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ESRF

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.

Once completed, the report should be submitted electronically to the User Office using the **Electronic Report Submission Application:**

http://193.49.43.2:8080/smis/servlet/UserUtils?start

Reports supporting requests for additional beam time

Reports can now be submitted independently of new proposals – it is necessary simply to indicate the number of the report(s) supporting a new proposal on the proposal form.

The Review Committees reserve the right to reject new proposals from groups who have not reported on the use of beam time allocated previously.

Reports on experiments relating to long term projects

Proposers awarded beam time for a long term project are required to submit an interim report at the end of each year, irrespective of the number of shifts of beam time they have used.

Published papers

All users must give proper credit to ESRF staff members and proper mention to ESRF facilities which were essential for the results described in any ensuing publication. Further, they are obliged to send to the Joint ESRF/ ILL library the complete reference and the abstract of all papers appearing in print, and resulting from the use of the ESRF.

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:** Coherent Diffraction Imaging of Dictyostelium Dicoideum	**Experiment number:** SC 2339
Beamline:	**Date of experiment:** from: 22.02.2008 to: 29.02.2008	**Date of report:** 25.08.2008
Shifts:	**Local contact(s):** Enju Lima	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

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The main goal of this experiment was to apply Coherent X-ray Diffractive Imaging (CXDI) in the hard X-ray regime on unstained freeze-dried and frozen-hydrated cells, especially of *Dictyostelium Discoideum*. This implied testing the relevant preparation techniques as well as the specially designed sample environment. As the application of CXDI to biological specimens in the hard X-ray regime has not been performed before, questions of feasibilty, required dose as well as achievable resolutions were also addressed. In addition, a new approach towards the problem of parasitic aperture scattering in CXDI was tested by generating a rotationally symmetric illumination function, which yields a highly localized far-field diffraction pattern in order to isolate scattering contributions from the sample, which fill the whole q-range.

