Improvement of Visibility of Diffraction Rings for Sorting Micrographs and Their Local Areas

S. Jonić[1], C. O. S. Sorzano[2,3], M. Cottevieille[1], E. Larquet[1], and N. Boisset[1]

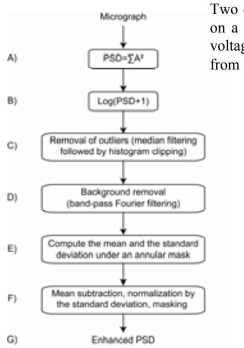
[1]Institut de Minéralogie et de Physique des Milieux Condensés, Université P et M Curie, UMR 7590, CNRS, 140 rue de Lourmel, 75015 Paris, France
[2]Escuela Politécnica Superior, Univ. San Pablo – CEU, Campus Urb. Montepríncipe s/n, 28668 Boadilla del Monte, Madrid, Spain
[3]Biocomputing Unit – National Center of Biotechnology (CSIC), Campus Univ. Autónoma s/n, 28049 Cantoblanco, Madrid, Spain

slavica.jonic@impmc.jussieu.fr, coss.eps@ceu.es, nicolas.boisset@impmc.jussieu.fr

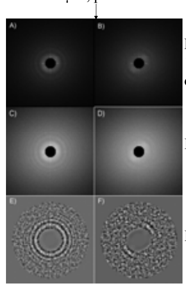
Abstract

In a context of automation of cryoelectron microscope observation, and of automation of image processing, the strategies for processing single particles and macromolecular assemblies are evolving toward iterative processing (3D alignment and CTF correction) of very large image data sets. However, quantity is not always related to quality, and some fast and efficient sorting must be applied to prevent the analysis of images that do not improve the global signal-to-noise ratio (for example, non-diffracting micrographs or their areas) or of those that introduce errors in the global 3D map (for example, drifted micrographs or charged areas). Also, images coming from very astigmatic micrographs/areas should be rejected when using algorithms that do not correct the astigmatism. In the present work, we developed a novel method for improving the visibility of diffraction rings of cryo-electron micrographs of vitreous ice (without carbon film or high concentration of diffracting material). We used these enhanced power spectra to automatically detect and remove the mentioned imperfect micrographs and/or local areas. In an experimental test case, we successfully sorted 142 out of 151 cryo-electron micrographs and 828 out of 866 local areas using the normalized cross-correlation between the enhanced power spectra and their copies rotated by 90°. This simple sorting scheme owes its success mainly to the novel pre-processing of power spectra, which simplifies also interpretation of the sorting results. We show that our algorithm can improve the visibility of diffraction rings of images of pure water and that this visibility depends on ice thickness. This algorithm is implemented in the Xmipp (open-source image processing package) and is freely available for implementation in any other software package.

1. Novel algorithm for enhancement of diffraction rings



Two out of 151 Glutamate Synthase (GltS) micrographs recorded on a JEOL JEM 2100F (ultra high-resolution pole piece, no tilt, voltage: 200 kV, Cs: 0.5 mm, magnification: X50000, defocus: from -1.7 µm to -3.2 µm, pixel size: 1.59 Å × 1.59 Å)



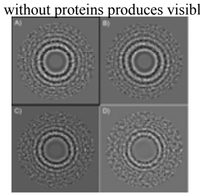
PSD=∑A² (averaging of spectra of about 1000 sub-areas of size 512 × 512 pixels overlapping by 50%)

Log(PSD+1)

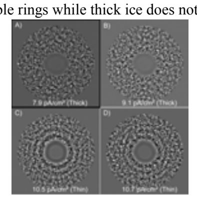
Enhanced PSD

2. Enhancement of diffraction rings in case of a low concentration of diffracting material

Hepes/KOH 25 mM, pH 7.5 buffer with EDTA 1 mM, DTT 1 mM → The buffer solution alone without proteins produces visible rings



Pure Micropore® water → Thin ice produces visible rings while thick ice does not



Remark: Water and buffer micrographs were recorded in similar cryo-EM conditions as with the GltS

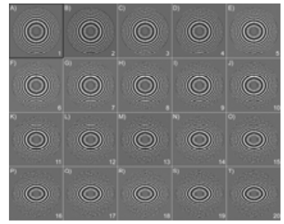
3. Normalized cross-correlation (NCC) for sorting based on anisotropy

$$\mathrm{NCC} = \max_{\mathbf{j}} \frac{\sum_{\mathbf{k}} \left(f_{\mathbf{k}} - \bar{f}\right)\left(g_{\mathbf{k}-\mathbf{j}} - \bar{g}\right)}{\sqrt{\sum_{\mathbf{k}} \left(f_{\mathbf{k}} - \bar{f}\right)^2} \sqrt{\sum_{\mathbf{k}} \left(g_{\mathbf{k}} - \bar{g}\right)^2}}$$

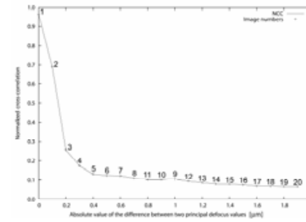
$f_{\mathbf{k}}, g_{\mathbf{k}}$: Sample of the enhanced PSD and of its 90°-rotated copy at the pixel coordinate **k,** respectively

$\bar{f}, \bar{g}$: Mean value of the enhanced PSD and of its 90°-rotated copy, respectively

4. Objective evaluation of the NCC measure



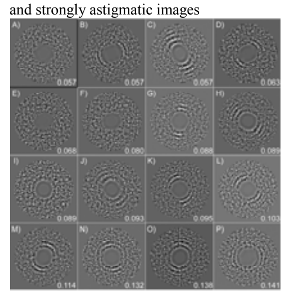
Enhanced PSDs of twenty synthesized images with simulated astigmatism (defocus varying along y-axis from -2.5 µm to -4.4 µm with a step of -1.0 µm**,** defocus along x-axis: -2.5 µm, angle of astigmatism: 0°, voltage: 200 kV, Cs: 0.5 mm, pixel size: 1.59 Å × 1.59 Å



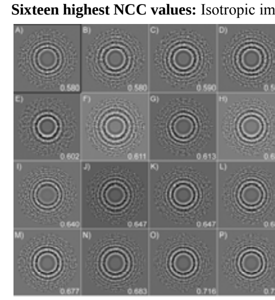
Results of sorting of images corresponding to the enhanced PSDs shown on the left

5. Sorting of 151 entire micrographs of the GltS

Sixteen lowest NCC values: Drifted, non-diffracting, and strongly astigmatic images

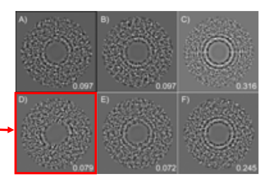


Sixteen highest NCC values: Isotropic images



6. Coexistence of isotropic and anisotropic local areas in a micrograph

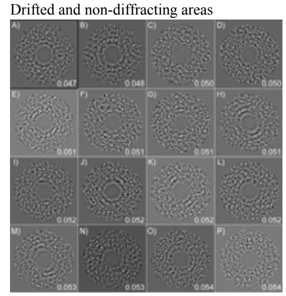
Charging effects



Enhanced PSDs of six local areas of size 3840 × 3840 pixels in a GltS micrograph. Each PSD was obtained by averaging the spectra of 225 sub-areas of size 512 × 512 pixels overlapping by 50%.

7. Sorting of 866 local areas collected from all GltS micrographs

Sixteen lowest NCC values:
Drifted and non-diffracting areas



Sixteen highest NCC values: Isotropic areas