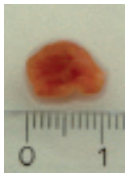
3rd International Bernd-Spiessl-Symposium, 14th - 16th June 2007, Basel, Switzerland

# The Transparent 3D Cell Culture - an Insight Image with SRμCT

B. Saldamli[1], J. Tübel[2], F. Beckmann[3], J. Herzen[3], C. Kober[4], P. Jürgens[1], J. Schauwecker[2], H.-F. Zeilhofer[1], R. Burgkart[2], and B. Müller[5]

[1]*Hightech-Research Center HFZ, University Hospital Basel, Switzerland*,
[2]*Department of Orthopedic Surgery, Klinikum rechts der Isar, TU Munich, Germany*,
[3]*GKSS-Research Center Geesthacht, Germany*, [4]*University of Applied Sciences, Osnabrück, Germany,*
[5]*Biomaterials Science Center, University of Basel, Switzerland*

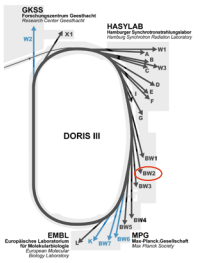
## INTRODUCTION



Cell cultures are established in vitro models for studying cellular processes. For many decades cells have been cultivated in 2D. More recently the importance of the third dimension in cell biology has been better understood. The growing knowledge has attracted further attention to 3D culturing. The characterization of 3D cultures includes methods, adapted from the ones established for 2D cultures. These methods, however, encounter certain restrictions, especially when it comes to the evaluation of the 3D spatial organization. Histological sectioning allows studying this aspect. Unfortunately, the extracted information is restricted to the rather arbitrarily selected slices. In addition, because of the destructive nature of the sectioning, further studies are often impossible. Hence non-destructive methods for the 3D characterization are highly desirable. Some pioneering studies have successfully applied synchrotron radiation-based micro computed tomography (SRμCT) for the visualization and even quantification of biological of biological meso- and microstructures, including scaffolds for tissue engineering. In this study we explored the potential of SRμCT for the characterization of scaffold-free, human osteoblast-derived histoids.

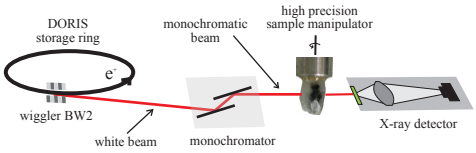
## SRμCT

The paraffin embedded histoid was measured by SRμCT in the absorption contrast mode at the beamline BW 2, HASYLAB at DESY, Hamburg, Germany, using a photon energy of 24 keV. The figure at the right shows the storage ring DORIS, where the synchrotron radiation is created by positrons travelling close to light-velocity through the wiggler of BW 2.

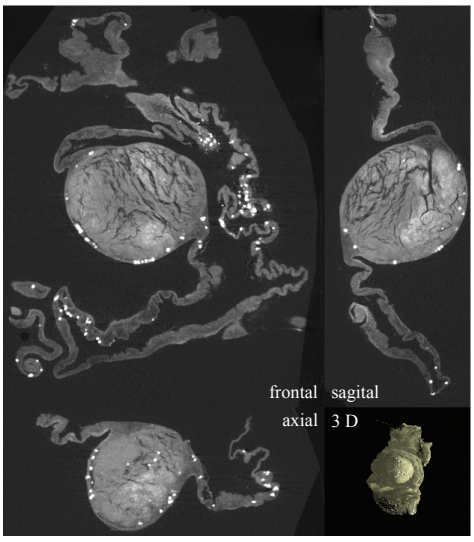



circumference
289.2 m

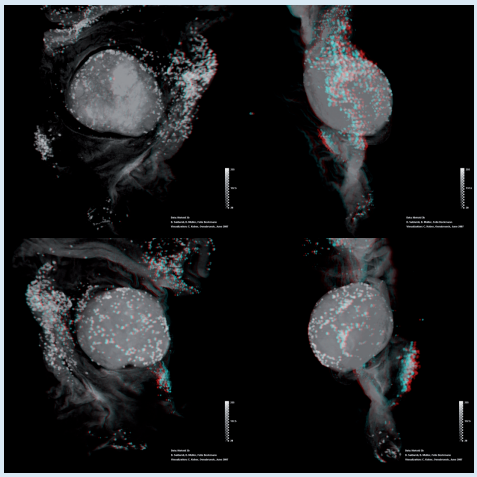
The tomography setup, operated by GKSS Center, is schematically represented below. The monochromator selects the desired photon energy out of the white spectrum of the synchrotron radiation. The monochromatic beam is attenuated by the histoid and the related image acquired on the 2D X-ray detector.



