

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 'untitled' micrographs to folder '00' and 'tilted' micrographs to '01.'

```
ln -s /legion/michael/10sep12a/rawdata/*en_00.mrc 00/  
ln -s /legion/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**:

Global parameters

Working subdirectory:

Delete working subdirectory if it already exists? ☐ Yes ☒ No

Directory name from where to process all scanned micrographs

Which files in this directory to process

Name for the output micrograph selffile:

Initial conversion to raw

Perform micrograph conversion? ☒ Yes ☐ No

Which conversion to perform? ☐ Tif2Raw ☒ Mrc2Raw ☐ Spi2Raw ☐ Raw2Raw ☐ Ser2Raw ☐ Dm32Raw

Downsampling

Perform downsampling? ☒ Yes ☐ No

Downsampling factor

CTF estimation

Perform CTF estimation? ☐ Yes ☒ No

Microscope voltage (in kV)

Spherical aberration

Magnification rate

Scanned pixel size (in um)

Amplitude Contrast

CTFFIND

Use N. Grigorieffs CTFFIND instead of Xmipp? ☐ Yes ☒ No

Location of the CTFFIND executable

CTF phase flipping

Perform CTF phase flipping on the micrographs? ☐ Yes ☒ No

Parallelization issues

distributed-memory parallelization (MPI)? ☐ Yes ☒ No

Number of MPI processes to use:

MPI system Flavour

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:

xmipp_protocol_particle_pick

GUI for Xmipp xmipp_protocol_particle_pick
Executed in directory: /local/michael/TFIID_ILA_SCP1/Negative_stain/OTR

Global parameters

Working subdirectory:

Preprocessing

Browse

Help

Selffile with all micrographs to pick particles from:

Preprocessing/tiltSel.sel

Browse

Is this selffile a list of untilted-tilted pairs?

Yes

No

Help

Name of the position files (or family name)

Common

Help

Perform automatic particle picking

Yes

No

Help

Parallelization issues for automatic particle picking

Number of (shared-memory) threads?

1

Help

Use distributed-memory parallelization (MPI)?

Yes

No

Help

Number of MPI processes to use:

1

MPI system Flavour

Help

Close

Show Expert Options

Load

Save

Save & Execute

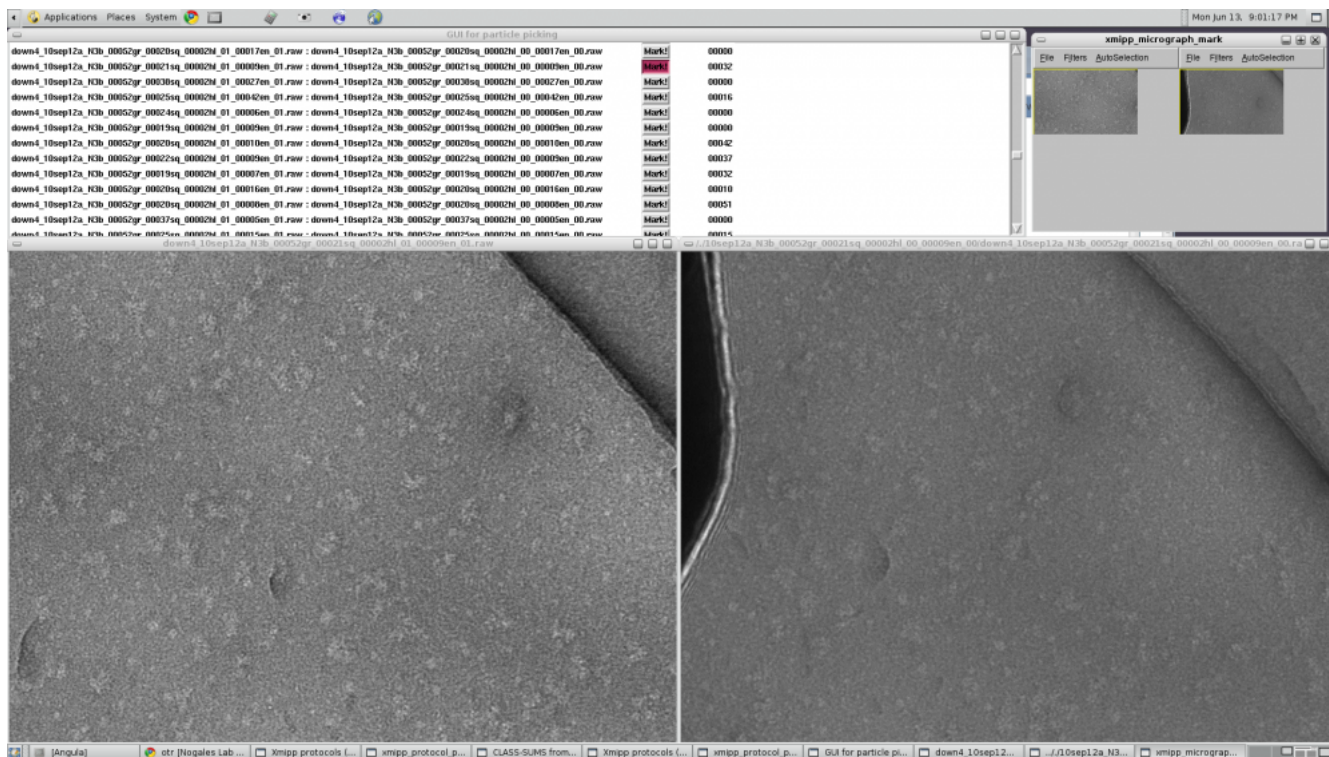
Click **Save & Execute** to get to xmipp_mark (<http://xmipp.cnb.csic.es/twiki/bin/view/Xmipp/Mark>).

GUI for particle picking

GUI for Xmipp particle picking
