National Center for Biothecnology

Biocomputing Unit

Introductory Tutorial

Scipion Team

Intended audience

This tutorial provides a general introduction to Scipion, an image processing framework to obtain 3D models of macromolecular complexes using Electron Microscopy (EM). It is designed to introduce 3D image processing in EM to people without any prior knowledge of Scipion, only limited knowledge about 3D-EM image processing, and with basic computer skills.

We'd like to hear from you

We have tested and verified the different steps described in this demo to the best of our knowledge, but since our programs are in continuous development you may find inaccuracies and errors in this text. Please, let us know about any error you find, as well as your suggestions for future editions by writing to scipion@cnb.csic.es.

Contents

1 General Introduction

1.1 Download and Install

The first step before start working on your projects is to download and install Scipion and all the related programs. At Scipion's website there is all the information needed to download and install the package.

2 Reconstruction of a viral capsid

In this demo, we have used the single particle analysis approach to obtain a 3D reconstruction of a *B*ovine Papillomavirus. The EM images have been collected at 300 kV and a calibrated magnification of 56,588, giving a pixel size of 1.237 Å(Wolf et al., 2010). Data have been kindly provided by Dr. Grigorieff's Lab.

2.1 Getting started

The data you will work on may be downloaded using the following command:

```
scipion testdata --download
```

The data will be download in *scipion_folder/data*. After download, you must launch the MAIN GUI by typing: `scipion`

Then, create a new project by clicking **Create project** button, type a *project name* and click OK. The GUI main project window will be launched. The left panel contains different protocols grouped in categories. Clicking on a group will display a menu with protocols that can be selected to launch the corresponding GUI.

2.2 Preprocessing

2.2.1 Import micrographs

The first step is to import the micrographs to your scipion project. To do this, press on **Import Micrographs** button. In *Pattern* you must indicate where your micrograph files are stored (clicking on **browse** button). The complete pattern are: (*$SCIPION_HOME/data/tests/xmipp_tutorial/micrographs/*.mrc*).

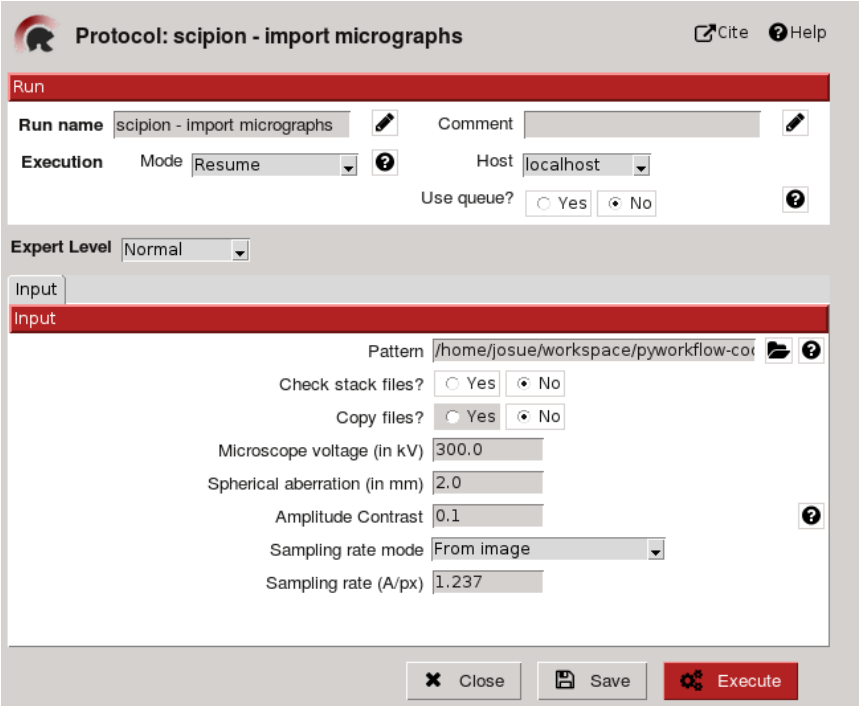

Figure 1: Import Micrographs protocol GUI.

Modify the parameters of the Import Micrographs protocol according to the ones shown in figure 1.

