



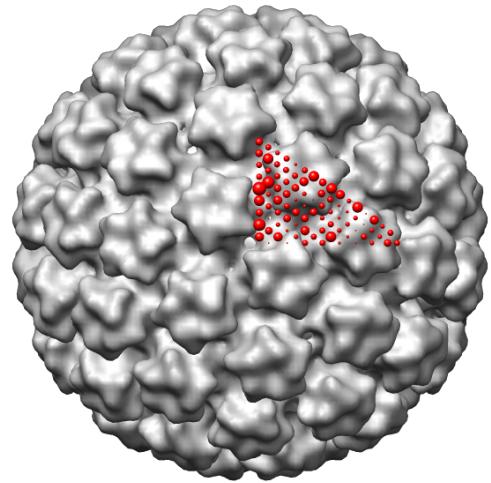
Scipion Tutorial Series

NATIONAL CENTER FOR BIOTECHNOLOGY  
BIOCOMPUTING UNIT

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## Introductory Tutorial

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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, some limited knowledge about 3D-EM image processing, and 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 errors, as well as your suggestions for future editions, by writing to [scipion@cnb.csic.es](mailto:scipion@cnb.csic.es).

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# 1 Software Installation

The first step before starting to work on your projects is to download and install SCIPION and related software packages. We describe briefly the process in the next sections. For the full documentation please refer to <http://scipionwiki.cnb.csic.es/bin/view/TWiki/NewInstallation>.

## 1.1 Xmipp

One of the main components of SCIPION is the Xmipp package. To get the latest version:

```
git clone http://git.code.sf.net/p/newxmipp/code xmipp
```

then change to the `xmipp` directory, and move to branch `xmipp_scipion` with

```
git checkout xmipp_scipion
```

Finally, run the installation with

```
./install.sh
```

## 1.2 Scipion and Extra Packages

To get the latest version of SCIPION run

```
git clone http://git.code.sf.net/p/pyworkflow/code scipion
```

and then change into the `scipion` directory. Finally run:

```
./scipion install --with-all-packages --with-xmipp=$XMIPP_HOME
```

where `$XMIPP_HOME` points to the directory with your local installation of Xmipp.

## 2 Reconstruction of a Viral Capsid

In this demo, we have used the *single particle analysis* approach to obtain a 3D reconstruction of a *Bovine 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). The data have been kindly provided by Dr. Grigorieff's Lab.

### 2.1 Getting Started

You can download the data with the following command:

```
scipion testdata --download xmipp_tutorial
```

It will be downloaded to `$SCIPION_HOME` › `data` › `tests`. After downloading, launch the scipion browser by typing: `scipion`

Then create a new project by clicking the `Create Project` button, type a *project name* and click `OK`. The 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 Importing Micrographs

The first step is to import the micrographs to your scipion project. To do this, double-click the `Import Micrographs` button. In *Pattern* indicate where your micrograph files are stored (for example by clicking on the browse button, the one that looks like a folder). The complete pattern is:

```
$SCIPION_HOME/data/tests/xmipp_tutorial/micrographs/*.mrc
```

Modify the parameters of the Import Micrographs protocol according to the ones shown in figure (1). Then click on the `Save` and `Execute` buttons. After executing the protocol, the project window will present the new information as shown in figure (2).

If we press the `Analyze Results` button a new dialog will appear that shows the different imported micrographs (not shown).

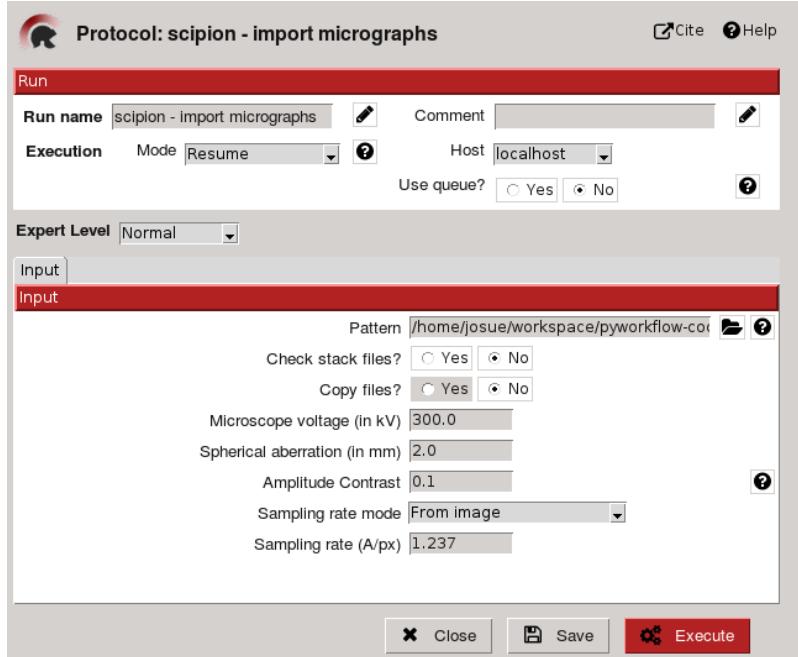


Figure 1: Import Micrographs protocol dialog.

### 2.2.2 Downsampling Micrographs

After importing the micrographs to your SCIPION project, you can perform the next step: **Preprocess Micrographs**. This protocol combines multiple Xmipp programs in order to perform several operations over the micrographs. Double-click on the protocol name and fill the dialog with the options shown in figure (3). To fill the *Input micrographs* field, simply click on the magnifying glass button and select the output from the previous step.

### 2.2.3 CTF Estimation

The next step is to estimate the CTFs (Contrast Transfer Functions) of the micrographs. There are currently two protocols in SCIPION to estimate the CTF: *CTFFind* (Mindell and Grigorieff, 2003) and *Xmipp CTF estimation*. You don't need to do any extra work to use both programs, like changing the extension of the micrographs, because in SCIPION the inputs and outputs that belong to the same type of protocol

