Confocal Microscope Alignment of Nanocrystals for Coherent Diffraction Imaging

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Abstract. We have installed and tested an Olympus LEXT confocal microscope at the 34-ID-C beamline of the Advanced Photon Source (APS). The beamline is for Coherent X-ray Diffraction (CXD) experiments in which a nanometre-sized crystal is aligned inside a focussed X-ray beam. The microscope was required for three-dimensional (3D) sample alignment to get around sphere-of-confusion issues when locating Bragg peaks in reciprocal space. In this way, and by use of strategic sample preparations, we have succeeded in measuring six Bragg peaks from a single 200nm gold crystal and obtained six projections of its internal displacement field. This enables the clear identification of stacking-fault bands within the crystal. The confocal alignment method will allow a full determination of the strain tensor provided three or more Bragg reflections from the same crystal are found.

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INTRODUCTION

In nanoscience, the bulk concepts of lattices and crystal defects must be reconsidered in order to explain why nanomaterials have new and exciting properties. We are interested in the strains that appear in small crystals, considering that these strains are the origin of the special properties of nanomaterials. For this application we have developed the method of Coherent X-ray Diffraction to obtain quantitative three-dimensional maps of the deformation of a crystal from its equilibrium lattice spacing [1]. We can presently work with single crystals in the range of 80-2000nm in size. A convenient signal level is generated with crystals of heavy elements around 200nm in size. The lower size limit is when we run out of flux, given that the system is mechanically stable for only a few hours at best. The upper size limit is given by the limited coherence of the source [2].

The 34-ID-C beamline has been optimised for this purpose. It consists of a U33 undulator, primary slits, horizontal filter mirror (29m), a Si(111) monochromator (46m), a coherence-defining aperture, Kirkpatrick-Baez (KB) focussing mirrors, a goniometer stage with high-precision XYZ sample translations and a detector arm with two angular axes able to extend out to 3m over a wide range of Bragg angles. The primary slits (28m) define a beam of 150microns in the horizontal and are open (guarding) in the vertical. The coherence-defining slits (50m) are usually set to 50x20 microns (VxH) since the vertical coherence length (90μm) is determined by the source size, the horizontal (25μm) by the primary slit and the longitudinal (0.5μm) by the monochromator [2]. Only the coherent beam reaches the KB mirrors, which focus it down to a 1x1 micron spot at the sample. There are beamline windows at 25m and 50m between the source and the sample, which is located at 51m. No other optics or slits are used as these can modify the coherence of the beam in an undesirable way. The coherent flux at 9keV is around 10^9 photons per second.

The measurement consists of the alignment and centring of a nanocrystal in the focussed beam. A Charge-Coupled Device (CCD) area detector is then placed far enough away from the crystal that the fringes arising from

the finite size can be resolved. The crystal is then rocked through the Bragg peak in steps small enough that each fringe is fully measured on the CCD. The collection of CCD frames is a complete 3D data set of the Bragg peak, oversampled in all three directions with respect to the spatial Nyquist frequency, given by the size of the crystal. In practice, correct oversampling means that each fringe be more than two pixels wide on the CCD and extending over more than two adjacent frames of the rocking curve. "Three pixels per fringe" is the rule-of-thumb we use to be sure of the oversampling, which turns out to be sufficient for the phasing to work (see below). A full strain measurement requires this procedure to be repeated at multiple Bragg peaks for the same crystal, which is why we have developed the confocal microscope setup.

Image Reconstruction

To invert the diffraction, we solve the crystallographic 'phase problem' by taking advantage of the oversampling using a support-constrained Hybrid Input-Output (HIO) algorithm [3]. The algorithm uses Fast Fourier Transforms (FFT) to convert the 3D data array between real and reciprocal space. In reciprocal space the diffraction amplitudes are updated to the measured values of every cycle of the algorithm. In real space the image of the crystal being measured is constrained to be bounded by a support box and the values are updated by the HIO procedure [3]. For simple cases without too much deviation from an ideal lattice, *i.e.* small strains, the algorithm converges quickly in about 50 iterations to a reproducible solution.

