

# A hybrid method coupling normal mode analysis with projection matching to study structural flexibility of macromolecular assemblies

S. Jonić<sup>1</sup>, C. O. S. Sorzano<sup>2</sup>, F. Tama<sup>3</sup>, E. Larquet<sup>1</sup>, and N. Boisset<sup>1</sup>

<sup>1</sup>IMPMC-UMR 7590, CNRS, Universités Paris 6 et Paris 7, IPGP, 140 rue de Lourmel, 75015 Paris, France

<sup>2</sup>Centro Nacional de Biotecnología - CSIC Campus Univ. Autónoma s/n, 28049 Cantoblanco, Madrid, Spain

<sup>3</sup>University of Arizona, 1041 East Lowell Street - BSW 448, 85721 Tucson, Arizona, AZ, USA

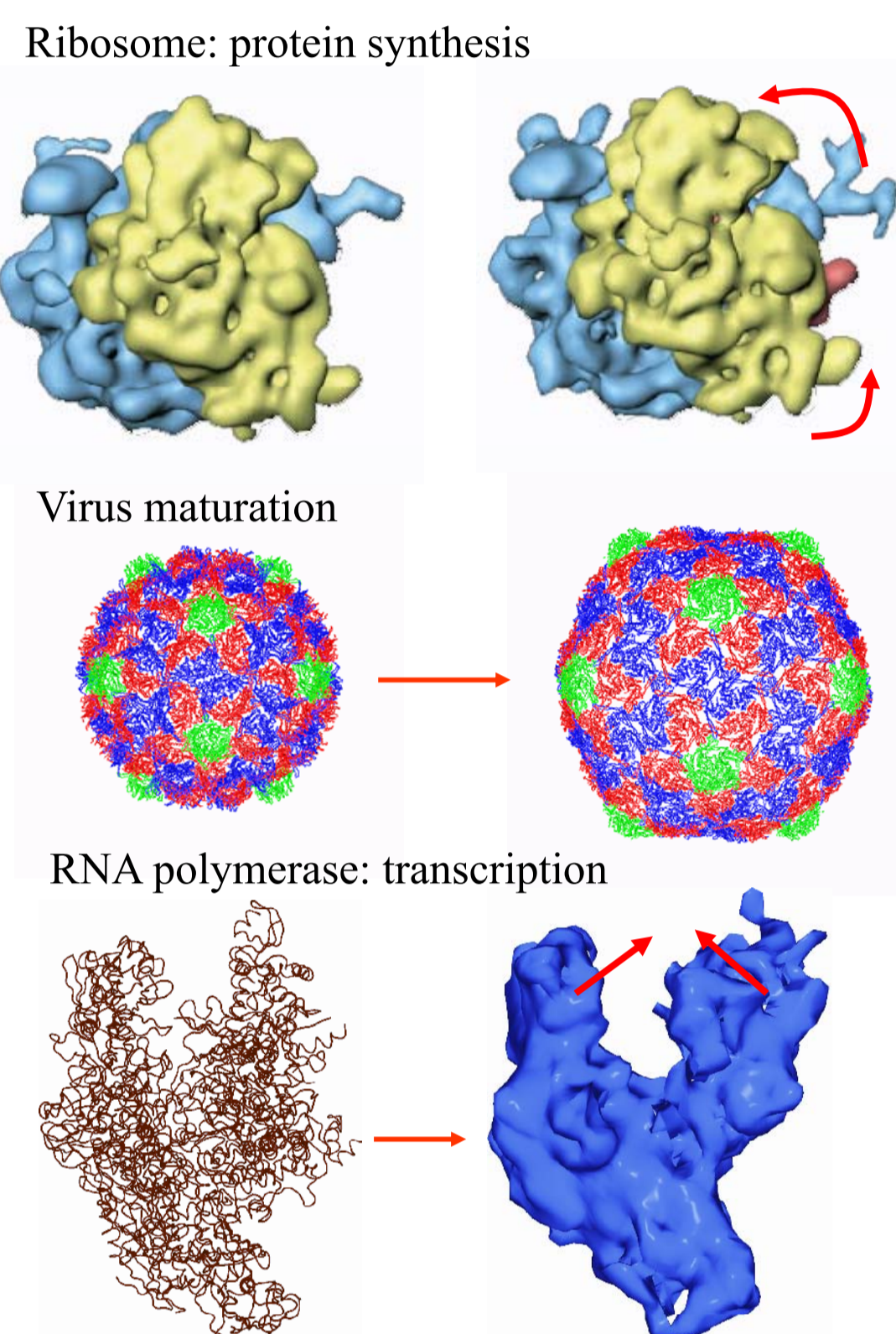
Correspondence: Slavica.Jonic@impmc.jussieu.fr

