Tilt Validation in EMAN2 Tutorial

Getting Started:

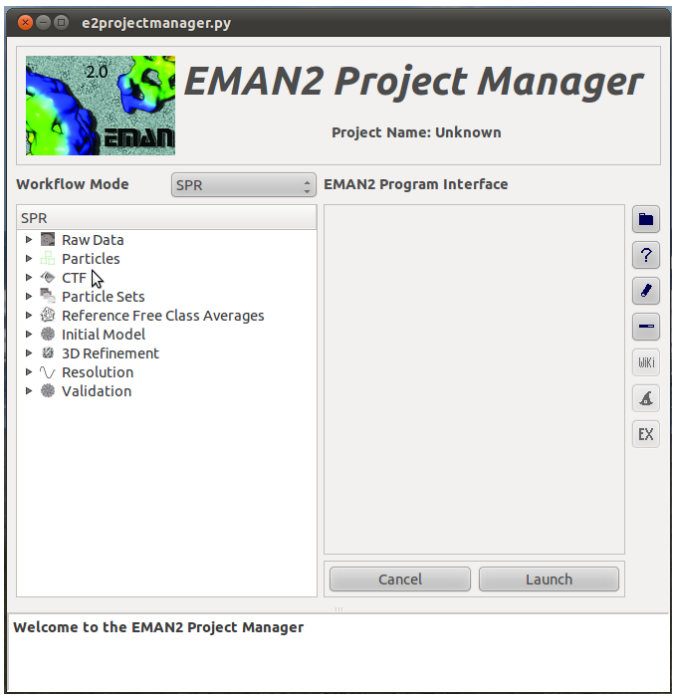
Make sure you have the latest version of EMAN2. For now, you will need the nightly build available for download at http://blake.bcm.edu. In the future, EMAN2.1 will be sufficient. You will also need do download the test data associated with this tutorial, and then untar it to get the Ip3R and PRD1 test data. For this tutorial you will chose to work with either PRD1, a 66 MDa virus, or Ip3R a 1.3 MDa membrane protein.

Introduction:

EMAN2 is an image processing software package directed towards electron microscopy data. It is composed of a C++ core and a suite of python programs that implement higher-level data processing functions. On top of this infrastructure, there is a GUI based data workflow framework, which will be used and introduced in this tutorial.

Initializing a new project using e2projectmanager.py

To begin a new project move to your project directory and launch e2projectmanager.py, the new EMAN2 GUI. A window should pop up as shown below:

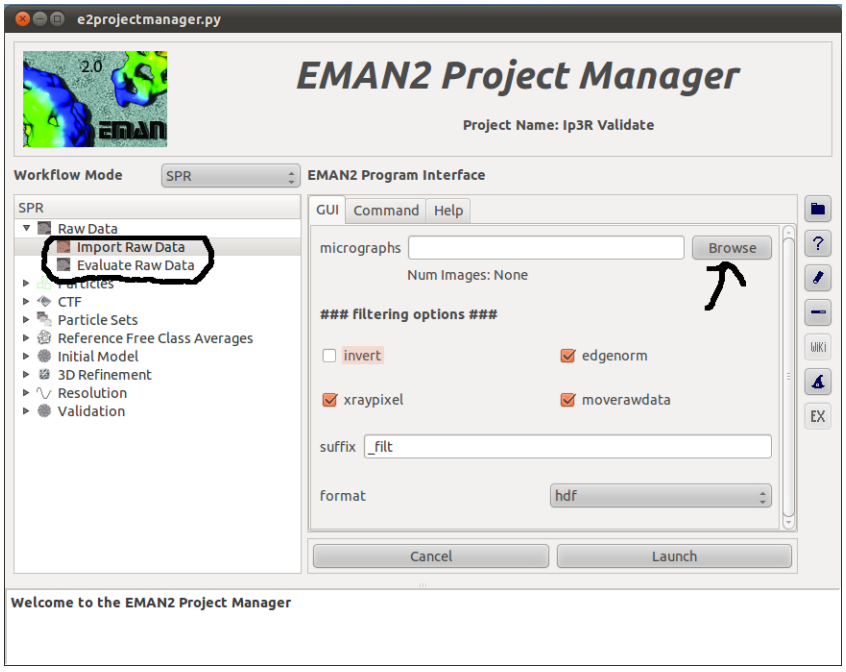


First you need to set the project parameters, so click the Menu item(in the menu bar location, which is platform dependent): Project->Edit
A dialog box will pop up. Here set the project parameters, according to what ever data set you wish to use (then click OK):