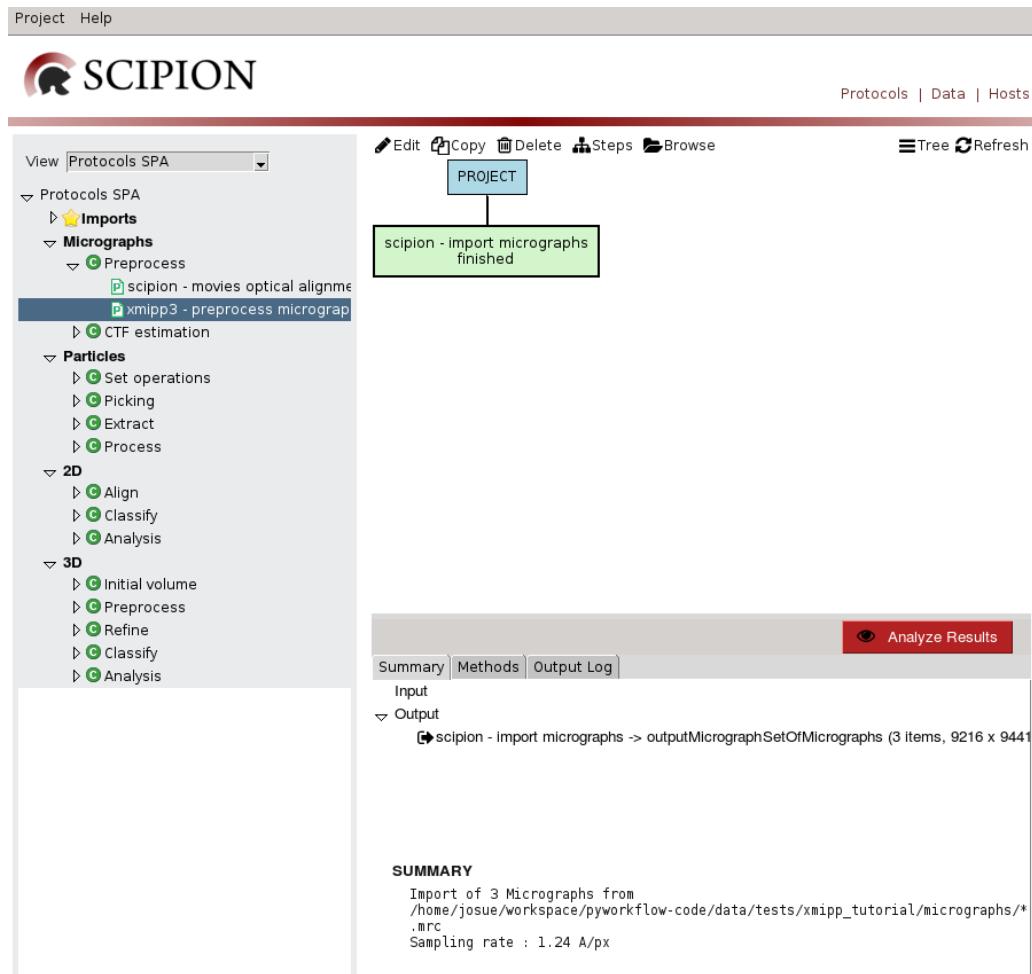


Figure 2: Scipion project GUI after processing Import Micrograph protocol.

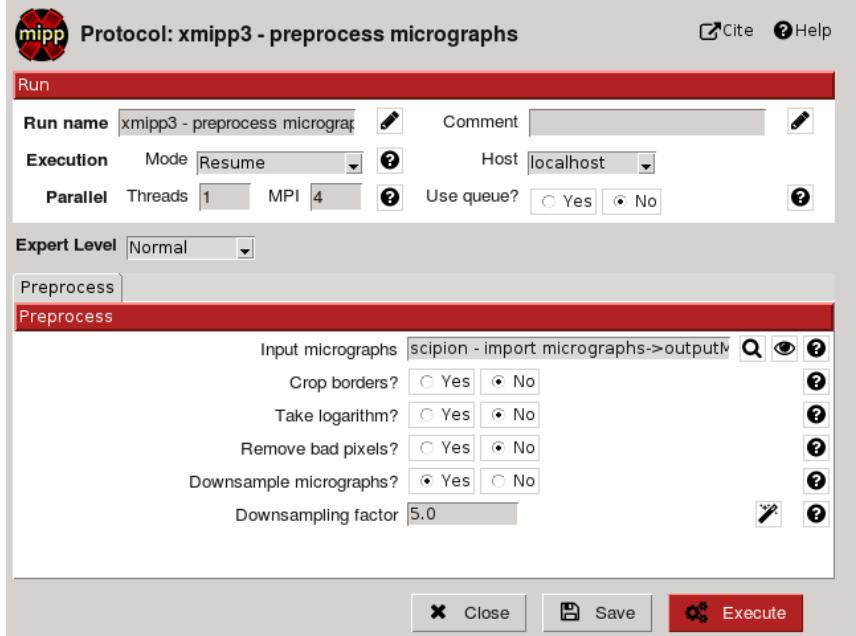


Figure 3: Preprocess Micrographs dialog.

are standarized.

These protocols estimate the PSD (Power Spectral Density) of the micrographs to then estimate the parameters of the CTF (defocus U, defocus V, defocus angle, etc). They cut the micrographs into plenty of images with the desired size. After that, they compute the Fourier Transform of each image and make an average.

## CTFFind

To estimate the CTF with CTFFind, you will need some parameters describing the frequency region to be analyzed. The parameters shown in figure (4) are the proper ones for this example. The limiting frequencies must be such that all zeros of the PSD are contained within those frequencies. There is a wizard, shown in figure (5), that helps in choosing those frequencies. To see the full available options, choose the **Rxpert Level** in the dropdown menu. Please note that the range of defocus search is usually larger than the one used in this example, and it must be chosen according to your data set.

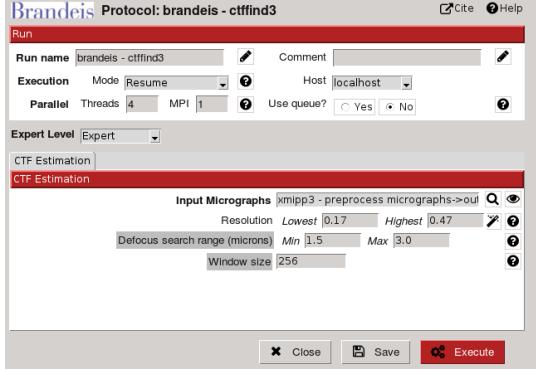


Figure 4: CTFFind protocol dialog.

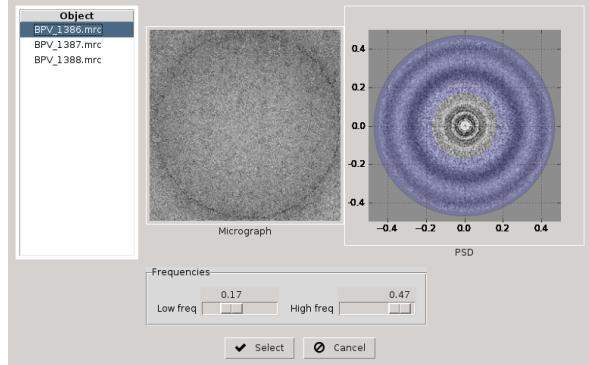


Figure 5: Wizard to choose the frequencies.

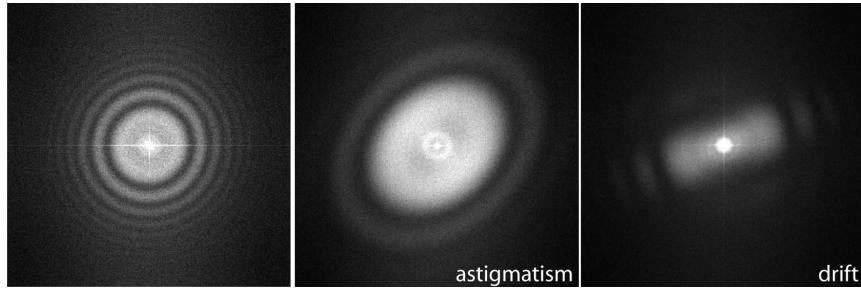


Figure 6: CTFs of good, astigmatic and drift micrographs respectively.

### Xmipp CTF estimation

If you prefer, you can estimate the CTF with the Xmipp CTF estimation. The parameters used in this protocol are the same as the ones in the CTFFind protocol, explained above.

The CTFs of good micrographs typically have multiple concentric rings, shown in figure (6) 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. A reason to discard micrographs may be the presence of strongly asymmetric rings (astigmatism, figure (6) center) or rings that fade in a particular direction (drift, figure (6) right).

