
SParx for HIgh Resolution Electron Microscopy

# SPHIRE Practical

## UTMB Workshop

## May 8, 2019

# SPHIRE Pipeline

## PROJECT
Project Settings

## WINDOW
crYOLO Particle Picking
WINDOW Particle Extraction

## VIPER
RVIPER Initial 3D Modeling

## SORT3D
3D Variability Estimation
SORT3D 3D Clustering

## MOVIE (optional)
Micrograph Movie Alignment
Drift Assessment

## CTER
CTER CTF Estimation
CTF Assessment

## ISAC
ISAC2 2D Clustering
Beautifier

## MERIDIEN
MERIDIEN 3D Refinement
3D Refinement Assessment
PostRefiner

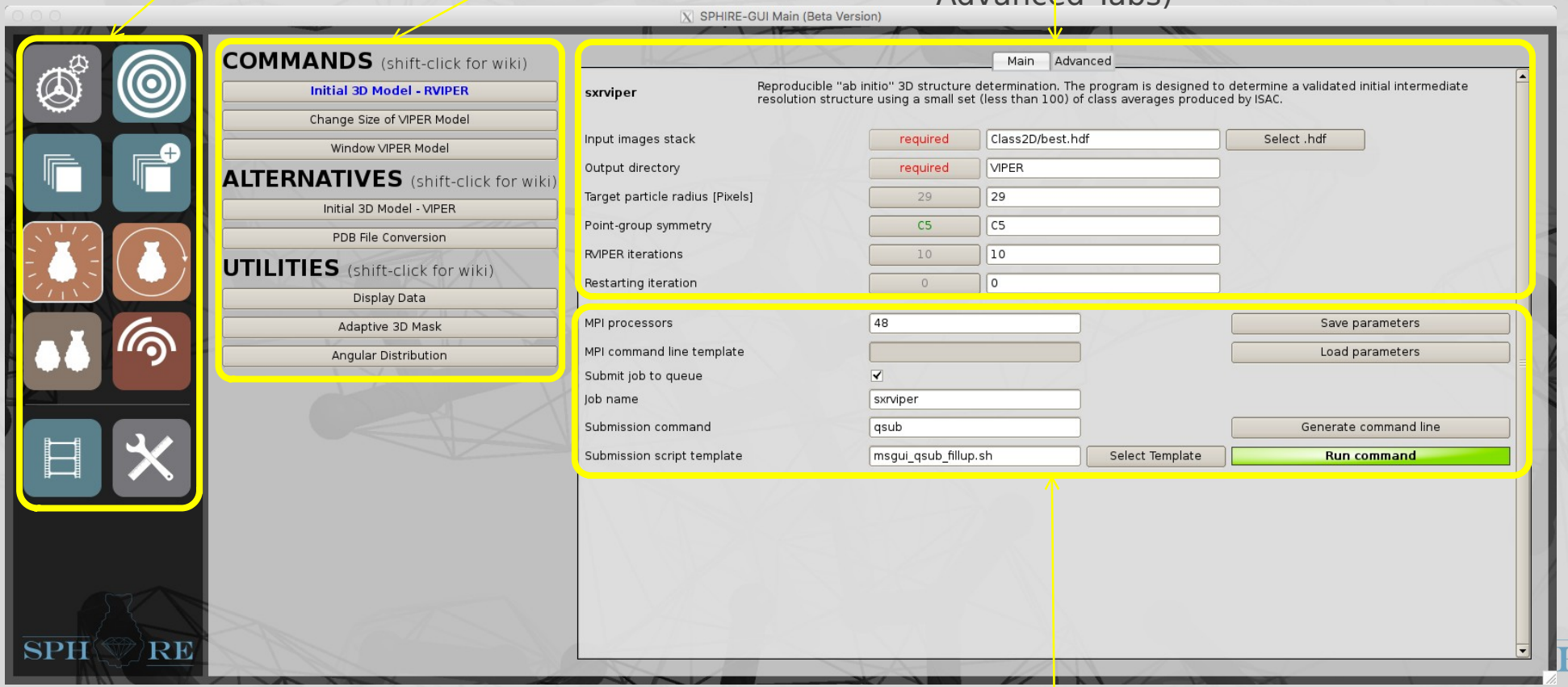
## LOCALRES
Local Resolution
3D Local Filter