	Ip3R	**PRD1**
Project Name	Ip3R validate	PRD1 Validate
Project Icon	Use default	Use Default
Mass	1,300	66,000
CS	2.0	2.0
Voltage	200	200
APix	1.88	2.8

Importing data into your new project:

In the left box of the GUI click on *'raw data'* in the SPR workflow tree. This box lists the workflow and all options for two types of EM data processing, SPR and tomographic data. For this tutorial we will be using the SPR workflow. After clicking on *'raw data'* tab in the workflow, a list of raw data processing options will appear. You need to click on '*Import raw data'*. This will cause a e2program GUI interface to be displayed in the right box of the project manager GUI. Next, in the e2program GUI interface, click on 'Browse' , which will display the new EMAN2 file browser. Using this browser, select the files to import (you can multi select by holding shift or range select using control). Once the files to import are selected, click 'Ok' in the file browser. The list of files to import will now be listed in the e2program GUI interface.

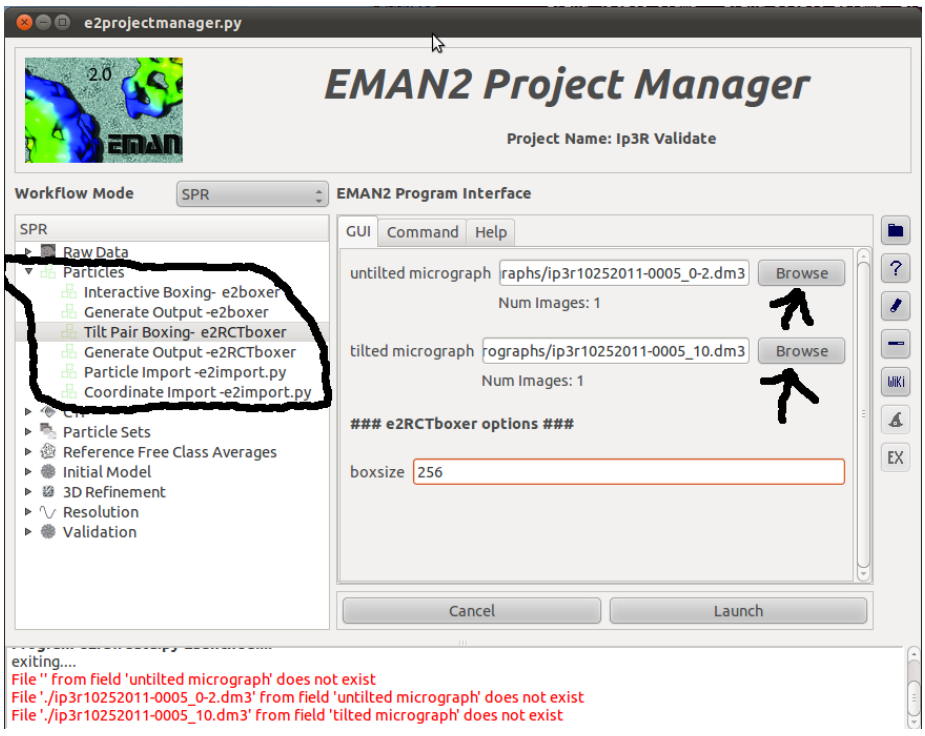


In addition to importing the particles, this program also preprocesses the micrographs. You can invert, remove X-ray pixels, etc. The recommended output format is HDF.

For all e2programs that projectmanager can run, in addition to displaying a GUI interface for the e2programs, you can also click on the command tab in the e2program interface, and the command that will be run is displayed. You can edit this command to tailor the e2program, that will be launched after clicking *'launch'*. You can also click on the help tab to list e2program specific help information. Once you have the selected files for import , click on 'Launch', this will cause e2projectmanger.py to run the e2program. In this case files will be imported to your project.

Picking particle pairs using e2RCTboxer.py

Once you have imported your particles, you need to extract particle pairs. This is accomplished using e2RCTboxer.py. To use e2RCTboxer.py with e2projectmanager.py, click on the particles tab in the SPR workflow tree. This will display a list of e2programs that can be used for particle extraction. Click on Tilt Pair Boxing to load the e2RCTboxer.py GUI interface into e2projectmanager.py.