To resolve the full strain tensor in these images it is necessary to measure three or more non-coplanar Bragg peaks from a single crystal of nanometre dimensions within a micron-sized beam. Without assistance, this is beyond the capability of any diffractometer ever constructed because the sphere of confusion is in the range of tens of microns at best. In this paper, we demonstrate that it is best to use a confocal microscope because the vertical positioning required is greater than that obtained with a normal light microscope.

Measurement of Multiple Bragg Reflections from Au Nanocrystals

An Olympus LEXT OLS 3100 laser scanning confocal microscope was installed in situ at beamline 34-ID-C at the APS. The microscope was mounted on a supporting arm attached to the diffractometer. This allowed the microscope to be positioned directly above the sample stage with a working distance of 1-10 cm (see Fig. 1), with enough space for the stage to be moved freely and so that there were no collisions with the KB focussing optics. The microscope itself has three modes of operation – confocal mode (with z-plane selection pinhole), non-confocal mode (without the pinhole) and camera mode. During normal operation three objective lenses were fitted to the microscope (5x, 10x and 20x) allowing a total magnification of 120x to 2880x. This proved vital in order to position 200nm crystals accurately. It was necessary to be able to operate the microscope from both inside and outside the experiment hutch so remote desktop software was used.

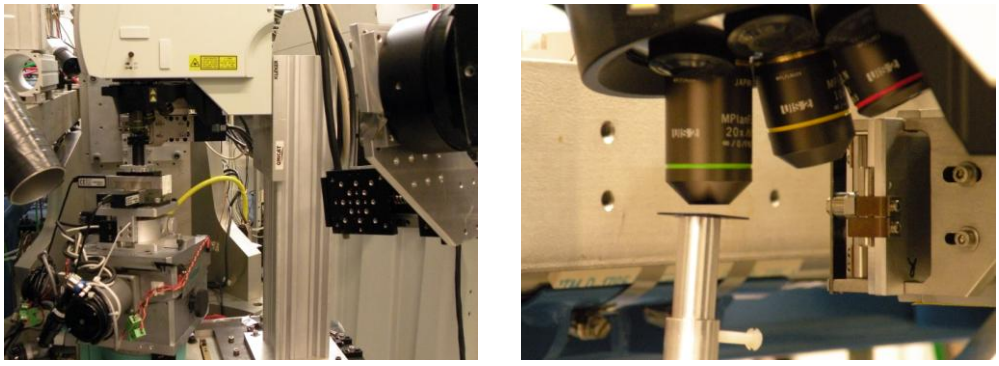

FIGURE 1. Left: confocal microscope in-situ on 34-ID-C diffractometer. Confocal can be seen suspended above the sample stage by a support arm Right: Confocal microscope in-situ on diffractometer at beamline 34-ID-C, Advanced Photon Source.

In order to measure multiple 3D Bragg reflections from a single 200nm crystal it was first necessary to determine the diffractometer centre of rotation. The sample stage angles phi and chi (tilts) were set such that the sample was flat. The confocal microscope (in camera mode) was then used to locate a feature on the sample, which was observed as the sample was rotated to -180º, -90º, 0º and 90º in theta. The intersection of these four new positions

gave an accurate (to 2 μm) centre of rotation. The next stage was to then align the highly focussed x-ray beam so that it passed through this centre of rotation. The sample was replaced with a phosphor screen, which converts the x-ray beam into visible light. This was placed on the diffractometer and brought into focus by varying the confocal microscope height. This confocal height was then used as a height reference point for the remainder of the experiment. So that visible light from the phosphor screen could be seen, the confocal microscope was set to "camera mode", where the confocal microscope acts as a regular light microscope. The sample height (z) was then varied until the x-ray beam was seen to shift into the previously determined centre of rotation. Alignment of the x-ray beam with the known centre of rotation was further refined by varying the horizontal translation positions of the KB focusing optics. This alignment procedure has been found to provide positioning of the beam with 1 μm accuracy.

