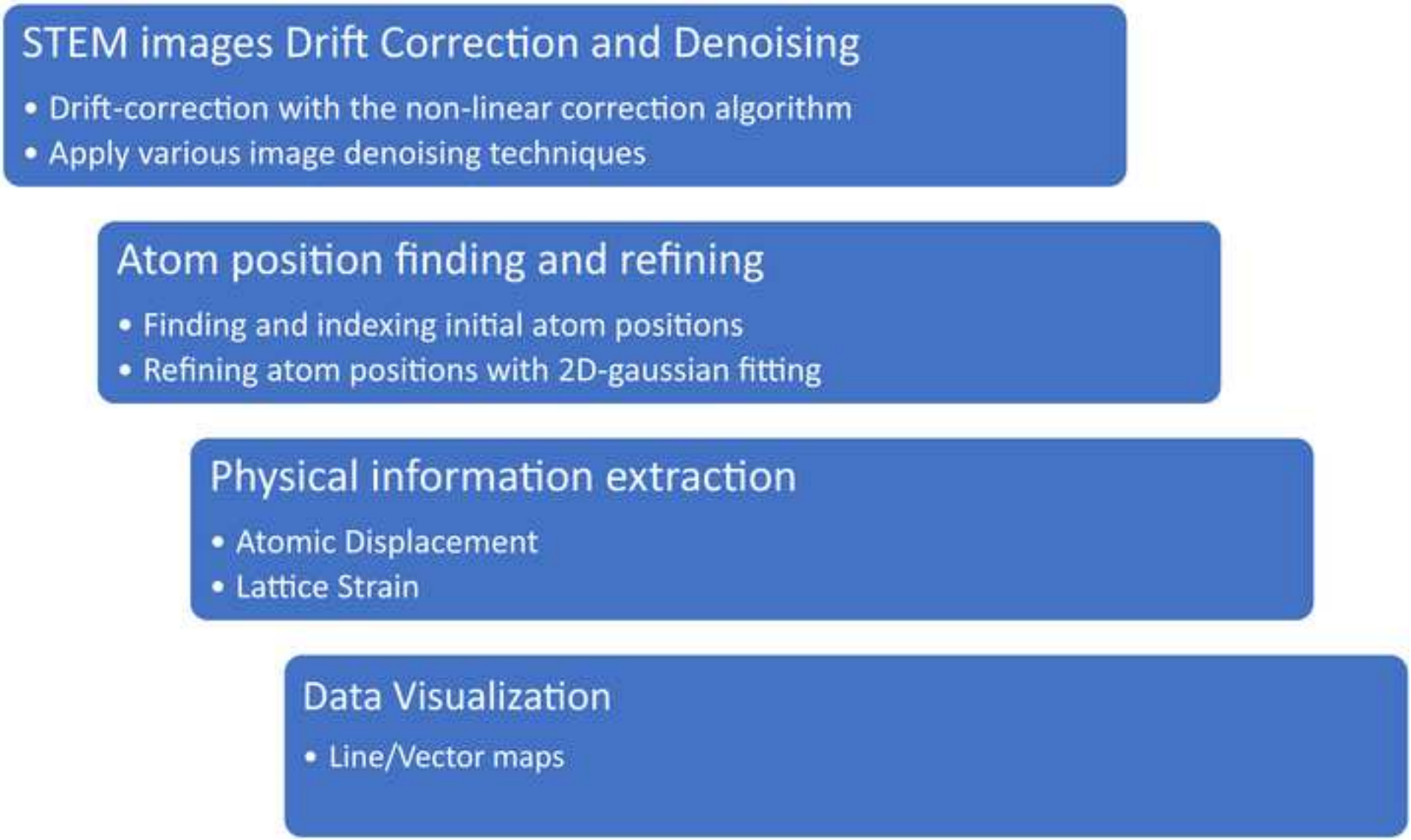

Figure 2

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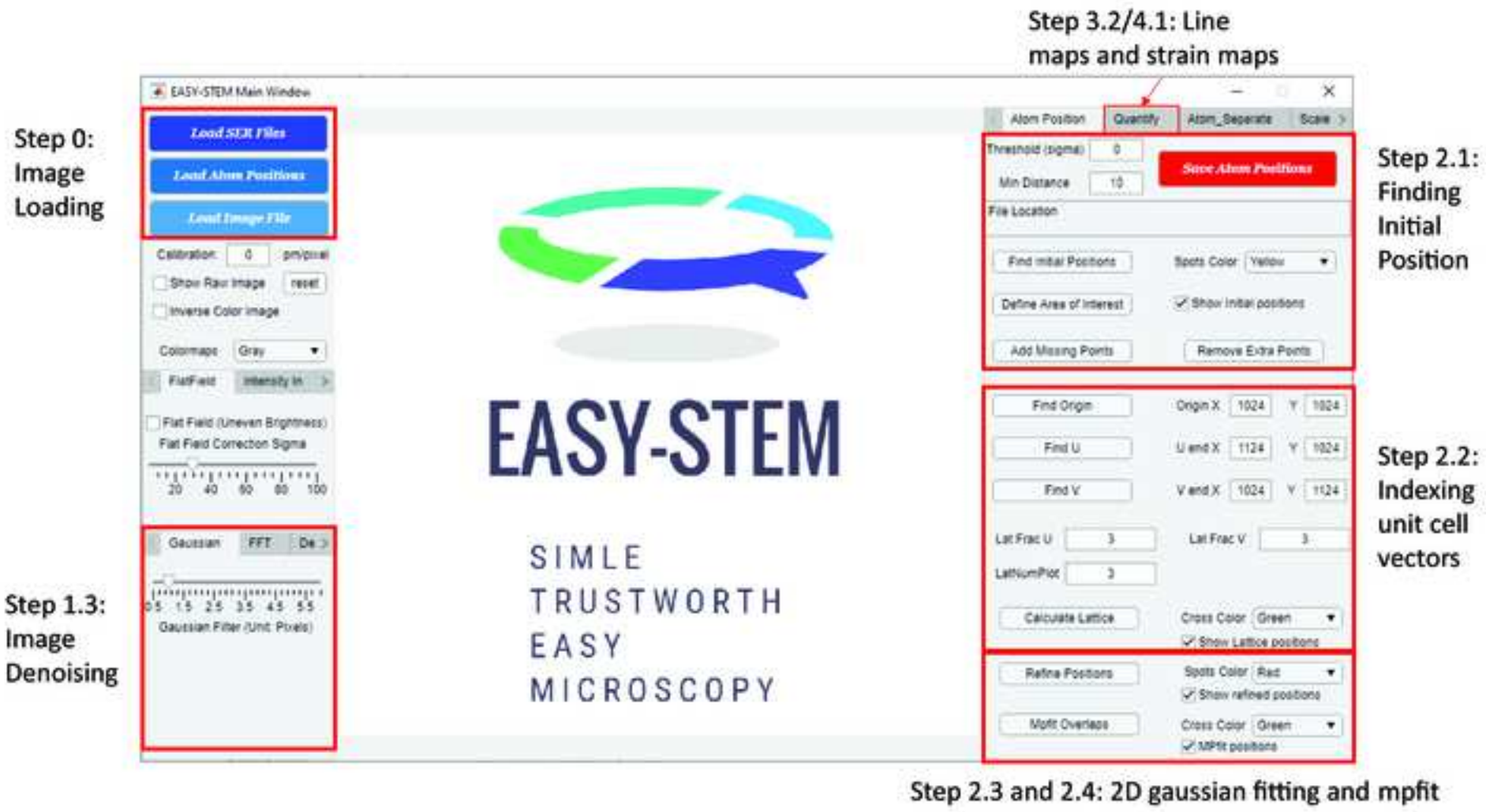

