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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.

Once completed, the report should be submitted electronically to the User Office via the User Portal:

https://wwws.esrf.fr/misapps/SMISWebClient/protected/welcome.do

Reports supporting requests for additional beam time

Reports can 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:** 3D density and DNA packing in bacterial nucleoids by Cryo-Tomo-CDI	**Experiment number**: LS2235
Beamline: Id10	**Date of experiment**: From: 03.07.2013 to: 09.07.2013	**Date of report**: 26.02.2014
Shifts: 18	**Local contact(s)**: Y. Chushkin	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

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Report: We report on Coherent Diffractive Imaging (CDI) experiment on isolated biological specimens of the bacterium *Deinococcus radiodurans* (*D. radiodurans*) aiming at a reconstruction of the 3D density distribution of the frozen, (fully) hydrated bacterial nucleoid under cryo-protecting conditions. DNA compactification in bacterial cells is an important biophysical problem for which the three-dimensional density distribution at the sub-cellular level has to be imaged with quantitative contrast values. For example, a simple unknown quantity relevant to the understanding of DNA compactification in bacterial nucleoids is the local mass density. This cannot be solved by electron microscopy or fluorescence microscopy studies, not for reason of resolution but of contrast. The bacterium *D. Radiodurans* [1-5] investigated in this experiment has received much interest due to the bacterium's extraordinary resistance to high doses of ionizing radiation. Its resistance has been postulated to result from DNA repair mechanisms which may take advantage of a particular structural arrangement of its nucleoid.
Towards the goal of this quantitative imaging experiment, 2D CDI data has been collected on test structures, freeze-dried biological samples and, finally, frozen-hydrated samples, including an angular series.
Materials and Methods: The experiments were carried out using monochromatic undulator radiation of 7keV photon energy at the (upgraded) EH2 of the ID10 beamline. Using a comparably large distance between sample and detector of about 7 metres which ensures the oversampling condition for large clusters of multiple bacterial cells and large samples. At this distance a real space pixel size of about 45 nm can be achieved under optimal conditions when using the MAXIPIX 2x2 detector (Lost 3 shifts due to problems with the MAXIPIX detector).
Results: In a first step, the information content of recorded diffraction patterns was analysed by studying the diffracted signal of a binary Au test structure under varying incident photon fluxes. According to [6] a criterion for uniqueness in the reconstruction takes the form that the number of photons, per pixel of contrast in the image, exceeds a certain minimum. Here, the analysis is can be made on statistical models that make use of the contrast characteristics of the test structure. Recorded diffraction patterns of this experiment are shown in figure 1. The analysis is still in progress.

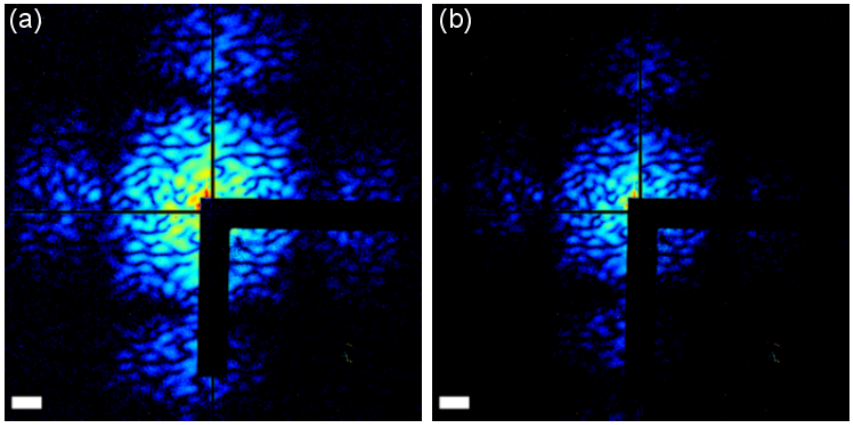

FIG. 1. (a), (b) Recorded diffraction patterns on test structure using different incident photon fluxes. (a) full beam, (b) attenuated beam. Scalebars denotes 10 /µm of scattering vector.

Next, the CDI method was tested on other test structures. A reconstruction of an electron-beam lithographically fabricated, gold representation of the 'nano world' is presented in figure 2 (b). The diffraction data necessary for obtaining the image is shown in figure 2 (a).
The main CDI experiment was performed on freeze-dried, biological samples such as cells of the bacterium *Deinococcus radiodurans* and the endospores of the bacterium *Bacillus subtilis.* A successful 2D reconstruction of *D. radiodurans* cells has been be obtained and can be seen in figure 2 (c).

