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Experiment Report Form

The double page inside this form is to be filled in by all users or groups of users who have had access to beam time for measurements at the ESRF.

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Should you wish to make more general comments on the experiment, please note them on the User Evaluation Form, and send both the Report and the Evaluation Form to the User Office.

Deadlines for submission of Experimental Reports

- 1st March for experiments carried out up until June of the previous year;
- 1st September for experiments carried out up until January of the same year.

Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report, in English.
- include the reference number of the proposal to which the report refers.
- make sure that the text, tables and figures fit into the space available.
- if your work is published or is in press, you may prefer to paste in the abstract, and add full reference details. If the abstract is in a language other than English, please include an English translation.

ESRF	**Experiment title:** Nanocrystallography of biological cells	**Experiment numbers**: MX-1192
Beamline: ID13-2	**Date of experiment**: from: 21/09/2011 to: 24/09/2011	**Date of report**: 27/02/2012
Shifts: 12	**Local contact(s)**: Manfred Burghammer	*Received at ESRF:*

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Report:

Experimental setup. The experiment was performed at the ID13 microfocus hutch (EH-2) using a Fresnel lens with 200 μm aperture during hybrid machine operating mode. The monochromatic beam (λ=0.0982 nm) was focused at about 140 mm from the optics to a spot of ~600(h)*300(v) nm. A Frelon CCD detector with 2Kx2K pixels and 16 bit readout was used for data collection. The pixel size used for data analysis of the 2x2 binned detector is 101.7x100.7 μm^2. The patterns were corrected for distortion and dark current. A grain of Ag-behenate was used for distance calibration[1]. Data analysis was performed by Christian Riekel using FIT2D[2].

Samples. Eosinophils were isolated from healthy donors/hypereosinophilic patients. Granules containing crystals were isolated from the cells with established methods, frozen and defrosted just before the measurements directly at the ESRF in Grenoble. Additionally, crystalline cores were extracted from granules upon treatment with a mild detergent (Triton 0.1%) and frozen in a cryoprotectant solution. Both granules and cores were thawed at the ESRF and spun in a table-top centrifuge, resuspended in a cryoprotectant solution and collected with a loop. We used both Mitigen Kapton loops as well as nilon loops. Silicon nitride membranes were employed for some experiments (Silson). In this case, the samples were applied over the membrane and dryed before freezing.

Results. We used MiTiGen Kapton loops for granules and isolated core-particle suspensions in ethylene glycol/sucrose/water mixtures. The diffraction patterns contained a significant amount of high energy scattering which will have to be reduced in future setups (Fig. 1).

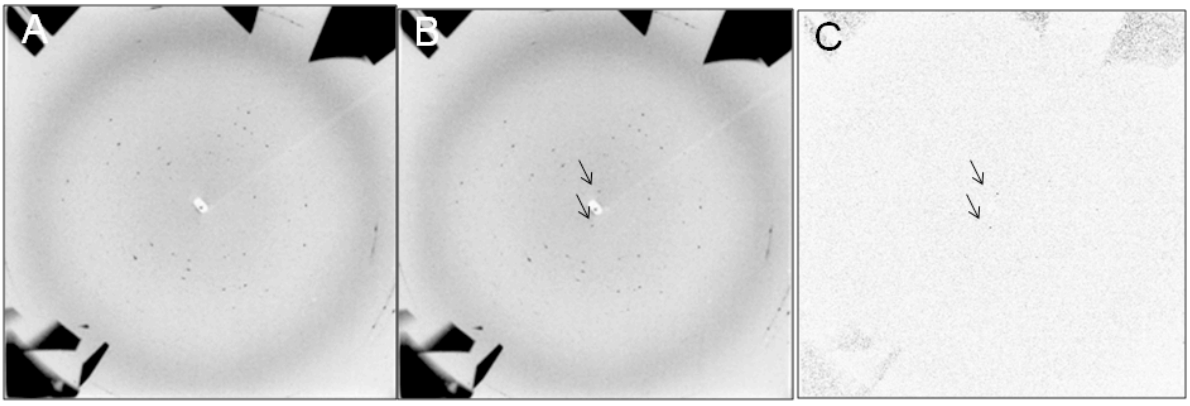

Fig. 1. Selected patterns recorded during a mesh-scan across *Eosinophil* core-particle aggregates. **A:** Pattern used as background; **B**: Neighboring pattern showing reflections indicated by arrows. Most of the spots are due to high energy background. **C**: Difference pattern after background subtraction.