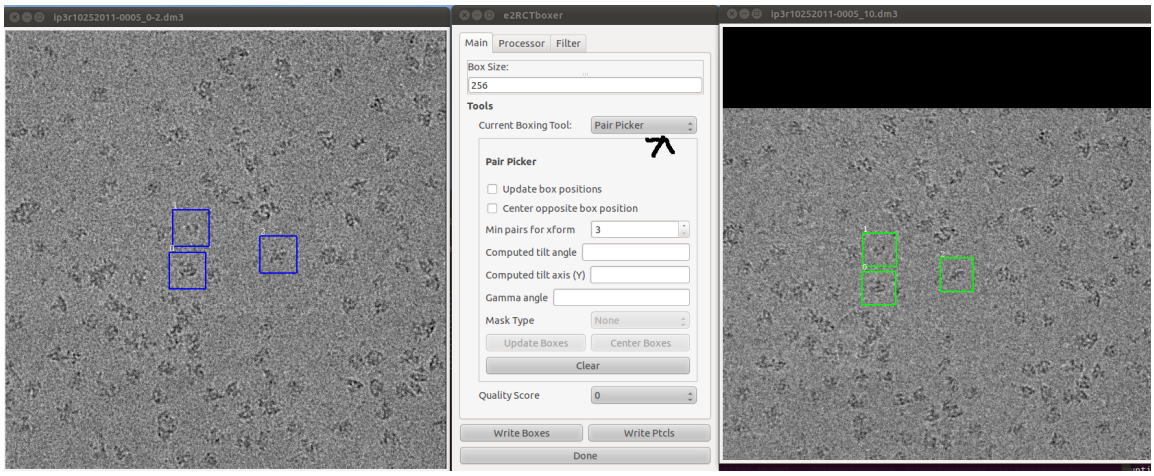


As before, click on browse to load filenames to be processed. Click on browse, to obtain to untilted micrograph (I suggest ip3r10252011-0005_0-2_filt.hdf), using the file browser. As before click 'ok' in the file browser. Next click on browse to obtain to tilted micrograph (I suggest ip3r10252011-0005_10_filt.hdf) using the file browser.

Once you have selected two micrographs for tilt pair extraction, set the boxsize to 256(for Ip3R), and click on 'Launch' to start e2RCTboxer.py.

e2RCTboxer.py will show four windows: a untilted micrograph window, a tilted micrograph window, a GUI control window, and a particles window. E2RCTboxer has two modes, Manual mode (default) and Pair picker mode). You should use Pair Picker mode, by clicking on the combo box and selecting this (see diagram below).

Before semiautomatic particle selection can begin, you need to select 3 anchor pairs, so starting on the untilted micrograph, pick any particle and then in the tilted micrograph pick this same particle. Repeat 3 times. The numbers at the upper left corner of the particles denote particle pairs. For example 1, and 1 are the same particle at different tilts. Now pick a particle in the untilted micrograph, and the corresponding particle will be automatically picked, though it might be a little off center, so you'll need to manually center it. Continue until you have picked all particle pairs. One useful tip is to change the 'mask type' in e2RCTboxer GUI to Line Mask, which will demarcate the areas the two micrographs share.

