

Extending lab-based X-ray nanotomography of low Z and porous materials to larger sample volumes without compromising resolution

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In this study, we present a method to extend the 3D volume reconstructed by X-ray nanotomography (Nano-CT) in a lab-based ZEISS Xradia 810 Ultra system while keeping the spatial resolution close to (50 nm)³. Furthermore, we demonstrate the improvement of image and segmentation quality for different low Z and porous materials. The advantage of this method is corroborated by determining the 3D resolution through Fourier ring/shell correlation (FRC/FSC).

Microscopy techniques, by their nature, cover a specific range of sample size and spatial resolution, both being closely related. Transmission electron microscopy (TEM), combined with electron tomography (ET), provides a very high resolution down to the atomic scale but is typically limited to a sample size of some hundred nanometers to a maximum of few micrometers, depending on the material [1]. At this point, lab-based X-ray microscopes such as the ZEISS Xradia 810 Ultra can extend the accessible sample volume for morphological studies to dimensions of (16 μm)³ (high-resolution (HR) mode) and (64 μm)³ (large field-of-view (LFOV) mode) at a spatial resolution of 50 nm and 150 nm, respectively. To enhance contrast at high resolution, the Xradia 810 Ultra uses quasi-monochromatic X-rays of rather low energy (5.4 keV, Cr K_α). As result, the absorption length for medium to high Z materials at this energy level is usually limited to a few microns and, therefore, often constrains the sample size. However, for low Z and/or highly porous materials, the maximum sample size is typically not limited by absorption but rather by the field of view (FoV) of the X-ray optics. For these materials, Zernike phase contrast imaging can be used to enhance material (and pore) contrast. Here, we present a method to enlarge the investigated sample size by stitching several adjacent tilt series, following the concept proposed in [2].

The extended tomography approach combines multiple tilt series of overlapping sample areas (see Figure 1a). The imaging parameters for tilt series acquisition are optimized with respect to the enlarged investigated volume by using an increased number of projections and extended acquisition time. An important prerequisite for extended tomography is a sufficient depth of field which, for the Xradia 810 Ultra, enables at least a doubling of sample size without considerable loss of resolution. Focusing on the highest possible resolution, in HR mode the depth of field results in 22 μm. This leads to a spatial resolution down to 60 nm, considering a sample with the size of (32 μm)³ (two times the FoV). Utilizing the stage position, temperature shift tracking and motion compensation [3] of the microscope, and by further performing feature-based image correlation in Python, the single images are converted into a stitched tilt series (see Figure 1c). Reconstructing the stitched-HR tilt series using an in-house Python script based on the Astra-toolbox [4] employing the simultaneous iterative reconstruction technique (SIRT) enables us to obtain improved stitched-HR reconstructions in comparison to single LFOV or HR reconstructions. FSC is utilized to confirm the spatial resolution of the HR mode being maintained in the extended HR reconstruction. In the next step, features of interest in the samples are segmented and analyzed according to their size, position, and spatial distribution (see Figure 2b). Depending on the sample system, the quality and reliability of the segmentation are improved by employing machine learning and virtual reality analysis.

The two investigated exemplary samples to demonstrate the stitching workflow are Supported Catalytically Active Liquid Metal Solutions (SCALMS) and macroporous MFI-type zeolite particles. In the case of SCALMS (see Figure 1c), we utilized the extended tomography approach to analyze the distribution of metal particles in a macroporous glass network and select features by site-specific focused ion beam milling for closer analysis using TEM [5]. Regarding the zeolite sample, a single macroporous zeolite particle of a few μm in size was investigated in a correlative way by ET and Nano-CT, enabling an improved, ET-informed segmentation of the pore structure in the HR Nano-CT datasets [1]. Based on this, the analysis was expanded to a cluster of several hundred particles using the extended tomography approach (see Figure 1b).

The extended tomography approach demonstrates that the accessible sample size can significantly be increased beyond the standard FoV without compromising spatial resolution. The 3D resolution of stitched volumes in HR mode is measured and compared utilizing FSC (see Figure 2c) [6]. The comparison of the stitched volumes to single representative reconstructions in HR and LFOV mode was accomplished regarding four aspects: image quality, segmentation results, 3D resolution of FSC, and the achievable theoretical resolution. Matching virtual slices through several dozen particles in the stitched-HR and LFOV reconstructions display the improvement of the image quality (Figure 2a). In this regard, we analyze the effect of the improved resolution on the segmentation quality for larger samples that exceed the original HR FoV of $16\ \mu\text{m}$. The 3D resolutions resulting from the FSC at 1-bit and $\frac{1}{2}$ -bit criteria of two independent stitched volumes (one above the other with an overlapping region) being compared to the 3D resolution of single-HR mode (equal resolution) and LFOV mode (similar reconstructed FoV) volumes (see Figure 2c). Finally, we determine the limits according to the depth of field and Crowther criterion of the possible total size and number of projections needed to achieve the highest possible resolution in the extended tomography datasets.