It was, however, possible to efficiently suppress the background by subtracting a pattern measured outside the loop. A single pattern with diffraction peaks is shown in Fig. 2A. Azimuthal integration reveals two orders corresponding to a 2.12 nm repeating unit (Fig. 2B).

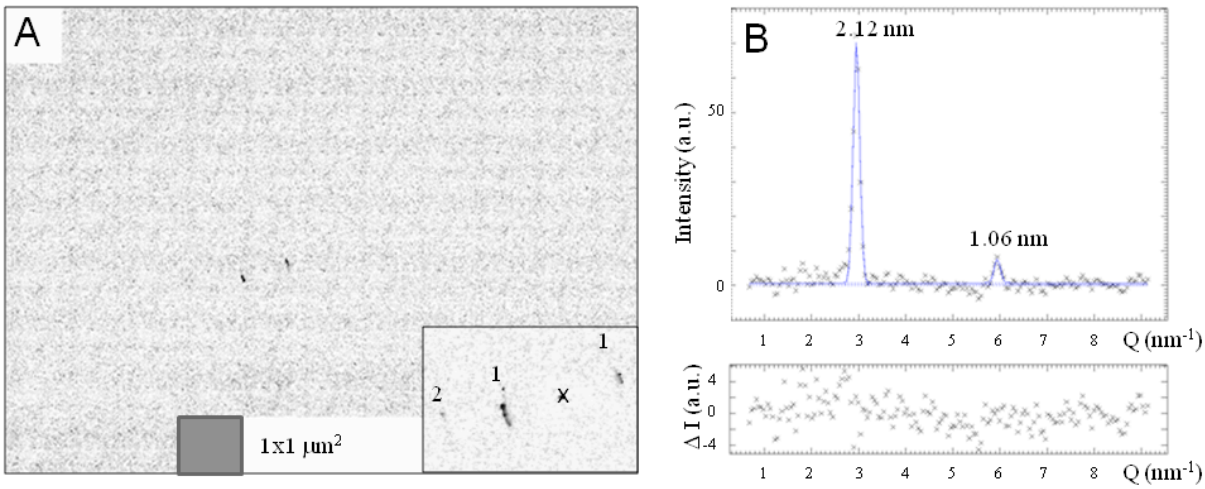

Fig. 2. A: Mesh-scan *Eosinophil* granule slush with 1x1 μm^2 raster and 10 s/pattern. The 1st pattern of the mesh-scan has been subtracted from all subsequent patterns. The zoom shows the single pattern in the center of the mesh-scan with 2 orders. The position of the beam center is marked by "x". **B**: Azimuthally averaged pattern. Two Gaussian profiles and a 0-order polynomial background have been fitted (blue lines).

A composite of all the images acquired on an isolated cores sample is shown in Fig. 3A. As evident from visual inspection, several diffracting particles can be identified. The logarithmic intensity plot of a single pattern shows two orders of diffraction (Fig. 3B). The azimuthal integration process is shown in Figure 3C. The 1D intensity profile containing the 1st order peak has been fitted with a Gaussian peak and a 0-order polynomial background (Fig. 3D). The 1st order lattice spacing shows a variability across a few analyzed patterns of d=3.43 (± 0.05 nm). The two peaks are attributed to in-plane spacings of the stacked crystalline eosinophil sheets. The sheets must aggregate to stacks of a certain thickness so that the peaks become observable by X-ray diffraction. The observation of 2.12/1.06 peaks (Fig. 2A) suggests 3D lattice order and

an epitaxial stacking. Whether the aggregation step occurs during the separation of the core-particles from the granules or during the drying of core-particle suspension on the hydrophobic SiN membranes is not clear at present. In addition, we measured the radiation stability of the diffracting particles. The decay of the 3.43 nm peak at 100 K was determined by a loop-scan with 10 s exposure per pattern. The sample loses its crystallinity without evidence for the transition into an intermediary phase. A half-time of decay of about 75 s is obtained.