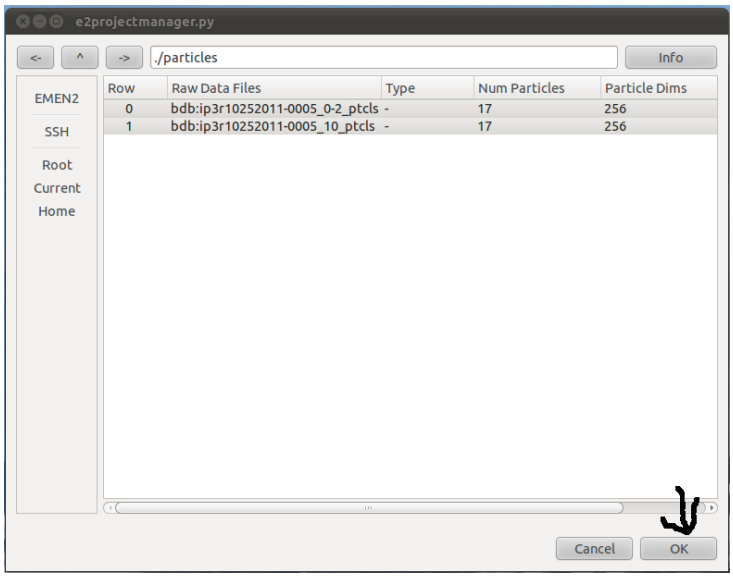


After you have finished picking particle pairs. Click on *Generate-Output-e2RCTboxer* in the particles tab of the e2projectmanager (SPR workflow mode). This will load a GUI interface into projectmanager to extract particle pairs from the micrographs using the stored box coordinates (cords are stored automatically). In addition, to particle extraction, particle contrast can be inverted, particle density can be normalized, and the particles can be shrunk in size. For the tutorial, you'll need to ensure that the invert contrast check box is checked, so that the particle's contrast is inverted. Also set shrink to 2 to speed later processing. Click 'Launch' as usual to run this e2program.

Correcting CTF

CTF correction for untilted micrographs is straightforward, however, CTF correction for tilted micrographs is non-trivial since there is a CTF gradient across the micrograph. However, if the tilting is small < 10 degrees, CTF can be approximately corrected, usually enough for the purposes of tiltvaldation, which is by nature a low to mid resolution technique.

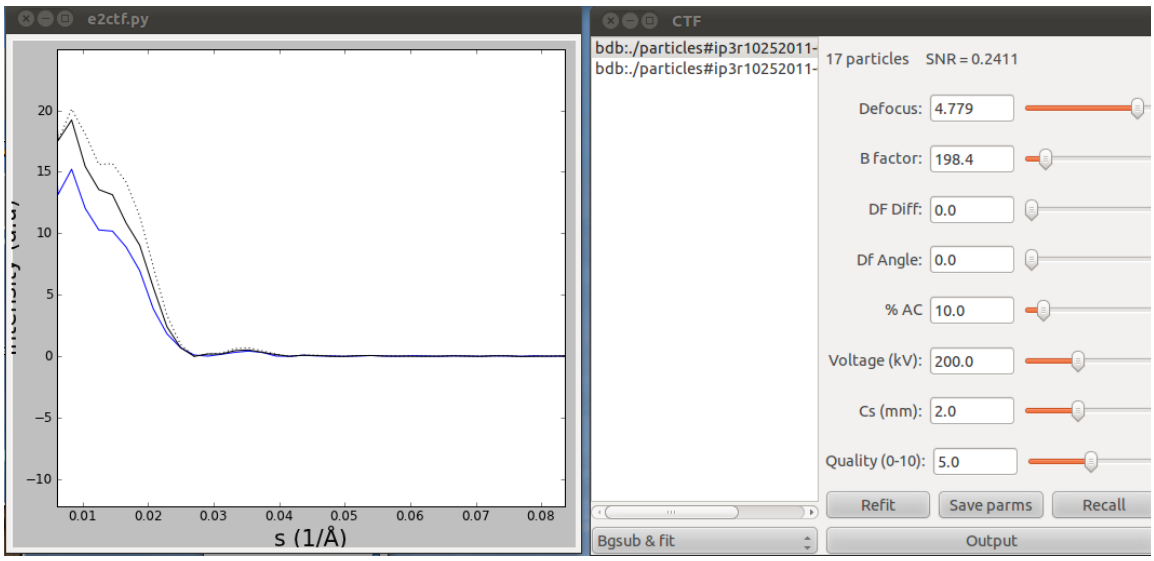
In e2projectmanager, click the CTF tab, listing CTF processing options. First, click on *'Automated Fitting-e2ctf'* to load the e2ctf.py GUI interface into the project manager. Once loaded, click browse, and select the particle stacks to CTF correct(see below). For the tutorial, there should be two particle sets listed, select them both, and click 'OK'.



This will load particle stacks to be processed into the e2CTF GUI. Microscope parameters should be listed in this GUI (derived from project parameters we set when we began this project). You will need to change the apix from 1.88, to 3.76 to reflect the fact that we shrunk the particles by a factor of 2 during the previous particle extraction step (1.88*2 = 3.76). Click Launch as usual, to automatically CTF fit the particle stacks.