## Utilities
Utility commands

SPHIRE

# GUI

1. Pipeline Steps Blocks (Icon Buttons)
2. Step Associated Commands (Buttons)
3. Command Associated Parameters (Main & Advanced Tabs)




4. Job Submission (Only in Main Tab)

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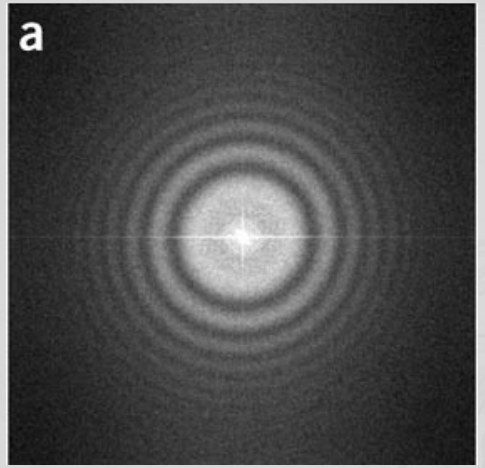
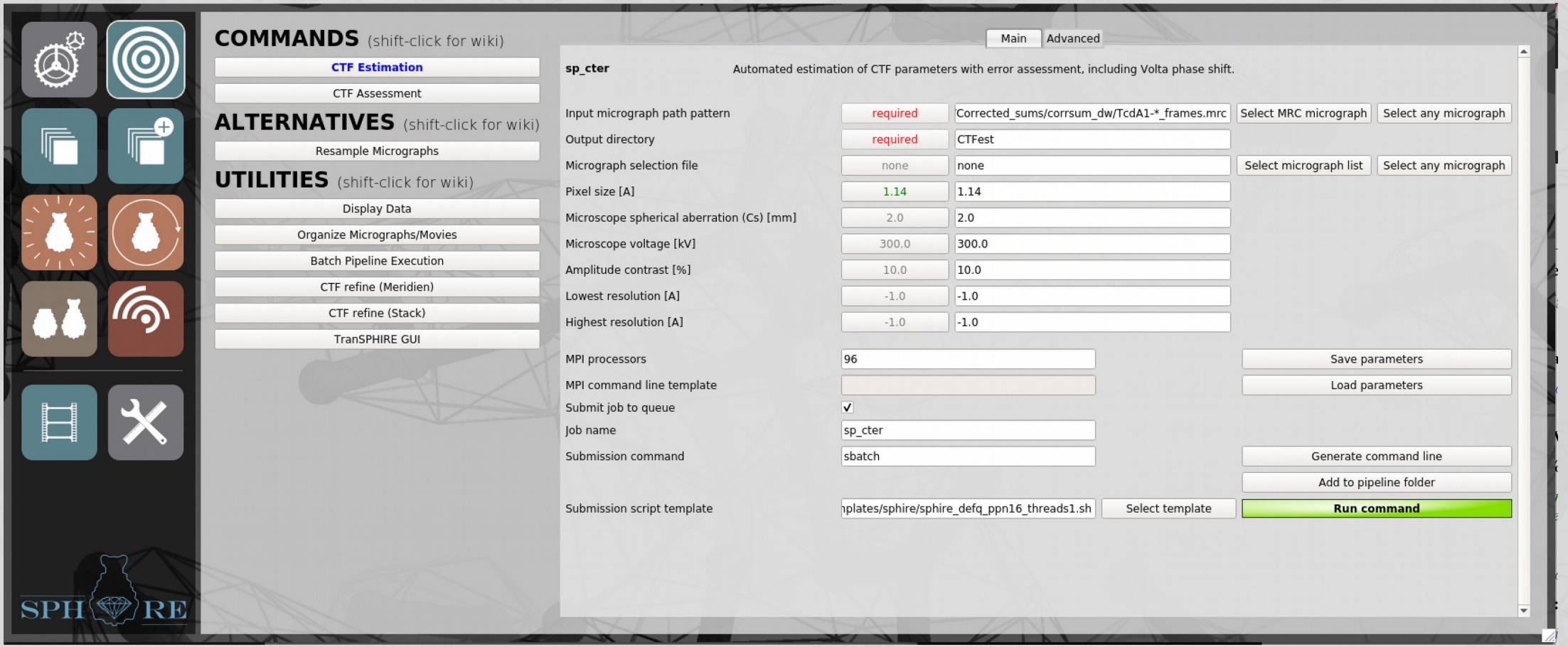