When the protocol (either CTFFind or Xmipp CTF estimation) is finished you may click on the **Analyze Results** button (figure (7)). To discard micrographs with bad

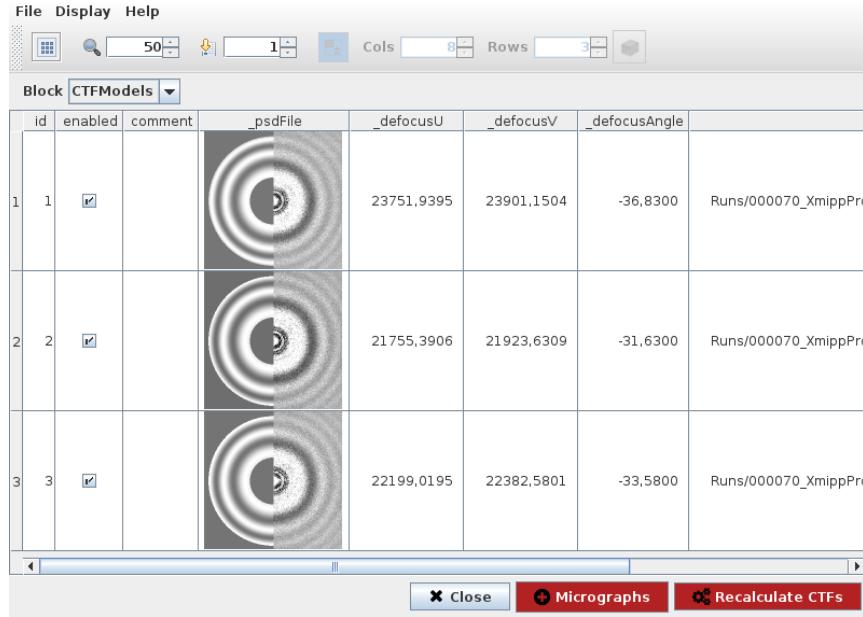


Figure 7: Output visualization of the CTFFind protocol that shows the CTFs of all the micrographs, and different related parameters.

CTFs you may click with the mouse right button and press **Disable**. Once you finish the selection, press on the **Micrographs** button to create your subset of micrographs with only the enabled ones.

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 on the image with the mouse right-button and choosing **Recalculate CTF** on the menu that appears. A graphical interface will help you to correctly identify the CTF. You must provide the first CTF zero and the limits, and then press **OK**. When you finish, press the **Recalculate CTFs** button.

#### 2.2.4 Particle Picking

Now you are ready to pick the particles. You can pick the particles in the micrographs with either *Xmipp manual picking*, *Eman boxer* or the *bshow* protocol. These protocols are in interactive mode, i.e. you can create different sets of coordinates within

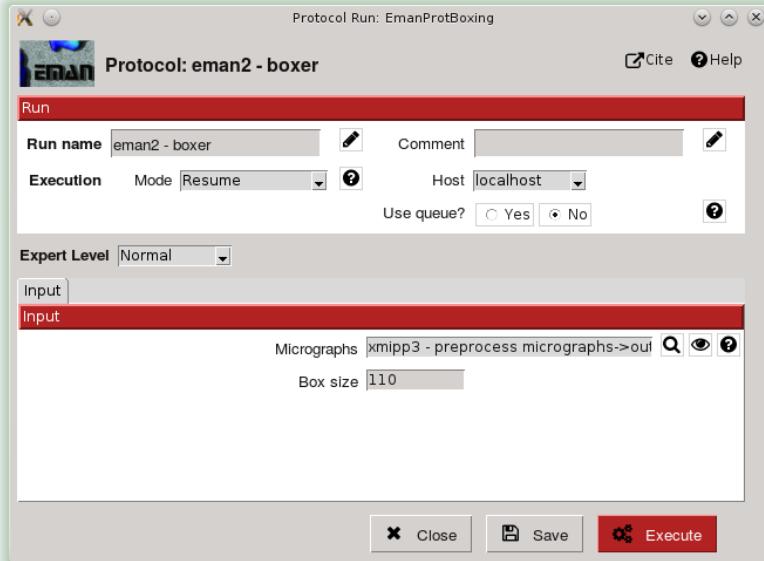


Figure 8: EMAN boxer protocol dialog.

the same protocol.

### EMAN boxer

Double-clicking on eman2 boxer will launch the window shown in figure (8). Set the parameters as they appear in the figure.

Once you press the **Execute** button, several windows will open (figure (9)). This protocol has several modes of selection, please refer to its webpage for further information. When you are done picking particles press **Done** and you will be asked if you want to create the output. If you choose **No**, you can continue picking and the selected particles will be added to the same Set, until you repeat the operation and say **Yes**.

### Xmipp particle picking

You could instead double-click on the *Xmipp supervised picking* protocol. It will launch a dialog as in figure (10). After selecting the set of micrographs to use and clicking **Execute**, it will launch the window shown in figure (11).

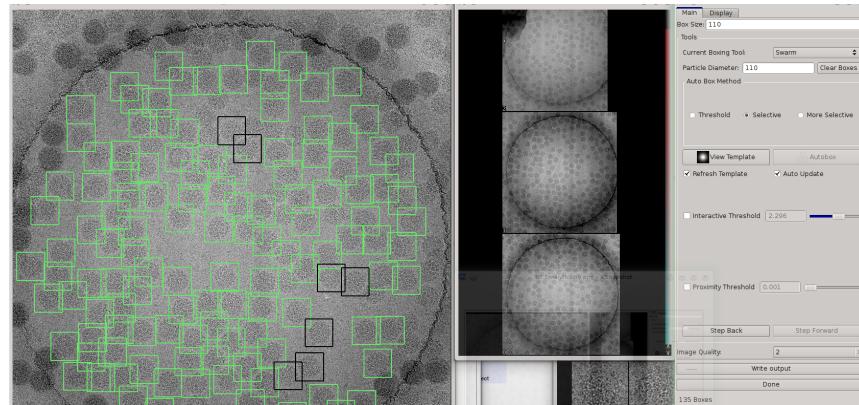


Figure 9: EMAN boxer, an interface for particle picking.

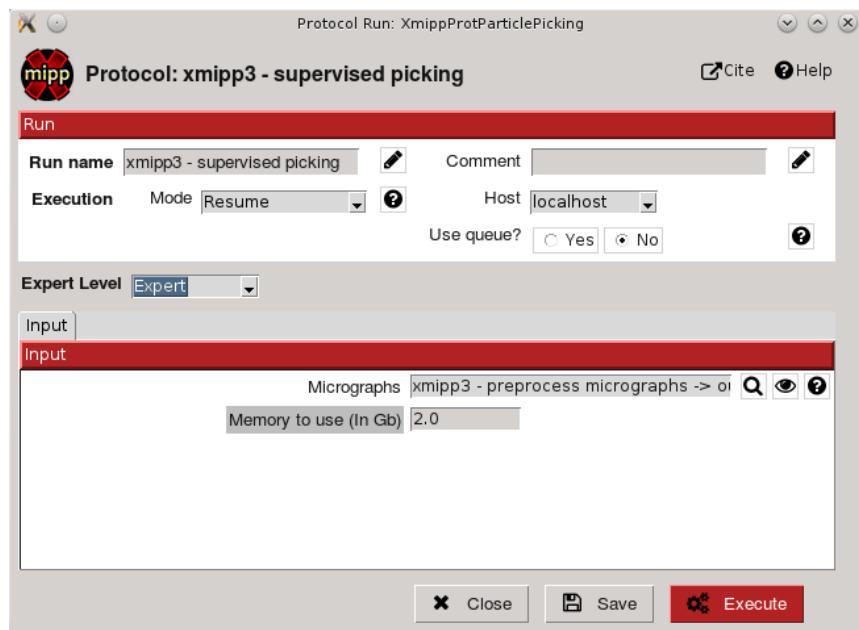


Figure 10: Xmipp supervised picking dialog.

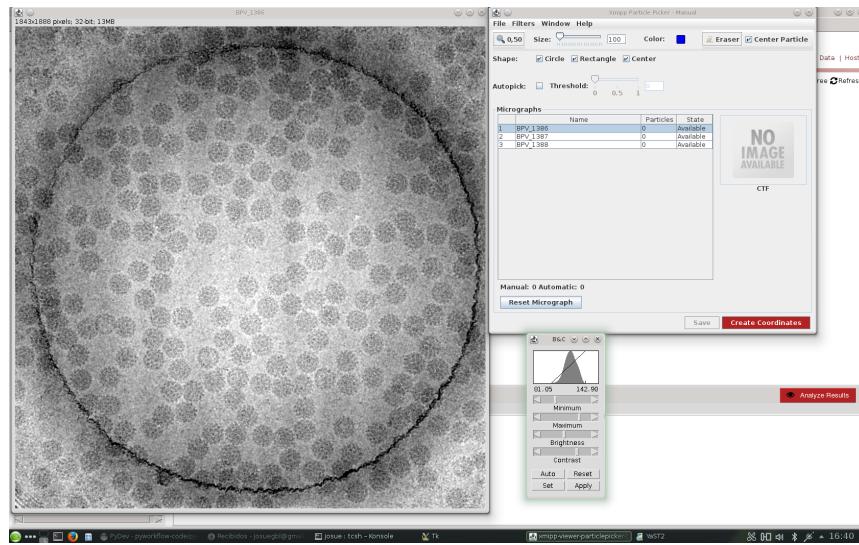


Figure 11: Xmipp supervised picking, a different interface for particle picking.