Figure 3

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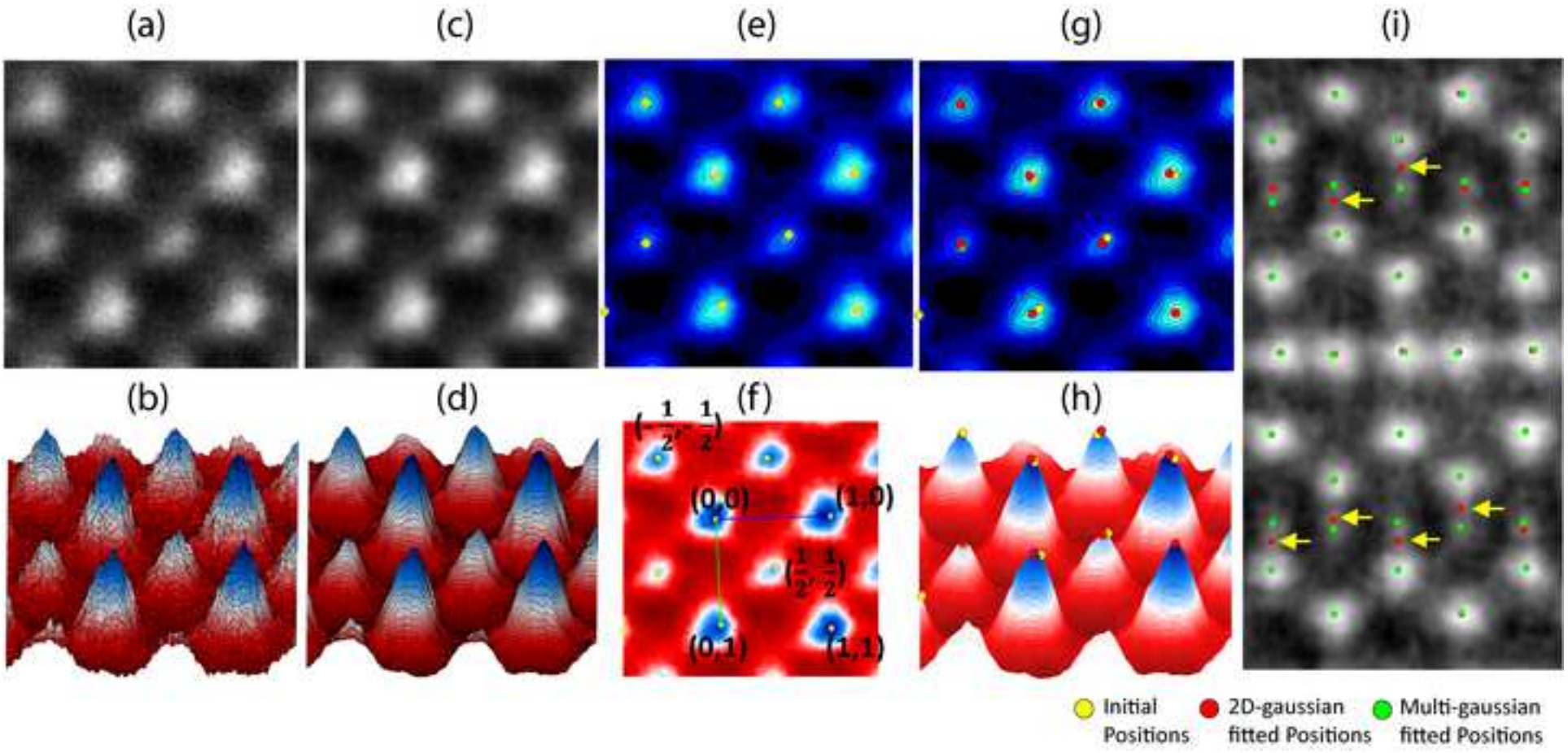