After automatic CTF fitting, it is best to confirm the fitting results. In the CTF tab of the projectmanager, click on *'Interactive Tuning-e2ctf'*, nothing needs to be changed here, all the particle stacks will be imported from the previous step. Click 'Launch' to being interactive tuning. Four windows will appear (two are shown below), a 1D rotationally averaged power spectrum window, a GUI window, a 2D power spectrum window, and a window showing images. The power spectrum window shows power spectrum (black line), and the fit CTF (blue line). Ensure that the blue line matches the black line, at least with regards to matching CTF undulations and CTF minima. The fit CTF defocus and B factor can be adjusted using the GUI control window. If any changes are made, you must click 'save params' to save your

changes. Next move on to the next particle stack and do the same. After finished close all windows.

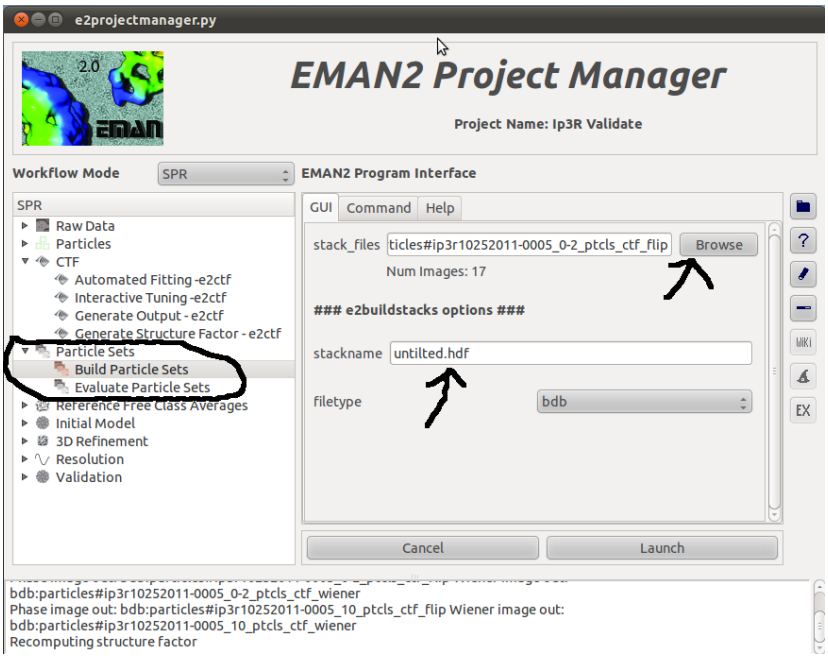


By now, you have determined the CTF parameters for both the untilted and tilted image stacks (4.8 and 5.2 respectively for Ip3R). You need to CTF correct your images by phase flipping. In the CTF tab, click *'Generate Output-e2ctf.'* Again everything should be ready to go, so just click 'Launch' to run the program. In most circumstances, it would be good to compute 1D structure factors for your particle using '*Generate Structure Factor',* but you need at least 4 stacks of particles, so we will skip this step (not necessary for our purposes)

Building particle stacks

Click the Particle Sets tab, listing options to build particle stacks for processing. Click *'Build particle Sets'*, loading the program GUI to build stacks for processing.

Click browse to search for stacks to build. The file browser will launch. For this tutorial, you will need to select just one stack (in larger projects you would select multiple stacks). Select bdb:ip3r10252011-0005_0-2_ptcls_ctf_flip (for Ip3R), and click 'Ok'. In the e2program GUI interface, set the name of the stack to 'untilted' As usual click 'Launch' to run this program. Next, rerun this program using the steps described above, only select bdb:ip3r10252011-0005_10_ptcls_ctf_flip (for Ip3R), and set the stack name to 'tilted'.

