Electron Tomography in the SEM via scanning-transmission imaging for biological and physical sciences

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The implementation of electron tomography in the SEM, operated in the scanning-transmission imaging mode (T-SEM), is reported and demonstrated for the investigation of nanosized heterostructures such as graphene-ZnO nanoparticles as well as collagen fibrils in biological tissue. According to the complete workflow developed in TEM tomography, a specific Si-based detection system for transmitted electrons was fabricated and integrated with a sample rotating holder with the purpose to record a series of projection images at different tilt angles. The adaptation of the imaging strategy (beam energy, geometry, and combinations of sectors in the detector) fulfills the basic requirement of a monotonic variation of image contrast upon increase of the projected thickness and atomic number in the specimen. This allowed to retrieve the three-dimensional spatial arrangement of the constituents by calculation of the tomogram and to perform an iterative refining of the reconstruction by exploiting the *a-priori* knowledge according to a compressed sensing (CS) approach [1]. The performance of the detector allowed one to record the image series at suitable speed and limited dose, making the technique feasible and offering an effective complement to the slice-and-view methods presently implemented in the SEM platform.
We report on the reconstruction of graphene-ZnO nanorods hybrid structure. Such a sample is not suitable for a conventional serial sectioning approach, while is readily transparent to the electron beam for the T-SEM imaging. The projection images have been recorded in dark-field (DF) imaging mode (Fig. 1, left), attaining at 27 keV beam energy the highest T-SEM signal and visibility for the details [2]. The 3D reconstruction of the sample, clearly presenting the arrangement of ZnO nanorods (Fig. 1, right), has been achieved from 50 projections obtained through single-axis rotation with 2° steps, between -50° and +50°.

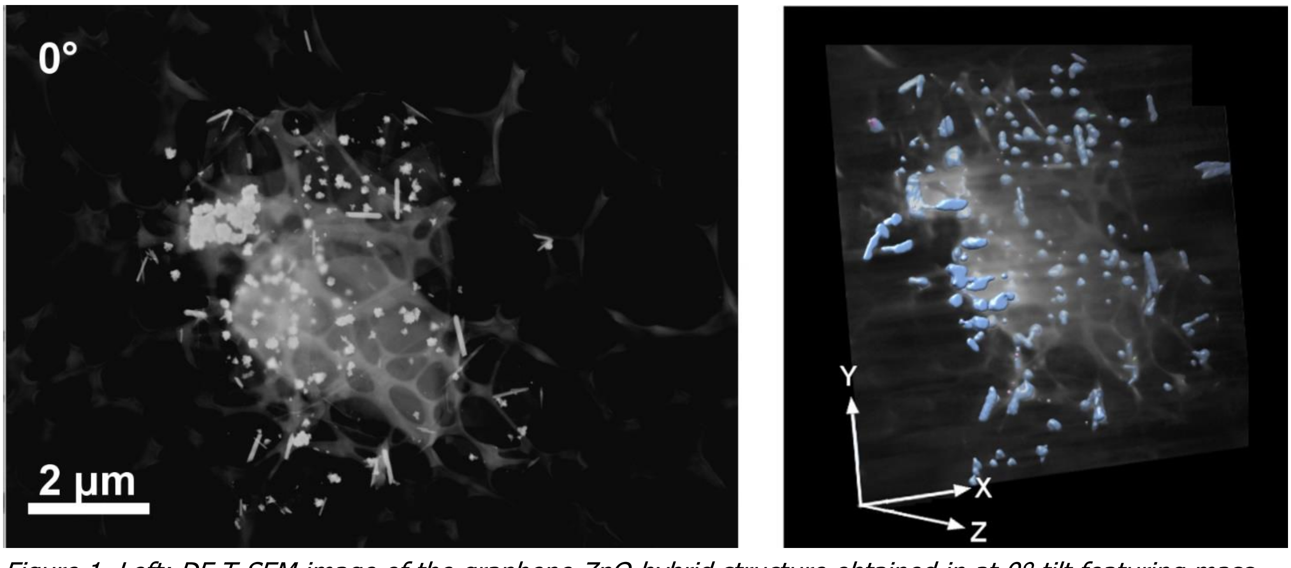

Figure 1. Left: DF T-SEM image of the graphene-ZnO hybrid structure obtained in at 0° tilt featuring mass-thickness contrast. Right: 3D reconstruction of the graphene-ZnO hybrid structure from the tilt series of T-SEM projections (light blue structures are ZnO nanostructures).