In order to select particles:

- Use **[↑] + [mouse wheel]** in the overview window to zoom in and out.
- Mark particles with the **[mouse left]** button. You may move its position by clicking the left mouse button on the selected particle and dragging it to the new position.
- Use **[↑] + [left mouse]** over a selected particle in order to remove it.
- You can apply filters to the micrographs, so that you may see the particles better. Filters are added into a queue, so whenever you change the visualized area, they are applied again. Select the menu **Filters** in the overview window and add as many filters as you like.
- You can clean the filter queue, if you want to return to the original image.
- You can create the set of coordinates or just save the picked particles to continue picking later.

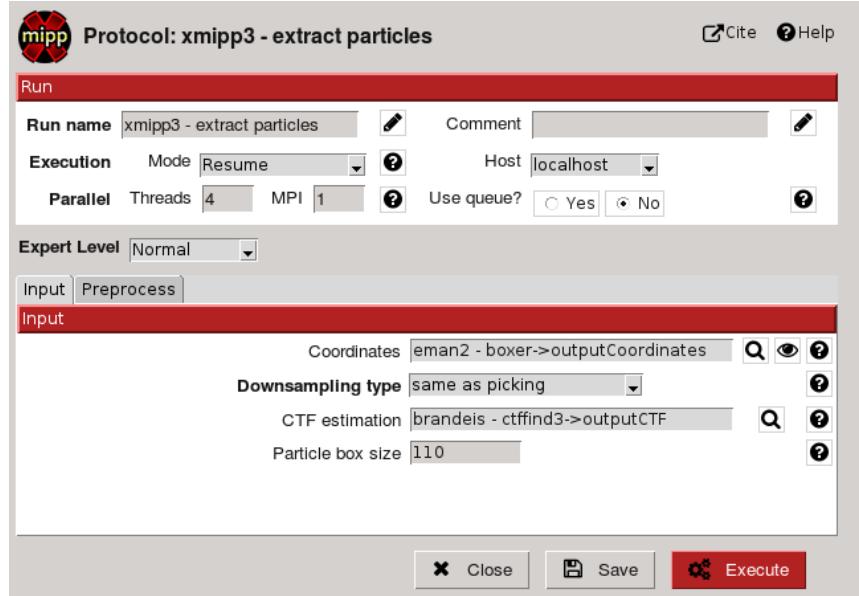


Figure 12: Extract particles protocol dialog (tab Input)

### 2.3 Extract Particles

Click on `Particles >> Extract >> xmipp3 - extract particles`. This will launch the dialog of our next protocol that will allow you to extract, normalize and correct the CTF phase of your picked particles, among other things. Modify the default parameters according to the ones shown in figure (12) and (13). They will contain:

- The *coordinates* of the particles in the micrographs, which are taken from the results of the previous step. Also in the same tab, the *particle box size* in pixels (in this case 110 px).
- The *invert contrast* flag. If activated, bright regions become dark regions and the other way around. This flag should be set so that the extracted particles are white over a dark background.
- The *phase flipping* flag. If activated (recommended), the protocol corrects the CTF phase of your particles.

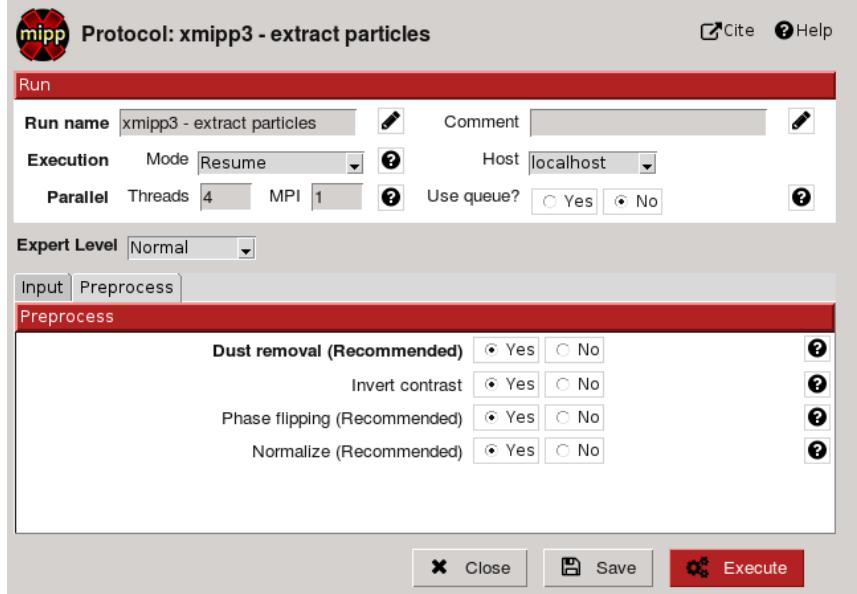


Figure 13: Extract particles protocol dialog (tab Preprocess)

- The *normalize* flag. If activated (recommended), the particles are normalized to have zero mean and a standard deviation of one for the background pixels.

The protocol also sorts the particles based on general statistics assigning to each particle a z-score value. Particles with low z-score are reliable and the ones with large z-score are outliers. Press the **Analyze Results** button in the main window to check the extracted and normalized images.

### 2.3.1 Particles Selection

By default, the visualization of the particles are sorted by z-score. If you want to remove some particles because they are outliers and are not good particle images, press the mouse right button on them and select **Disable**. When you are done press the **Particles** button to create a new SetOfParticles (figure (14)).

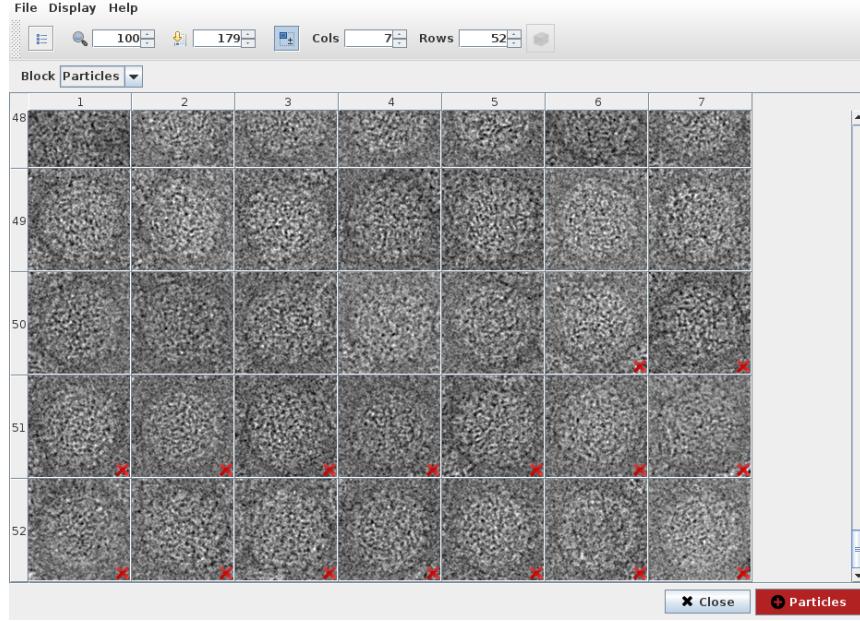


Figure 14: Analyze Results window of the Extract Particles protocol.