Executed in directory: /local/michael/TFIID_ILA_SCP1/Negative_stain/OTR/Preprocessing

down4_10sep12a_N3b_00052gr_00024sq_00003hi_01_00013en_01.raw	: down4_10sep12a_N3b_00052gr_00024sq_00003hi_00_00013en_00.raw	Mark!	00054
down4_10sep12a_N3b_00052gr_00039sq_00002hi_01_00017en_01.raw	: down4_10sep12a_N3b_00052gr_00039sq_00002hi_00_00017en_00.raw	Mark!	00034
down4_10sep12a_N3b_00052gr_00020sq_00002hi_01_00021en_01.raw	: down4_10sep12a_N3b_00052gr_00020sq_00002hi_00_00021en_00.raw	Mark!	00053
down4_10sep12a_N3b_00052gr_00019sq_00002hi_01_00008en_01.raw	: down4_10sep12a_N3b_00052gr_00019sq_00002hi_00_00008en_00.raw	Mark!	00048
down4_10sep12a_N3b_00052gr_00019sq_00003hi_01_00012en_01.raw	: down4_10sep12a_N3b_00052gr_00019sq_00003hi_00_00012en_00.raw	Mark!	00065
down4_10sep12a_N3b_00052gr_00019sq_00002hi_01_00002en_01.raw	: down4_10sep12a_N3b_00052gr_00019sq_00002hi_00_00002en_00.raw	Mark!	00000
down4_10sep12a_N3b_00052gr_00038sq_00002hi_01_00014en_01.raw	: down4_10sep12a_N3b_00052gr_00038sq_00002hi_00_00014en_00.raw	Mark!	00033
down4_10sep12a_N3b_00052gr_00019sq_00003hi_01_00007en_01.raw	: down4_10sep12a_N3b_00052gr_00019sq_00003hi_00_00007en_00.raw	Mark!	00013
down4_10sep12a_N3b_00052gr_00038sq_00002hi_01_00020en_01.raw	: down4_10sep12a_N3b_00052gr_00038sq_00002hi_00_00020en_00.raw	Mark!	00038
down4_10sep12a_N3b_00052gr_00021sq_00002hi_01_00012en_01.raw	: down4_10sep12a_N3b_00052gr_00021sq_00002hi_00_00012en_00.raw	Mark!	00000
down4_10sep12a_N3b_00052gr_00025sq_00002hi_01_00002en_01.raw	: down4_10sep12a_N3b_00052gr_00025sq_00002hi_00_00002en_00.raw	Mark!	00000

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 *raw2boxdb.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 batchboxer for making the stack.

Extract particles and calculate tilt angles

Symbolically link all of your micrographs to your working directory:

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

To simultaneously extract particles while also generating a per-particle CTF & tilt angle file (from CTFTILT [http://www.ncbi.nlm.nih.gov/pubmed/12781660]), copy *ctftilt.py* to your working directory and edit the beginning of the file.

If you'd like to perform **Ace2 Wiener Filtering** a la APPION, run the script *ctftilt_ace2_weiner.py* instead of *ctftilt.py*.