## Abstract

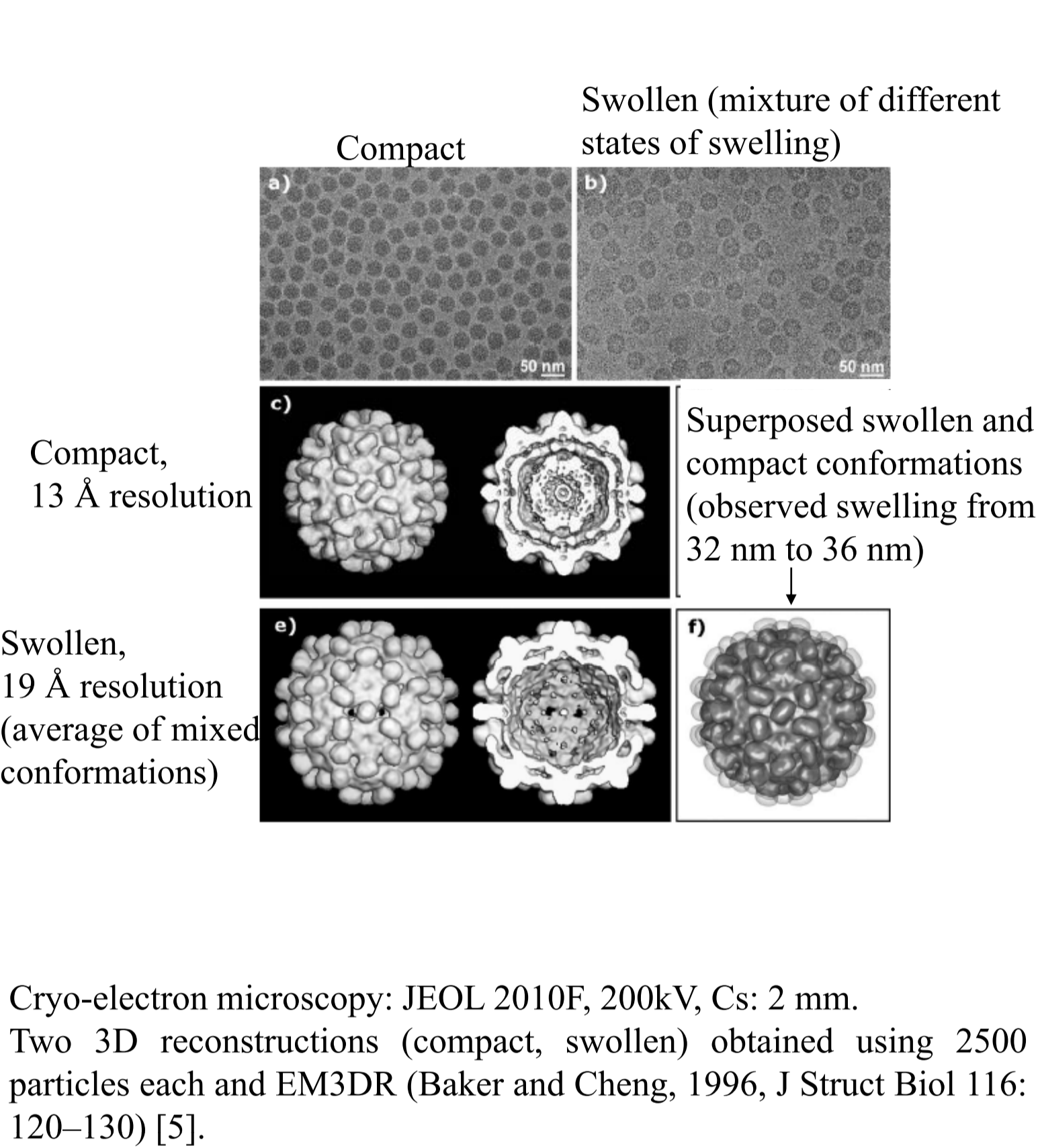
A hybrid approach that integrates Normal Mode Analysis (NMA) into a rigid-body, projection matching method has been developed to study conformational changes in macromolecular assemblies (large-scale structural rearrangements) [1,2,3,4]. The novelty of this approach is that it allows exploring all relevant intermediate conformations contrary to the existing methods that explore only the extreme conformations. It applies the normal modes on three-dimensional (3D) atomic coordinates of a macromolecular complex to determine the deformation of the 3D model giving the projection that matches best with the experimental two-dimensional (2D) image. The deformation parameters and the 3D-to-2D alignment parameters are determined simultaneously. This method is currently used to study the swelling mechanism of the Tomato Bushy Stunt Virus (TBSV) [5]. In this poster, we describe the NMA-based 3D-to-2D alignment method and show the results of its tests using synthetic data as well as preliminary results on experimental, cryo-electron microscopy data of the TBSV at different stages of swelling. In the future work, this method will be tested on dynamical studies of other macromolecular complexes.

