

# Scanning Transmission Electron Microscopy Through-Focal Tilt-Series on Biological Specimens

Trepout S<sup>1,2,3</sup>, Messaoudi C<sup>1,2,3</sup>, Perrot S<sup>4,5</sup>, Bastin P<sup>4,5</sup>, Marco S<sup>1,2,3</sup>

<sup>1</sup>Institut Curie, Centre de Recherche, 26 rue d'Ulm, 75248 Paris Cedex 05, France

<sup>2</sup>INSERM U1196, Bât. 112, Centre Universitaire, 91405 Orsay Cedex, France

<sup>3</sup>CNRS UMR9187, Bât. 112, Centre Universitaire, 91405 Orsay Cedex, France

<sup>4</sup> Institut Pasteur, Trypanosome Cell Biology Unit, 25 rue du Docteur Roux, 75015 Paris, France

<sup>5</sup> INSERM U1201, Institut Pasteur, 25 rue du Docteur Roux, 75015 Paris, France

Since scanning electron beam can produce high signal-to-noise ratio bright field images of thick specimens, scanning transmission electron microscopy is emerging as the method of choice to study thick ( $\geq 500$  nm) biological samples by tomographic approaches. However, in a convergent beam configuration, the depth-of-field can be rather limited, only a thin portion of the specimen (from a few nanometres to tens of nanometres depending on the convergence angle) is imaged at focus. A way of solving this difficulty is take advantage of raster scanning to get focused images line by line by dynamic focus [1]. However, in our experience, the recovery of full-focused images by dynamic focus, useful for tomographic reconstruction of cells, is impaired at high tilt angles ( $>45^\circ$ ) in very thick samples ( $>0.5$   $\mu\text{m}$  thickness). To circumvent this limitation we have developed an acquisition scheme and an image processing method in which we reconstruct full-focused images from STEM images recorded at different defocus. The method was applied to compute the 3D reconstruction of the base of the flagellum in the protist *Trypanosoma brucei brucei*. Our results demonstrate the improvement in the accuracy of the observed details as supported by the analysis of qualitative (visual) and quantitative [2] image descriptors (entropy, RMS contrast and Michelson's contrast).

## References:

1. Feng J, Somlyo AP, Somlyo AV, Shao Z. Automated electron tomography with scanning transmission electron microscopy. *J Microsc.* 2007 228:406-12.
2. Peli, E., 1990. Contrast in complex images. *Journal of the Optical Society of America. A, Optics and image science* 7, 2032-2040.

## Acknowledgements:

This work was funded by an ANR grant (ANR-11-BSV8-016). We also acknowledge the PICT-IBiSA for providing access to chemical imaging equipment.