Figure 4

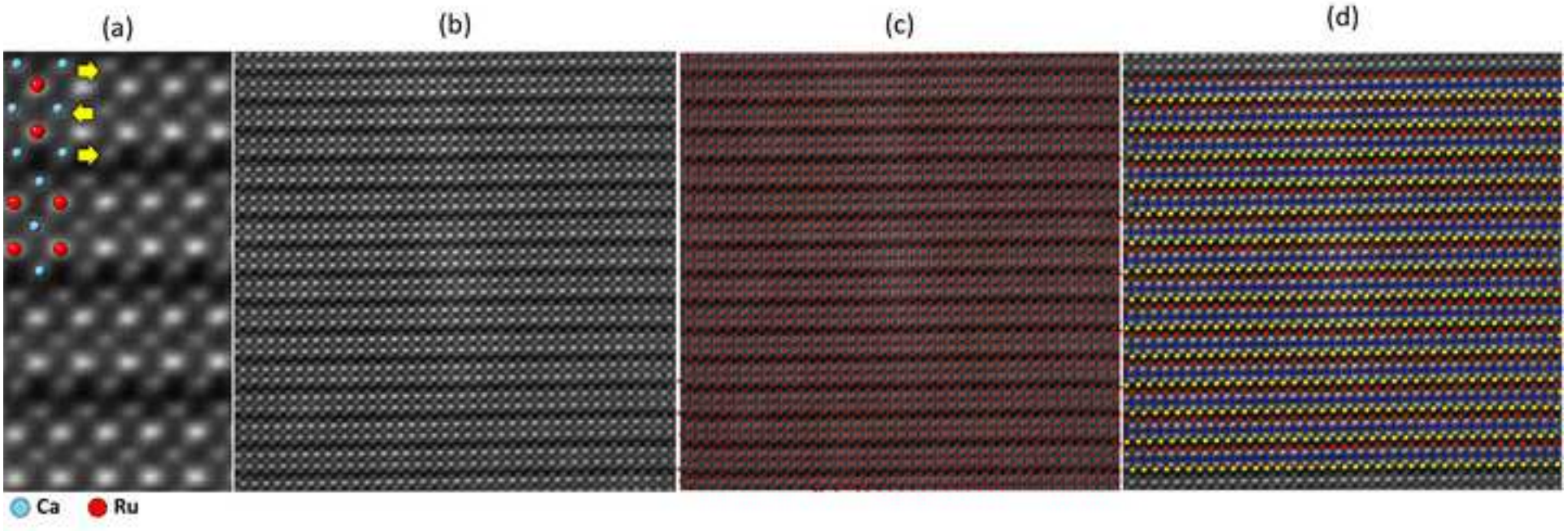

Figure 5

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