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Automating 3D Imaging of Inorganic Nanoparticles

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Three dimensional imaging of inorganic nanoparticles has developed rapidly since first experiments in the early 2000s (Koster et al., 2000; Midgley &Weyland, 2003). The advent of aberration correctors and developments in electron tomography have led to three dimensional reconstructions in which individual atoms can be straightforwardly resolved (Yang et al., 2017; Van Aert et al., 2013). However, experiments in which atomic resolution is required in inorganic nanoparticles are typically long and arduous and require expert input in both acquisition and processing.

In contrast, three dimensional imaging of viruses and proteins has recently been developing in to an automated tool that can be used by a wide array of structural biologists (Tan et al., 2016; Xie et al., 2020). Automated routines for both acquisition and processing of data and the development of user-friendly software has widely disseminated the technique and this has contributed to a boom in its use.

We aim to emulate the progress made in cryo-EM by developing automated workflows for 3D imaging of inorganic nanoparticles. This encompasses acquisition of atomic resolution images of nanoparticles, segmentation of individual nanoparticles, atom counting along atomic columns and single particle reconstruction routines.

As a first step towards automated acquisition of high angle annular dark field (HAADF) images, we have developed a DigitalMicrograph script that allows automated acquisition of atomic resolution images (Slater, 2019). The script handles sample movement, automated checking of focus and re-focusing, checking the presence of nanoparticles and the acquisition of images. Hundreds of atomic resolution images can be acquired with no human input beyond initial setup (Figure 1).

Once images of nanoparticles are acquired, they are first segmented and analysed using the ParticleSpy python package (Slater, 2020), which we have developed to support our work on inorganic nanoparticles. ParticleSpy allows straightforward segmentation in an automated pipeline using either simple thresholding methods or a machine-learning based trainable segmentation.

Once particles have been segmented, three dimensional reconstructions can be obtained using one of two methods. In the first method, 3D images are obtained using the same single particle reconstruction algorithms used in structural biology. That is, each particle image is treated as a 2D projection of a 3D structure and, after determining the relative orientation of each projection, a simple backprojection algorithm is used to obtain the structure in 3D. If this approach is applied to the population as a whole the reconstruction is poor, due to inhomogeneity in the inorganic particles. To combat this issue, we cluster sets of particles images in terms of their properties (e.g. projected area, total HAADF intensity and a number of shape measures) using functions in the ParticleSpy package.

As an initial example, a sample containing three distinct particle populations was investigated to determine whether the workflow could distinguish between significantly different particles (Slater et al., 2020). A clustering algorithm was found that not only separated the three known populations, but also split these into sub-populations based on size and morphology differences. A 3D reconstruction of each sub-population could then be obtained using well known single particle reconstruction software.

The reconstructions we have so far obtained of inorganic nanoparticles have a spatial resolution on the order of 1 nm. In order to obtain reconstructions at the atomic level the particle populations must be near atomically identical, and this sets a major constraint on the particles on which this technique can be used. The second method which we have employed does not have this constraint. We use the well-developed atom counting technique to count the number of atoms along atomic columns in images of nanoparticles oriented along a major zone axis.

To provide an automated pipeline, we must first identify particles on a major zone-axis using an FFT based routine. Once ideal particles have been found, atom locations are found using the atomap python package (Nord et al., 2017). This package also contains code for performing quantification of atomic column intensities using either a detector-normalised or a statistical approach. An example of a Pd nanoparticle analysed via the statistical approach is shown in Figure 2.

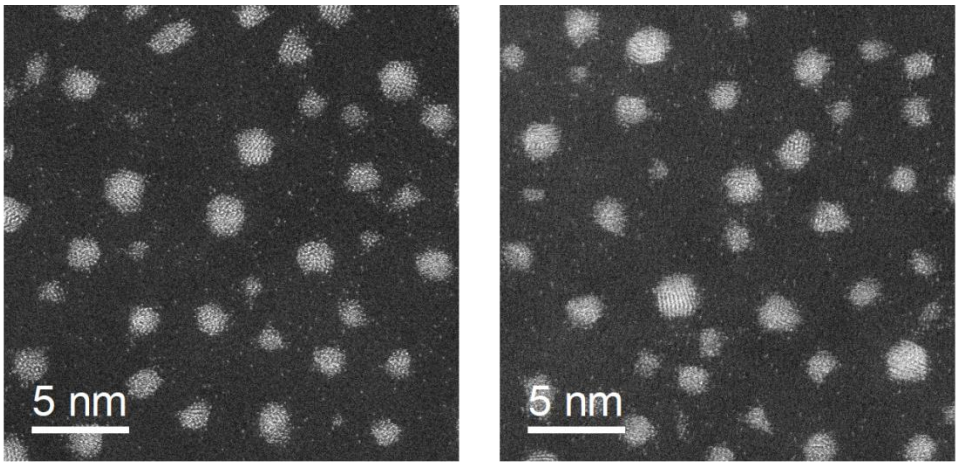

Figure 1. Figure 1. HAADF-STEM images of sputtered Au clusters collected using the autoSTEMDigitalMicrograph script. The images were acquired over an hour apart with no operator input.

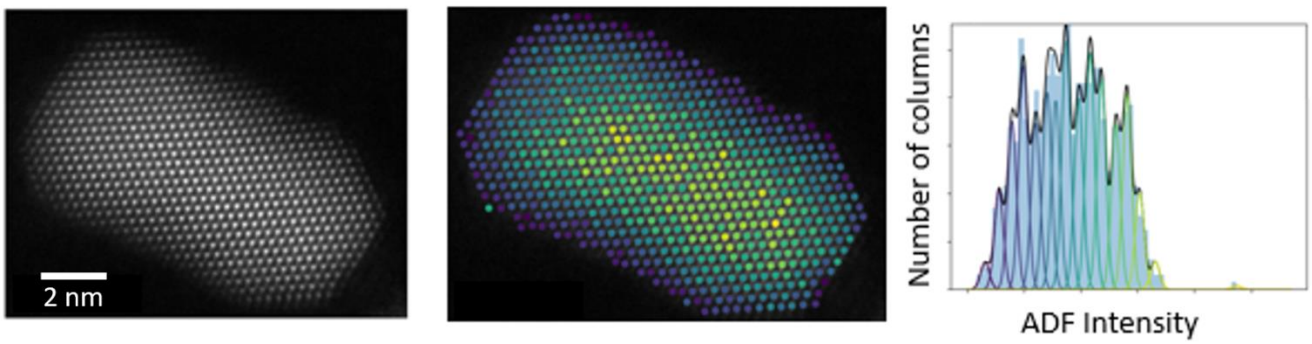

Figure 2. Figure 2. Demonstration of the statistical approach to atom counting on a HAADF image of a Pd nanoparticle. The histogram of the HAADF image (image on the left) is fitted with a Gaussian mixture model (on the right) in order to assign numbers of atoms to each atomic column (as shown in the centre).

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