When you have completed the form, click on the **Save & Execute** button. After executing the protocol, it will appear in the main Scipion project GUI the new information shown in figure 2

If we press in the **Analyze results** button it will appear a new pop-up GUI that shows us the different imported micrographs (not shown)

2.2.2 Downsampling micrographs

After importing the micrographs to your Scipion project, you can perform the next processing step that consists in preprocess micrographs. This protocol uses several xmipp programs in order to perform several operations over the micrographs. In this example we use the options shown in figure 3. Please, put the values as same as

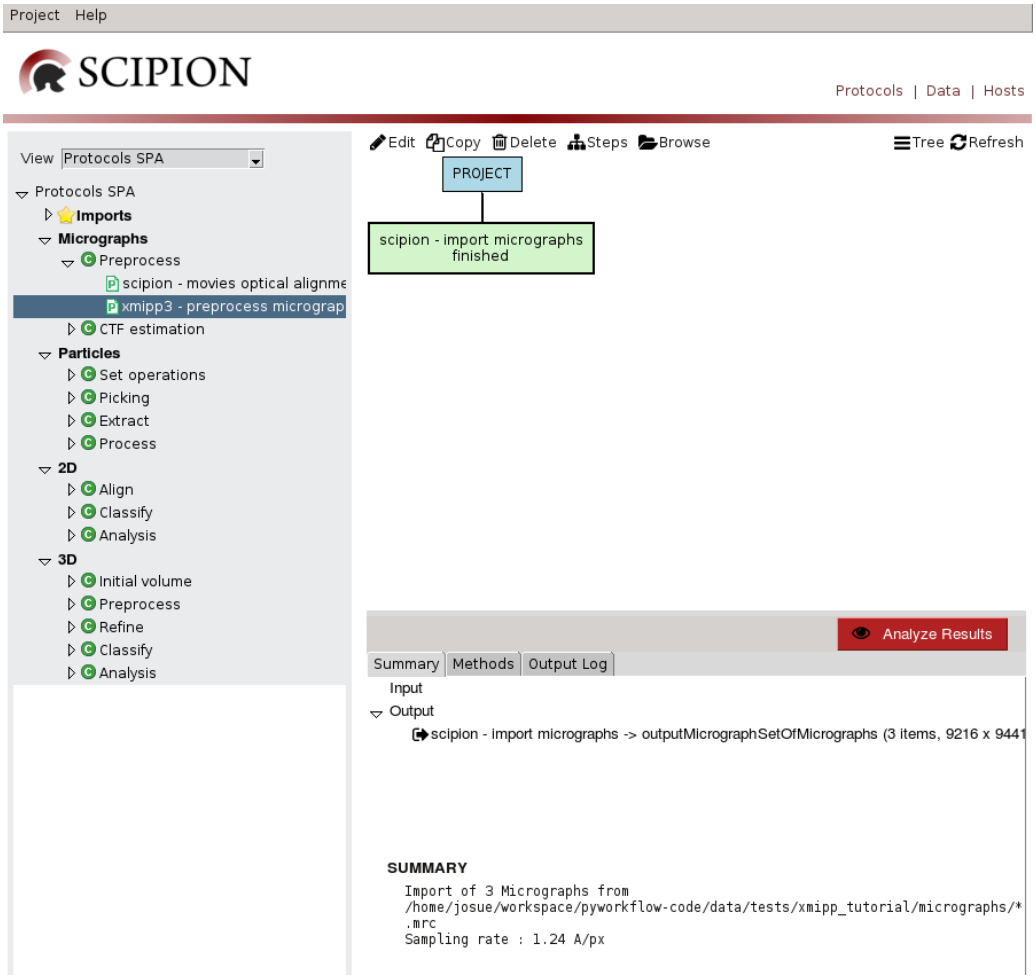

Figure 2: Scipion project GUI after processing Import Micrograph protocol.