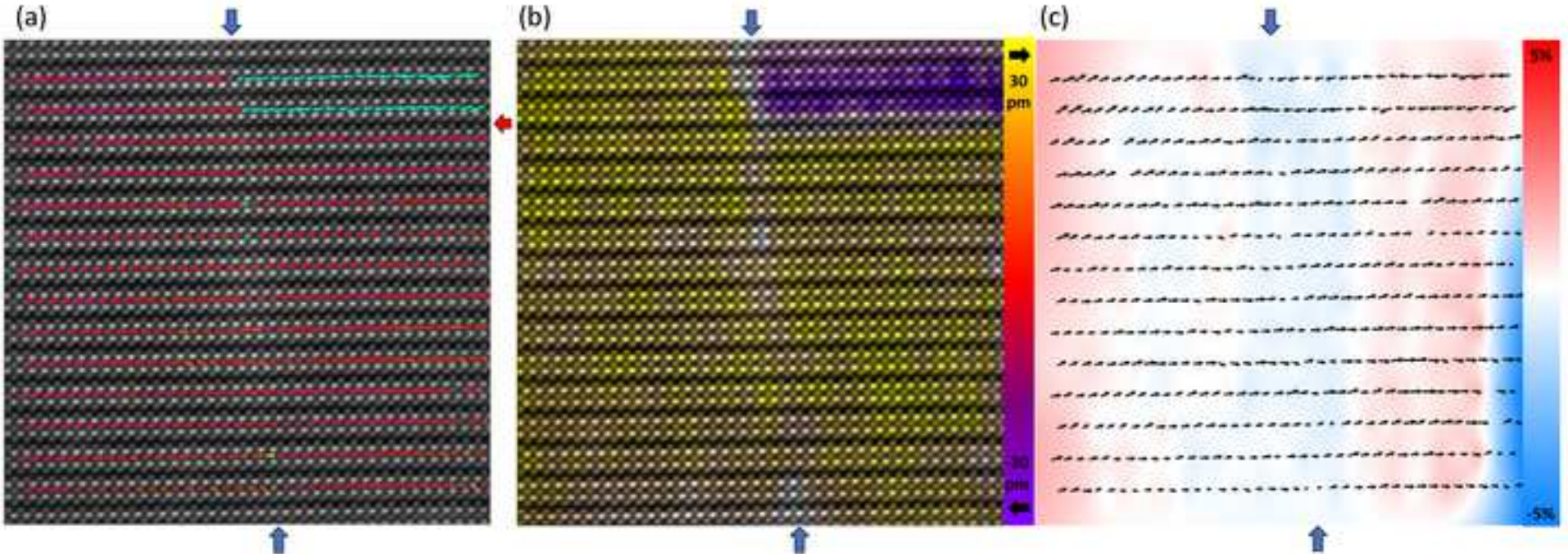

Figure 6

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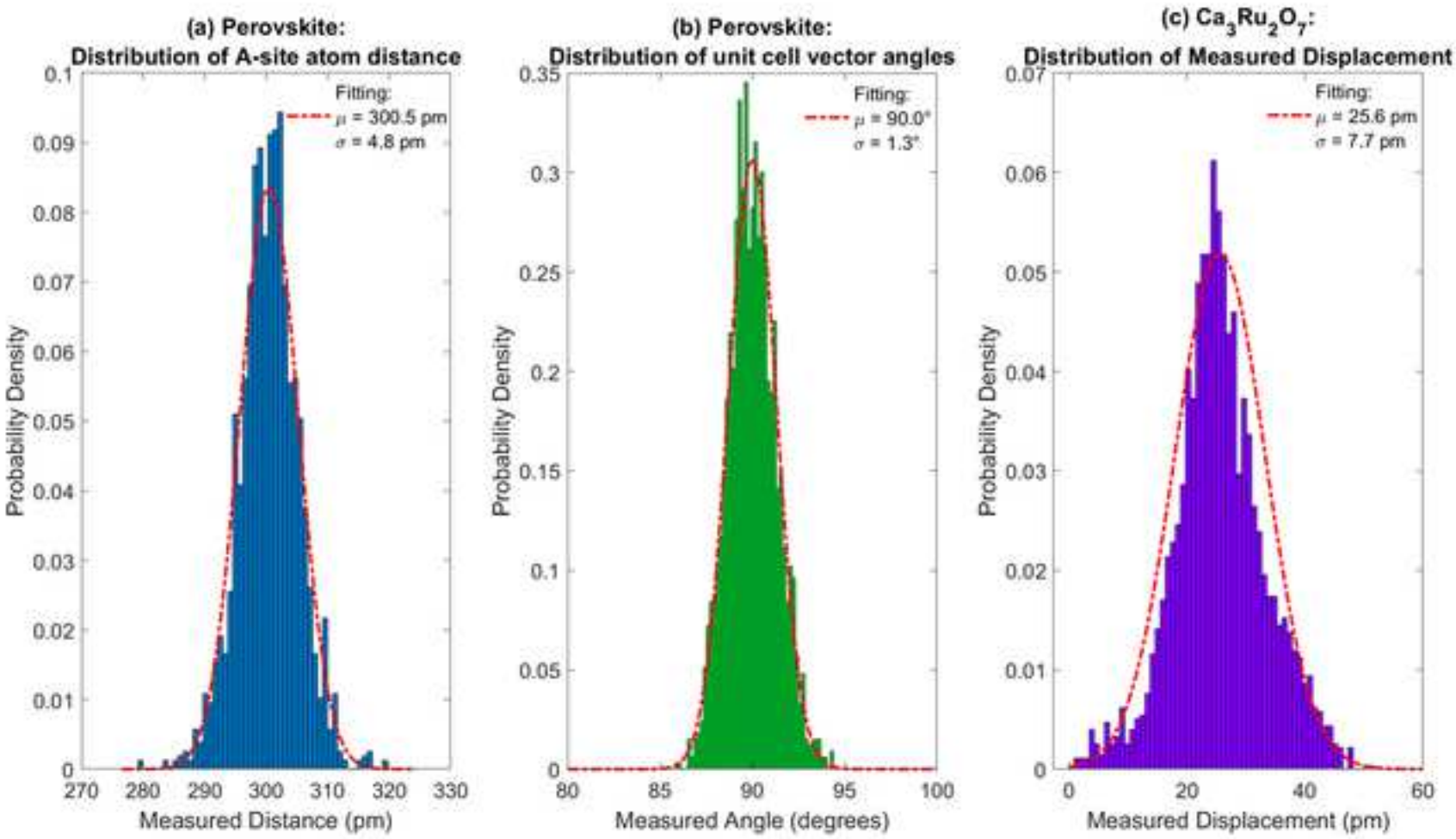