SPHIRE

# CTER


COMMANDS (shift-click for wiki)
CTF Estimation
CTF Assessment
ALTERNATIVES (shift-click for wiki)
Resample Micrographs
UTILITIES (shift-click for wiki)
Display Data
Organize Micrographs/Movies
Batch Pipeline Execution
CTF refine (Meridien)
CTF refine (Stack)
TranSPHIRE GUI
Main
Advanced
sp_cter
Automated estimation of CTF parameters with error assessment, including Volta phase shift.
Input micrograph path pattern
required
Corrected_sums/corrsum_dw/TcdA1-*_frames.mrc
Select MRC micrograph
Select any micrograph
Output directory
required
CTFest
Micrograph selection file
none
none
Select micrograph list
Select any micrograph
Pixel size [A]
1.14
1.14
Microscope spherical aberration (Cs) [mm]
2.0
2.0
Microscope voltage [kV]
300.0
300.0
Amplitude contrast [%]
10.0
10.0
Lowest resolution [A]
-1.0
-1.0
Highest resolution [A]
-1.0
-1.0
MPI processors
96
Save parameters
MPI command line template
Load parameters
Submit job to queue
Job name
sp_cter
Submission command
sbatch
Generate command line
Add to pipeline folder
Submission script template
nplates/sphire/sphire_defq_ppn16_threads1.sh
Select template
Run command

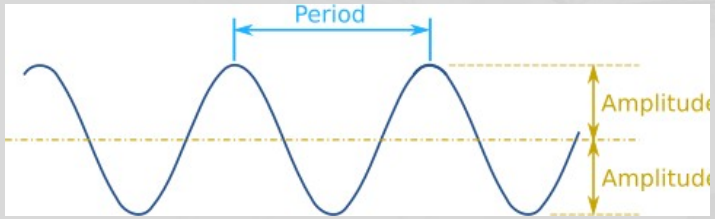
# CTER

Main | Advanced

**sp_cter** Automated estimation of CTF parameters with error assessment, including Volta phase shift.

| | | | | |
|---|---|---|---|---|
| Input micrograph path pattern | required | Corrected_sums/corrsum_dw/TcdA1-*_frames.mrc | Select MRC micrograph | Select any micrograph |
| Output directory | required | CTFest | | |
| Micrograph selection file | none | none | Select micrograph list | Select any micrograph |
| Pixel size [A] | 1.14 | 1.14 | | |
| Microscope spherical aberration (Cs) [mm] | 2.0 | 2.0 | | |
| Microscope voltage [kV] | 300.0 | 300.0 | | |
| Amplitude contrast [%] | 10.0 | 10.0 | | |
| Lowest resolution [A] | -1.0 | -1.0 | | |
| Highest resolution [A] | -1.0 | -1.0 | | |

