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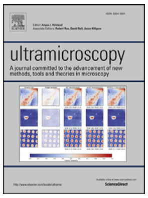

Image registration of low signal-to-noise cryo-STEM data

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ABSTRACT

Combining multiple fast image acquisitions to mitigate scan noise and drift artifacts has proven essential for picometer precision, quantitative analysis of atomic resolution scanning transmission electron microscopy (STEM) data. For very low signal-to-noise ratio (SNR) image stacks – frequently required for undistorted imaging at liquid nitrogen temperatures – image registration is particularly delicate, and standard approaches may either fail, or produce subtly specious reconstructed lattice images. We present an approach which effectively registers and averages image stacks which are challenging due to their low-SNR and propensity for unit cell misalignments. Registering all possible image pairs in a multi-image stack leads to significant information surplus. In combination with a simple physical picture of stage drift, this enables identification of incorrect image registrations, and determination of the optimal image shifts from the complete set of relative shifts. We demonstrate the effectiveness of our approach on experimental, cryogenic STEM datasets, highlighting subtle artifacts endemic to low-SNR lattice images and how they can be avoided. High-SNR average images with information transfer out to 0.72 Å are achieved at 300 kV and with the sample cooled to near liquid nitrogen temperature.

1. Introduction

Imaging atomic structures with sub-angstrom resolution and sub-picometer precision is now possible in modern scanning transmission electron microscopes (STEMs). While advances in aberration correction have enabled sub-angstrom electron probes [1–3], making full use of these narrow electron beams has required optimizing the stability of the microscope, sample stage, and room environment [4]. To minimize the effect of any remaining mechanical, electromagnetic, thermal, and acoustic instabilities and to improve the signal-to-noise ratio (SNR) of the final image, a variety of post-processing algorithms have been developed, and have proven essential for high precision, quantitative STEM analysis [5–9].

STEM imaging of samples cooled to liquid nitrogen temperatures (cryo-STEM) opens the possibility of characterizing the atomic structure of electronic materials across phase transitions, probing processes at solid-liquid interfaces, examining the structure of cells and other biological systems across a wide range of sample thicknesses, or controlling carbon contamination effects [10–14]. Currently, cooling a sample while preserving the ability to align along a crystallographic axis is only possible with side entry cryo holders, in which the sample stage is in thermal contact with a liquid nitrogen bath, resulting in increased stage drift and additional noise due to cryogen bubbling. Bubbling can be min-

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imized by ensuring good thermal isolation between the cryogen and the environment, and maintaining a clean dewar to prevent bubble nucleation. Drift can be minimized by allowing sufficient time for the stage to settle, however, is difficult to fully eliminate. The effect of sample drift can be mitigated by acquiring many images with very short frame times, and subsequently registering and averaging the resultant stack of images [15]. However, the frame times required (often <1 s) can yield very low-SNR data, complicating image registration. Particularly challenging datasets, such as nearly perfectly translationally symmetric images (e.g. featureless lattices), can exacerbate the problem by inducing unit cell misalignments between image pairs. This precise situation arises in many solid state systems where picometer-precision atomic position fitting is most relevant for probing the underlying physics, much of which only emerges in low temperature phases, including multiferroics, charge density wave systems, and high temperature superconductors [16–18].

Here, we present an image registration approach that is optimized for difficult, low-SNR cryo-STEM images, which often cannot be registered successfully by other means. We introduce an approach which does not rely on a single reference image, but instead uses all possible combinations of image correlations to determine the optimal shifts. Incorrect correlations, which plague low-SNR data, are then identified and handled by enforcing physical consistency using the surplus of information present in registrations of all image pairs. Our approach accounts for sampling errors which can result in unit-cell jumps in translationally symmetric data, minimizing the possibility of these artifacts. As difficult datasets often involve exploring multiple combinations of registration parameters, our implementation is designed to be both fast and flexible, allowing straightforward variation of real space boundary condition handling, Fourier space masking, choice of correlation function (cross correlation, mutual correlation, phase correlation [19,20]), correlation maximum determination, and outlier removal methods. The implementation outputs a brief report on each registration performed which facilitates quick determination of success or failure, both qualitatively and quantitatively.