## 2.4 3D Reconstruction: Projection Matching

Having the right angles of each projection is crucial for making a 3D reconstruction. However, in 3DEM you don't know a priori the angles and you have to estimate them as part of the problem. The most popular way of estimating them is by comparing somehow the projections of a volume that is similar to the volume to be reconstructed (initial model) with the images obtained from the microscope.

A possible approach is to generate equidistant projections from the initial model. The experimental data set is then compared (for example, by cross correlation) to each reference projection. A “similarity” coefficient (for example, crosscorrelation coefficient) is generated between each experimental particle and reference projection. Each individual experimental particle is matched to the reference projection that gave the highest “similarity” coefficient. Therefore, it is assumed that this experimental particle was projected with the same Euler angles as the reference projection.

As an initial map is necessary, you need to import a volume. Press on **Import Volume** and set in *Pattern* parameter the following path:

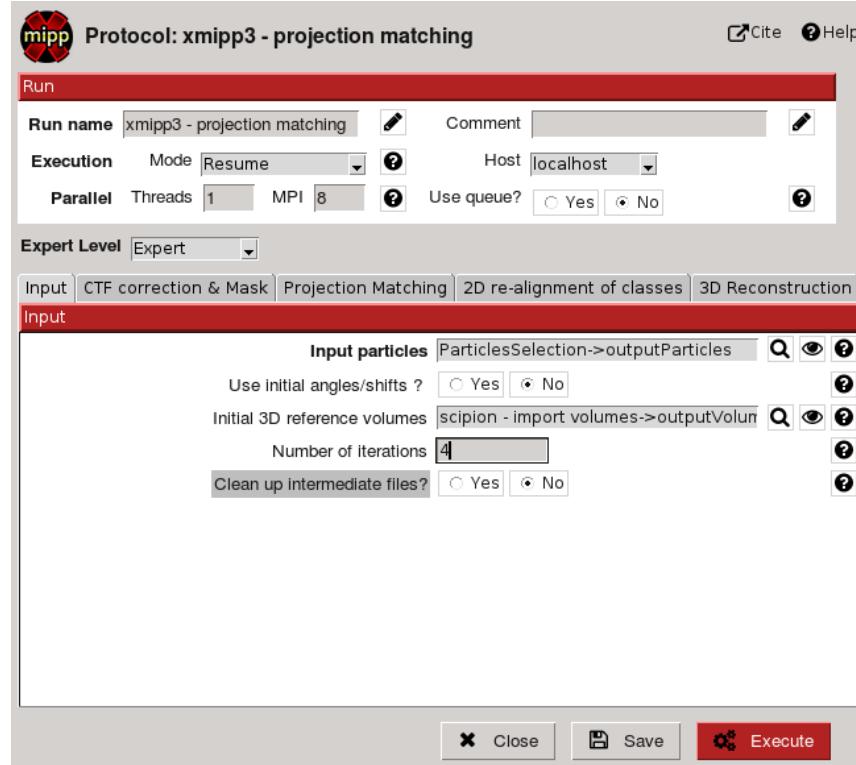


Figure 15: Projection matching parameters I

```
$SCIPION_HOME/data/tests/xmipp_tutorial/micrographs/BPV_scale_filtered_windowed_110.v
```

Please, set 6.185 on the *sampling rate* parameter.

In order to lauch the projection matching protocol click on **refine ↗ projection matching** and set the parameters as the ones shown in figures (15), (16), (17) and (18). Choose the SetOfParticles that has been selected previously in subset selection protocol.

Finally, in order to visualize the obtained results click on **Analyze Results** button. You will see a GUI as the one shown in figure (19).

To see the volumes you can press **eye** button in the field *Display reconstructed volume?* and you will see the reconstructed volume as shown in figure (20) or in chimera if you select Chimera checkbox (figure (21)).