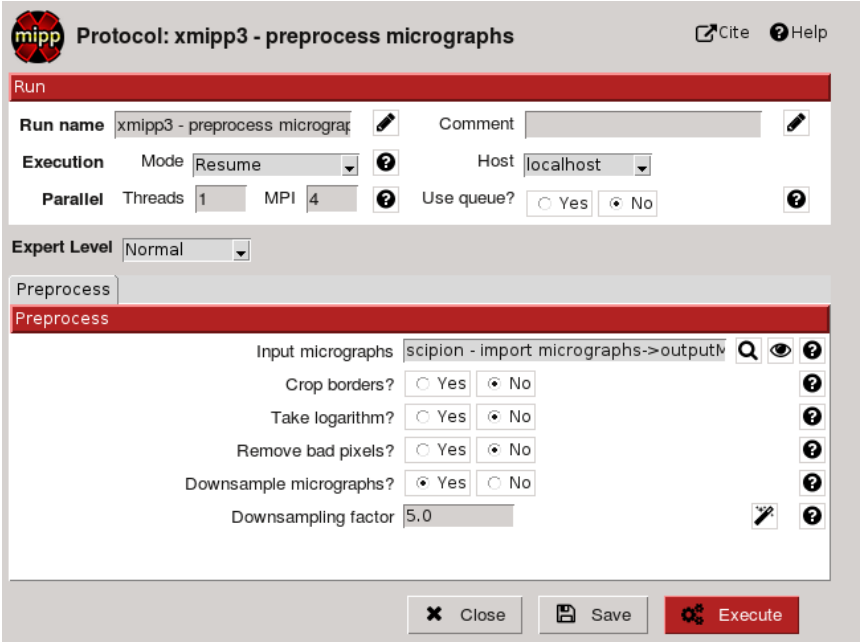

Figure 3: Preprocess Micrographs GUI.

figure 3.

2.2.3 CTF estimation

The next step is to estimate the CTF of the micrographs. In Scipion you can estimate the CTF with (at the moment) CTFFind (Mindell and Grigorieff, 2003) and Xmipp CTF estimation. You don't have to do anything extra, e.g. change the extension of the micrographs, to use both programs because in Scipion the inputs and outputs that belongs to same type of protocols are standarized.

This protocols estimate the PSD (Power Spectrum density) of the micrographs to estimate the parameters of the CTF (defocus U, defocus V, defocus angle, etc). They cut the micrographs into a plenty of images with a desire size. After that, calculate the Fourier transform to each image and averaged.

The CTFs of good micrographs typically have multiple concentric rings, shown in figure 4 left, extending from the image center towards its edges. Bad micrographs may lack rings or have very few rings that hardly extend from the image center.

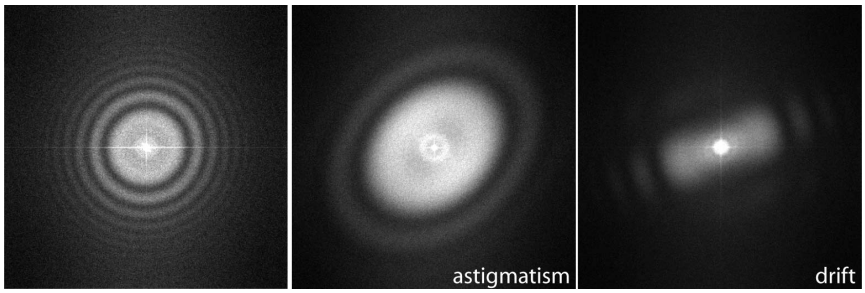

Figure 4: CTF of good, astigmatic and drift micrographs respectively

A reason to discard micrographs may be the presence of strongly asymmetric rings (astigmatism, figure 4 center) or rings that fade in a particular direction (drift, figure 4 right).

When the protocol is finished you may click on **Analyze Result** button (figure 5). To discard micrographs with bad CTFs you may click with the mouse right button and press **Discard** button. Once you finish the selection, press on **Micrographs** button (figure 5).

Sometimes, the CTF estimation algorithm may fail to find the rings even if they can be seen by eye. If this is the case, you may help the algorithm to find the rings by clicking with the mouse right button and pressing **Recalculate CTF** in the corresponding row of the Output visualization. A graphical interface will help you to correctly identify the CTF. You must provide the first CTF zero and the limits and press OK. When you finish, press **Recalculate CTFs** button (figure 5).

CTFFind In order to estimate the CTF with CTFFind, you will need some parameters describing the frequency region to be analyzed. The parameters shown in figure 6 7 are the adequate ones for this example. Note: In a real case, the limit values from frequencies must be adequate so that all zeros of the psd are contained within those frequencies. There is a wizard (figure 7, right) to choose those frequencies. The range of defocus to search is usually larger than the ones used in this example and according to your data set.