Table of Materials

Click here to access/download;Table of Materials;JoVE_Materials.xls

Name of Material/ Equipment	Company	Catalog Number
EASY-STEM	Nasim Alem Group, Pennsylvania State University	
JoVE article example script	Nasim Alem Group, Pennsylvania State University	
Matlab Optimization Tool Box	MathWorks	
Matlab	MathWorks	
Matlab: Image Processing Tool Box	MathWorks	

Comments/Description

Matlab app for STEM image processing;
Download link:
https://github.com/miaoleixin1994/EASY-STEM.git

Example Script for sorting atoms in unit cells

Optimization add-on packge in Matlab
Numerical calculation software
Image processing add-on packge in Matlab

PENNSTATE

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MATERIALS RESEARCH INSTITUTE

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March 25th 2021

Dear Dr. Vineeta Bajaj,

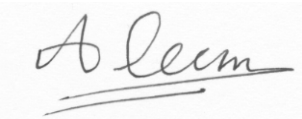
Attached please find our revised manuscript (Manuscript ID: JoVE-62164) entitled "Picometer-precision atomic position tracking through electron microscopy" by Leixin Miao, Adrian Chmielewski, Debangshu Mukherjee and Nasim Alem.

We have made a number of updates in response to the reviewers' comments. In our response to reviewers, the reviewers' comments are numbered, and our responses follow below, in blue. Corresponding changes are highlighted with Microsoft Word's Track Changes feature in the manuscript text in the revised file. A detailed response is attached, and we highlight several important points here.

1) We have provided a more detailed assessment of our atom position tracking methodology in order to demonstrate the precision for our measurements on the experimental data. We show explicitly that the precision in our atom tracking algorithm is at picometer level by performing the statistical quantification of the lattice parameter of the standard material. We then used the statistical quantification to further discuss the limitation of the measurement.
2) We have provided detailed explanations of some small details in data analysis, for example we explained the importance of the scanning directions for drift-correction, the operation of 'beam shower' during the sample cleaning and so on.
3) We did a major overhaul on the format of the protocol section of the manuscript and the video based on the detailed comment from the editors.
4) We have corrected the typos and grammar mistakes in the manuscript.

We believe that this work will be of broad interest to the diverse readership of Journal of Visualized Experiments. We reaffirm that none of the work included in this manuscript is under consideration to be published elsewhere. We thank you for handling our manuscript and look forward to hearing from you soon.

Sincerely,



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Manuscript Summary:
In this article, authors are presenting the workflow of the atomic position tracking in the atomic resolution transmission electron microscopy imaging using Matlab app (EASY-STEM). They finish with some tips for acquiring high-quality STEM images.

Overall, this is a very interesting work which will be of use to researchers to extract more quantitative analysis from their STEM outputs in the future. This manuscript describes the advanced STEM image quantification and analysis. The method is overall well-presented and the topic itself is very interesting. In the video, the example of atom indexing and quantifying the atom positions for CaRuO is very well-explained.

We appreciate the reviewer for the positive comments.

This method gives the users the ability to design and customize the data analysis for their unique material systems. However, some minor points should be considered before publishing the manuscript.

Major Concerns:
#1: First on the overall structure, the writing does not seem to flow well; the authors did not explain the topic in order (as the sequence in the abstract). I recommend firstly address the tips for acquiring high-quality STEM images as it is a vital step before analyzing the image.

We appreciate the reviewer to point this out. We agree with the reviewer that the tips for acquiring high-quality images should be addressed first in the protocol. We have moved the tips from the discussion section to step 1.1 in the protocol section. (from line 96 to line 148).

#2: There are some typos and some missing parts. In the first sentence of the abstract, the "transmission" is missed. Also, the spelled-out of some abbreviations are not presented when they are used for the first time, such as ADF. The fifth item in the abstract is numbered as 4. In protocol, Figure 3f is addressed before 3(a to e).

We thank the reviewer for noting this. We have fixed the typos and mistakes in writing. Namely, we added transmission in the first sentence of the abstract (line 30). Also, we fixed the numbering of the steps involved (line 34 - 40). Also, in Figure 3, we have fixed the issue by addressing the Figures based on the order they are mentioned.

#3: In Figure 4a, technically you are unable to observe oxygen atoms (due to the weak scattering). So, why are you showing oxygen positions in this figure?

We have removed the O label from the schematic in Figure 4a to avoid confusion.

#4: The authors mentioned some limiting factors - they should present and discuss these limiting factors in more detail.