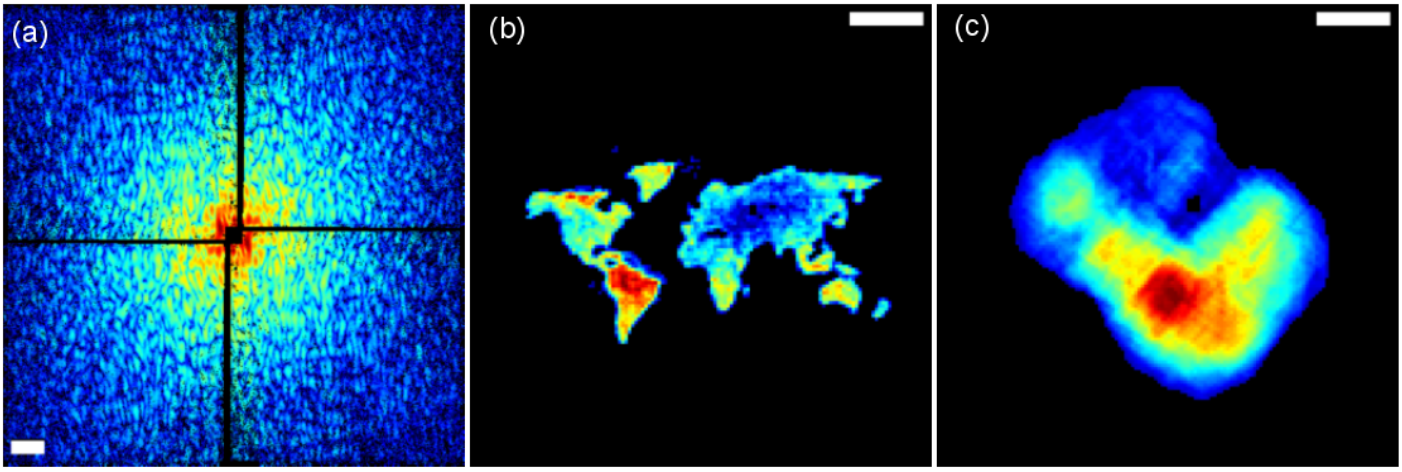

FIG. 2. (a) Example of a symmetrized diffraction pattern of the sample that is shown in (b). (b) Example of a CDI reconstruction of an Au test pattern. (c) CDI reconstruction of freeze-dried bacteria Deinococcus radiodurans. Scalebar in (a) denotes 10 /μm of scattering vector. The pixel size in both reconstructions (b), (c) is 45nm and the scalebars denote 1μm.

Finally, the experiment was performed on frozen-hydrated samples. Recorded diffraction patterns of cells of *D. Radiodurans* and of a bacterial spore of *B. subtilis* are shown in figure 3 (a) and (b), respectively. In this case the diffraction signal was lower in comparison to the case of freeze-dried samples making the reconstruction more difficult. The reconstruction analysis is still in progress.

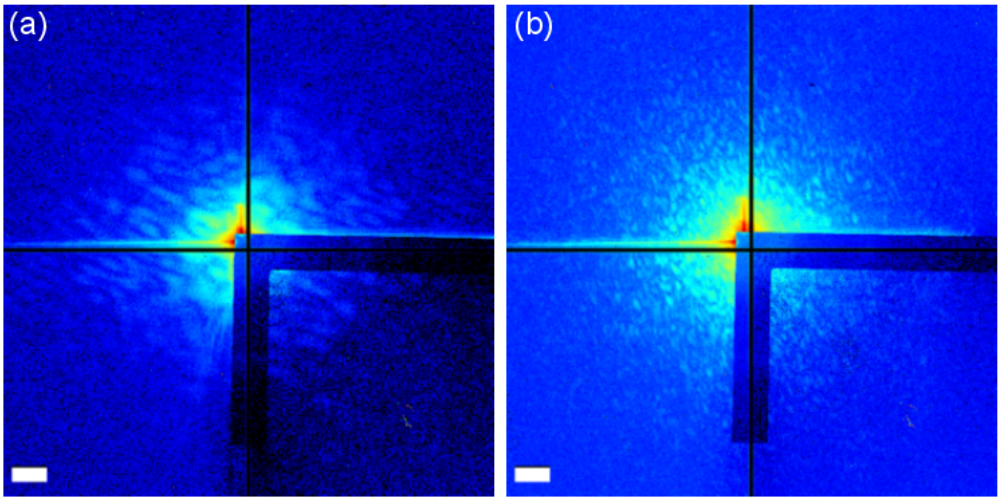

FIG. 3. Example of recorded diffraction patterns of frozen-hydrated samples: (a) cells of the bacterium Deinococcus radiodurans, (b) endospore of the bacterium Bacillus subtilis. The exposure times are 60 s and 600 s in (a) and (b), respectively. Scalebars denote 10 /μm of scattering vector.

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[2] Eltsov, M. and Dubochet, J., "Rebuttal: Ring-Like Nucleoids and DNA Repair in Deinococcus radiodurans," J. Bacteriol. 188, 6052 (2006).
[3] Levin-Zaidman, S. et al., "Ringlike Structure of the Deinococcus radiodurans Genome: A Key to Radioresistance?", Science 299, 254-256 (2003).
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[6] V. Elser and S. Eisebitt. 2011. New Journal of Physics 13, 023001