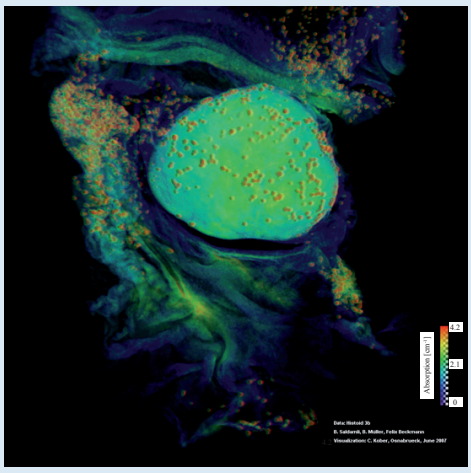

To investigate the sample five tomographical scans were performed and combined after reconstruction. The reconstructed 3D volume consists of 1535 x 1535 x 2037 voxels, representing a volume of 8.2 x 8.2 x 10.9 mm³. The measured spatial resolution corresponds to about 9.1 μm at a voxel length of 5.34 μm. The figure below shows 2D slices through the reconstructed volume and a 3D representation of the histoid.




## DIRECT VOLUME RENDERING STEREOGRAPHIC VISUALIZATION



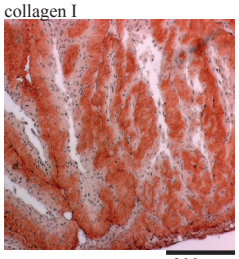
The stereographic visualization was performed at the University for Applied Sciences, Osnabrück by means of direct volume rendering (AMIRA) with high transparency. The figure above shows gray-scale images captured from a clockwise motion in 90° steps. For comparison, the figure below shows the histoid in color. In this intuitive visualization approach, every voxel is allowed absorbing or emitting light according to its x-ray absorption. With regard to color map, values corresponding to structures of low x-ray absorption are rendered in dark gray and scarab blue, whereas white and red refer to values corresponding to structures of high x-ray absorption, respectively.



## HISTOLOGY

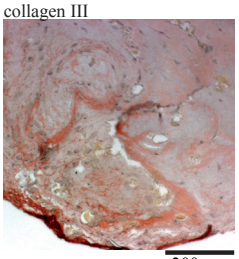
Thin sections (4 μm) of a formalin fixated and paraffin embedded histoid were characterized by histochemistry, immunohistochemistry and nick-translation.

collagen I



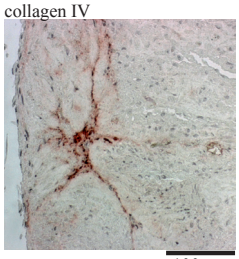
200 μm

collagen III



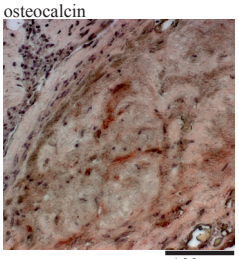
200 μm

collagen IV



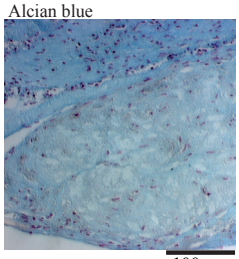
100 μm

osteocalcin



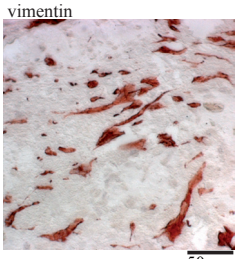
100 μm

Alcian blue



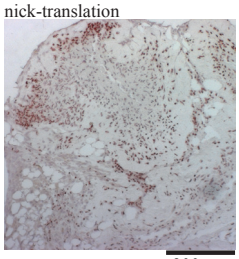
100 μm

vimentin



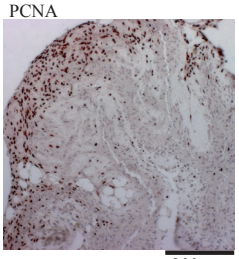
50 μm

nick-translation



200 μm

PCNA



200 μm

It was possible to obtain information about the composition of the ECM and the distribution of the type I, III and IV collagens, osteocalcin, and mucopolisaccharides. Variations in cell size, morphology, and even type were detected. Additionally the viability, proliferative activity, as well as the death of the cells have been analyzed.

## CONCLUSION AND ACKNOWLEDGEMENT

The SRμCT yields details about the 3D spatial organization of the histoids. Information about ECM-composition, cell morphology and type as well as cellular processes such as viability, proliferation and death, however, have still to be provided by histological methods. The SRμCT together with the advanced visualization techniques is a non destructive approach for the identification and selection of areas of interest in 3D space prior to histological sectioning. Our study of the millimeter-sized, scaffold-free histoids demonstrates that microarchitecture and the features on the cellular level can be made threedimensionally visible and quantified with high precision, combining SRμCT data with sophisticated computer vision tools.

The project was partially funded by the Bavarian Research Foundation and HASYLAB at DESY, Hamburg, Germany.