## 1. Type of structural flexibility to study: Large-scale conformational changes

Examples:



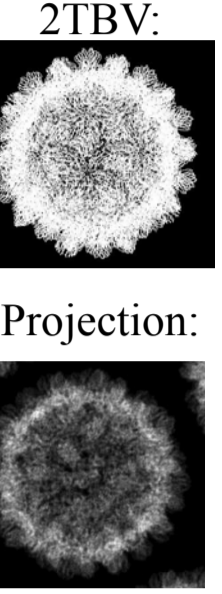
## 2. Swelling of Tomato Bushy Stunt Virus (TBSV) – Recalling the TBSV study from [5]



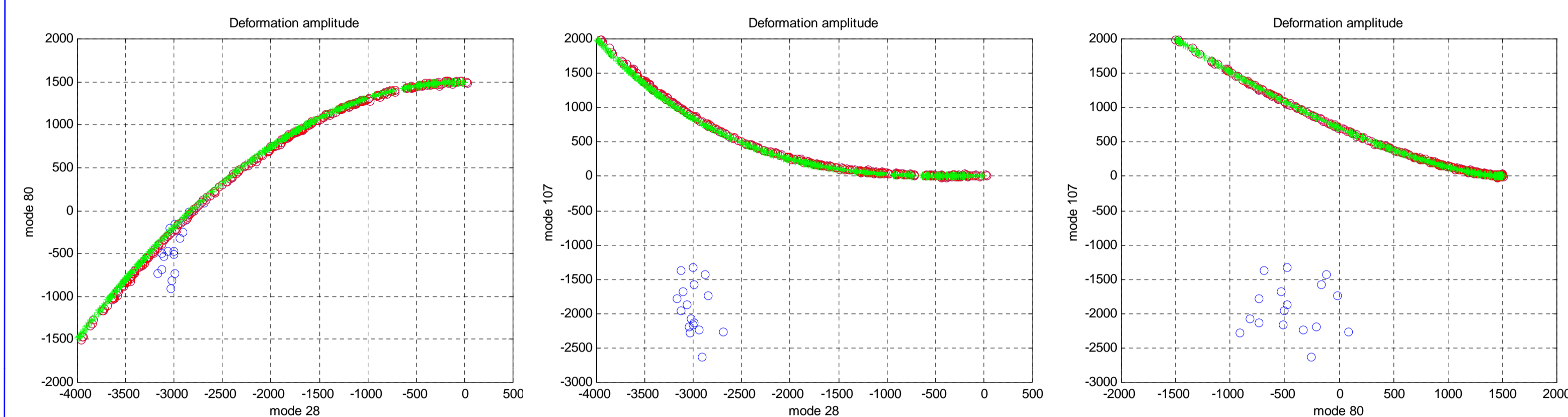
## 4. Tests with synthetic data computed using the TBSV atomic coordinates