The source code is available as a free, open source Python package with a modular, extensible structure, designed for either interactive use through the Jupyter notebook, or for automated batch processing. Code is freely available on github at https://github.com/bsavitzky/rigidRegistration.

1.1. Approaches to image correction

Rapid progress in aberration corrected STEM in the early and mid 2000s was followed by various approaches and implementations to correcting image artifacts or distortions. The earliest approaches involved deconvolution of the probe and object functions [21,22]. STEM and TEM images of identical regions were used to correct for non-orthogonal or continuously warped regions in the STEM data, in either reciprocal or real space [22,23]. Others determined and corrected for systematic distortions in their particular microscopes by examining the similarities in strain fields across many lattice images of many sample regions using geometric phase analysis [24,25].

Scan noise, offsets in the starting position of each scan line, is particularly difficult to diagnose and correct. Scan noise results in blurring of the Bragg peaks in fast Fourier transforms (FFTs) of lattice images along the slow scan direction, thus one approach to scan noise correction involves analyzing the phase information in these streaks to directly extract and correct for scanning offsets [26]. Alternative approaches include shifting pixels along the fast scan direction to maximize their cross correlation with a section of pixels in the adjacent rows, and rearranging rows of pixels vertically to ensure the intensity of each atomic column decreases monotonically from its center [27].

Methods to align, or register, images span electron microscopy, scanned probe miscoscopy, medical imaging, cartography, computer vision, and many other fields [28–30]. The fundamental limits of the general image registration problem have been explored at low- and high-SNRs for single and multiple image registrations [31–33]. Efficient, high fidelity registration is required for cryo-TEM [34–36]. In STEM, image registration and averaging tends to average out both scan noise and Poisson noise, and several approaches have been developed. Rotation of the scan direction has been used to diagnose and correct for constant or linearly varying sample drift [5]. Registration methods which allow for continuous, or ‘non-rigid’, distortion of the probe position during scanning have been developed and applied to obtain sub-pm precision identification of atomic positions [6,37]. Another rotating scan approach determines and corrects for shifts in the initial position of each scan line by leveraging the superior information transfer along the fast scan direction, comparing local information in scans rotated by 90 degrees, and ultimately weighting information in Fourier space more heavily along the fast scan direction of each image before averaging [7].

The approach here is comparatively simple. We begin with the assumption that all images in an acquisition series are identical, save for a translational offset due to drift of the sample stage. While this ignores the complicated and real effects of continuous image distortions from scanning offsets, or higher frequency stage position variations, we find that this simpler approach is well suited to low-SNR cryo-STEM imaging in which particular care is required to avoid subtle artifacts from incorrect registration. Here, we document such subtle artifacts, identify their sources, and present approaches both to avoid incorrect registrations and to confirm correct final registrations. Moreover, we find that assuming simple translational offsets is an excellent first order approximation which requires little sacrifice in the final quality of the reconstructed images. Using the acquisition and registration technique described here, we demonstrate cryo-STEM imaging with 0.72 Å information transfer, and clearly distinct atomic columns of disparate Z values at <2 Å spacing.

2. Theory

2.1. Referenceless correlation

The cross correlation of real valued functions f and g

$$(f\star g)(x)\equiv\int_{-\infty}^{\infty}f(y)g(x+y)dy \tag{1}$$

is interpretable as the overlap of f and g given some relative shift x. For a pair of identical, translationally offset images, the correct shift for an optimum registration is therefore given by the value of the argument x which maximizes the cross correlation – see, e.g., [29]. Typically, all images in a series are registered to a single image. Iterative schemes may then re-register using the output averaged image as a reference one or more times [6,38]. In low-SNR data, a single incorrect cross correlation can spoil an entire reconstruction. An ad hoc approach may be employed, whereby incorrectly registered images are discarded, or various images are tested as the reference. However, this approach may discard useful data, and requires significant and subjective user input. Moreover, incorrect correlations can introduce subtle artifacts which can be difficult to detect, but result in spurious analysis - see Fig. 3 and the associated text in the Results section.

The approach here is to correlate all pairs of images. This has numerous advantages. First, it is possible to calculate the optimum image shifts based on more complete information of all relative