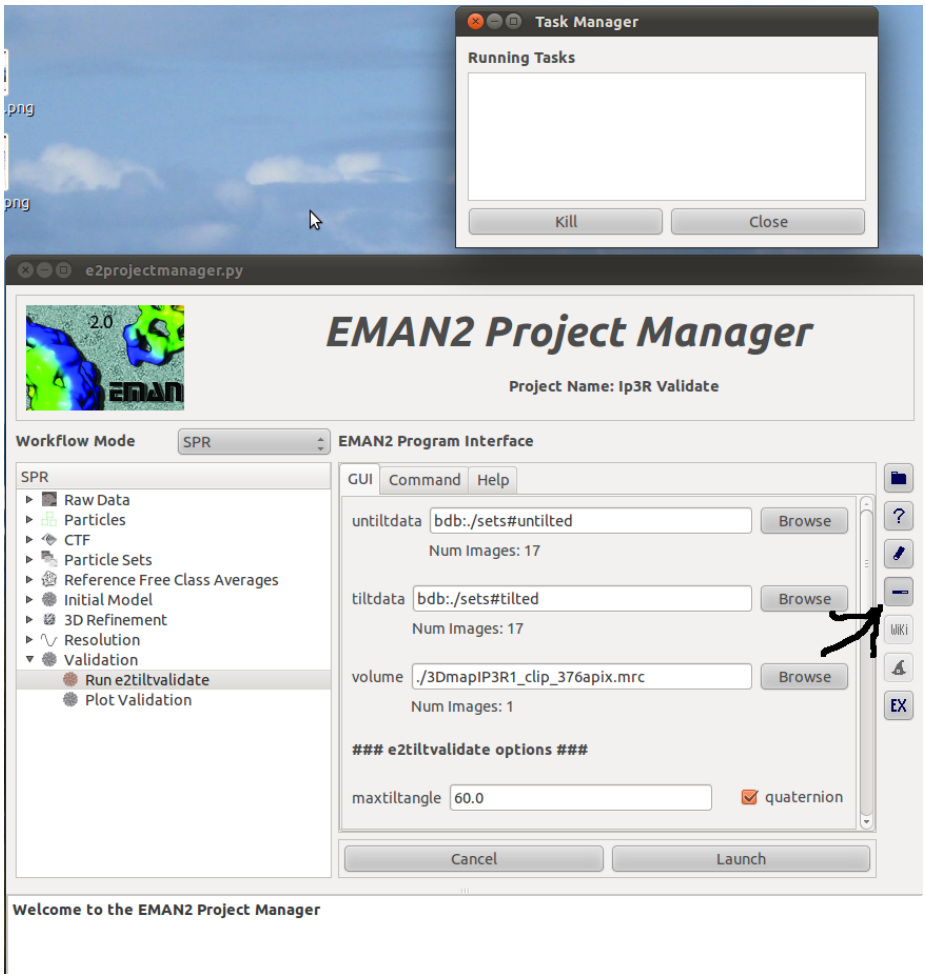


Validating the reconstruction using e2tiltvalidate.py

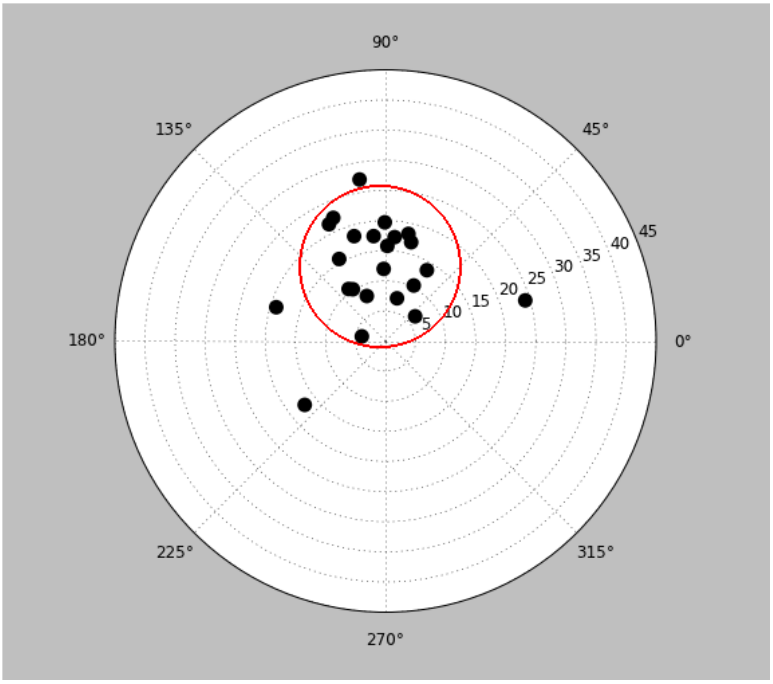
You have now a pair of tilt pair stacks, which can be used for tiltvalidation. Click on the validation tab in e2projectmanager, listing validation programs. Click *'Run e2tiltvalidation'* to load e2tiltvalidate GUI interface in the projectmanager (see figure below).
Click browse and load stacks for untileddata (file: untilted) and tiltdata (File: Tilted). Also Click browse to load the volume to validate. The default location is ./initial_models. Your model will not be here, so move to directory ., which should show the volume to validate, 3DmapIPmapIP3R1_clip_376apix.mrc. This can be accomplished by deleting 'initial_models' in the textbox at the top of the browser window, and replacing with just '.'. Once these files have been loaded (listed) in the GUI, you need the set the following parameters.

