TheNogalesLab
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Orthogonal tilt reconstruction (OTR)

Original reference: Leschziner & Nogales, 2006 [http://www.ncbi.nlm.nih.gov/pubmed/16431136]

Methods: Leschziner, 2010 [http://www.ncbi.nlm.nih.gov/pubmed/20888964]

Comparison with RCT: Chandramouli et al. 2011 [http://www.ncbi.nlm.nih.gov/pubmed/21536134]

Picking particles

There are two choices: APPION-based picking [http://www.ncbi.nlm.nih.gov/pubmed?term=tiltpicker] and manual picking. Since Web (SPIDER) does not work on our workstations we have been using XMIPP:

(1.) Place micrographs into working directories

Symbolically link 'untilted' micrographs to folder '00' and 'tilted' micrographs to '01:'

```
ln -s /leginon/michael/10sep12a/rawdata/*en_00.mrc 00/
ln -s /leginon/michael/10sep12a/rawdata/*en_01.mrc 01/
```

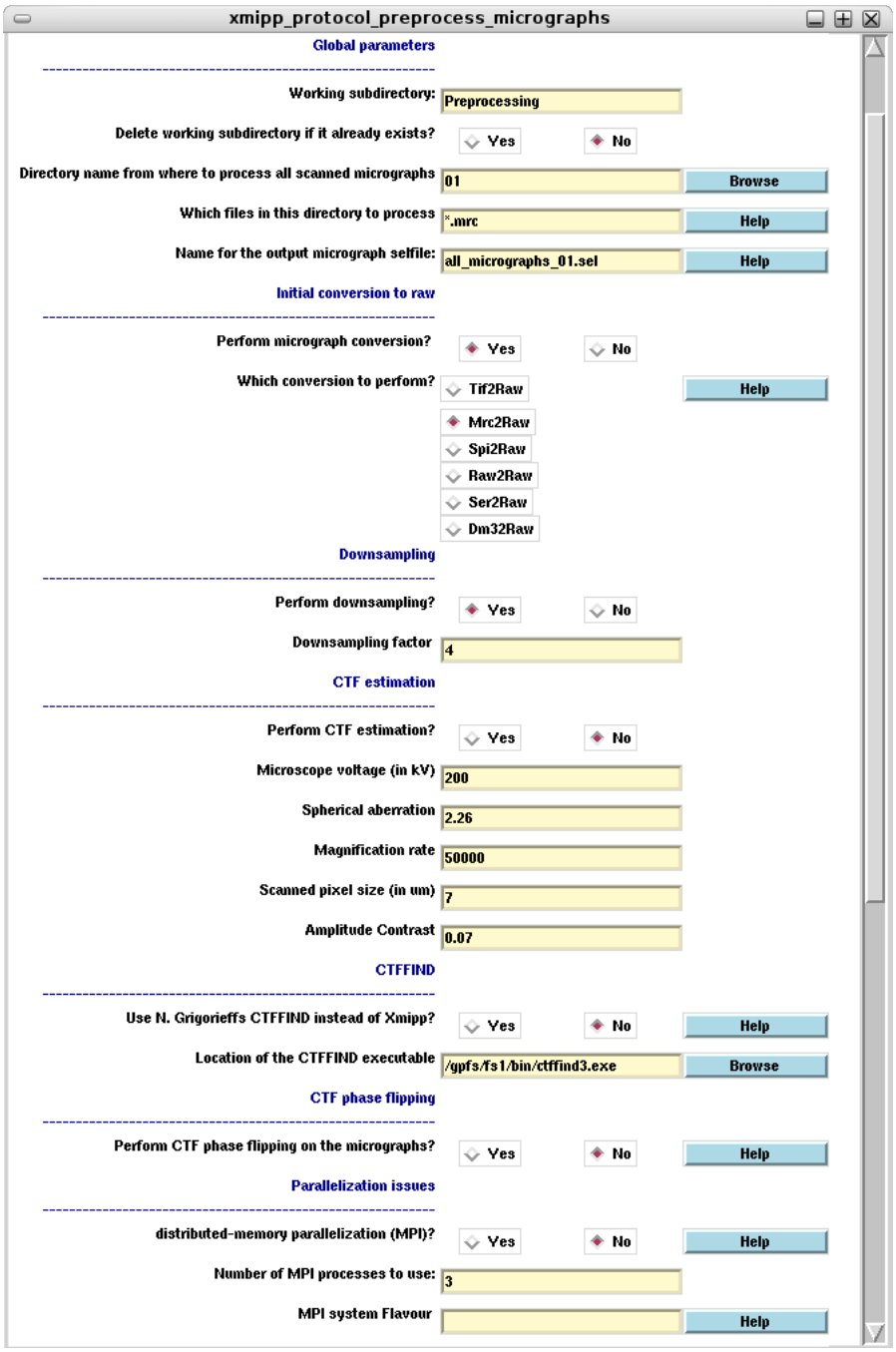
(2.) Import micrographs into XMIPP format