We appreciate the reviewer for pointing this out. To discuss the limitation in a much more detailed fashion, we added Figure 6 into the manuscript, which presents the statistical

quantification of the proposed atom position finding method. We added more details in the discussion section to stress the limitation of the proposed method and importance of measurement precision.

In the discussion section (line 520 - line 532), we added the following text:

To demonstrate the measurement precision, the statistical quantification of the atom position finding is presented in Figure 6. The measurement of cubic ABO_3 perovskite A-site distance distribution and unit cell vector angle distribution is plotted using histogram in Figure 6a and 6b respectively. By fitting the normal distribution curve to the distributions, the A-site distance distribution shows a mean of 300.5 pm and standard deviation of 4.8 pm and unit cell vector angle distribution shows a mean of 90.0 degrees and standard deviation of 1.3 degrees. The statistical quantification indicates the method proposed here enables picometer-level precision and can greatly alleviate the distortion due to drift during imaging. This result suggests that this measurement is trustworthy when the physical information to be measured is greater or equal to roughly 10 pm. For example, in the case of aforementioned CRO crystals, the measurement of the magnitude of the polar displacement is presented in Figure 6c. The measurement shows a mean of 25.6 pm, a standard deviation of 7.7 pm, and it shows that the polar displacement measurement in CRO STEM images is solid.

#5: Another evidence of defected crystal structure can be shown to describe the precision of this method. In addition, in order to allow the reader to understand how accurate this quantification study is, the authors should provide the standard deviation that can be associated with the measurement method, e.g. by repeatedly scanning different regions of the same sample.

We agree with the reviewer to provide the quantification of the measurement precision. We have added the standard deviation of the measurement of the polar displacement in CRO crystal in Figure 6c. We determined a mean displacement of 25.6 pm with 7.7 pm standard deviation and plotted the histogram of the polar displacement and the normal distribution fitting of the measurement.

#6: Quantitative numbers for aforementioned systems (perovskite and CaRuO) in the manuscript could be provided.

We also agree with the reviewer's suggestion to provide the quantitative numbers in the perovskite and CRO crystals. For the perovskite system, we added a distribution of measurement of the lattice parameter and angles of the unit cell vectors in Figures 6a and 6b. In Figure 6a, we fitted a normal distribution to all the measured A-site distances as an assessment for the measurement precision. The result shows a standard deviation of 4.8 pm which supports our claim of single picometer precision for our method. In Figure 6b, we measured the unit cell vector angle to assess the atom positioning precision and the benefit of drift-correction. Our result shows a 90 degree average and a 1.3 degree standard deviation. Given the prior knowledge that the perovskite unit cell is cubic, it shows the atomic positioning and drift-correction is effective. For the CRO, we have included the polar displacement measurement as a benchmark for the measurement precision in Figure 6c.

Reviewer #2:
This manuscript, written by Leixin Miao et al., introduces post-processing and quantification of aberration corrected STEM images. As this reviewer is also in related research field, I agree with the authors that although obtaining atomic-resolution STEM became much easier, advanced data analysis procedures and methods (denoising, drift-correction and quantification) are still not familiar with most of the researchers. Some groups have their own algorithms and codes about these, but most of the groups without experience about image processing or writing code face hard time on analyzing their images. Therefore, an article with detailed description is worth to be published. I suggest this manuscript to be published in JoVE after some revisions, especially this journal aims to provide protocols for the beginners.

We appreciate the reviewer for the positive comments.

My detailed comments are:

1. In line 101, can authors explain in more detail about why scanning beam direction should be avoided paraller or perpendicular to the lattice planes?

The reason to avoid the scanning direction to coincide with the lattice planes is to prevent the potential drift artifacts during the image registration while drift correcting the images. The drift correction algorithm typically uses the cross correlation for "patch matching" the input images. During patch matching, the features with both horizontal and vertical variations will be ideal, since shifting the patch window in any direction would yield large changes. If the scanning direction coincides with certain horizontal or vertical features (lattice planes, interfaces, etc..), it is possible that the highly directional features may cause the patch matching to produce artifact.

Based on your feedback, in the protocol 1.1.6 section (line 144-148) of the Manuscript, we added the following text to further clarify on this point in the manuscript:

Additionally, it is recommended that the scan direction and the low index lattice planes not maintain parallel or perpendicular directions with each other and instead maintain oblique angles. If the scan direction coincides with certain horizontal or vertical features (lattice planes, interfaces, etc..), the drift along the direction of the strong vertically/laterally varying features may cause artifacts during image registration.

2. As the authors already discussed about artifacts that may generated during the process, the artifacts may affect the quantification results like atomic displacement or strain measurement and bring errors. In such cases, researchers should determine whether these results are due to artifact, often subtle to recognize. If possible, can authors provide advice about this in discussion section?

Thank you for the excellent suggestion. One major source of the measurement artifact is a result of the limitation of measurement precision. We added Figure 6 to present a statistical quantification of the atom position measurement. The result suggests that our method is well capable of providing single picometer level precision in atom position measurement. In the discussion section (line 520 - line 532 and line 536 - line 538), we added a discussion on the precision requirement for the measurement.