	Ip3R	**PRD1**
maxtiltangle	60.0	20.0
quaternion	checked	unchecked
symetery	C4	ICOS
parallel	# cores on your machine	# cores on your machine
delta	2.0	1.0
shrink	2	2
simcmp	frc maxres=60	Frc maxres=50
simalign	rotate_translate	Rotate_translate_iterative
simaligncmp	ccc	ccc
simralign	refine	None
simraligncmp	dot	dot

To determine what these or any parameters listed in the e2program GUI interface, just mouse over the form entry to display a tooltip, which displays help information relating to this item. As usual, click 'Launch' to run the program. This may take 15-30 minutes, or longer. You can check the status of your job by clicking in the status bar in the projectmanager toolbar (far right side of the projectmanager).

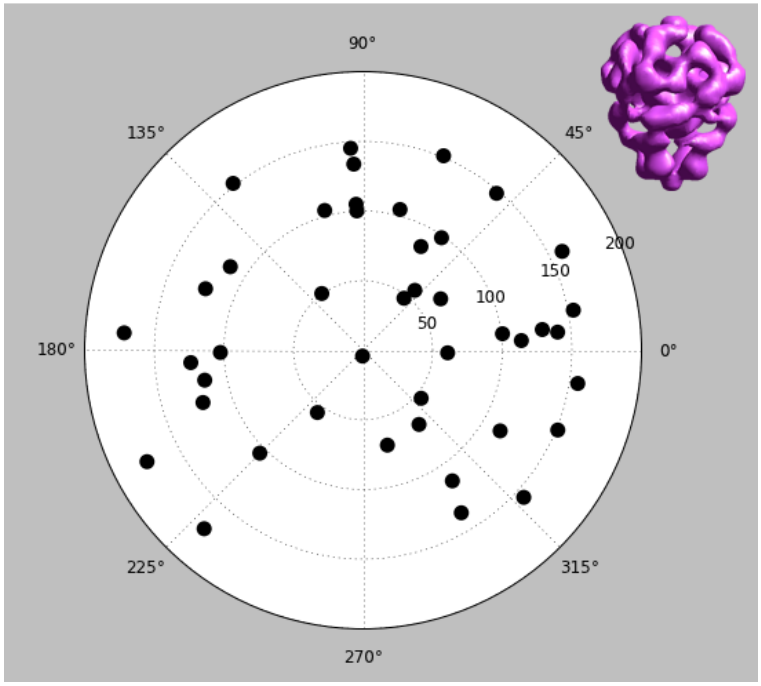


After the tiltvalidation program has run, you need to display the results. In the validation tab of the SPR workflow, click *'Plot Validation'* to load the GUI interface into projectmanager. There is a path option in this GUI listing directories containing results from e2tiltvalidate.py runs. Select the most recent run (highest directory index). Next set radcut to 45 (this mean don't plot anything above a radius of 45 degrees), set planethres to 0.3 (this rejects any solution whose out of planeess is greater than 0.3 in quaternion mathematics). As usual click 'Launch', which will display a polar plot of computed tilt geometry per particle pair.



If solutions cluster around the experimental tilt angle (radial polar coordinate) and experimental tilt axis (theta polar coordinate), the structure has been validated (above). On the other hand if the solutions are randomly distributed, then we have not validated the reconstruction (below). The red circle demarcating the cluster in the above figure is created interactively. To draw, left click in what you perceive to be the cluster center and then drag to enlarge the circle until it encompasses the cluster. After releasing the mouse button, the mean and STD tilt angle and tilt axis will be computed from points falling within the red circle. These stats will be displayed in the GUI.

You may notice, in the terminal window, a message saying ‘Cannot draw contour plot’. e2tiltvalidate.py is capable of also drawing a contour in addition to a scatter plot. To enable this, bring up the e2tiltvalidate.py GUI as before, and then click on the EX tool bar to move to expert mode. When in expert mode you have additional options for e2tiltvalidate.py. Click 'docontourplot’ if you want to generate contour plots, and run as before.


90°
135°
45°
200
150
100
50
180°
0°
225°
315°
270°