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An optimization procedure for spatial and density resolution in hard X-ray micro-computed tomography

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Abstract

The quality of the X-ray tomogram not only depends on the spatial resolution but also on the density resolution or contrast. Based on the theory of [Nucl. Instr. and Meth. 206 (1983) 541] it is concluded that the density resolution can be substantially improved by merging of pixels, referred to as binning, prior to reconstruction. We demonstrate that the quality of a given 3D-data set, i.e. the tomogram of stained biological cells seeded on a polymer multifilament yarn in phosphate buffered saline, can be optimized with respect to the product of spatial and density resolution – the image quality factor. This procedure improves or even enables the visualization and quantification of selected constituents in the tomogram.

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Using tomographic imaging the penetrating power of X-rays reveals the bulk architecture of materials. Recording the projections, i.e. the lateral spatial dependence of the absorption one can reconstruct the inner structure of the sample down to about one micron, a limit set by the currently available detection units without magnifying X-ray optics. The strong dependence of the absorption on the atomic number gives rise to high contrast in most cases. In some cases, however, the absorption of the constituents is quite similar and, therefore, the contrast too weak. In particular, biological cells consist mainly of water as does the surrounding medium. Consequently, cell cultures cannot be directly visualized by micro computed tomography in absorption contrast mode. By means of molecules containing species of high atomic number, which are selectively bound to specific parts of the cells, the cell's absorption is increased and, finally they become visible. One can estimate the concentration level necessary with high precision [1]. The staining agent, however, can influence the cell behavior. High concentration

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can further lead to background staining. Thus, the concentration level should be held as low as possible. Here, although the spatial resolution is one order of magnitude better than the size of the features to be detected, it may occur that the tomogram is quite noisy, and the segmentation of the cells becomes complicated. Grodzins has theoretically demonstrated that the contrast can be improved reducing the spatial resolution [2]. This means merging of pixels in the projection, termed binning, increases the contrast or the density resolution. Using the claim of Grodzins that the density resolution is indirectly proportional to the square of the voxel length and the spatial resolution scales with the voxel length, we hypothesize that for a given tomogram there exists a binning factor, where the quality of the tomogram described by the dimensionless product of density and spatial resolution is optimal. Such a choice means that both contributions are equally weighted in the dimensionless quality factor. The hypothesis implies that the density resolution for a certain feature size reaches a limit for high binning factors. In this communication, we evaluate how far the predictions of Grodzins can be applied to fibroblasts with moderate staining concentration in hydrated environment.

The sample investigated is an in vitro cell culture of rat tendon fibroblasts (3T3) seeded on a poly-ethylene terephtalate (PET) multifilament yarn of 32 filaments each about 20 μm in diameter. The cell seeding procedure including yarn preparation is described in detail in a previous publication [1]. Subsequently the cell culture was incubated in Dulbecco's modified Eagles medium containing 10% fetal bovine serum (FBS) and 1% broadband antibiotic antimytotic (ABAM) at 37 °C and 5% CO_2 for 3 days. Then the fibroblasts were fixated in 3% glutaraldehyde in PBS overnight, washed in PBS once, and post-fixed with 0.1% OsO_4 for 1 h. Finally, the sample was washed with PBS twice. For tomographic imaging the yarn was transferred into a 10 μm thin-walled glass capillary (diameter 0.7 mm) filled with PBS. 720 radiographic projections were recorded over the angular range between 0° and 180° using the photon energy of 9 keV at the beamline BW2 at Hamburger Synchrotronstrahlungslabor (HASYLAB) at Deutsches Elektronen-Synchrotron (DESY) [3] with the effective pixel size of $(1.45\ \mu m)^2$. The dose applied can only be roughly estimated. Due to the 180° scan the dose varied from about 0.66 to 1.44 Gy with a mean value of 1.04 Gy (standard deviation 0.13 Gy). The intense,