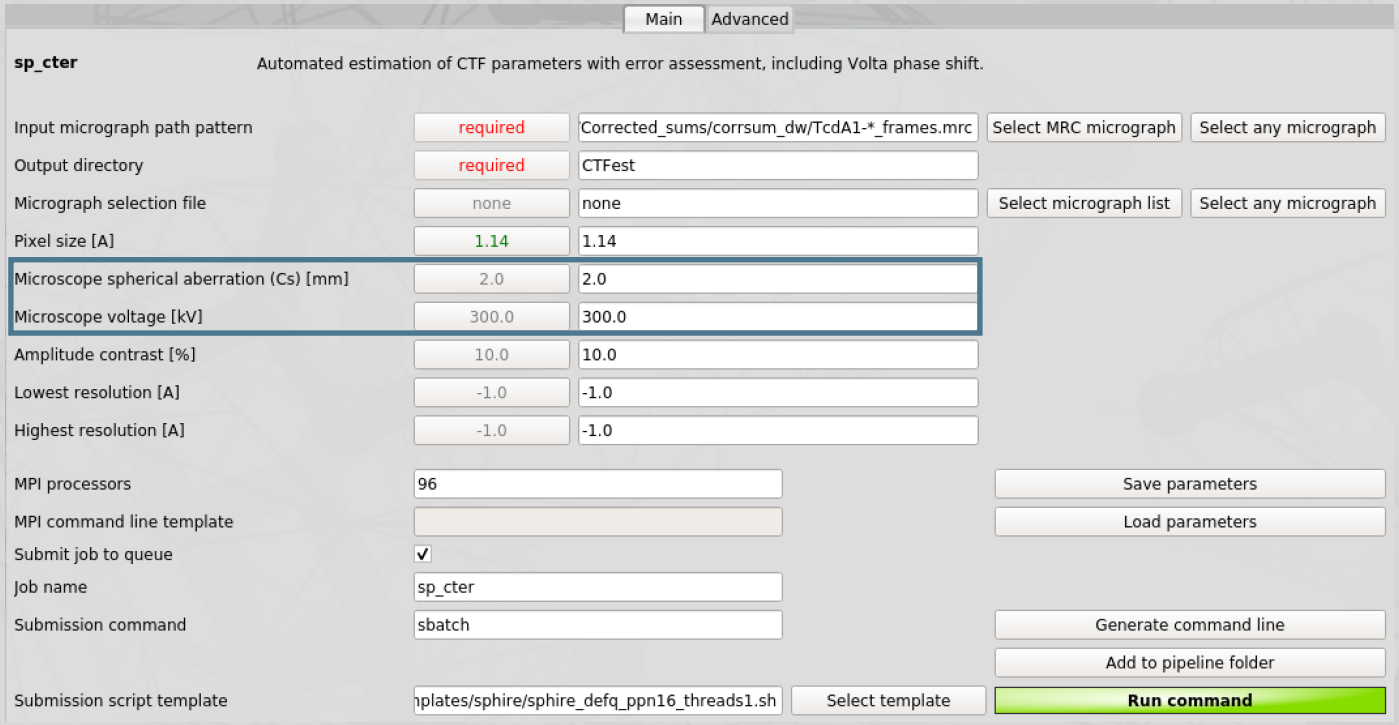
| | | | |
|---|---|---|---|
| MPI processors | 96 | | Save parameters |
| MPI command line template | | | Load parameters |
| Submit job to queue | ✓ | | |
| Job name | sp_cter | | |
| Submission command | sbatch | | Generate command line |
| | | | Add to pipeline folder |
| Submission script template | nplates/sphire/sphire_defq_ppn16_threads1.sh | Select template | **Run command** |