In the folder *above* 00/ and 01/, start xmipp_protocols:

```
[michael@angula manualOTR ]$ xmipp_protocols
 + Xmipp_protocols was never executed before in this directory.
 + Make sure you run xmipp_protocols only in your project directory.
 + You are in directory: /local/michael/TFIID_IIA_SCP1/Negative_stain/OTR/Preprocessing
 + Do you want to setup Xmipp protocols here? [y/n]:y
```

Click on **Preprocess Micrographs**

After inputing & selecting the options shown below, click **Save & Execute**:



Perform the same command for '00' with the **Preprocess Micrographs** GUI.

(3.) Create select file for tilt pairs

Copy create_tiltSelFiles.py from:

```
~michael/BATCHLIB/OTR_scripts/TiltPickingXMIPP/
```

Within the *PreProcessing* directory created by xmipp_protocols, run:

```
./create_tiltSelFiles.py > all_tilts.sel
```

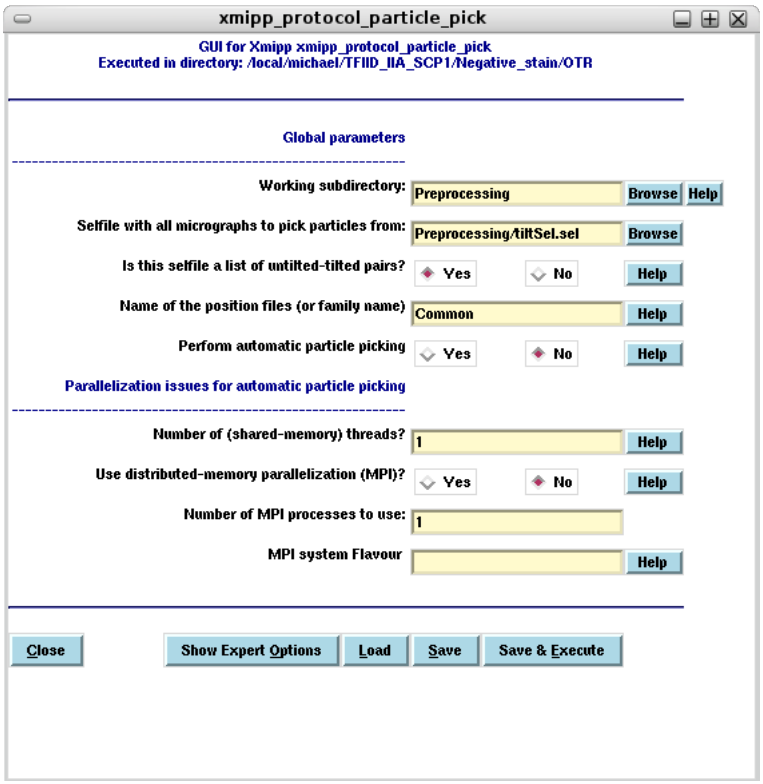
This outputs the names of tilt mates in the proper XMIPP format:

```
<tilt_00.raw>   <tilt_01.raw>   1
```

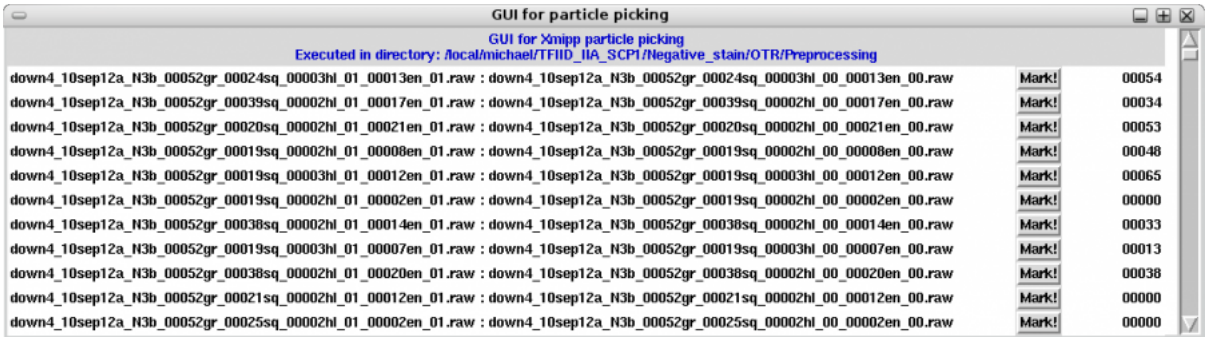
- 1 - denotes that the tilt mates are 'active' for picking