### Case 1: Images without noise and CTF influence

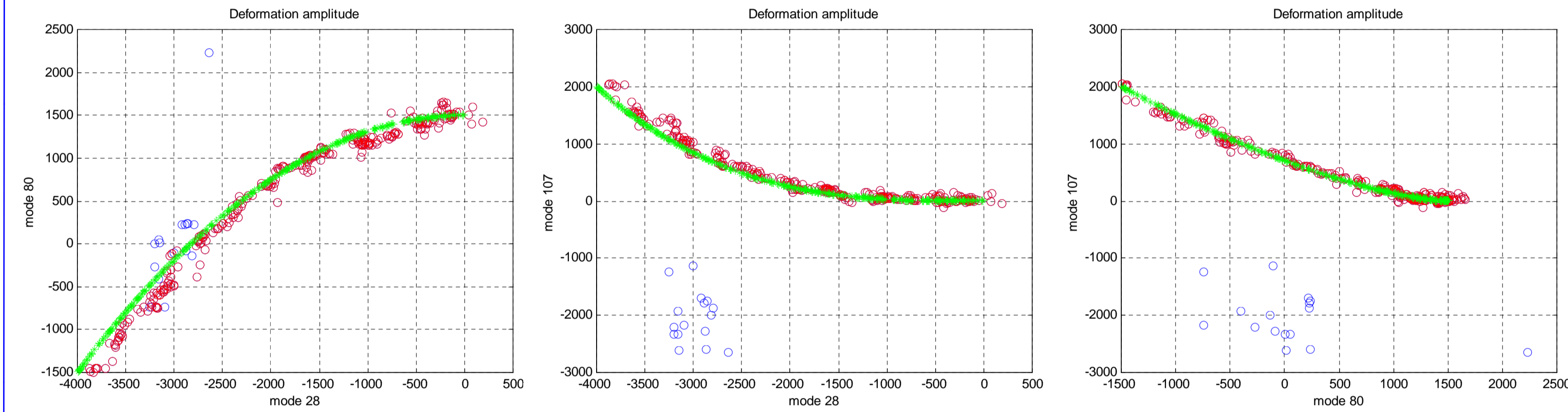
Normal mode analysis was performed on the TBSV atomic coordinates (PDB code: 2TBV) and low-frequency normal modes were computed [1,2]. The analysis indicated 3 normal modes along which deformations do not break the symmetries of the structure (modes: 28,80, and 107). The mode 28 is responsible for scaling. Deformations along the remaining modes do not preserve the symmetries. We deformed the atomic structure by applying a combined deformation along the three symmetric modes (random deformation was generated along the mode 28 while the deformation along the modes 80 and 107 follows a known (given) dependence on the mode 28). The deformed structure was converted into a volume of size 256x256x256 voxels with a sampling step of 1.6 Å. 300 projections of the volume with uniform random orientations and particle center positions were computed (distance between the particle center and the image center was between 0 and 10 pixels). Deformation amplitudes along the three modes, Euler angles and translations were estimated for each synthetic image using the NMA-based 3D-to-2D alignment method, and the estimated parameters were compared with their true values (the ground truth is in green).



With two pyramid levels (image sizes: 128 pixels and 256 pixels) and 10° angular step for computing reference images and discrete alignment. Green: True. Red and blue: Estimated. Blue: Outliers obtained by thresholding of the Mahalanobis distance.



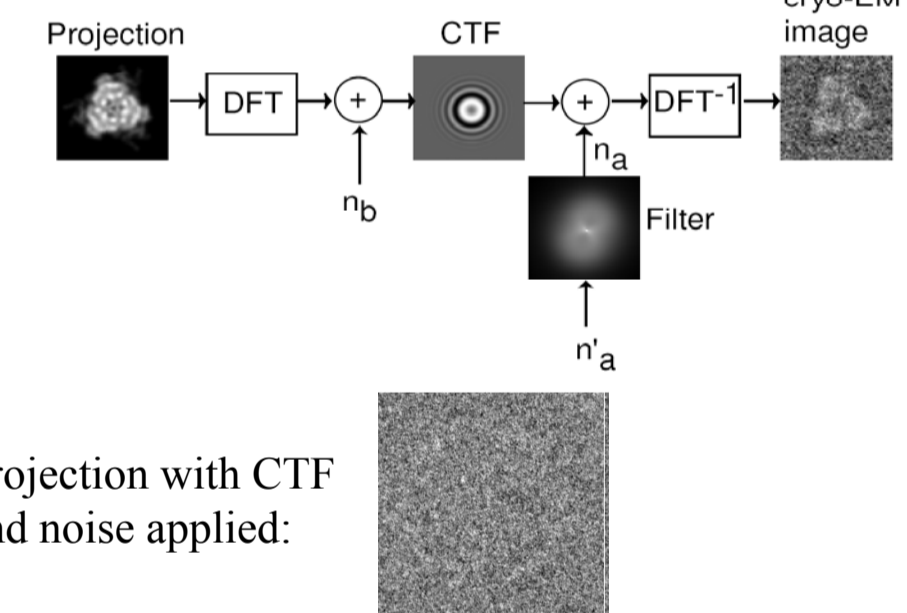
With initial image size reduction from 256 pixels to 128 pixels, two pyramid levels (image sizes: 64 pixels and 128 pixels), and 5° angular step for computing reference images and discrete alignment. Green: True. Red and blue: Estimated. Blue: Outliers obtained by thresholding of the Mahalanobis distance.