The sample was returned to the diffractometer stage and the sample height (z) was varied until the sample was in focus under the confocal microscope. This brought the sample into the focal point of the x-ray beam. Fine tuning of the vertical sample positioning was achieved using the microscope in confocal mode. In this setting, the microscope is extremely sensitive to focus in the z direction, especially when the sample is tilted (see Fig. 2c). As can be seen there is a narrow band of the sample surface which is in focus and correspondingly an equally small x-ray footprint. The combination of beam alignment with diffractometer centre of rotation and high accuracy vertical positioning of the confocal microscope is highly useful. It allows a Bragg reflection to be measured from a particular 200nm crystal and its reconstructed image to be compared with real-world SEM images of the same nanocrystal.

A set of samples were produced using e-beam lithography which consist of a square array of thousands of Au film 'dots' on a silicon substrate. When high temperature annealed, these film patches form individual nanocrystals whose size is dependent on the size of initial film deposit. A 0.25 μm² area film 'dot' has been found to routinely form a single 200-250nm crystal ideal for CXD. In order to measure multiple Bragg peaks from the same 200nm Au crystal it was first necessary to carry out the beam alignment procedure described earlier. Due to the small size of the nanocrystals, a 20x objective lens and a software magnification of 3x was used.

The sample was placed on the diffractometer and tilted to ensure a small beam footprint (8º angle of incidence). It was assumed that when the Au crystals are annealed, they form oriented with 111 pointing up, perpendicular to the substrate. This possible reflection was entered into beamline control software (SPEC), which then calculated the approximate motor positions to place another Bragg reflection on the CCD detector. The crystal of interest was brought into the x-ray beam focal point and rotated about theta in small steps until a Bragg peak was located on the CCD detector. The centre (brightest slice) of the Bragg reflection was then found by refining stage translation motor positions (x, y, and z) and angle motor positions (phi, chi, and theta). Then a full 3D Bragg reflection was measured by recording 2D images of the Bragg peak intensity as the sample is rotated through the Bragg peak in theta in steps of 0.02º. These slices are then stacked together to form a 'θ-stack' of the Bragg reflection. Once found, the precise motor positions of the reflection were entered into SPEC as the primary alignment reflection and further Bragg reflection motor positions can be calculated with increased accuracy. Two alignment reflections are sufficient to find all other peaks once the crystal is re-centred in the confocal microscope in x, y and z. This process of locating and recording multiple Bragg reflections is now routinely used to locate 6 reflections (or more) from the same 200nm Au crystal and is highly time efficient. Evidence that multiple reflections recorded are from the same nanocrystal can be seen in Fig. 2, where burn marks are clearly visible on the silicon substrate due to the x-ray beam incident upon the sample surface after measurement of six Bragg reflections.

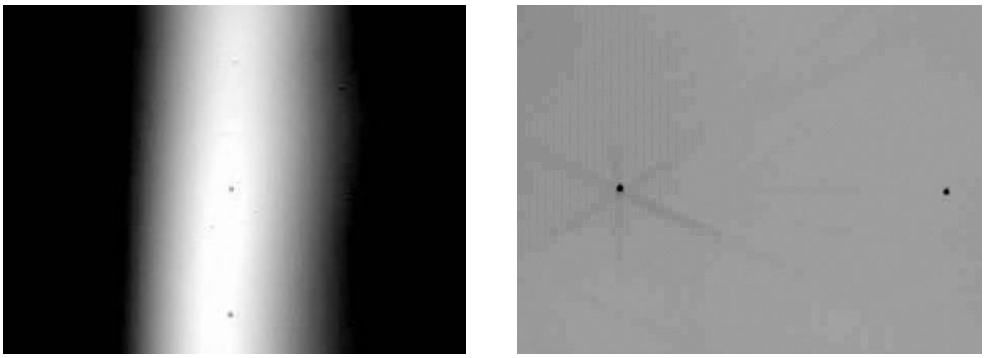

FIGURE 2. Left: confocal image of highly tilted sample under confocal microscope using 20X objective and zoom. Only the part of the (tilted) sample that is at the confocal height appears bright. Right: confocal image of Au nanocrystal array with x-ray burn marks due to multiple Bragg reflection measurement.