(4.) Picking tilt pairs

Click on **Particle Selection** from the main xmipp_protocols GUI and the following window opens:

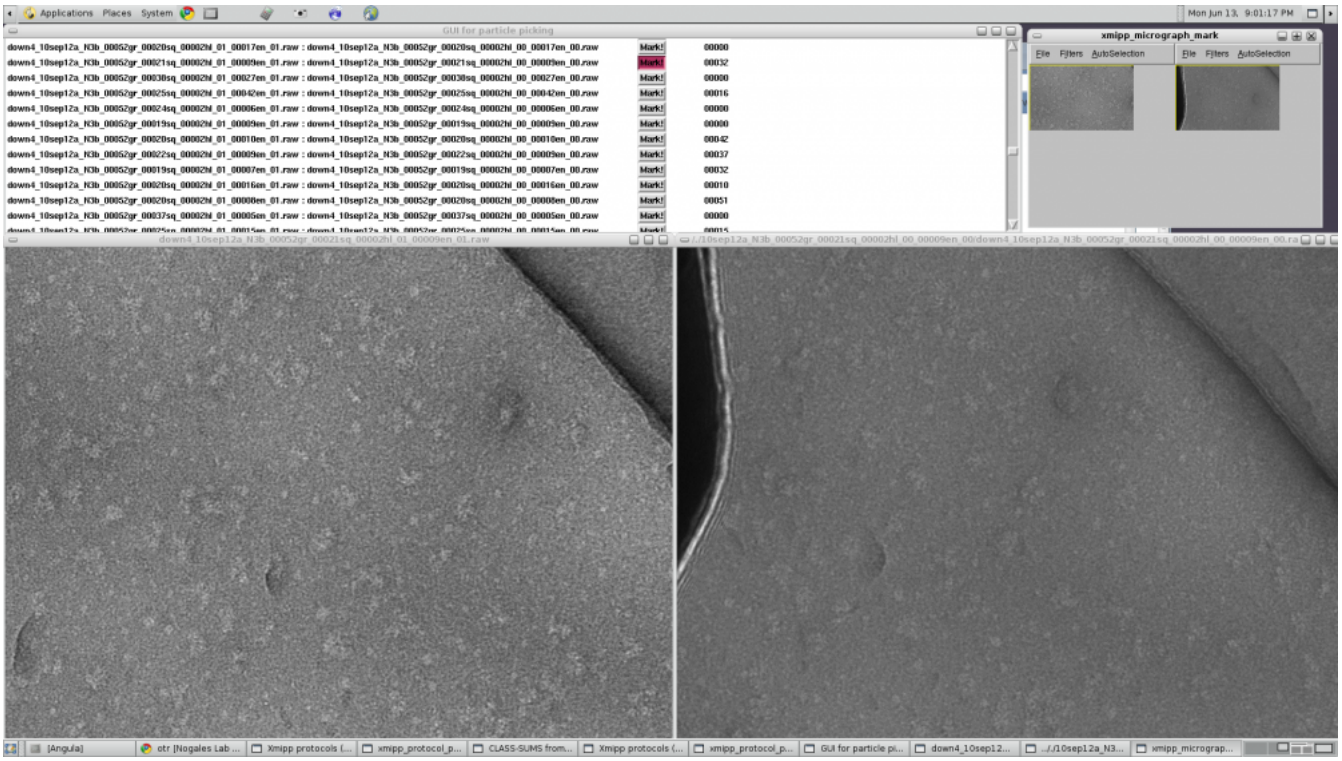


Click **Save & Execute** to get to xmipp_mark [http://xmipp.cnb.csic.es/twiki/bin/view/Xmipp/Mark].



This first GUI organizes all of the tilt pairs for picking, allowing you save your picking progress and come back anytime to finish picking.

Clicking on **Mark!** will open tilted micrographs for picking:



xmipp_mark will slowly learn the angle between the micrographs, getting better at picking tilt mates as you pick more particles.

(5.) Saving particle coordinates

When you are finished picking particles, be sure to save the coordinates & angles for **each micrograph** by clicking **File** -> **Save Coords** and **File** -> **Save angles**.