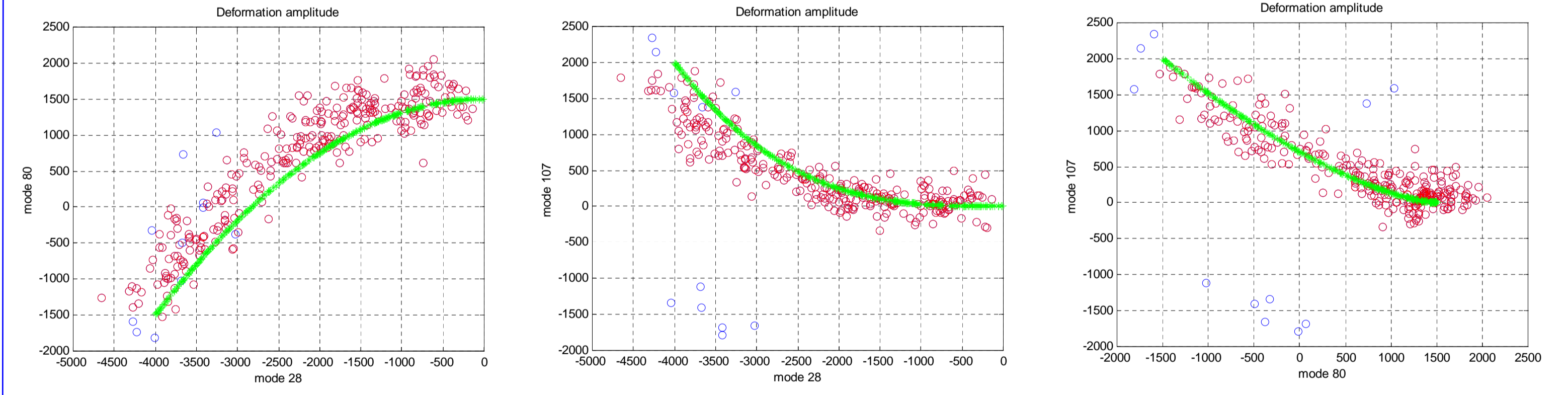
### Case 2: Images with simulated noise and CTF

The CTF and the noise were applied on the images computed for the case 1. The noise before the CTF ( $n_b$ ) was modeled by a Gaussian white noise with the standard deviation  $\sigma_n$ . The noise after the CTF ( $n_a$ ) was modeled by a low-pass filtered Gaussian noise with the standard deviation  $\sigma_a$  generated at the input  $n_a$ . The standard deviations were adjusted so that the signal-to-noise ratio was approximately equal to 0.1. The filter and the CTF were estimated simultaneously from a TBSV cryo-EM image for the following parameters of the electron microscope: accelerating voltage=200 kV, defocus=-1.76  $\mu$ m, spherical aberration=0.5 mm. The CTF, the background spectrum filter, and the synthesized cryo-EM images were of size 256 x 256 pixels.