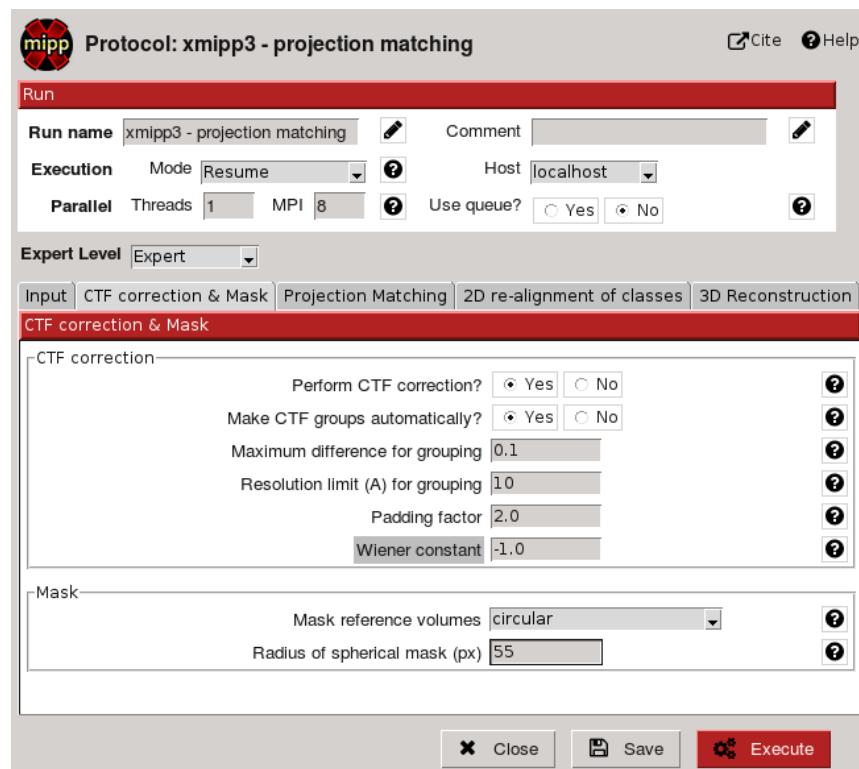


Figure 16: Projection matching parameters II

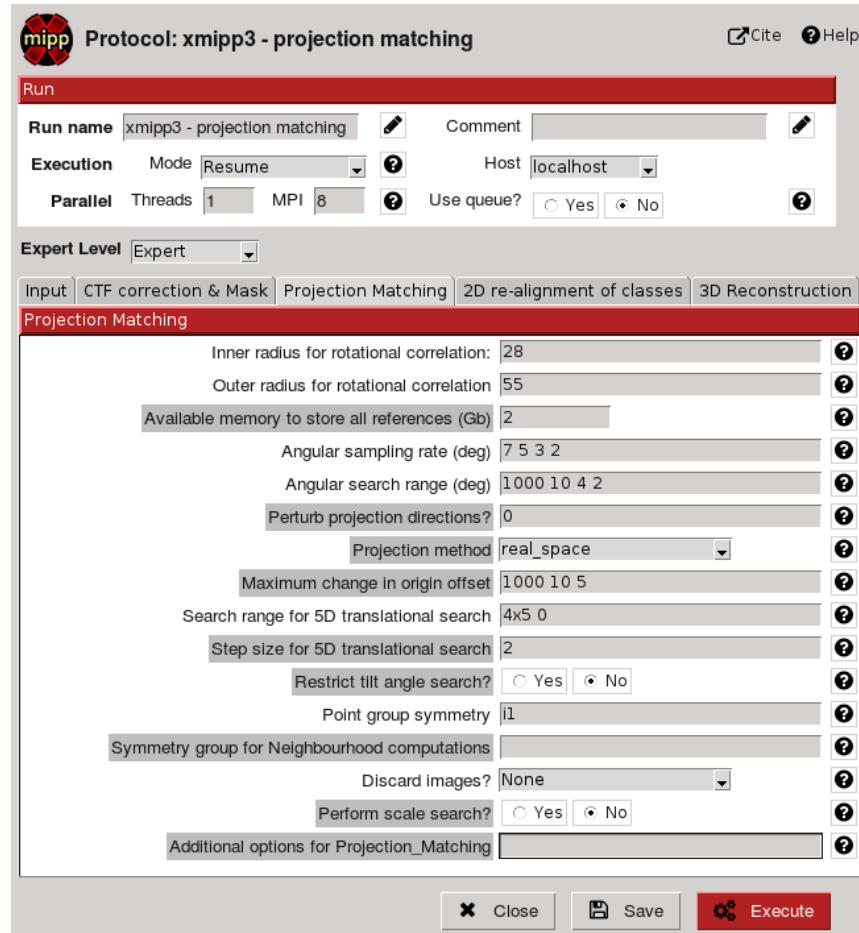


Figure 17: Projection matching parameters III

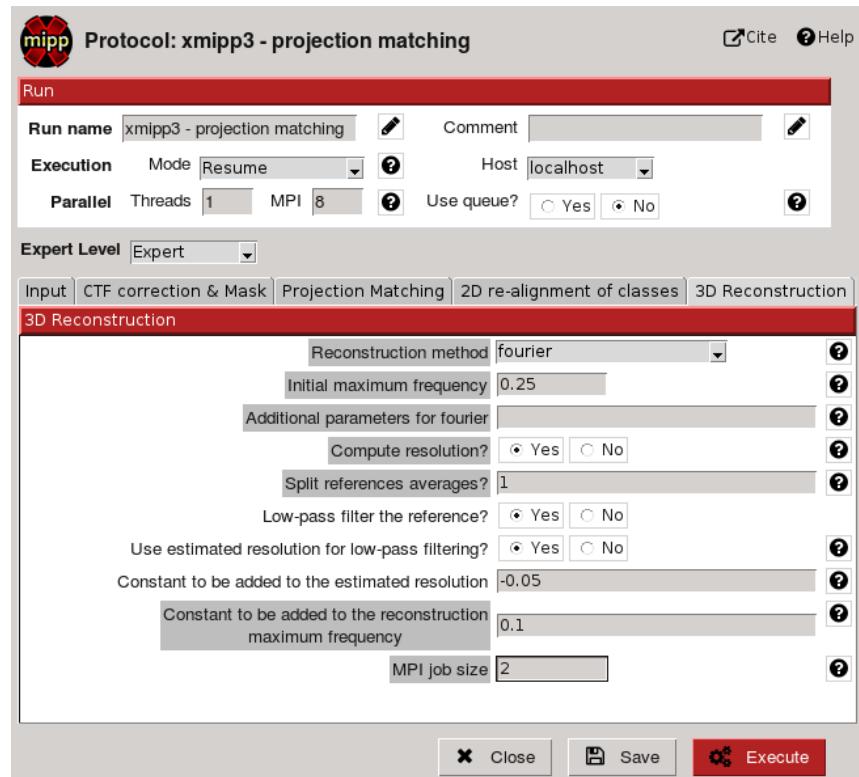


Figure 18: Projection matching parameters IV

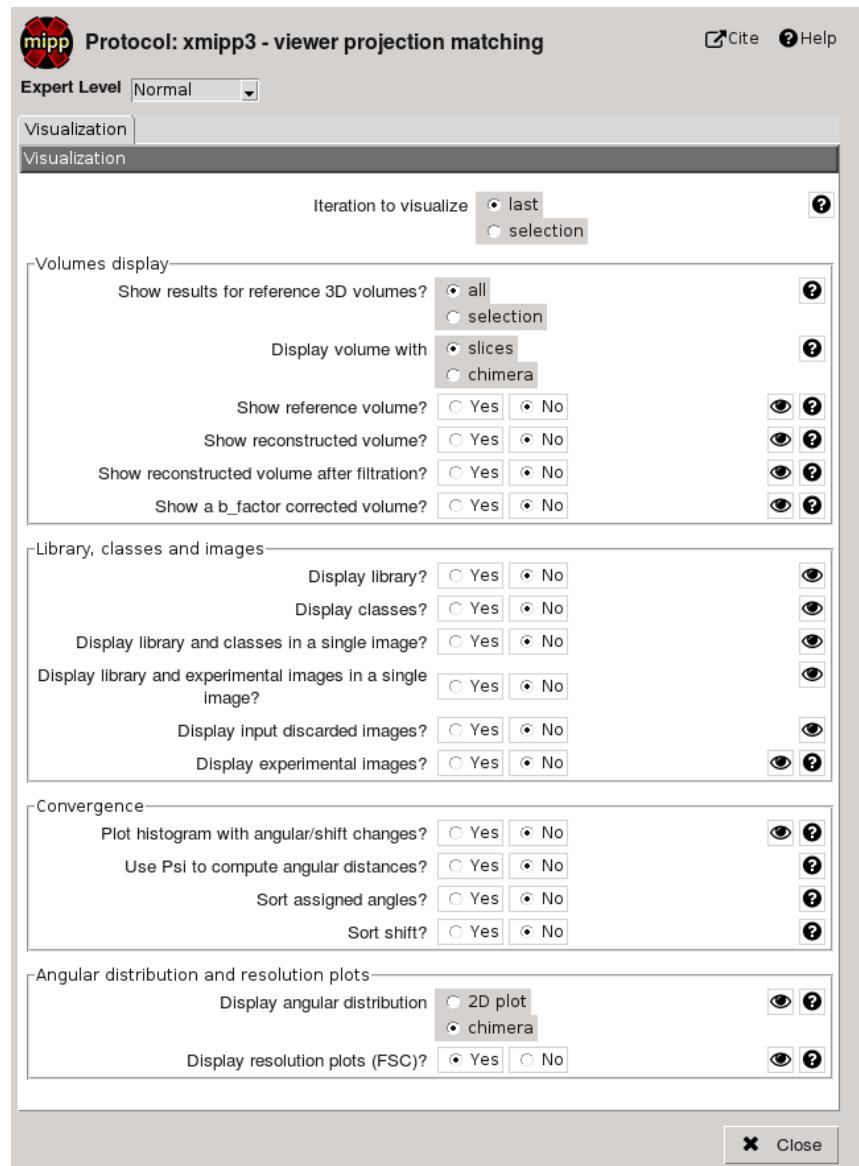


Figure 19: Projection matching viewer

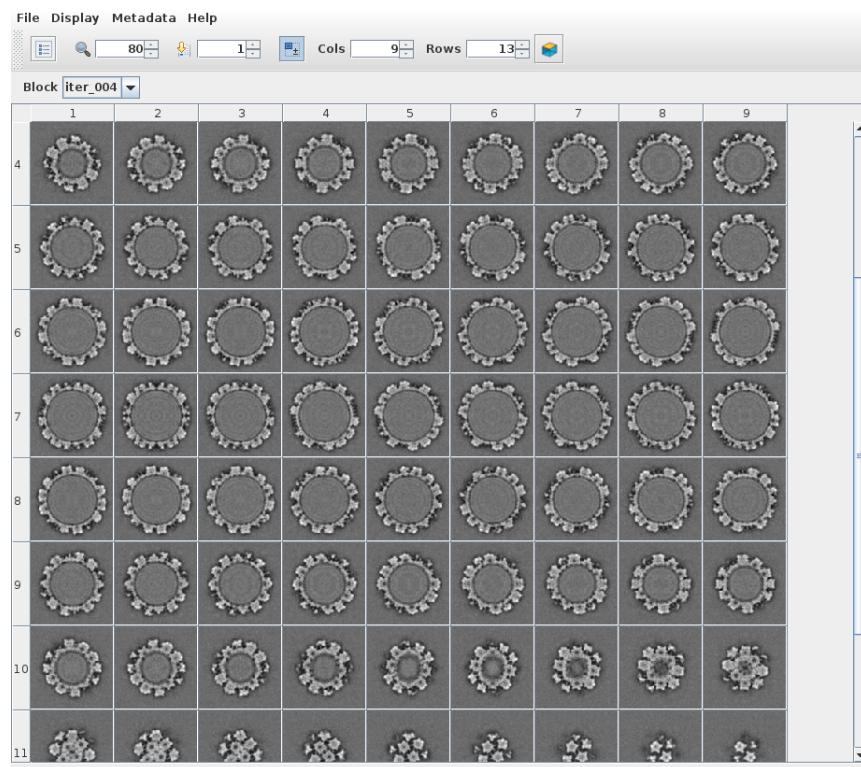


Figure 20: reconstructed volume

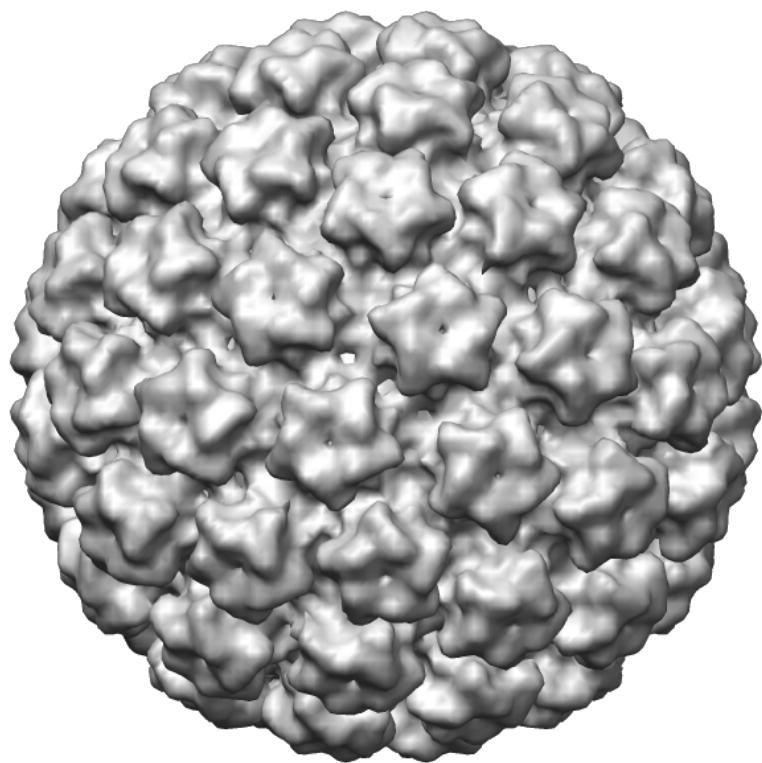


Figure 21: reconstructed volume with chimera

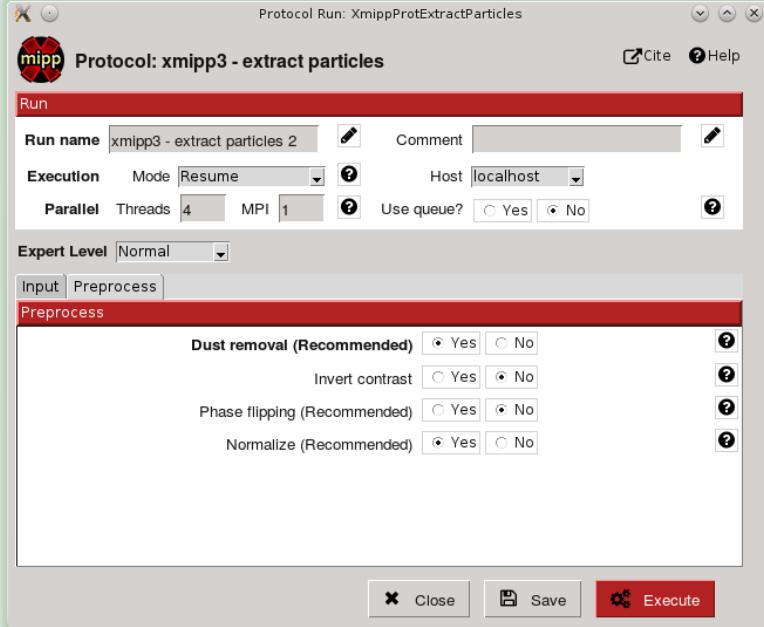


Figure 22: GUI of Extract Particles protocol II

## 2.5 Frealign Refinement

If you want to refine the output volume of projection matching, you can use Frealign refinement protocol. First, you need the "same" SetOfParticles that has been processes with Projection matching, but with the features needed to Frealign.

**Subset selection:** To do this, the firs step is extract the particles again, but Preprocess tab changed as shown in figure (22).

Once the particles are extracted, press on **Set operations → intersect sets** button. In first place, you set the last Extracted particles, and in second place the particles selected that has been used to refine a 3D reconstruction.

The new SetOfParticles is the input of Frealign refinement protocol. Please, set the parameters as shown in figures (23), (24), (25) and (26).