Max frequency is determined by **Nyquist**

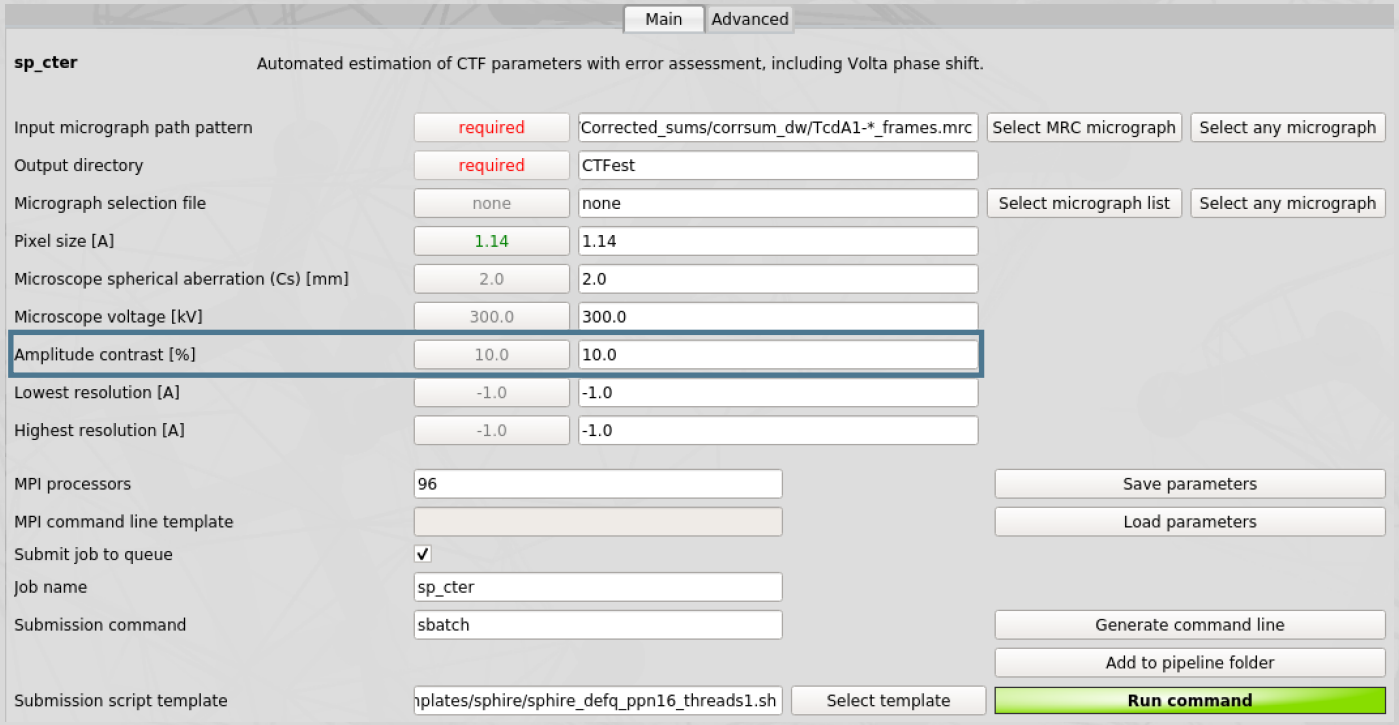
SPHIRE

# CTER



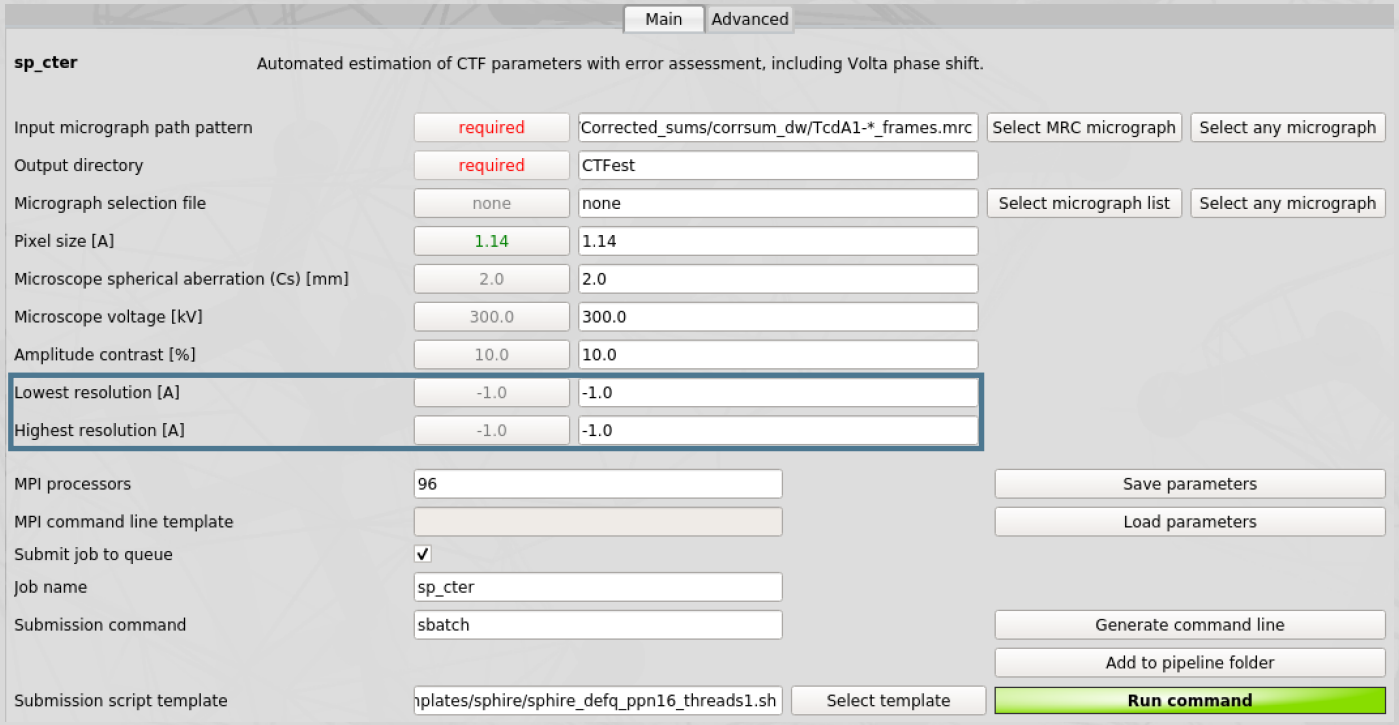

Constants of your microscope