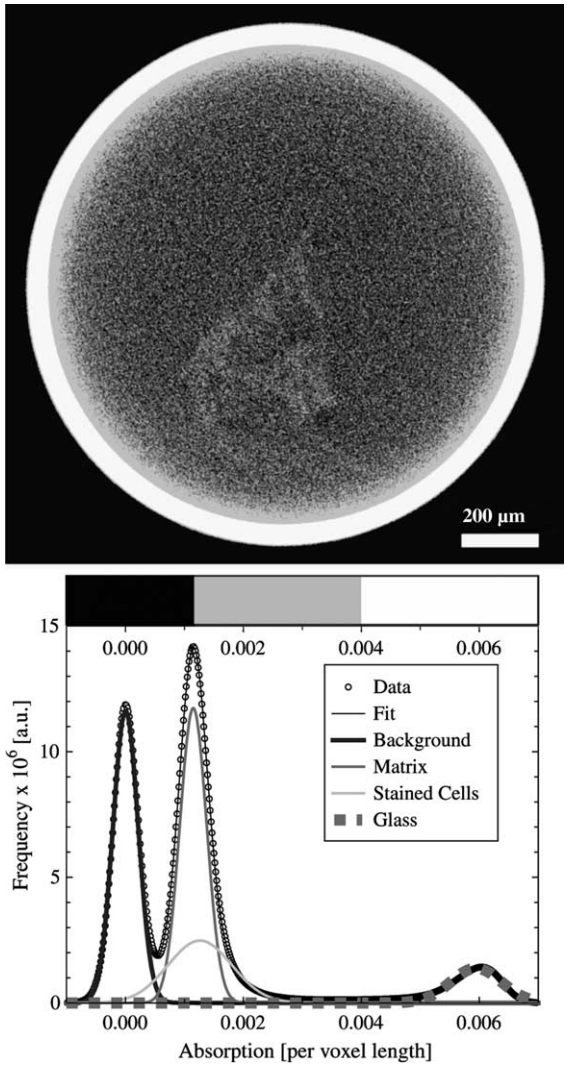

Fig. 1. Selected slice of the tomogram showing the container (white), the stained cells (gray), PBS and air (black). The stained cells become only visible due to the choice of the threshold well below the intersection of the Gaussians for PBS and stained cells. The related fits in the histogram are represented below.

monochromatic synchrotron radiation might modify the sample chemistry. Sometimes, we have observed the formation of bubbles but morphological damages of the samples we have never detected.

The spatial resolution of the entire system is characterized by the modulation transfer function (MTF) of a gold edge [4]. The intersection of the MTF with the 10% line is extracted by a linear fit using the ProFit code (Quantumsoft, Switzerland). The density resolution calculates from the histogram of the absorption values in the tomogram. The quantitative parameter is the half-width of the peak for the constituent of interest. As shown previously [5], the peaks have Gaussian shape and are, therefore, easy to fit. We have incorporated the ramp filter in the back-projection reconstruction algorithm to avoid deviations from the Gaussian shape, which is often observed when using more sophisticated filters such as Buter or Hanning (http://cfi.lbl.gov/cfi_software.html), Nuclear Medicine and Functional Imaging at Lawrence Berkely National Laboratory, USA). Here, Gaussians are fitted with the Levenberg-Marquardt algorithm (ProFit).

The tomogram of highest spatial resolution, as recorded, exhibits almost no contrast between cells and PBS, as shown in Fig. 1 qualitatively by a single slice and quantitatively by the histogram of the whole tomogram in the absorption range of interest. The Gaussian related to the cell absorption strongly overlaps with the PBS peak. Thus, the cells cannot be segmented from the matrix by means of a simple threshold algorithm. Furthermore, the absorption values of the PBS near the PBS–glass interface are identical to the ones of the