Extracting particles

All scripts in this section can be found:

```
~michael/BATCHLIB/OTR_scripts/extract/
```

(1.) Convert XMIPP coordinates (.pos) to BOXER coordinates (.box)

Copy all files (.Common.pos) within the Preprocessing folder to a new folder, along with *raw2boxdb2.pl* and *raw2boxdb_all.csh*. After editing the filenames within *raw2boxdb_all.csh*, run:

```
./raw2boxdb_all.csh
```

This will output .box filenames that will correspond directly to micrograph names within leginon/, allowing you to import the box files into APPION or use batcboxer for making the stack.

(2.) Extract particles using batch boxer

Symbolically link all of your micrographs to your working directory:

```
ln -s /leginon3/michael/12feb01b/rawdata/*en_??.mrc .
```

Extract particles:

```
./batchBoxer.csh 00 4 stack_00.hed
```

- 00 - extension of micrographs/box files
- 4 - scaling factor used while picking particles
- stack_00.hed - output stack name

Repeat for 01 particles.

Make sure that there are **no edges** or bad particles within either of the stacks. If you do find particles that you would like to remove run, copy down the EMAN number (when viewed using EMAN) into a .txt file. Then run:

```
proc2d stack_01_norm_dc4_64.hed stack_01_norm_dc4_64_sel.hed exclude=bad.txt
```

- bad.txt - list with EMAN numbers for 'bad' particles

Calculating tilts between micrographs with CTFTILT

Rename micrographs into a numbering scheme (this is the only way that CTFTILT will take filenames):

```
./makeNumberedMicros.py
```

Outputs:

- outputMicroList.spi - SPIDER list of micrograph numbers
- mrc_list.txt - list of files for ctftilt

Run CTFTILT:

```
./ctfTilt.py mrcList.txt ctfTiltPythonTest.txt.txt > ctfTiltparsed.txt
```

- mrcList.txt - list of micrographs without .mrc extension
- ctfTiltPythonTest.txt - template columns
- ctfTiltparsed.txt - output file with tilt info

To parse into a SPIDER friendly format:

```
./ctfTilt_Parser.py ctfTiltparsed.txt ctfTiltparsed
```

- ctfTiltparsed - output basename for [basename]_tilt.spi & [basename]_until.spi files

2D alignment of particles

Using the 'good' tilted particles or merged stack of both tilts, perform 2D reference-free alignment with autoAlign or MSA-MRA.

Calculate class volumes

(1.) Select iteration of 2D reference-free alignment from autoAlign or MSA-MRA that looks good

Output particle membership info and rotation/shifts. Rotation & shifts should come from **previous** iteration:

```
IMAGIC-COMMAND : header

  ** HEADERS (vs. 8-Oct-2010) welcomes you **


Options available:
  READ/LOOK  WRITE/SET  HOW_MANY   PLT_OUT    TAKE_OVER
  SORT       COMPARE    HISTOGRAM  MEANINGS
Please specify option [PLT]                        :

Read options available:
  ALIGNMENT               MOVE(ROTATION&SHIFT)
  MSA/CLASSIFICATION      THREED_RECONSTRUCTION
  ANGULAR_RECONSTITUTION  PROJECTION_MATCHING
  EULER_ANGLES            INDEX/LABEL
Please specify option [ALIGN]                      : MOVE
Input (header) file, loc#s [current_mra]           :
PLT output file to store values [rotAngles.plt] :

IMAGIC-COMMAND : header

  ** HEADERS (vs. 8-Oct-2010) welcomes you **


Options available:
  READ/LOOK  WRITE/SET  HOW_MANY   PLT_OUT    TAKE_OVER
  SORT       COMPARE    HISTOGRAM  MEANINGS
Please specify option [PLT]                        : plt

Read options available:
  ALIGNMENT               MOVE(ROTATION&SHIFT)
  MSA/CLASSIFICATION      THREED_RECONSTRUCTION
  ANGULAR_RECONSTITUTION  PROJECTION_MATCHING
  EULER_ANGLES            INDEX/LABEL
Please specify option [ALIGN]                      : MSA
Input (header) file, loc#s [current_mra]           :
PLT output file to store values [membership.plt] :
```

(2.) Convert each IMAGIC output into SPIDER readable format

```
cat membership.plt | gawk '{printf ("%5d %1d  %-6.2f \n",NR,1,$0)}' >> membership.spi

cat rotAngles.plt | gawk '{printf ("%5d   %1d   %-6.6f     %-6.6f     %-6.6f \n",NR,3,$2,$3,$4)}' >> rotAngles.spi
```