The set up has been challenged for the investigation of a biological specimen of collagen fibrils bundled in an embedding matrix, prone to a potential damage due to the electron beam. Here, the compressed sensing approach used for the reconstruction was proven to be effective in preserving the quality of the reconstruction in terms of resolution and artifact. Furthermore, the CS-refined tomogram of the collagen sample outperforms the conventional algorithms and maintains a resolution in the nanometric range, even if the number of projections is reduced from 91 to 31 (Fig. 2). In this case, electron tomography in SEM is demonstrated to be able to investigate up to 1 μm^3 volumes at nanometric resolution.

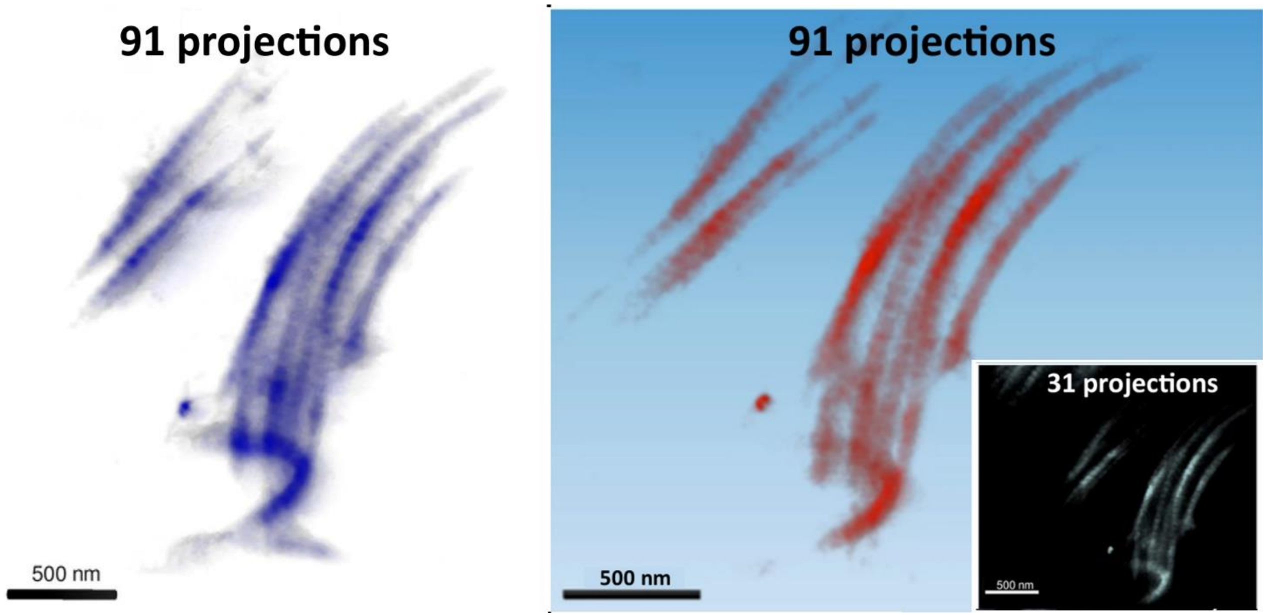

Figure 2. Comparison between conventional Algebraic Iterative reconstruction (left) and CS-refined tomogram of the collagen sample. In both cases, 91 projections have been used for the reconstruction. Inset: CS-refined tomogram obtained with 31 projection images, where the fibrils are still clearly reconstructed. (Adapted from ref. [2]).

In addition to a reduction of the number of projection images, the algorithmic approach supports the definition of the optimal acquisition strategy, in particular alternative projection schemes have been exploited thanks to the flexibility of the SEM platform. Departing from the single axis tilting scheme, a conical-tilt scheme was considered. In this way, the Fourier space percentage covered is higher than in single-axis tilt geometry and the signal on the detector could be more rapidly optimized.

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Reference List:

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