To demonstrate the measurement precision, the statistical quantification of the atom position finding is presented in Figure 6. The measurement of cubic ABO_3 perovskite A-site distance distribution and unit cell vector angle distribution is plotted using histogram in Figure 6a and 6b respectively. By fitting the normal distribution curve to the distributions, the A-site distance distribution shows a mean of 300.5 pm and standard deviation of 4.8 pm and unit cell vector angle distribution shows a mean of 90.0 degrees and standard deviation of 1.3 degrees. The statistical quantification indicates the method proposed here enables picometer-level precision and can greatly alleviate the distortion due to drift during imaging. This result suggests that this measurement is trustworthy when the physical information to be measured is greater or equal to roughly 10 pm. For example, in the case of aforementioned CRO crystals, the measurement of the magnitude of the polar displacement is presented in Figure 6c. The measurement shows a mean of 25.6 pm, a standard deviation of 7.7 pm, and it shows that the polar displacement measurement in CRO STEM images is solid. ... Our method is insufficient when the precision is required at the sub-picometer level, so a more advanced analysis routine is necessary if the feature to be extracted in the image is below a certain threshold.

The other source of error is the experimental limitation of signal-to-noise ratio. For example, the low signal-to-noise ratio when imaging some beam-sensitive samples can bring artifacts in atom position measurement. In the discussion section (line 532 - line 535), we added the discussion such issue:

Additionally, more caution needs to be taken in case of experimental limitations such as low signal-to-noise ratio when imaging beam-sensitive samples. In those cases, the measured atom positions need to be closely examined against the raw images to ensure the validity of the measurement.

3. In line 343, what does highly defocused beam mean? Does it mean weak beam with low dose? Please elaborate.

Dear reviewer, by the highly defocused beam, we mean spread the beam by adjusting the strength of the condenser lenses. This process is usually referred to as “beam shower”, and it is typically done at low magnification and with relatively high electron dose. The electron beam can push away hydrocarbon contamination on the TEM samples and thus reduce the accumulation of the contamination during imaging.

Based on the comment from reviewer #1, we moved the discussion related to the tips for cleaning the TEM sample from the discussion section to step 1.1.2 of the protocol. Based on your question, we elaborated the operation of ‘beam shower’ as the following text (line 110-113):

“... or irradiating the region of interest in the sample at low magnifications by spreading the electron beam after sample insertion into the microscope (‘beam shower’).”

4. In line 362, "Next, when determining the initial atom positions based on local maximum/minimum, try to adjust the restriction minimum distance between peaks to avoid

creating redundant positions between atomic columns. " is not clear. Did the authors mean try to avoid artifacts that can be generated between atomics columns? Please elaborate.

Yes, the redundant positions in the original text are the artifacts generated between atomic columns. We have further explained this point. In the manuscript line 482, we have added:

These redundant positions are artifacts generated due to the algorithm mistakenly recognizing the local maxima/minima in the image as atomic column.

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. Please use American English throughout.

   We appreciate the editor's comment. We have fixed the grammar issues and used American English throughout the manuscript.

2. Please ensure that all abbreviations are defined during the first-time use.

   We carefully ensured that the abbreviations are defined the first time they are stated in the manuscript.

   Detailed changes are:
   Line 33-34: *scanning transmission electron microscopy (STEM);*
   Line 72: we used cryogenic-STEM instead of abbreviated cryo-STEM;
   Line 97: annular dark field (ADF)/annular bright field (ABF);
   Line 244: 2D-Gaussian multi-peak fitting (mp-fit).

3. Please adjust the numbering of the Protocol to follow the JoVE Instructions for Authors. For example, 1 should be followed by 1.1 and then 1.1.1 and 1.1.2 if necessary. Please refrain from using bullets, alphabets, or dashes.

   Thank you for pointing this out. We have fixed the numbering in the protocol section based on your suggestion. We also removed all bullets in numbering to be consistent. Please see the protocol section from line 91 to line 394.

4. Please ensure that all text in the protocol section is written in the imperative tense as if telling someone how to do the technique (e.g., "Do this," "Ensure that," etc.) with all possible details associated with it. The actions should be described in the imperative tense in complete sentences wherever possible. Avoid usage of phrases such as "could be," "should be," and "would be" throughout the Protocol. Any text that cannot be written in the imperative tense may be added as a "Note."

   Thank you for the comment. We have made the changes based on your comment. All sentences in the protocol section are now in the imperative tense. The usage of "could be", "should be" and "would be" is avoided. The only place we used the phrase "should be" is in the note of step 1.1.6 (line 140 and line 143) to suggest to the reader the optimal action.

   We wrote:
   "The images should be taken using the same imaging condition except for the scanning directions. ", and "... one should be mindful of beam damage on the sample during successive scanning of the same region."

5. The Protocol should contain only action items that direct the reader to do something.

   As mentioned in the response to comment #4, only the imperative tense is used when writing the protocol step. Only actions are written in the protocol sections, and all sentences that are not specific action items are written as "note" to complement the actions.

6. Please add more details to your protocol steps. e.g., how do you perform the alignment?

   Regarding the TEM alignment in comment #6, we have made general comments regarding the general alignment of the microscope as well as minimizing the aberrations. TEM alignment is a very complicated and lengthy process and involves numerous steps. In addition, the alignment process can be slightly varied from one instrument to the other based on the manufacturer. Therefore, we did not provide details on the alignment step and mainly stated the overall steps.

