SLS Symposium on

Imaging

Tuesday, March 12, 2013

10:00 to 12:15, WBGB/019

10:00 Development of microtomographic in vivo imaging to study lung dynamics at the μm-scale
G. Lovric, R. Mokso, David Haberthür, Sébastien Barré, J.C. Schittny, M. Roth-Kleiner and M. Stampanoni

10:30 4D Materials Science: In Situ X-ray Synchrotron Tomography of Deformation in Metallic Materials
J.J. Williams, S.S. Singh, X. Xiao, F. De Carlo and N. Chawla

11:00 Coffee

11:15 NanoXAS - Combining Scanning Probe and X-Ray Microscopy for Nanoanalytics
N. Pilet, P. Warnicke, J. Raabe, R. Fink, H. Hug, C. Quitmann

11:45 Cryo-electron microscopy reveals the high resolution details of axonemal microtubule doublet
Aditi Maheshwari, Khanh Huy Bui, Keitaro Shibata, Yoko Y. Toyoshima and Takashi Ishikawa

Development of microtomographic in vivo imaging to study lung dynamics at the µm-scale

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Lung failure represents the leading cause of morbidity and mortality worldwide and is the fourth leading cause of death in Switzerland [1,2]. Despite the fact that recent decades have brought forth a huge clinical progress in treating lung injuries, including e.g. the immediate postnatal treatment of very preterm infants, two hypotheses on the structural alterations in the gas-exchange area during breathing are still under debate: a heterogeneous distention pattern of different lung areas and a homogeneous cyclic opening-and-collapse of all alveoli. Current techniques for performing lung imaging with small animal models at synchrotrons [3,4], however, were unsuccessful in answering these questions either by only applying 2D imaging or due to unsufficient temporal and/or spatial resolution. Thus, in-vivo X-ray tomography with micrometer spatial and sub-second temporal resolution has yet to be developed.

We present our recent progress in developing 3D in-vivo lung imaging by combining a new ultra-fast endstation with a novel data acquisition and post-processing paradigm. Results from first pilot experiments at the TOMCAT beamline with freshly prepared ex-vivo mouse samples will be presented. We describe our approach to image formation and biological interpretation, aiming at optimal image quality in terms of contrast, resolution and deposited radiation dose. We show the application of recently developed quantitative data evaluation tools [5], allowing for the first time a detailed analysis of lung tissue at the level of alveoli. Our strategy for deciphering lung filling and air recruitment patterns as well as a future outline for in vivo imaging are described. Finally, comparative values of the radiation dose are given.
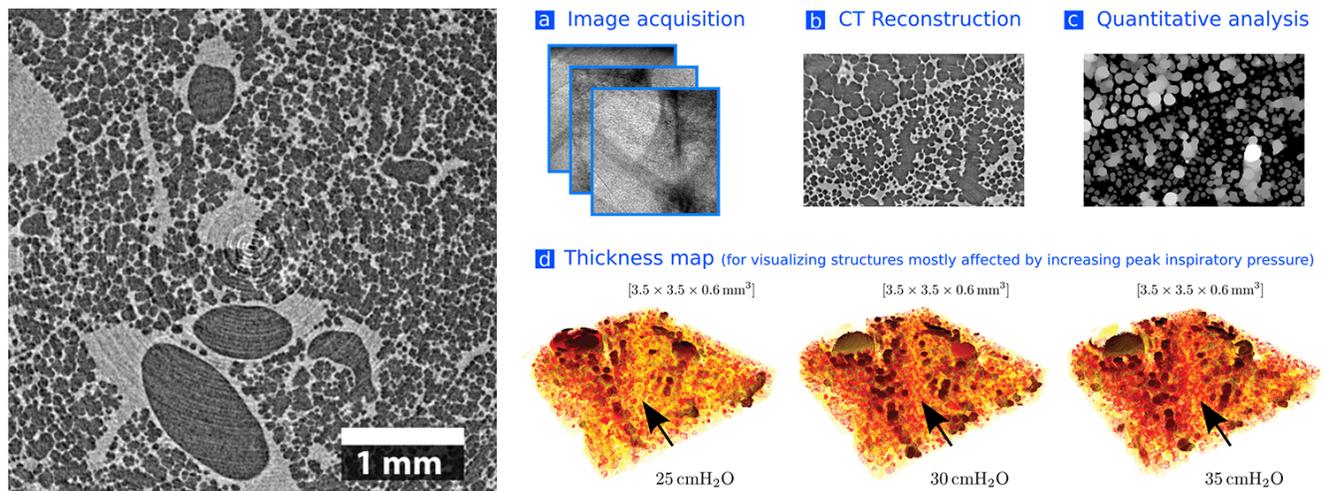

Fig. 1: (Left) Tomographic slice of a 14 days old mouse lung, acquired in 0.3 s for a full 180-degrees tomographic scan with 901 projections. The total dose for one tomographic scan is 5 Gy. (Right) Flowchart for further analysis of the 3D lung data.