Procedure for simulating noise and CTF (Velazquez-Muriel et al., 2003, Ultramicroscopy 96: 17–35):

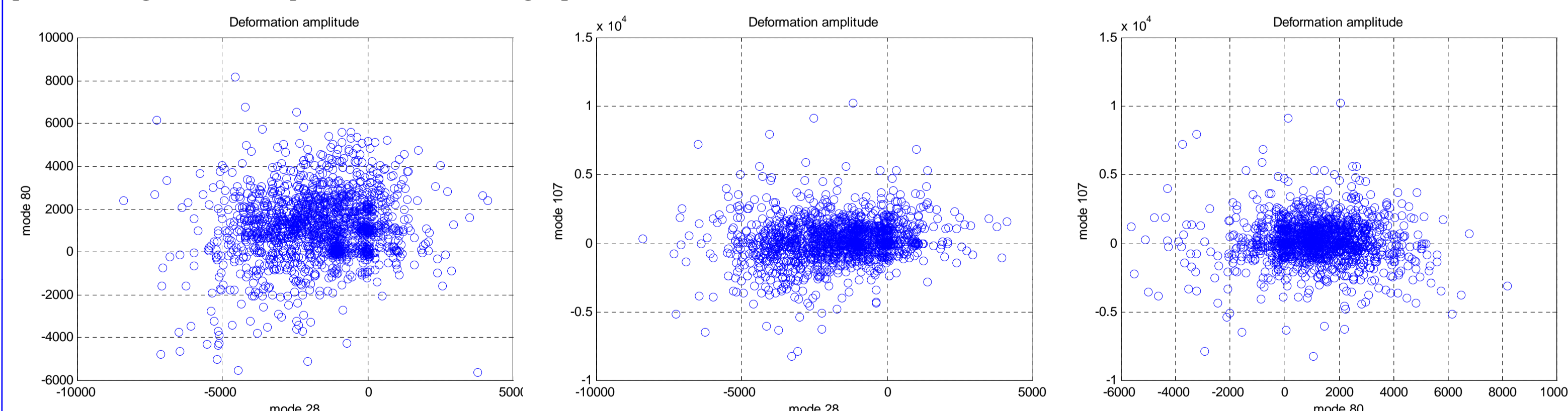


With initial image size reduction from 256 pixels to 128 pixels, two pyramid levels (image sizes: 64 pixels and 128 pixels), 5° angular step for computing reference images and discrete alignment, and phase flip for CTF correction. Green: True. Red and blue: Estimated. Blue: Outliers obtained by thresholding of the Mahalanobis distance.