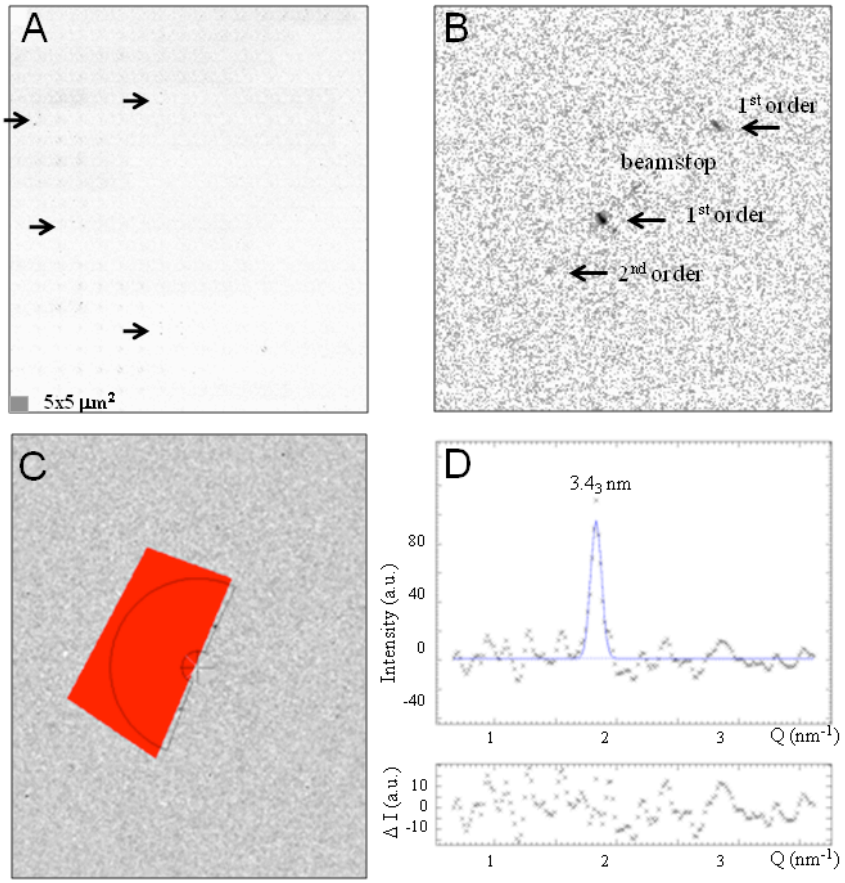

Fig. 3. A: Part of 5x5 µm^2 mesh scan with 20 s/pattern showing several *Eosinophil* peaks indicated by arrows; **B:** Logarithmic intensity display of a single pattern showing two orders. **C**: Pattern showing integration limits for azimuthal averaging of pattern. The red area was excluded for the integration; **D**: Azimuthally averaged pattern with fitted Gaussian profile and 0-order polynomial background.

Preliminary conclusions. The current data on human eosinophil crystals reveal a lattice spacing of d=3.43 nm. This can be attributed to an in-plane spacing of stacked crystalline sheets in view of the aggregate-morphology. A further lattice spacing of d=2.12 nm does not agree with an in-plane spacing. The fact that this lattice spacing is not present for the isolated cores but only for the granules could be due to the more random orientation of granules. If the 2.12 nm spacing would be due to the separation of thin lattice planes observed in TEM data[3], one might be able to observe this by rotating the core particle containing membrane to 90° at the beamline. We could detect 2nd orders to the 3.43 and 2.12 reflections. Other reflections have, however, not yet been observed. The quality of the present data will have to be improved by averaging over several patterns and performing oscillation experiments, which seem feasible given the relative stability of the specimen in this setup.

References

[1] Blanton, T. N. *et al.* JCPDS-International Centre for Diffraction Data round robin study of silver behenate. A possible low-angle X-ray diffraction calibration standard. *Power Diffraction* **10**, 91-95 (1995).

[2] The FIT2D Home Page (ESRF, Grenoble, 2009).

[3] Miller, F., Harven, E. D. & Palade, G. E. The Structure of Eosinophil Leukocyte Granules in Rodents and in Man. *The Journal of Cell Biology* **31**, 349-362 (1966).

Note: This report is based on the document “Nanodiffraction of eosinophil leukocyte particles” redacted by Christian Riekel (25/10/2011).