References

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4D Materials Science:
In Situ X-ray Synchrotron Tomography of Deformation in Metallic Materials

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Advances in experimental methods, analytical techniques, and computational approaches, have enabled the development of three dimensional (3D) analyses. The study of 3D microstructures under an external stimulus (e.g., stress, temperature, environment) as a function of time (4D) is particularly exciting [1-4]. Furthermore, advances in 3D and 4D computational tools and methods have enabled the analysis of large experimental data sets, as well as simulation and prediction of material behavior. X-ray synchrotron tomography provides a wonderful means of characterization damage in materials non-destructively. In this talk, I will describe experiments and simulations that address the critical link between microstructure and deformation behavior, by using a three-dimensional (3D) virtual microstructure obtained by x-ray synchrotron tomography. The approach involves capturing the microstructure by *in situ* deformation (tension, shear, cyclic fatigue) in an x-ray synchrotron, followed by x-ray tomography and image analysis, and 3D reconstruction of the microstructure. Quantitative analysis and incorporation of the microstructure into a powerful finite element modeling code for simulation can also be conducted. Case studies on fundamental deformation phenomena in aluminum alloys and metal matrix composites will be presented and discussed.
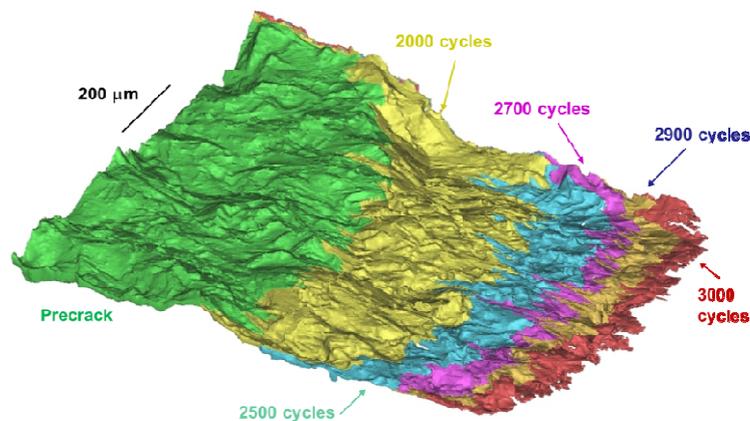

Figure 1. 3D reconstruction of fatigue cracking in 7075 Al alloy as a function of cycles.

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NanoXAS - Combining Scanning Probe and X-Ray Microscopy for Nanoanalytics

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NanoXAS is a novel x-ray microscope combining x-ray spectroscopy with scanning probe microscopy [1, 2]. This instrument uses Fresnel zone plates to focus x-rays (270 – 1800 eV) onto a semi-transparent sample which is raster scanned through the beam. A scanning probe microscope (SPM) is looking on the down stream side of the sample. In complement to the material contrast arising from the x-ray transmission chemical sensitivity, topology, magnetic forces, elasticity, friction, conductivity may be successively assessed using the SPM. Furthermore scanning transmission x-ray microscopy (STXM) images have been recorded using the atomic force microscopy (AFM) tip to probe the transmitted x-rays allowing the record of the topological image in mean time. A few results will be shown to demonstrate the principle. For example the use of such imaging technique in polymer science will be illustrated (see Figure 1).
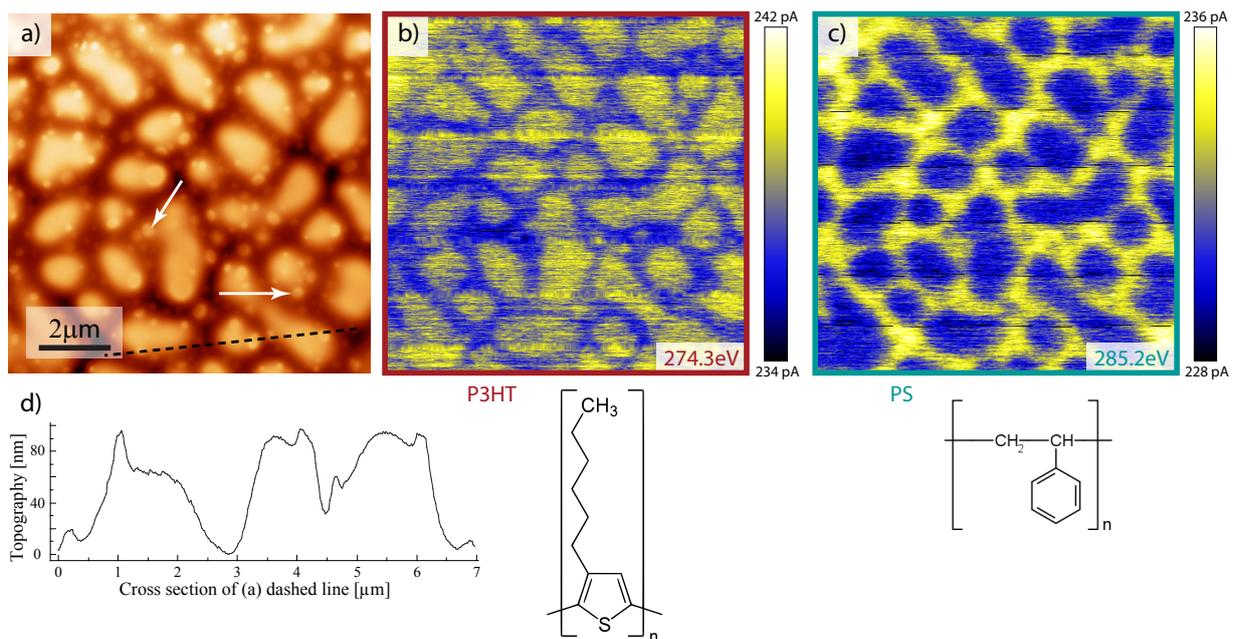