## 5. Test with cryo-EM images containing the TBSV at different stages of swelling, and using three « symmetric » modes (28,80, and 107)

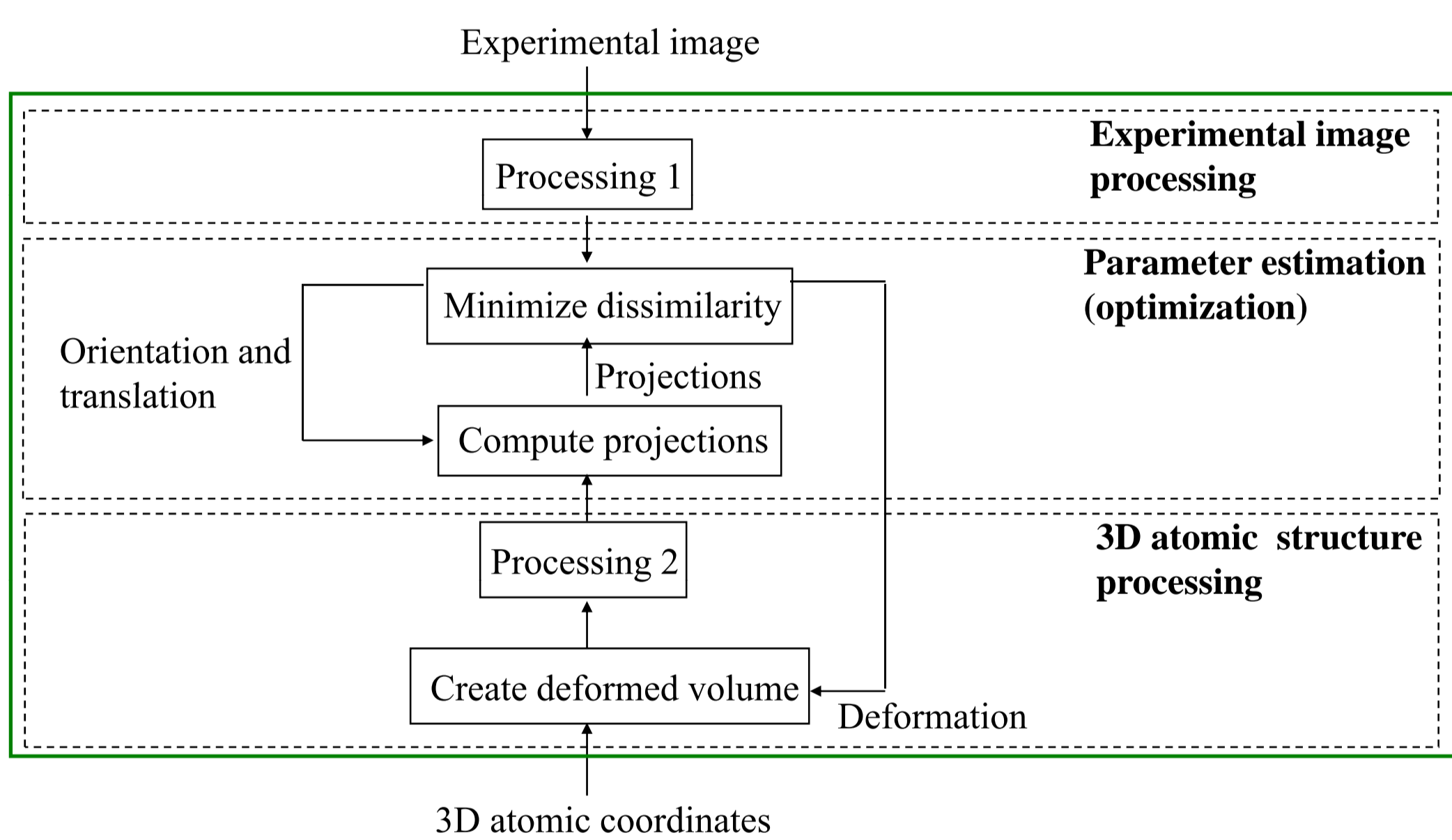
JEOL JEM 2100F (200kV, Cs=0.5 mm). Magnification: x50,000. Holey carbon film. Low-dose: 10 e<sup>-</sup>/Å<sup>2</sup>. Sampling step: 1.6 Å. Size of single particle images: 256x256 pixels. Number of single particles: 1518.