# CTER




Initial Ac value for optimization.

# CTER



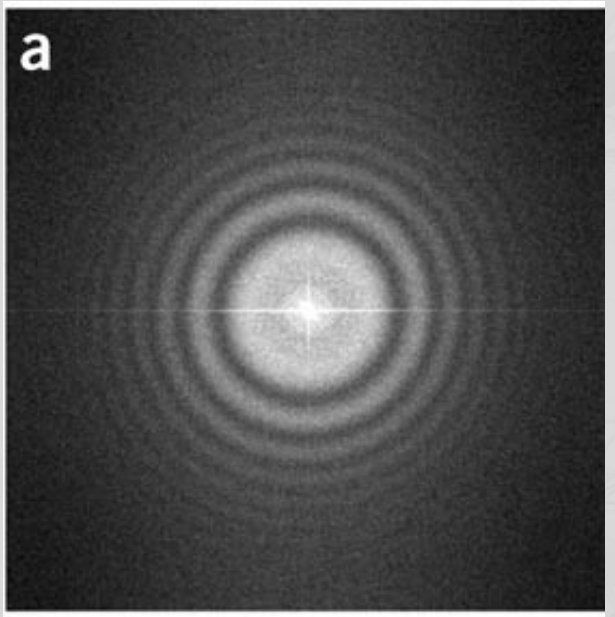

Frequency range during optimization. Fitting is done in this range. CTER estimates it automatically.

