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. In extension of our previous work on coherent projection images of unsliced freeze-dried *Deinococcus radiodurans* bacteria [1], we have here used tomography to reconstruct the full 3D density distribution. In contrast to ptychographic tomography, which has also been demonstrated with the P10 KB nanofocus [2], the present approach was to use x-ray waveguide based projection imaging, in order to take advantage of a full field method. The basic concept is sketched in Fig.1.

Figure 1: The 13.8 keV (Si(111)) undulator beam at P10 was focused by two KB mirrors onto the system of two crossed planar waveguides (WG). The sample was placed on a tomographic stage at a distance $z_1$ behind the WG exit. The area pixel detector (Maxipix) was placed at a distance $z_2 \gg z_1$ away from the sample to record the diffraction pattern. The inset shows an optical micrograph of the sample. Sample alignment is facilitated by on-axis optical microscopes at the beamline. Phase reconstruction for each projection followed by 3D tomographic reconstruction yields a 3D data set with quantitative electron density values. The approach was demonstrated to be particularly dose efficient [3].

Experimental details: Cells of *Deinococcus radiodurans* wild-type strain were be suspended on Si$_3$N$_4$-foils, vitrified by cryogenic fixation in ethane (cryo plunging), and subsequently kept at cryogenic temperatures using a cryo jet sample environment, or alternatively further treated by a freeze drying process. The waveguide system consisted of two crossed planar waveguide slices, each with a sputtered thin film sequence Ge/Mo/C/Mo/Ge, with amorphous C acting as a guiding layer [4, 5]. Phase reconstruction of the waveguide data was based on a robust, dose-efficient and quickly converging iterative reconstruction scheme which takes photon noise effects into account [6]. While scanning diffraction based on the Kirkpatrick-Baez (KB) focused beam was successfully carried out under cryogenic conditions ( 7.9 keV and 13.8 keV, Si(111), spot size of typ. $\approx 200 \times 350$ nm with an intensity of $\geq 10^{11}$ cps), the full tomographic tilt series was do date only possible in a freeze dried state, since the cryogenic sample in the jet turned out to be incompatible with the instrumentation of waveguide holder. This issue is currently addressed by improving the sample environment and sample holders. At the same time the pronounced structure of the DNA rich regions in the bacterial nucleoid have been confirmed by comparing freeze dried to cryogenic (vitrified) samples in single projections [7]. Fig.2 shows the experimental results. The density
distribution was reconstructed at extremely low average fluence of $\leq 3 \cdot 10^5$ photons/µm$^2$ per projection to a real-space half-period of $\approx 150$ nm, underlining the dose efficiency of the method. In fact, for the gram-positive bacterium *Deinococcus radiodurans*, single projections can in principle be obtained at dose values well below the lethal dose. Ongoing instrumental improvements and experiments on bacterial cells have in the meantime yielded a significantly increased spatial resolution (analysis in progress, data not shown). The work was funded by Sonderforschungsbereich 755 *Nanoscale Photonic Imaging*.

![Figure 2](image)

Figure 2: Results of a tomographic study at P10 carried out on freeze-dried *D. radiodurans* cells: (a) Direct volume rendering of the three-dimensional effective mass density. The coloring indicates densities from 0.8 g/cm$^3$ (blue) to 1.2 g/cm$^3$ (red). (b) Surface rendering obtained by choosing 0.75 g/cm$^3$ as a threshold value. (c) Combined direct volume rendering (1.15 – 1.25 g/cm$^3$) and surface rendering obtained by clipping the dataset with a plane. (d) Histogram of the effective mass density inside the surface shown in (b), corresponding to the volume occupied by the cells. The limits used for direct volume rendering in (a) and (c) are indicated by solid green and dashed red lines, respectively. Adapted from [3].

**References**


