Functional Imaging of Cell Clusters

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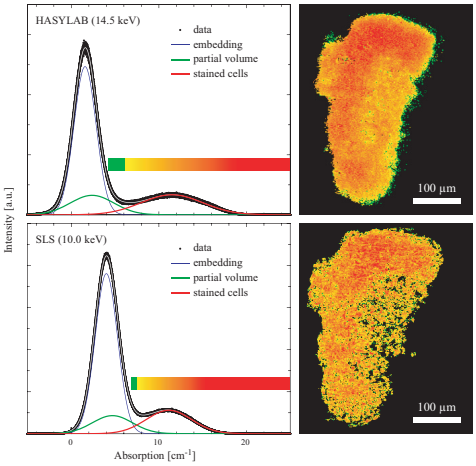
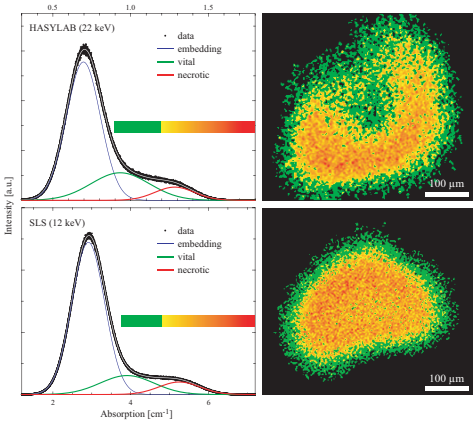
INTRODUCTION

Synchrotron radiation-based micro computed tomography (SRμCT) allows nondestructively determining the local X-ray absorption. Since biological cells consist mainly of water as the surrounding medium, stains have to be applied to uncover the structures of interest. Human embryonic kidney (HEK 293) cell clusters were stained using osmium tetroxide and osmiumammine-B. The retrieved tomograms clearly show that the osmium tetroxide membrane staining gives rise to homogeneous absorption throughout the cell clusters, whereas the RNA/DNA-stained cell clusters exhibit a core of higher and a rim of lower density that correlate with results of fluorescence microscopy and histology. The inner part of the clusters is associated with necrotic cells as the result of insufficient oxygen and nutrition supply surrounded by about 6 layers of vital cells.

THRESHOLDING

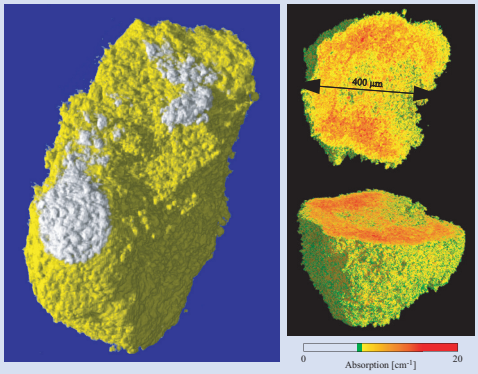


The histograms of the local absorption values are perfectly fitted by 3 Gaussians - white line on top of the black dots characterizing the data. Note the similarities between both diagrams obtained from tomograms of 2nd and 3rd generation sources at different photon energies. The slices have not exactly in the same orientation, but clearly indicate the better image quality of the SLS data. Here, in areas of lower cell density features with a diameter of about 10 μm associated with individual cells might be extracted.

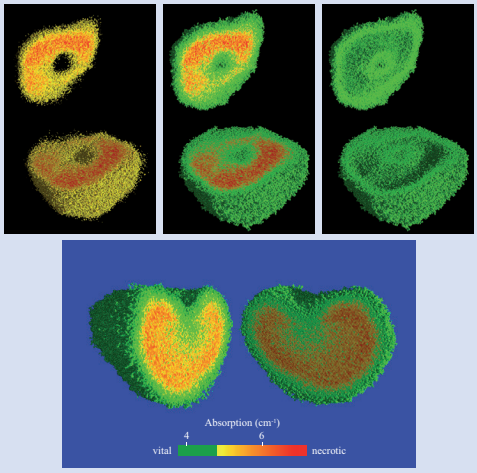


The RNA/DNA staining using osmiumammine-B results in a transition range, green colored, which is related to the area at the cluster's periphery about 50 to 100 μm wide.