Fig. 1: Successive measurements performed on a P3HT/PS polymer blend. (a) AFM shows the topography of the sample. (b, c) STXM images recorded in situ at the same sample position at two different energies highlight the P3HT and the PS rich phase.

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Cryo-electron microscopy reveals the high resolution details of axonemal microtubule doublet

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The axoneme is conserved yet complex bending machinery. It has nine microtubule doublets and a number of binding proteins. Detail of binding of these proteins to microtubule doublets has never been described at the resolution of tubulin monomers. This study is based on results from the high resolution 3D structure of *Tetrahymena* MTD obtained by single particle cryo-EM analysis and medium resolution structure of β-tubulin specific kinesin decorated microtubule doublet obtained by cryo-electron tomography. The results enabled us to establish the fact that α- and β-tubulins form B-lattice arrangement in the entire MTD with a seam placed closer to the outer-junction. Furthermore, we found that the inner arm dynein tails are anchored on the MTD in the similar manner with one exception of dynein e. One radial spoke (RS3) has different way of binding from the other two. We discovered two new sub-classes of microtubule-inner proteins and reveal the unique art of how MIPs bind, connect and bridge protofilaments.
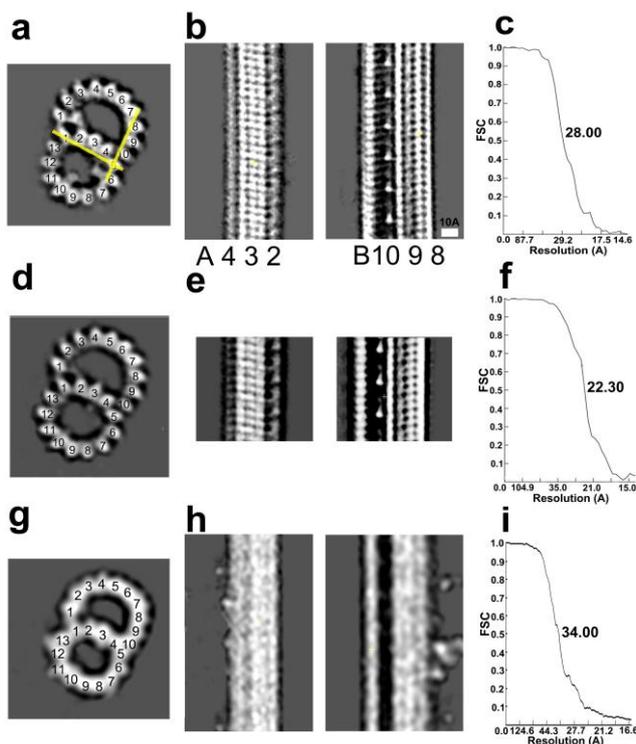
Figure: Comparison of axonemal microtubule doublet (MTD) structure from cryo-single particle analysis and cryo-ET

a,b,c,d,e,f) Single particle analysis, g,h,i) Cryo-ET. a,d,g) cross-section of the axonemal doublet (seen from the proximal end), b,e,h) longitudinal sections of PFs of the A- and B-tubule of the double (top: distal end; bottom: proximal end). c,f,i) The Fourier shell correlation curve showing the resolution at the 0.5 sigma cut-off. 28 Å and 22 Å resolutions were achieved with single particle analysis and further averaging with 16nm periodicity, respectively, the PFs are well resolved and also the tubulin monomers can be clearly distinguished. At the 34 Å resolution attained with cryo-ET, the PFs are not well resolved, illustrating the resolution limitation of cryo-ET.