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Robust, automated detection of defects in direct images of periodic structures

Konrad Bojar, *Industrial Research Institute for Automation and Measurements, Warsaw, (Poland).* E-mail: kbojar@piap.pl

We present a novel algorithm for robust, automated detection of point defects, dislocations, and other defects in direct microscopic images of periodic structures like crystal lattices [1,2], graphene layers [3], or biological membranes [4]. The algorithm accepts images registered by a number of microscopic imaging techniques, including SEM, TEM, HRTEM, STEM, Z-contrast, and AFM. The only constraint imposed on image acquisition method is that all visible primitive cells of the lattice are of almost identical dimensions. Small rotations, translations and scalings of particular cells are acceptable and are not treated as defects, so there is no need to "unbend" the structure [5] or to remove spherical aberration of the apparatus [6]. Also, to obtain correct results the contrast transfer function does not need to be corrected [7] and no background normalization is necessary.

The first step of the algorithm is to determine lattice spatial dimensions in pixels by means of a procedure imported from the *2dx* package [4]; this procedure performs analysis of peaks in the power spectrum of numerical diffractogram of the input image *i*. Once the unit cell size is known, a few cells are randomly selected, and for each cell its clockwise and counterclockwise rotations are computed. Then, for each rotated and unrotated pattern several upscaled and downscaled patterns are calculated. For every pattern *p* obtained in this way the map of statistical correlation coefficient $\rho_p(x,y)=cov(p,i_{x,y})/(var(p)var(i_{x,y}))^{1/2}$ is computed, where $i_{x,y}$ is a subimage of *i* located at *(x,y)* and of size matching that of *p*. This coefficient is invariant under affine remappings of gray levels of *p* and *i*; it has been proven to be a robust measure in pattern matching [8] and can be efficiently computed using the fast Fourier transform and the running sums algorithm [9]. Next, step all maps ρ_p coming from rotated patterns *p* are derotated to unify coordinates; there is no need to account for scaling at this step. Then, a list *L* of all local maxima in all maps ρ_p is created. Each element of *L* is inspected by a classifier deciding its correspondce to a true cell of the lattice. Once locations of all defect-free lattice nodes are known, for every node its neighborhood is determined. We obtain an adjacency graph whose vertices are labeled with spatial positions of lattice nodes. This graph is used to find crystallographic defects: vacancies and voids, interstitial defects, Frenkel pairs, edge dislocations, distinct impurities and clusters of these, or Stone-Wales defects. The final result is an image in which all defects are drawn and labeled. In future the algorithm can be extended to produce LaTeX-formatted output in the Kröger–Vink notation for point defects.

The algorithm proved robust, fast, and efficient in automatic localization of defects when tested on images of graphene layers, crystal lattices, and biological membranes; all defects have been localized successfully. It can be easily incorporated into public-domain open-source software packages for crystallographic analysis, especially in the quality assurance domain.

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Novel Combination of 3DXRD and Grain Boundary Tracking for Mapping Polycrystaline Al-alloys

Darren J. LeClere,[a] Yoshikazu Ohkawa,[a] Hiroyuki Toda,[a] Masakazu Kobayashi,[a] Yoshio Suzuki,[b] Akihisa Takeuchi,[b] Kentaro Uesugi,[b] [a]*Departament of Mechanical Engineering, Toyohashi University of Technology, Toyohashi, Aichi (Japan).* [b]*Japan Synchrotron Radiation Research Institure, Sayo-gun, Hyogo (Japan).* E-mail: darren@four-d.me.tut.ac.jp

A novel method that can provide accurate analysis of individual grains has been produced by combining X-Ray Diffraction (XRD) microscopy with grain boundary tracking (GBT) [1]. Since XRD is a non-destructive technique for characterizing bulk materials, *in-situ* analysis of metals can be performed both with and without applied loads. Loads can also be applied close to the point of fracture whilst still producing practical data. This combination of techniques, known as GBTXRD, provides accurate information about individual grain orientations from near field XRD analysis, whilst the grain boundary tracking accesses 1 micron level analysis of grain morphologies. The experiments used to derive GBTXRD analysis, further developments and possible applications, shall be presented.