Xmipp CTF estimation

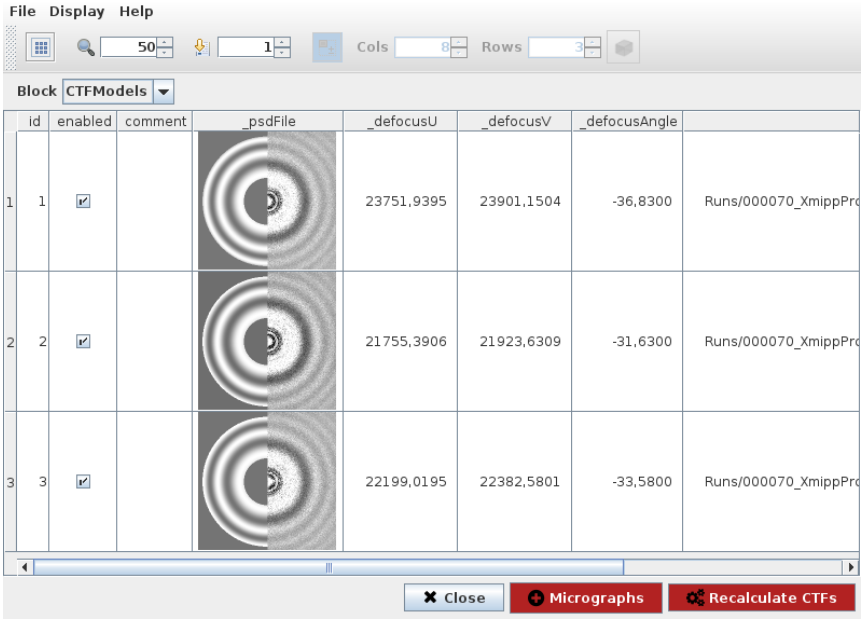

Figure 5: Output visualization of CTFFind protocol that shows the CTF of all the micrographs and different parameters

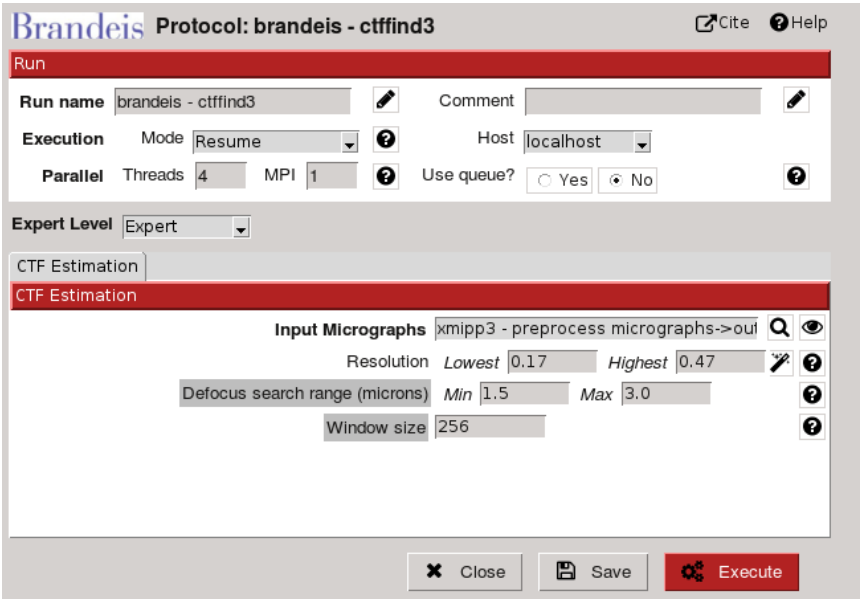

Figure 6: Output visualization of CTFFind protocol that shows the CTF of all the micrographs and different parameters

References

Mindell, J. A. and Grigorieff, N. (2003). Accurate determination of local defocus and specimen tilt in electron microscopy. *JSB*, 142:334 – 347.

Wolf, M., Garcea, R. L., Grigorieff, N., and Harrison, S. C. (2010). Subunit interactions in bovine papillomavirus. *Proc Natl Acad Sci U S A*, 107(14):6298–6303.