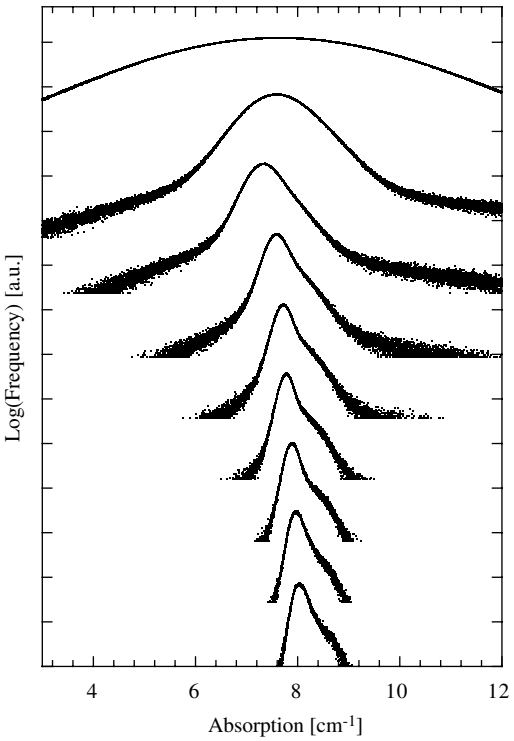

Fig. 2. Histograms of the reconstructed data sets after selection of a cylindrical region of interest with increasing binning factor from 1 to 9 (top to bottom). The main peak becomes steeper and steeper. The shoulder at the right of the main peak evolves with increasing binning. It is associated to the stained cells.

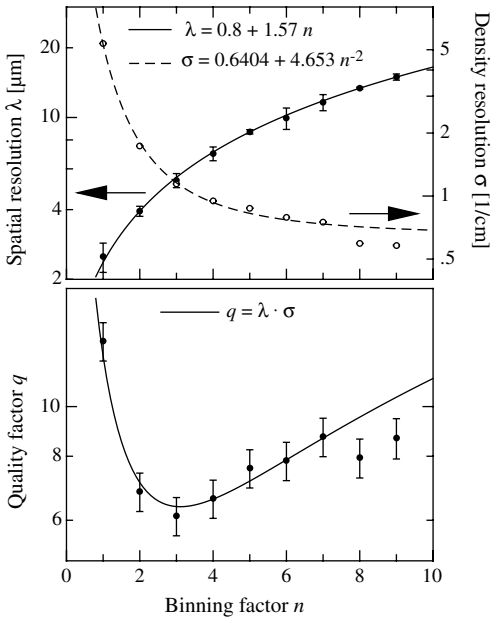

Fig. 3. The spatial and the density resolution versus binning factor show the predicted behavior as verified by the fitted curves. The errors of the fit parameters are of the order of magnitude of the last digit. The error bars are directly retrieved from the fitting procedure. The individual values of the dimensionless quality factor q and the product of the fitted curves shown above exhibit a minimum at a binning factor of about 3.

stained cells. Presumably this phenomenon is the result of the total X-ray reflection at the glass surfaces. This effect, however, could be overcome by the selection of a region of interest (ROI). Here, we extracted a cylinder containing the cells on the yarn in PBS. Nevertheless, the constituents give rise to broad Gaussians (cp. Fig. 2). In succession, the raw data are binned in steps of integers up to the binning factor of 9 by means of the interactive data language – IDL (Research Systems Inc., USA) prior to tomographic reconstruction. Note that the formalism [2] does only apply if raw, uncorrected projections including the corresponding dark and background images are binned. Again cylindrical ROIs are extracted. The improved density resolution is clearly demonstrated by the histograms in Fig. 2. With increasing binning factor the peak becomes steeper, and the shoulder representing the stained cells develops to its right. It should be mentioned that the matrix peak shifts with increasing binning factor, first to lower values and then to higher ones. In general, one expects that the peak is related to a certain absorption value and, therefore, its mean value remains constant. The reconstruction procedure based on the incorporation of filters results, however, in an offset of the retrieved absorption histograms, which is best seen for the background