(3.) Generate angular file for each particle

Run the following for MSA-MRA: (or run shiftRotImagicParams_autoAlign.spi if you used autoAlign)

```
[michael@angula reconstruction]$ spider spi @shiftRotImagicParams

 \__`O O'__/        SPIDER  --  COPYRIGHT
 ,__xXXXx___        HEALTH RESEARCH INC., ALBANY, NY.
   __xXXXx__
 /  /xxx\  \        VERSION:  UNIX  18.10 ISSUED: 03/23/2010
   /     \          DATE:     14-JUN-2011    AT  16:58:34



 Results file: results.spi.0
 Running: spider
 .?Input the rotation & shift file from IMAGIC?: rotAngles
 .?Input the class membership file from IMAGIC?: membership
 .?Input particle-micrograph list?: micrographs_list
 .?Input basename from ctfTilt_Parser.py?: ctfTiltparsed
```

Output

The output ctfTiltparsed_angular.spi will have the euler angles calculated by taking into account the in-plane rotations from MRA with the tilt angles between micrographs (see Leschziner, 2010 [http://www.ncbi.nlm.nih.gov/pubmed/20888964] for equations to calculate euler angles):

```
    1 3   3.7200      -94.700       165.19
    2 3   3.7200      -94.700       96.541
    3 3   3.7200      -94.700       8.4850
    4 3   3.7200      -94.700       157.41
    5 3   3.7200      -94.700       223.97
    6 3   3.7200      -94.700       153.73
    7 3   3.7200      -94.700       87.393
    8 3   3.7200      -94.700       204.88
    9 3   3.7200      -94.700       291.60
```

(5.) Calculate class volumes

By combining the per-particle euler angles with select files of particles in each classum, you can calculate the 3D volumes of each class:

```
[michael@angula reconstruction2]$ spider spi @bp3f_filter_OTR_volumes

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   __xXXXx__
 /  /xxx\  \        VERSION:  UNIX  18.10 ISSUED: 03/23/2010
   /     \          DATE:     14-JUN-2011    AT  17:09:18



 Results file: results.spi.3
 Running: spider
 .?Input basename of select files?: sel_class
 .?Input tilted stack?: OTR_norm_dc4_sel3_01
 .?Number of classums?: 23
 .?Angular file?: ctfTiltparsed_merge_angular
 .?Pixel size?: 6
```

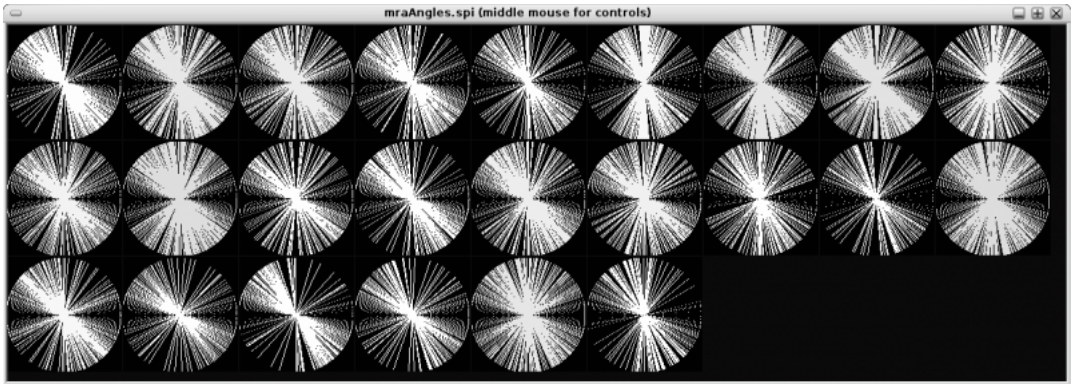
And if you would like to center the particles against the initially calculated volume, run *bp3f_filter_refine_OTRvols_FSC.spi*.

Evaluating model quality

Please see Chandramouli et al. 2011 [http://www.ncbi.nlm.nih.gov/pubmed/21536134] for a more detailed discussion in evaluating OTR model quality.

(1.) Angular bias

This script will plot the distribution of in-plane rotations within classums:



To run:

```
[michael@angula reconstruction_classsums9]$ spider spi @createMRAplot

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   __xXXXx__
 /  /xxx\  \        VERSION:  UNIX  18.10 ISSUED: 03/23/2010
   /     \          DATE:     14-JUN-2011    AT  18:56:47



 Results file: results.spi.5
 Running: spider
 .?Input MRA angle output from IMAGIC?: rotAngles
 .?Input select file basename?: classsums9_class_
 .?Number of averages?: 23
 .?Name of output stack?: MRAplot
```

And can be found:

```
~michael/BATCHLIB/OTR_scripts/evaluate/
```

(2.) Fourier Ring Correlation between models & reference-free class averages

See Model Validation by 2D reference-free averages.

E-mail to Webmaster