An X-ray pencil beam was employed to perform the analysis of two types of materials: Al single crystal wire and Al-4mass%Pb alloy. The single crystal wire was used for alignment purposes and to determine a relationship between the wire's diameter and the diffraction spots it produced. However, relationships generated employing the data obtained from the single crystal wire were ambiguous; this was believed to be due to extinction factors. A preliminary 3-dimensional X-Ray diffraction (3DXRD) grain map of the Al-alloy was produced at the European Synchrotron Radiation Facility (ESRF) [2], after which XRD and computer tomography (CT) was carried out at Spring-8 Synchrotron in Japan. The 3DXRD grain map was used to determine the orientation of the grains so that their respective diffraction spots could be located. The morphology of the grains were then determined from CT images of the sample subjected to a liquid metal wetting method using Ga. These CT images were exploited for grain boundary tracking, which provided a more accurate description of the position and morphology of the grains than can be achieved through 3DXRD. Upon determining which diffraction spots were related to which grain, analysis of the relationship between various parameters describing both the grain and its relevant diffraction spots were determined. Correlation coefficients where calculated for all the groupings of these parameters. By combining results from the parameters with the three best correlation coefficients using the data acquired at Spring-8, it was possible to describe the grain misorientation with an accuracy of 100%.

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Masquerade: Improving Data Quality with Masks for Beryllium Rings

Jonathan A. Coome, Andrés E. Goeta, Judith A. K. Howard, Michael R. Probert, *Department of Chemistry, University of Durham, South Road, Durham, DH1 3LE, (UK).* E-mail: jonathan.coome@durham.ac.uk

The XIPHOS system in Durham is now operational, allowing crystal structures to be determined routinely at temperatures down to 2K. [1] In order to reach these temperatures, the sample is isolated from the environment using vacuum shrouds made from beryllium. These shrouds contain unevenly distributed crystalline parts, resulting in powder rings of non-uniform intensity. When collecting data with a weakly diffracting crystal where the reflections from the beryllium are strong in comparison to those reflections from the crystal, the uneven intensities from the rings can distort the intensities calculated by integration programs.

This can be avoided, and improved data quality obtained by masking out the regions of the diffraction image which are affected by beryllium scattering. Any reflections which overlap with the masked region are discarded by the integration program. As the scattering from the beryllium shrouds and the crystal originates from different positions, overlapping reflections can be separated by changing the detector distance. By using masks and recovering the lost data with these different detector distances, data quality can be improved compared with data obtained at one distance and without masking.

Masquerade has been written to generate the mask files required for this technique quickly and accurately. The positions of the beryllium rings are calculated using an *ab-initio* model of the position of the shrouds in 3D space, and include goniometer rotations as well as centring offsets for the shrouds themselves. The program is written in C++ and uses multithreading to make full use of modern multi-core processors. It runs on a variety of platforms and can generate thousands of mask images for a full data collection in minutes.

The use of this program along with the data collection at multiple detector distances has been found to improve the data quality obtained from weakly diffracting crystals, showing clear improvements in R_1, wR_2 and Rsigma values over data integrated without the masks.

