Simple Phase Contrast Imaging (SPCI): An ImageJ plug-in for phase contrast.

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Identification of nanometric sized objects is of paramount importance in many scientific and industrial applications. Electron transmission microscopy permits efficient analysis at this small scale. Indeed, very relevant information for nano-objects is extracted using phase contrast imaging. Phase contrast imaging, achieved by attaching phase-plate hardware to the microscope, provides an efficient tool to quantitatively study nano-particles. However, in TEM, other methods, which sometimes require additional hardware are able to retrieve phase images [1]. In recent developments sophisticated algorithms have been proposed to provide quantitative phase images at a very high resolution [2]. We propose in this study an alternative approach relying on a simple processing of focal series. This simplified method is based on [3] and needs the acquisition of two defocused images. The numerical treatment involves few parameters: most of them describe the microscope configuration and only one parameter that can be tuned to enhance the contrast of the phase image. As a consequence, the method is advantageous to improve contrast and automatic detection of nano-structures with weak contrast. However, the main drawback with this method is the loss of quantitative information in the resulting phase image.

A computer program has been developed in the form of a plug-in for the popular ImageJ scientific image processing software. The plug-in interest is to propose a user friendly interface with a minimum set of parameters (only one parameter is adjustable, the others are describing the microscope configuration). This plug-in can be used as a convenient segmentation treatment that helps the user identify their elements of interest.

Unlike other existing approaches based on focal-series with many terms, our method provides phase images of limited resolution, typically 1 nm for a 100 nm defocus. Though limited, such resolution can be sufficient for many applications. Indeed, retrieving phase images from as few as two defocused images allows reducing the electron dose and therefore preserves the sample. When added to the contrast enhancement through the numerical post treatment achieved by the SPCI plug-in, the method may be of specific interest for studying biological samples. Additionally, several examples of applications including material science nano-structures and life science examples are presented (figure).

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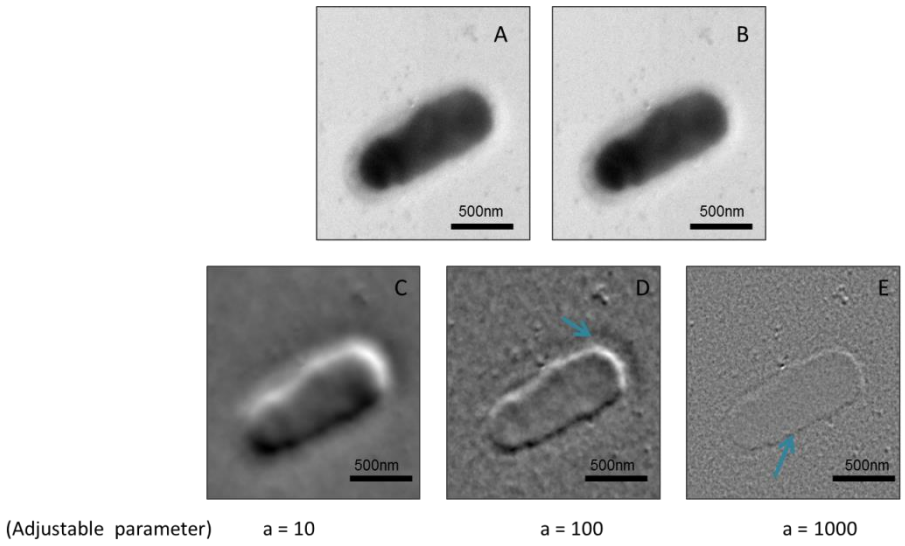

Figure : The images A and B show two images defocused at respectively +2496nm and -2496nm of the bacteria *Escherichia coli*. The sample is absorbed and dehydrated on grids without contrast agent. The images C D and E are the result of the application of SPCI plug-in for several values of the adjustable parameter. The image at a=100 shows the bursting of the bacteria and the image at a=1000 shows the demarcation of the bacteria.