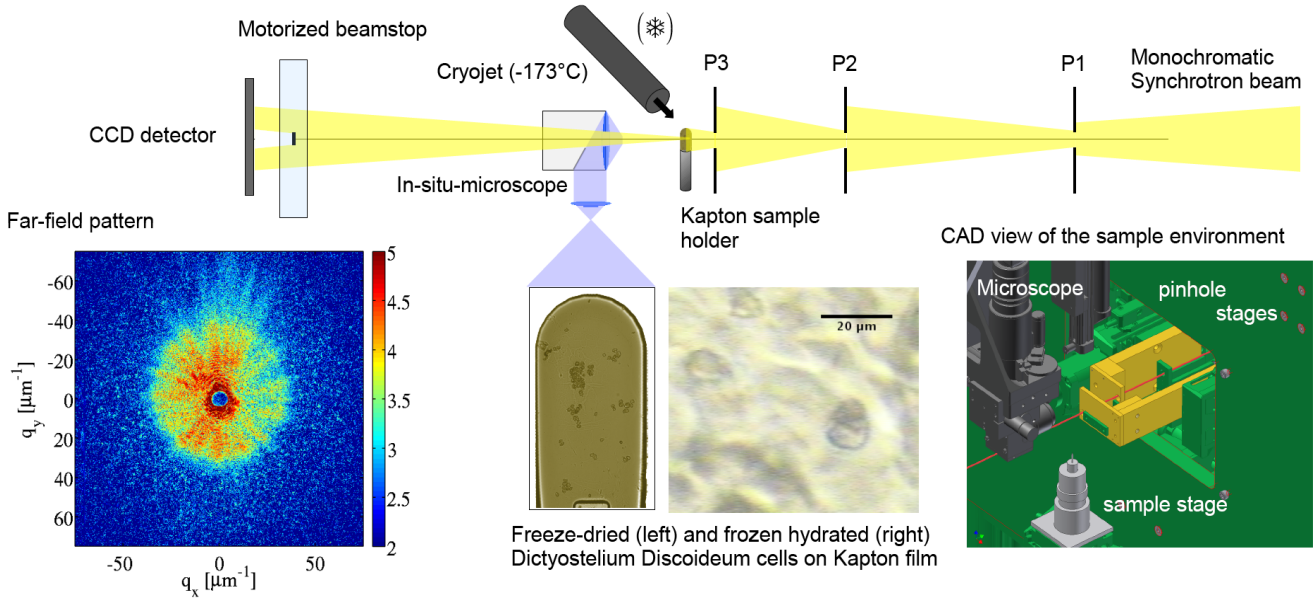

Figure 1: Experimental setup used for multi-pinhole coherent diffractive imaging on biological cells. The pinhole positions were controlled by 8 translation stages with tens of nm accuracy (cf. lower right inset). Using an optical microscope (Accel/Maatel, Germany/France) with a drilled lens the sample on a thin Kapton holder (MiTeGen, USA) could be monitored during the experiment with 1µm resolution. The detector was placed 2.75 m away from the sample and was protected by a circular beamstop (different sizes were available) mounted on Kapton films on a motorized in-vacuo-stage. The diffraction pattern (lower left inset, absolute value of difference of diffraction with and without the sample) was collected by illuminating a group of freeze-dried Dictyostelium cells (cf. middle inset). Although the scattering signal from the beam-defining apertures could be restricted to a certain q-range and speckles outside this range from the sample are visible, the pinhole scattering should have been much more localized according to simulations based on numerical Fresnel propagation.

In the first part of the experiment a multi-pinhole illumination function was generated by placing a system of three circular apertures (Pt, thickness 100-200µm, ∅ = 5-10µm) into the mirror-focused monochromatic undulator beam of the ID10C end station. Our simulations had shown before that the generation of highly

localized intensity distribution in the detector plane is feasible with a system of three pinholes, increasing in diameter towards the sample, so that one pinhole cuts into the first or second Airy minimum of the preceding one suppressing higher-order contributions. Unfortunatelly, in contrast to our previous experience and although all pinholes had been prepared very carefully by Focused Ion Beam polishing, the far-field diffraction pattern even of a single pinhole was strongly distorted by non-isotropic contributions in the exiting wave-field. Similarly, strong distortions of the wavefront by the monochromator and focusing mirrors were observed in pinhole line scans through the incoming wavefield and have most probably contributed – in combination with partial coherence effects and the small distance of the sample from the optical elements – to the non-ideal scattering from the pinhole apertures. The localization of the far-field diffraction pattern was partly achieved (cf. Fig. 1), however, it is concluded here that designing a special illumination function to separate the scattering from the sample and the illuminating aperture should be experimentally possible, but needs individual investigation and highly optimized conditions to provide a better plane-wave illumination of the aperture system.