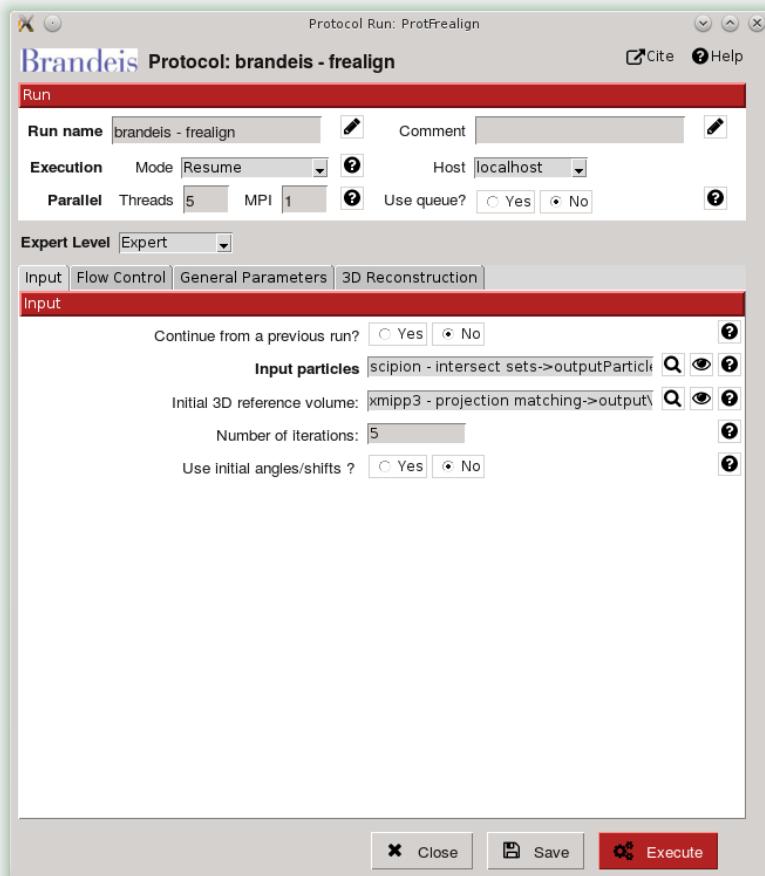


Figure 23: Frealign parameters I

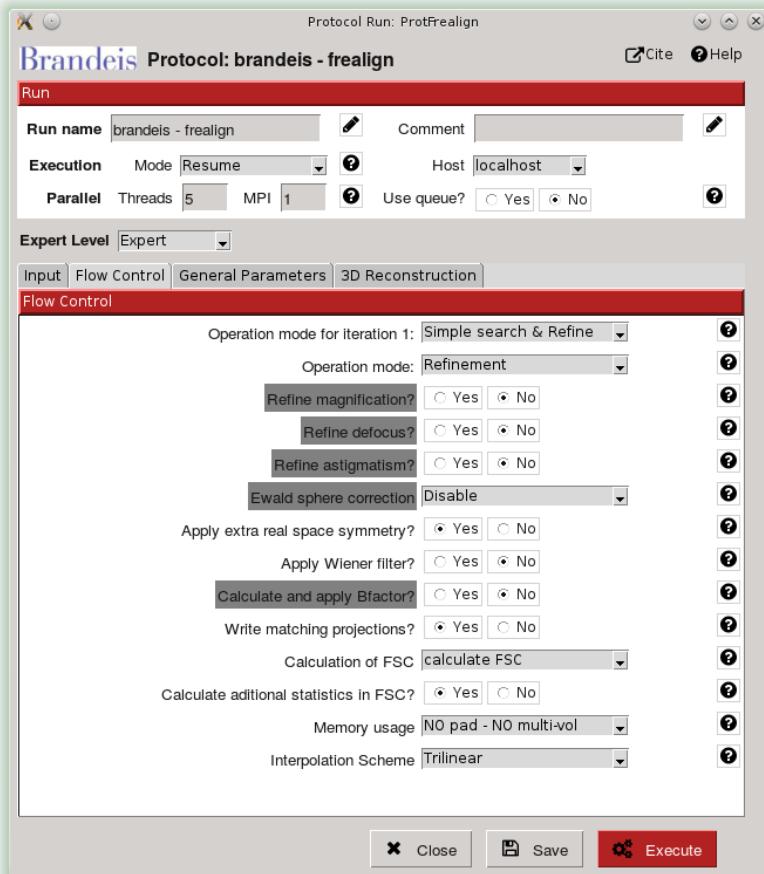


Figure 24: Frealign parameters II

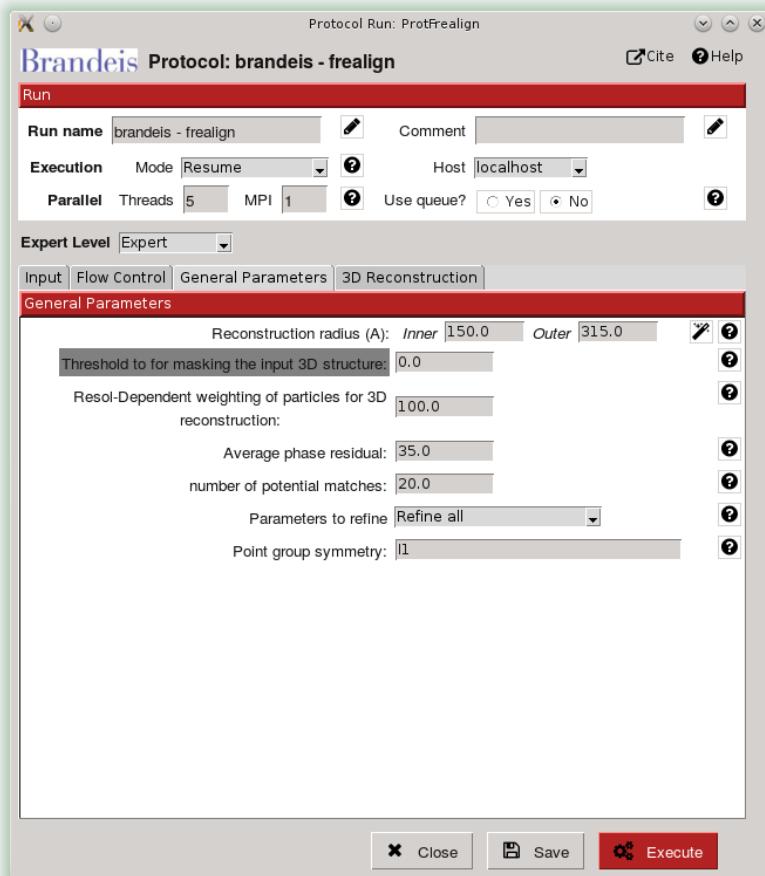


Figure 25: Frealign parameters III

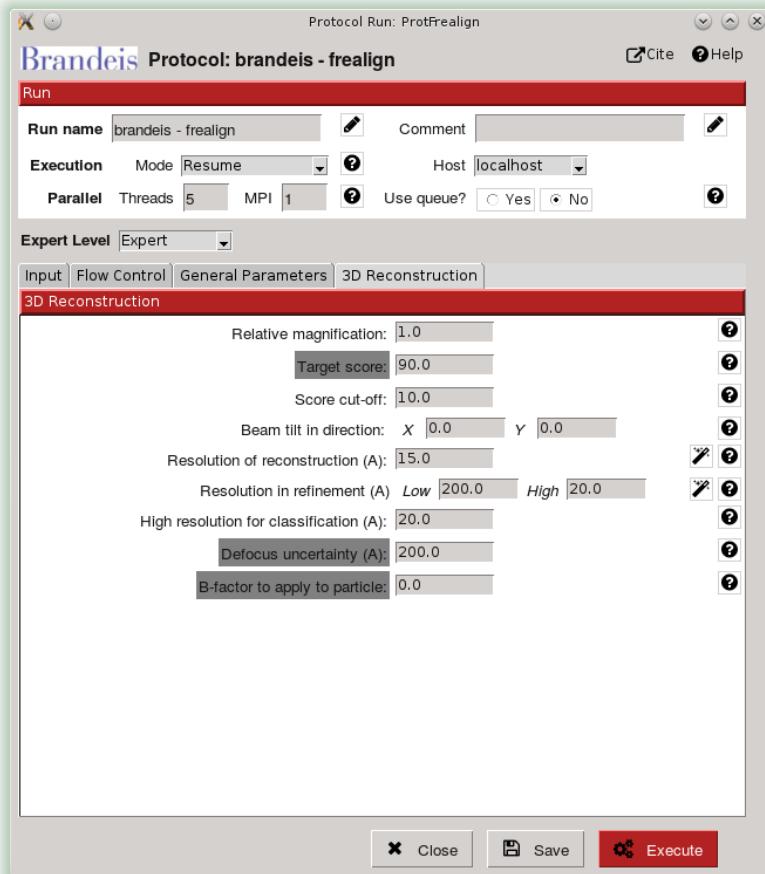


Figure 26: Frealign parameters IV

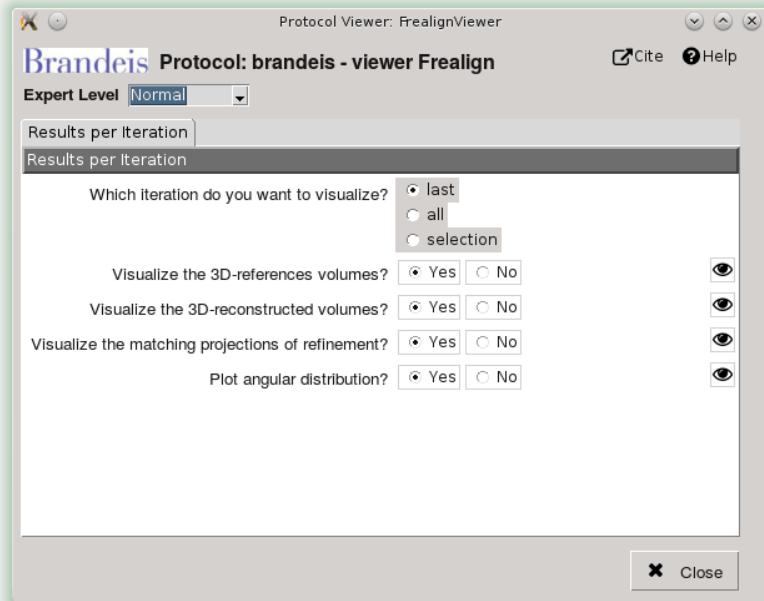


Figure 27: Frealign viewer

Again, in order to visualize the obtained results click on **Analyze Results** button. You will see a GUI as the one shown in figure (27).

## 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.