CLUSTER VISUALIZATION

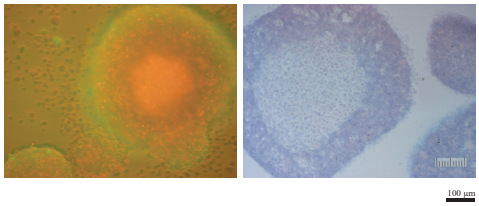


The cluster of 10^4 to 10^5 HEK 293 cells about 0.5 mm long shows that SRμCT might uncover cell-like structures. The white areas instead of the yellow color should support the impression. The data are visualized from a tomogram obtained at the materials science beamline 4S (SLS, PSI, Switzerland) using the photon energy of 10.0 keV. To increase the density resolution (contrast) the data are binned by the factor of 3.

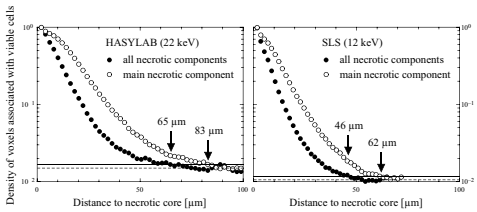


The colors of the segmented cell cluster correspond to the local absorption values given by the color bars of the SLS data. On the left the core and on the right the shell of the cluster are extracted. In the lower part, the virtual cut perpendicular is given.

SHELL THICKNESS



The HEK 293 cell clusters are characterized by 2D images of fluoroscopy and phase-contrast microcopy after slicing for classical histology. Both images illustrate the separation of the cell cluster in core and shell (rim). The fluoroscopic image proves the viability of the cells at the periphery by the green FDA color from the esterase activity. The cores of the relatively large cell clusters exhibit the PI-color related to the non-vital cells. The phase-contrast image of the histological slice colored by hematoxylin/eosin.shows small, bright areas in the center and a well-separated darker outer part of the spheroid. Hematoxylin leads to the violet color in RNA-rich areas. Consequently, the core has the lower RNA-density with respect to the shell.



The largest component of the necrotic part, which can be easily identified (98.7% - SLS - and 98.2% - HASYLAB - of the entire cluster's core), is voxel-wise expanded (open circles) until the noise level (dashed line) is reached. If all parts associated with the necrotic part are voxel-wise expanded, one obtains the closed circles. These data are (17 ± 1) μm lower then the calculation for the main component. The noise level can be determined with high precision by counting voxels, thresholded for vital cells, in a region well apart from any cell cluster. The crossing of the data with the full line, which is 10% above the noise level, is selected as the shell thickness. The dots are the lower limit and the open circles the higher one. The exact value should be closer to the main component analysis, as indicated by the first crossing of dots and circles.

Assuming a typical diameter of 10 μm for the HEK 293 cells, the shell of the cluster investigated corresponds to (6 ± 1) layers of vital cells.

CONCLUSION AND ACKNOWLEDGEMENT

SRμCT is used to extract and quantitatively analyze the non-vital core of cell clusters in 3D space. SRμCT allows functional imaging of biological matter on the cellular level, which is of key importance in biotechnology and tissue engineering. The optimal HEK 293 cluster diameter, i.e. the maximum cluster size of viable cells without the formation of a necrotic core, corresponds to maximal 120 μm for the selected culture condition. Consequently, SRμCT provides the possibility to correlate cell cluster morphology and cell viability to the prefrerred culture conditions. With further development and adaptation of promising stains for functional imaging, using SRμCT other features of interest in biological cells can be detected and visualized in 3D space.

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