In conclusion, we demonstrate a versatile stitching tomography workflow for laboratory Nano-CT. This method enables the 3D investigation of samples with a size of tens of up to potentially a few hundred micrometers without compromising spatial resolution. Stitched Nano-CT thereby creates the possibility to assess improved statistics regarding essential material properties and features, e.g., particle and pore size distribution, in a 3D reconstruction. [7]

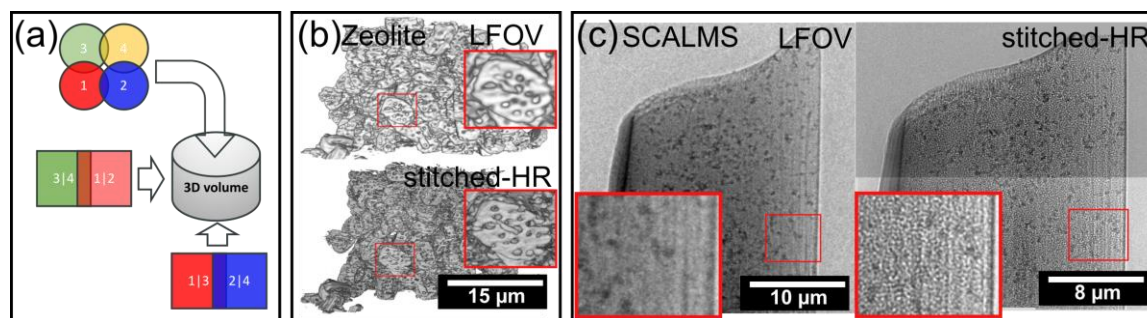


Figure 1. Stitched Nano-CT: (a) Acquisition scheme of four tilt series with about 10 % area overlap. The tilt series are stitched before reconstruction resulting in a 3D volume without gaps, which would occur if stitching would be performed after the 3D reconstruction of single tilt series. (b) Qualitative comparison of 3D volume renderings of LFOV and stitched-HR reconstructions of the same volume of a macroporous zeolite particle agglomerate. (c) Phase contrast Nano-CT projections of (left) a single LFOV tilt series and of (right) two stitched-HR tilt series of the same area of interest in a SCALMS sample. For each of the two stitched-HR tilt series, four overlapping tilt series (blue areas) according to (a) were acquired. In both modes, LFOV and stitched-HR, the Pd/Ga particles are revealed with darker contrast in the porous glass

network. However, since the pore size is in the range of 150 nm, the LFOV reconstruction reaches its limits regarding resolution, whereas the stitched-HR volume clearly resolves the pore network of the pillar with $\sim 30 \mu\text{m}$ diameter.

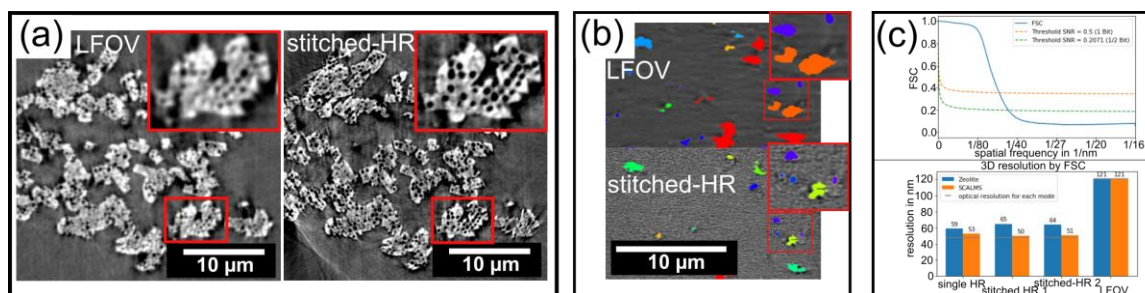


Figure 2. Comparison of stitched-HR to LFOV Nano-CT: (a) Matching virtual slices through the same area in LFOV and stitched-HR reconstructions of the macroporous zeolite particle agglomerate of Fig. 1b: the particles and the pore network appear more precise, sharper and with improved contrast. (b) Segmented Pd/Ga particles in the glass network of the SCALMS sample in Fig. 1c after greyscale threshold segmentation by machine learning in Arivis Vision4D: both segmentations detect the same total number of particles, but the stitched-HR volume shows a more detailed segmentation of the individual particles. (c) (top) Exemplary 3D resolution analysis by FSC for the stitched-HR volume: the intersection of the FSC function and the signal-to-noise curve (SNR) of 0.5 (so-called 1-bit criterion) determines the 3D resolution of 51 nm. Whereas the $\frac{1}{2}$ -bit criterion for a lower SNR shows a slightly better resolution of 45 nm. (bottom) The bar plot compares the spatial resolutions according to the 1-bit criterion determined for the single-HR, LFOV and the two independent stitched-HR reconstructions, respectively. The optical resolutions were measured in 2D at a star pattern. The stitched volumes exhibit about two times better resolution than the LFOV reconstruction of the same sample and the same resolution compared to a single-HR reconstruction.

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