   Here is what we wrote in protocol 1.1.3 (line 115 - 120):

   1.1.3 Align the microscope and tune the aberration correctors to minimize the lens aberrations as much as possible.

   Note: try to test the resolution by acquiring a few S/TEM images on a standard sample to confirm the spatial resolution can resolve the specific crystal structures and further fine-tune the aberrations in the image.

7. What is the sample used in your experiment? How do you apply various image deionizing techniques in your experiment, how do you extract atom position and perform refinement, etc.
8. Please ensure you answer the "how" question, i.e., how is the step performed? This can be done by mechanical actions, button click in the software ("Click", "Select", etc.), knob turns (turn "On" the red knob, etc.).

   Dear editor, here is a combined response to your comments 7 and 8 since they are closely related. In comment 7, you suggested adding more details to the protocol steps. Based on your comment, we included the specific actions mainly needed for data processing and details on operation in the note section. The specific actions in the protocol focus on the "how" question as you suggested in comments 7 and 8. We have included details on the operation in the note section which is stated below:

   1) the precautions: the cautions needed for operations. For example, in step 1.1.6, we first wrote the action for taking STEM images (line 136):

"1.1.6 Acquire STEM images with different scanning directions to correct for potential drift during acquisition. First, acquire an image and then take the second one from the same region immediately after rotating the scan direction by 90°.

Then we noted the precautions during the acquisition for readers to get the optimal results (line 140 - 148):

"The images should be taken using the same imaging condition except for the scan directions. The purpose of this step is to feed the rotated images to the drift correction algorithm developed recently[17]. One can also input more than two images with more varying scanning directions (with arbitrary angles) into the algorithm. However, successive scanning of the same region may lead to lattice damage or drift in that area. Additionally, it is recommended that the scan direction and the low index lattice planes not maintain parallel or perpendicular directions with each other and instead maintain oblique angles. If the scan direction coincides with certain horizontal or vertical features (lattice planes, interfaces, etc..), the drift along the direction of the strong vertically/laterally varying features may cause artifacts during image registration"

2) the explanations: the reason or theory behind the operations. For example, in step 2.3, we first wrote the action "Click" for refining atom positions by fitting a 2D-Gaussian (line 231):

"2.3 Click on the "Refine Positions" button in the EASY-STEM app to refine atom positions with 2D-Gaussian fitting.."

Then we noted the algorithm for this Gaussian fitting and how to define the refined atom positions (Line 236):

"Note: After obtaining the initial atom positions and indexing the atoms in the image, a 2D-gaussian fitting around each atomic column needs to be applied to achieve the sub-pixel level precision in the analysis. Using this algorithm, it is possible to first crop an area in the image around each initial atom position in the image and then fit a 2D-gaussian peak in the cropped image. We then use the centers of the fitted 2D-gaussian peaks as the refined atom positions."

9. Please include a single line space between each step, substep, and notes of the protocol section.

Thank you for the note. We have included a single line space between each step, sub-step, and notes.

10. Please do not abbreviate the journal titles in the manuscript text.

Thank you for the note. We will not abbreviate the journal title in the manuscript text.

Regarding the video

1. Please increase the homogeneity between the video and the written manuscript. Ideally, all figures in the video would appear in the written manuscript and vice versa. The video and the written manuscript should be reflections of each other.

   We appreciate the suggestion from the editors. We have improved the homogeneity between the video and the manuscript and ensured that all figures appear in both the manuscript and the video.

2. Furthermore, please revise the narration to be more homogenous with the written manuscript. Ideally, the narration is a word-for-word reading of the written protocol.

   Thank you for your suggestion. We made a major overhaul of the narration in the video and drastically increased the similarity between the narration and the written protocol. The narration is now almost a word-for-word matching of the protocol section.

3. Please do not use personal pronouns in the narration.

   We removed all use of personal pronouns in the narration.

4. Please ensure that protocol subheadings are the same both in the text protocol and the video protocol.

   We have ensured the consistency of the subheading text between the text protocol and video protocol.

5. Please incorporate jump cuts/crossfades (as applicable) instead of bringing in the shots from the left.

   Thank you for the note. All transitions between the clips in the video are changed to jump cuts and crossfades.

6. Please ensure that the narration matches the shots being shown in the video.

   Thank you for the note. We carefully examined the video and made sure the narration matches the shots.

7. Please ensure the representative result section is the same in the video and in the text. Please ensure all figures showing representative results are present both in the text and in the video.

   Thank you for the note. We have ensured the figures showing the results are present both in the text and in the video.

8. Voice-over sounds very compressed, especially in the results section. Please clean it up if possible.

   Thank you for the note. We remade the audio files in the video and used an upgraded microphone for the recording. The audio should be much cleaner than the previous version.

Example Atom Sorting Script

EASY-STEM Matlab Script file

EASY-STEM Matlab app install package

Click here to access/download

Supplemental Coding Files

EASY STEM_1065.mlappinstall