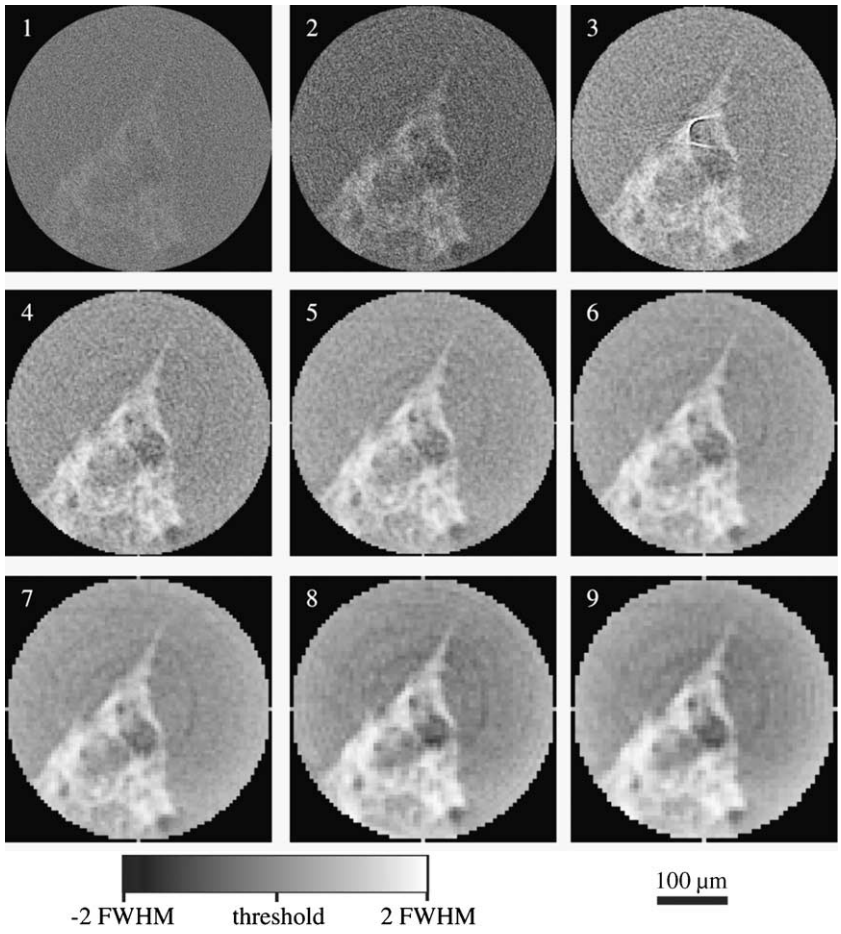

Fig. 4. Selected slice for different binning factors as indicated. The visibility of the cells increases up to the binning of 3 or 4. With further increasing binning factors details are lost as the result of the partial volume effect. Around the binning factor of 6 the scattering at the PBS–glass interface comes to light.

peak (air). Therefore, we have shifted this peak to the absorption value of air for the tomograms. This procedure, however, does not explain the phenomenon that the peaks of the constituents do not stick to a certain value independent of the binning factor. We believe that it is related to the partial volume effect. Upon binning the voxel values at interfaces get closer to the mean absorption value of the related constituents.

The evolution of spatial and density resolution versus binning is shown in Fig. 3. According to Grodzins [2] the density resolution σ is indirectly proportional to the square of the voxel length. This behavior fits very well to our data, when an offset is considered for the fit of full-width-at-half-maximum (FWHM) values (Fig. 3). The offset characterizes the limit for the density resolution given by partial volume and volume discretization effects. The exceptions are the data for the binning factors 8 and 9, which are below the fitted curve. This deviation is explained by the X-ray reflection and scattering at the glass–PBS interface, which starts to influence the ROI. Qualitatively the effect becomes clear in the thresholded 2D slices shown in Fig. 4. A half-moon shaped feature evolves at the circular boundary at the area closer to the glass wall.

The spatial resolution is proportional to the voxel length. It is extracted from the linear fits of the MTF values between 5% and 15%, quite an arbitrary choice. Consequently, the error bars in Fig. 3 for the spatial resolution are averaged, whereby the other error bars plotted in Fig. 3 are directly retrieved from the fitting procedures, just to give the reader a suitable estimate.

The solid curve in the lower part of Fig. 3, which describes the quality factor q is simply the product of the two fits shown in Fig. 3 above. It reaches a local minimum at a binning factor of about 3, where the voxel length corresponds to 4.5 μm. This should be the optimum for our imaging task. This voxel length corresponds well to a typical dimension of the fibroblast cells (cell height, 3–6 μm [6]). The result is validated by the slices shown in Fig. 4. Obviously, the stained cells are best visualized at binning factors of 3 and 4, respectively. At higher binning factors information is lost as details vanish. At lower binning factors the details are not or hardly detectable.

In summary, the data analysis of the tomogram underlines the validity of the trade-off between spatial and density resolution. The derived dimensionless quality factor does reach a minimum, related to the optimal binning factor, of about 3, for visualization and data analysis. We propose that this technique allows a hierarchical visualization approach, correlating the datasets at different binning factors. In particular, one might have a closer look to selected features comparing the tomograms at higher density resolution (higher binning factor) and higher spatial resolution (lower binning factor).

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