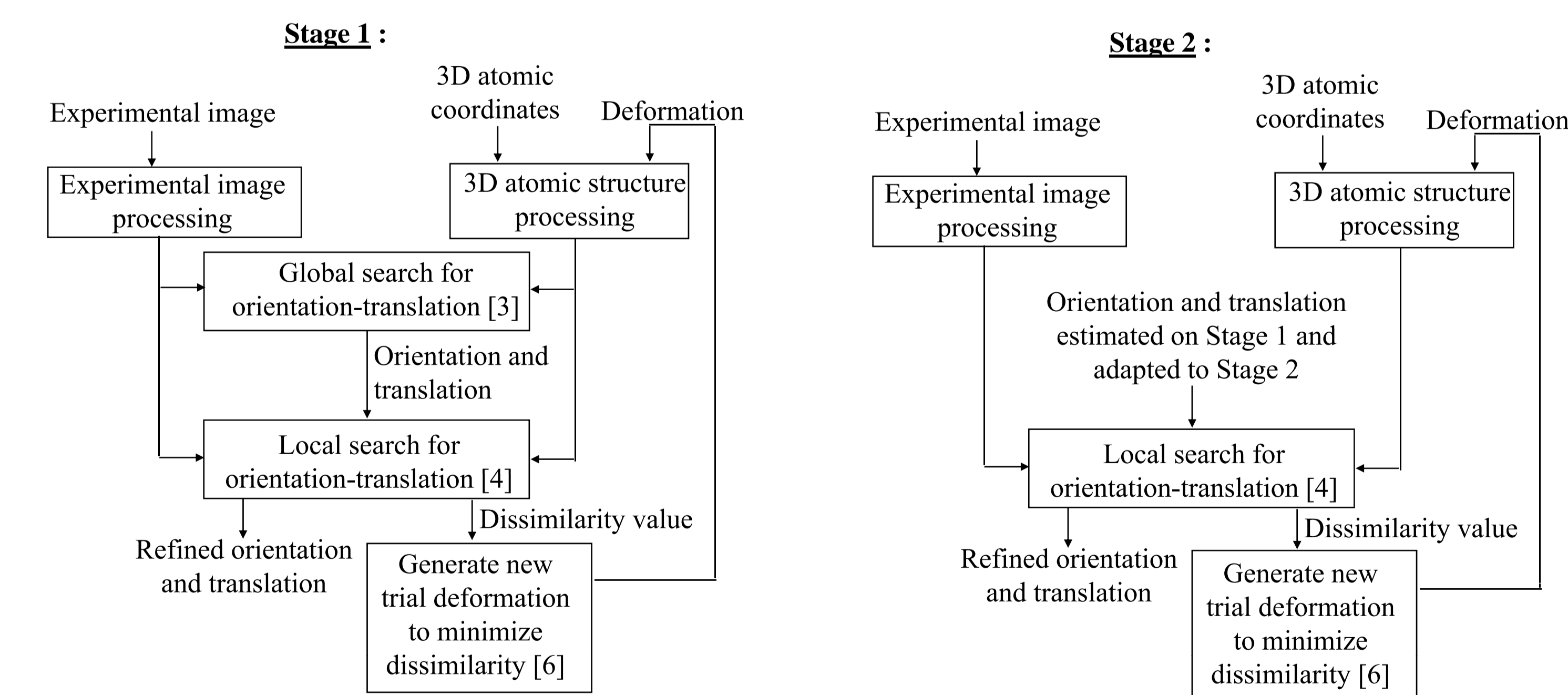
## Literature

- [1] Tama F, Miyashita O, Brooks 3<sup>rd</sup> CL (2004a). J Struct Biol.147: 315-326.
- [2] Tama F, Miyashita O, Brooks 3<sup>rd</sup> CL (2004b). J Mol Biol 337: 985-999.
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- [5] Aramayo R., C. Mérigoux C, Larquet E, Bron P, Pérez J, Dumas C, Vachette P, Boisset N (2005). Biochimica et Biophysica Acta 1724: 345-354.
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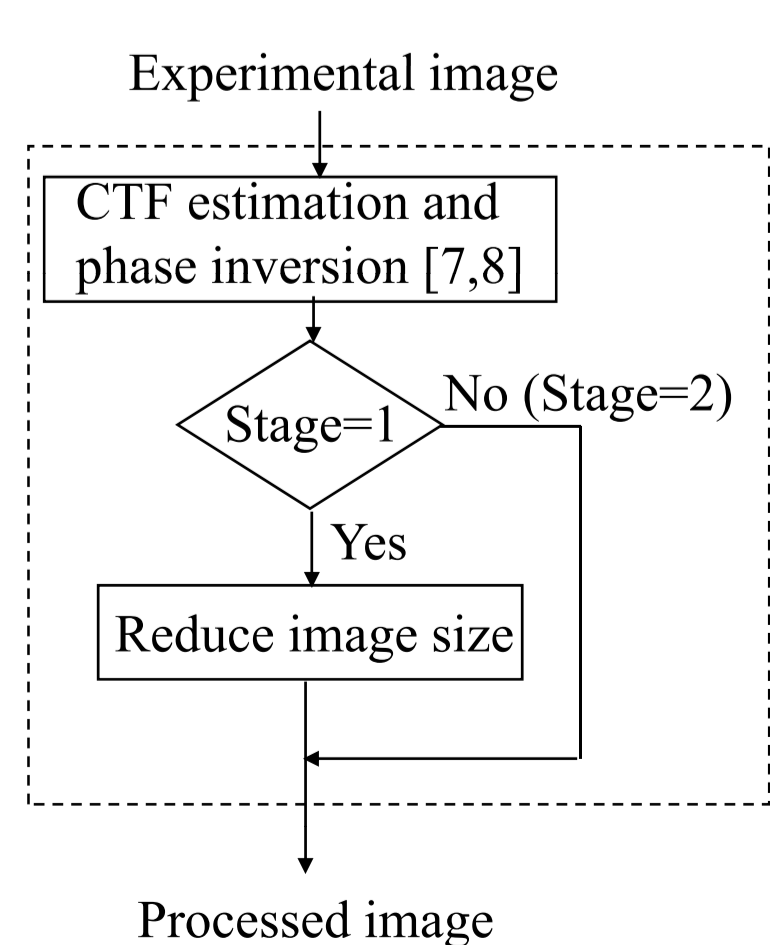
## Simplified algorithm:



## Details of the algorithm:



### Experimental image processing:



### 3D atomic structure processing:

