

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

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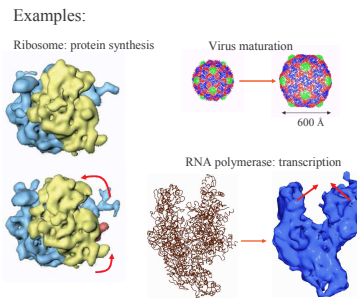
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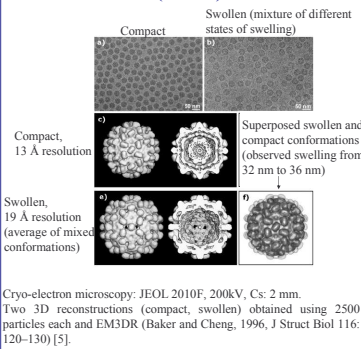
## Abstract

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

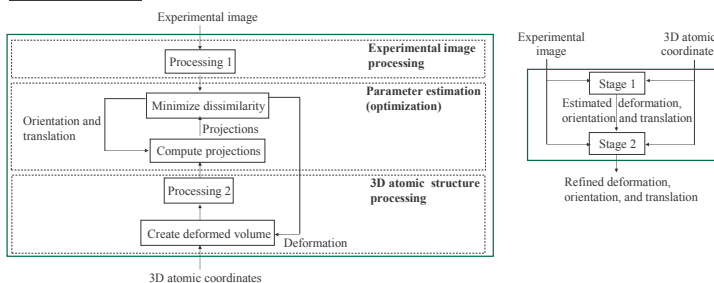


## 2. Swelling of Tomato Bushy Stunt Virus (TBSV)

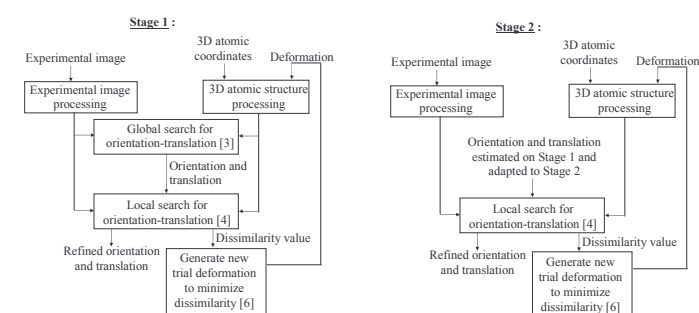


## 3. Algorithm

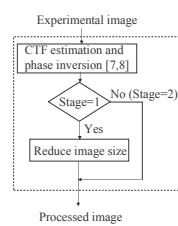
### Simplified algorithm:



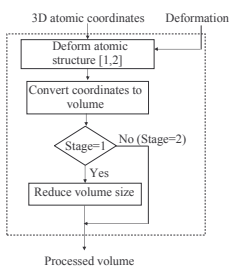
### Details of the algorithm:



### Experimental image processing :



### 3D atomic structure processing :

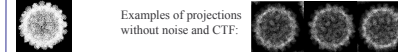


## 4. Tests with synthetic data computed using the TBSV atomic coordinates

### 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 a sequential application of three deformations with the following deformation amplitudes: -4000 along the mode 28, -1000 along the mode 80, and 1000 along the mode 107. The deformed structure was converted into a volume of size 256x256x256 voxels with a sampling step of 1.6 Å. 125 projections of the volume were computed with random orientations determined by a set of three Euler angles uniformly distributed on the asymmetric unit, and with uniformly distributed random translations (the standard deviation of noise applied on the coordinates of the particle center was 10). Deformation amplitudes along the three symmetrical modes (28, 80, and 107), 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.

PDB: 2TBV



### Results:

Global angular distance: 0.005°

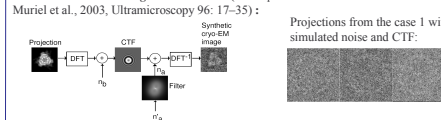
Deformation amplitude RMS difference:

34 (mode 28), 13 (mode 80), 8.8 (mode 107)

### 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  $\sigma_b$ . The noise after the CTF ( $n_c$ ) was modeled by a low-pass filtered Gaussian noise with the standard deviation  $\sigma_c$  generated at the input  $n_b$ . The standard deviations were adjusted so that the ratio between the power of the signal and the power of the total noise was approximately equal to 1/3. 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 nm. 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):



### Results:

Global angular distance: 0.4°

Deformation amplitude RMS difference:

223 (mode 28), 206 (mode 80), 154 (mode 107)

## 5. Tests with experimental cryo-EM images of the TBSV: Preliminary results

Examples of the compact-form TBSV

Examples of the swollen-form TBSV

JEOL JEM 2100F, 200kV, Cs=0.5 mm

Defocus: -3.5 to -4.6  $\mu$ m (microscope), -1.7 to -3.2  $\mu$ m (computed)

Magnification: x50,000, Holey carbon film, Low-dose: 10 e<sup>-</sup>/Å<sup>2</sup>

Image size: 256x256 pixels, Sampling step: 1.6 Å

Results of a test on a micrograph of the compact-form TBSV (measured defocus=-4.5  $\mu$ m, computed defocus=-2.3  $\mu$ m):

Deformation amplitude RMS difference: 620 (mode 28), 30 (mode 80), 65 (mode 107)

Results of a test on a micrograph of the swollen-form TBSV (measured defocus=-4.5  $\mu$ m, computed defocus=-2.5  $\mu$ m)

	Mode 28	Mode 80	Mode 107
1	104	2193	200
2	-4184	1605	360
3	-2834	-1056	-1185
4	-4531	225	-254
5	-2514	1489	234
6	-4558	-475	-716
7	-2105	1224	804

## Literature

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