```
#Output filenames
paramOUT1 = 'parameter_00.par' #param filename for untilted particles
paramOUT2 = 'parameter_01.par' #param filename for tilted particles
stack1 = 'stack00' #stack name for untilted particles
stack2 = 'stack01' #stack name for tilted particles

#Inputs
shrink = 4 #Binning factor for final particle stack
new = 64 #Box size for binned particles
scale = 4 #Binning factor used for micrograph from which the particles were picked

#CTFTILT inputs
parm3 = "2.2,120.0,0.2,99993,15,2\n" # !CS[mm],HT[kV],AmpCnst,XMAG,DStep[um]
parm4 = "128,400.0,8.0,2000.0,30000.0,500.0,30,5\n" #!Box,ResMin[A],ResMax[A],dFMin[A],dFMax[A],FStep
```

Then run:

```
./ctftilt.py &
```

Be patient, this will need ~2 minutes per micrograph (for CTFTILT) as it simultaneously determines the CTF of your images & makes your particle stacks. This will make the particle stacks and parameter files as specified above. The format of the parameter file is:

DEF1	DEF2	ASTIG	TILT ANGLE
5879.46	6073.91	34.13	-3.07
5879.46	6073.91	34.13	-3.07
5879.46	6073.91	34.13	-3.07
5879.46	6073.91	34.13	-3.07
5879.46	6073.91	34.13	-3.07

Inspecting each particle stack, check to see if there are any edges that have been boxed out. If there are, write down the number of the particle in a text file using the EMAN numbering scheme ($i = i - 1$). Chances are that you'll be inspecting the particles using EMAN.

To exclude the bad particles:

```
proc2d stack00.hed stack00_sel.hed exclude=bad.txt
```

To exclude corresponding lines from parameter files:

```
./select_params.py
Usage: select_params.py -p <parameter> -b <exclude>

Options:
-h, --help      show this help message and exit
-p FILE         Parameter file with per-particle CTF information from ctftilt.py
-l FILE         List of particles (numbered in EMAN format)
-o FILE         Output filename
-b             Flag if particle list is for particles to EXCLUDE
-g             Flag if particle list is for particles to INCLUDE
-d             debug
```

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'__/\
, _xxxxx_   SPIDER -- COPYRIGHT
/_xxxxx_    HEALTH RESEARCH INC., ALBANY, NY.
/_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
```

```

\__`O O'__/      SPIDER -- COPYRIGHT
, _xxxxx_      HEALTH RESEARCH INC., ALBANY, NY.
, _xxxxx_
/ _/xxx\__      VERSION:  UNIX  18.10  ISSUED: 03/23/2010
/ _/xxx\__      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 classsums?: 23
.?Angular file?: ctftiltparsed_merge_angular
.?Pixel size?: 6

```

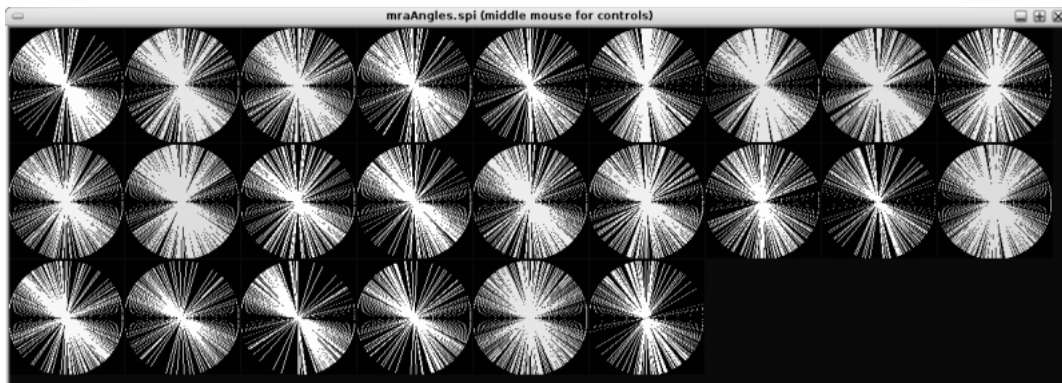
And if you would like to center the particles against the initially calculated volume, run `bp3f_filter_refine_OTRvols_FSC.spi`.

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 classsums:



To run:

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

```

\__`O O'__/      SPIDER -- COPYRIGHT
, _xxxxx_      HEALTH RESEARCH INC., ALBANY, NY.
, _xxxxx_
/ _/xxx\__      VERSION:  UNIX  18.10  ISSUED: 03/23/2010
/ _/xxx\__      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](#).

otr.txt - Last modified: 2012/06/07 16:18 by michael

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