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X-ray-induced cooperative atomic movements in protein crystals

Tatiana Petrova,[a,b] Stephan Ginell,[a] Andre Mitschler,[c] Youngchang Kim,[c] Vladimir Y. Lunin,[b] Grazyna Joachimiak,[a] Alexandra Cousido-Siah,[c] Isabelle Hazemann,[c] Alberto Podjarny,[c] Krzysztof Lazarski, [a] and Andrzej Joachimiak,[a] [a]*Structural Biology Center, Biosciences Division, Argonne National Laboratory, Argonne, Illinois, 60439 (USA).* [b]*Institute of Mathematical Problems of Biology, Russian Academy of Sciences, Pushchino, 142290 (Russia).* [c] *Département de Biologie Structurale et Génomique, IGBMC, CNRS, ULP,INSERM, 1 rue Laurent Fries, B.P. 163, 67404 Illkirch, (France).* E-mail: petrova@impb.psn.ru

A specially designed diffraction experiment with controlled radiation damage to proteins makes it possible to investigate X-ray-induced changes in protein molecules and their crystals at the atomic level. We present the results of several experiments in which multiple data sets corresponding to increasing absorbed doses were collected from the same crystals at atomic resolution ([1], [2]). A comparison of the refined models of a protein molecule that corresponded to different levels of damage allowed us to study progressively increasing local and overall radiation damage effects. It was shown that irradiation causes displacements of big parts of the protein molecule. These movements have a cooperative character: big parts of protein molecules are displaced approximately as rigid bodies. The collective movement of the atoms of the protein leads to the expansion of the protein globule, which occurs synchronously with, and in the same direction as, the expansion of the unit cell.

It was also shown that water molecules in the vicinity of protein surface move in the same direction and in concert with the nearest protein atoms. It appears as if the atoms of the expanding protein molecule pull the hydrogen-bonded network.

Displacements of protein domains occur simultaneously with the X-ray-induced damage to protein residues that participate in the contacts between different domains (decarboxylation of Asp and Glu residues, disruption of S-S bridges). An analysis of atomic ADPs revealed that, for the atoms with initially isotropic ADPs, an increase in anisotropy of ADPs occurs in the same directions as atomic displacements. This indicates that radiation-induced atomic displacements occur with different speed in different cells of the crystal and lead to an additional increase in crystal static disorder.

We studied the radiation-induced local and global damage not only at 100K, which is a usual temperature of data collection at modern synchrotrons, but also at as low as 15K. In the last case, crystals were cooled by a cold helium stream. Both kinds of radiation damage, local and overall, evolve on approximately the same time scale and similarly diminish as temperature decreases from 100K to 15K. Lowering the temperature from to 100 to 15K decreases the disulfide-bond deterioration and atomic displacements, the decrease being somewhat greater than twofold.

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Characterization of radiation damage in macromolecular crystals at cryo and room temperature

Ricardo M. F. Leal,[a] Silvia Russi,[b] Gleb P. Bourenkov,[c] Alexander N. Popov,[a] [a]*ESRF, Rue Jules Horowitz, BP 220, Grenoble (France).* [b]*EMBL Grenoble, rue Jules Horowitz, BP 18, Grenoble (France).* [c]*EMBL Hamburg, Notkestraße 85, Hamburg (Germany).* E-mail: ricardo.leal@esrf.fr

Radiation damage caused by ionising radiation is a constant source of concern when collecting X-ray data from biological samples. Its effects are sample dependent and are proportional to the absorbed dose. Accurate dose estimation is essential to determining the crystal dose tolerance and data collection strategy. A reliable and reproducible method to automatically characterise the radiation sensitivity of macromolecular crystals based on a preliminary experiment sacrificing a whole or part of a sample has been recently developed [1]. The method was implemented at the ESRF beamlines through an automated procedure using the EDNA online data analysis framework [2] and the MxCuBE data collection control interface [3]. The information extracted from this procedure is directly used for optimal planning of data collection by strategy software program BEST [4]. Using test crystals with well known radiation sensitivity, the procedure can also be used at the beamlines to verify and calibrate X-ray flux and beam size.

The method has been recently applied to a systematic study of radiation damage at room temperature. Reproducibility of the measurements, dose rate effects, radiation damage correlation with crystal properties and the diffraction intensity degradation model have been investigated, analysed and compared with the results obtained at cryo-temperature.