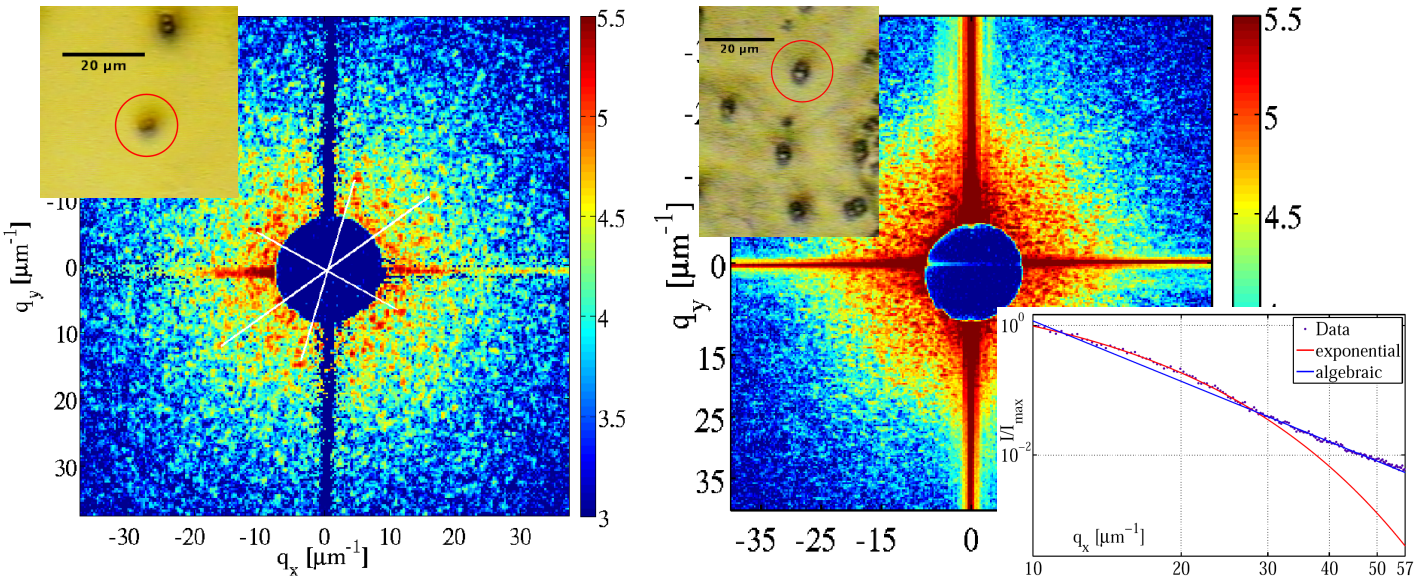

Figure 2: (left) Coherent, background- and slit-scattering-corrected far-field intensity distributiuon (exp. time 400 sec, total intensity appr. $1.3 \cdot 10^8$ photons) of an *unstained freeze-dried* radiodurans cell shown in the small inset (in-situ image during the experiment; imaged cell is marked with a red circle). As expected for a photon energy of 8 keV the pattern shows significant rotational symmetry, suggesting to a good approximation a pure phase distortion of the incoming wavefront by the sample. (right) Coherent, background-corrected far-field intensity distribution of an *unstained frozen-hydrated* radiodurans cell shown in the small inset (in-situ image at -173°C during the experiment; imaged cell is marked by a red circle). No ice crystals are visible in the optical microscopy image suggesting a successful vitrification. This is supported also by a speckle size similar to the case of freeze-dried cells. An angular integration in the lower right quadrant of the right graph shows that the descrease of intensity at high scattering angles can be described by a power law ($\sim q^{-3.1(1)}$).

As a consequence the optical setup was changed, i.e. the focusing mirrors were removed and two pairs of rectangular polished slits were inserted into the beam path (beam defining aperture was 10x10µm²). Sample cells had been prepared by rapid freezing in liquid ethane on thin Kapton films (d = 10-20µm) mounted on metal holders (MiTeGen, USA) and could be visualized *in-situ* during the experiment by an on-axis microscope (Accel, Germany), mounted coaxial with the beam path (cf. Fig. 1). Now smaller and more localized freeze-dried cells of the procaryotic species deinococcus radiodurans were introduced into the beam (cf. Fig. 2). As visible in Fig. 2 the smaller cell size allowed for sufficient oversmapling with a speckle size of 3-5 pixels, yielding a cell size of ca. 2-5µm, which is very consistent with the optical micrographs.

In summary, it could be shown that unstained prokaryotic and eukaryotic cells could be prepared in freeze-dried and frozen-hydrated state and reciprocal space data could be collected in the relevant q-space up to an angle of q = 50 µm^{-1} yielding a real-space half-period of ca 60 nm. From an extrapolation of the algebraically decaying scattering intensity it could be concluded that a resolution of 10 nm would require a total flux of 10^{10} photons at 12 keV. This is an important experimental result towards answering the question of an optimum wavelength to minimize the dose at a given resolution. It was shown that biological specimens act as nearly pure phase objects at 8keV photon energy and a resolution of 50nm and higher with hard X-ray CDI on unstained biological materials should feasible. Reconstruction attempts based on iterative procedures have not been successful yet mainly due to reasons of lacking q-data and partial coherence. However, by combination with the optical images from the in-situ-microscope this might become possible.