Results

Figure 3 (a, b and c) show isosurface views of three reconstructions from different reflections (11-1, -111 and 020) measured from the same 260nm Au crystal. The reconstructions show the recovered amplitude of the complex density obtained using the support constrained HIO methods described in the Image Reconstruction section. The reconstructions have been re-oriented into the same coordinate frame as in Fig. 3 (d). Figure 3 (d) is an SEM image of the crystal from which the multiple reflections were measured. As can be seen in two out of the three reconstructions (-111 and 020) there is a section of missing amplitude corresponding to a defect in the crystal structure. The same defect can be seen in the SEM image as a stripe across the top surfaces of the crystal. The defect is not present in the reconstruction from the 11-1 reflection.

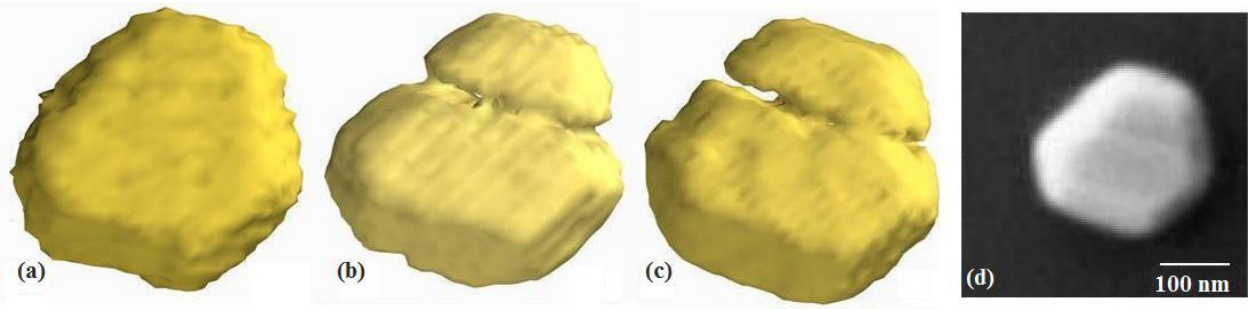

FIGURE 3. Amplitude isosurface views of three reconstructions obtained with different Bragg reflections (a) 11-1, (b) -111 and (c) 020, measured from the same 260nm Au crystal. (d) SEM image of the same Au nanocrystal.

Discussion

The defect shown in Fig. 3 is not present in one out of the three reflections and is visible in the SEM image. One explanation is that the defect is due to a stacking fault in the crystal structure. Each reconstruction from a Bragg reflection carries information mostly about the perfection of the crystal in the direction of its Q vector [4]. If the 11-1 is perpendicular to the direction of the stacking fault, then the Bragg reflection and its corresponding reconstruction would then be insensitive to this particular crystal defect. The faulted slab of crystal still contributes to the 11-1 Bragg peak but not the others. There is no missing density in the SEM image, which is not sensitive to the Bragg planes, yet a jog where the crystal is faulted can be identified.

The most useful confocal operating mode was found to be alignment of the x-ray beam with the diffractometer centre of rotation, then the use of a static confocal microscope height as a reference point. This allows the crystal of interest to be placed squarely in the x-ray beam for multiple Bragg reflection measurement. Due to high beam stability and small mechanical drift in the confocal setup, realignment is rarely required.

The combination of Au nanocrystal array samples and use of a confocal microscope allows the same Bragg reflection from the same crystal to be located again, months after the initial measurement. This opens up the possibility of measuring multiple reflections before and after strain has been purposefully induced in a crystal in order to study its effects. This will also allow further investigation of a particular defect if found in reconstructions. If a reconstruction has a volume of missing amplitude due to a section of crystal with different crystallographic orientation, it should be possible to return back to the crystal and measure further Bragg reflections which fill in the missing piece in further reconstructions. This will allow a complete image of the crystal to be constructed.

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