# CTER

Main | Advanced

**sp_cter** Set advanced parameters

| | | |
|---|---|---|
| CTF window size [Pixels] | 512 | 512 |
| Number of CTF estimates per micrograph | 16 | 16 |
| X overlap [%] | 50 | 50 |
| Y overlap [%] | 50 | 50 |
| Edge x [pixels] | 0 | 0 |
| Edge y [pixels] | 0 | 0 |
| Check consistency of inputs | NO | ☐ |
| Enable debug mode | NO | ☐ |
| Volta Phase Plate Dataset | NO | ☐ |
| Minimum defocus search [um] | 0.3 | 0.3 |
| Maximum defocus search [um] | 9.0 | 9.0 |
| Defocus search step [um] | 0.1 | 0.1 |
| Minimum phase search [degrees] | 5.0 | 5.0 |
| Maximum phase search [degrees] | 175.0 | 175.0 |
| Phase search step [degrees] | 5.0 | 5.0 |
| Use PW spectrum | NO | ☐ |
| Skip calculation of 2D power spectra | YES | ☑ |




How fine the range between 0 and Nyquist is sampled in the spectra is determined by the **CTF window size**

SPHIRE

# CTER

Main | Advanced

**sp_cter** Set advanced parameters

| | | |
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| Edge y [pixels] | 0 | 0 |
| Check consistency of inputs | NO | ☐ |
| Enable debug mode | NO | ☐ |
| Volta Phase Plate Dataset | NO | ☐ |
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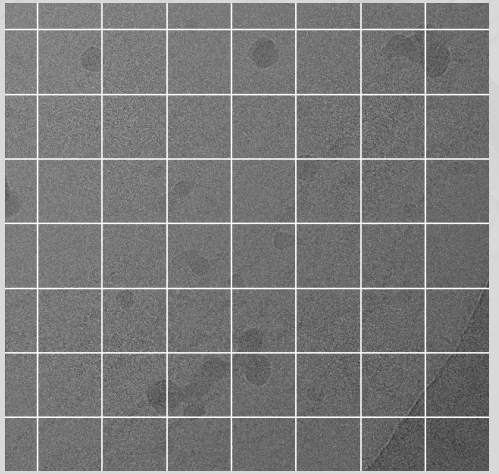
| Phase search step [degrees] | 5.0 | 5.0 |
| Use PW spectrum | NO | ☐ |
| Skip calculation of 2D power spectra | YES | ☑ |



To estimate the error for the estimated parameters CTER does the estimation multiple times for random subsets of the tiles.

# CTER

Main | Advanced

**sp_cter** Set advanced parameters

| | | |
|---|---|---|
| CTF window size [Pixels] | 512 | 512 |
| Number of CTF estimates per micrograph | 16 | 16 |
| X overlap [%] | 50 | 50 |
| Y overlap [%] | 50 | 50 |
| Edge x [pixels] | 0 | 0 |
| Edge y [pixels] | 0 | 0 |
| Check consistency of inputs | NO | ☐ |
| Enable debug mode | NO | ☐ |
| Volta Phase Plate Dataset | NO | ☐ |
| Minimum defocus search [um] | 0.3 | 0.3 |
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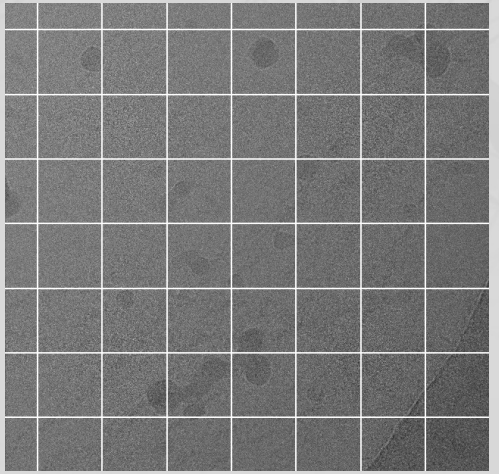
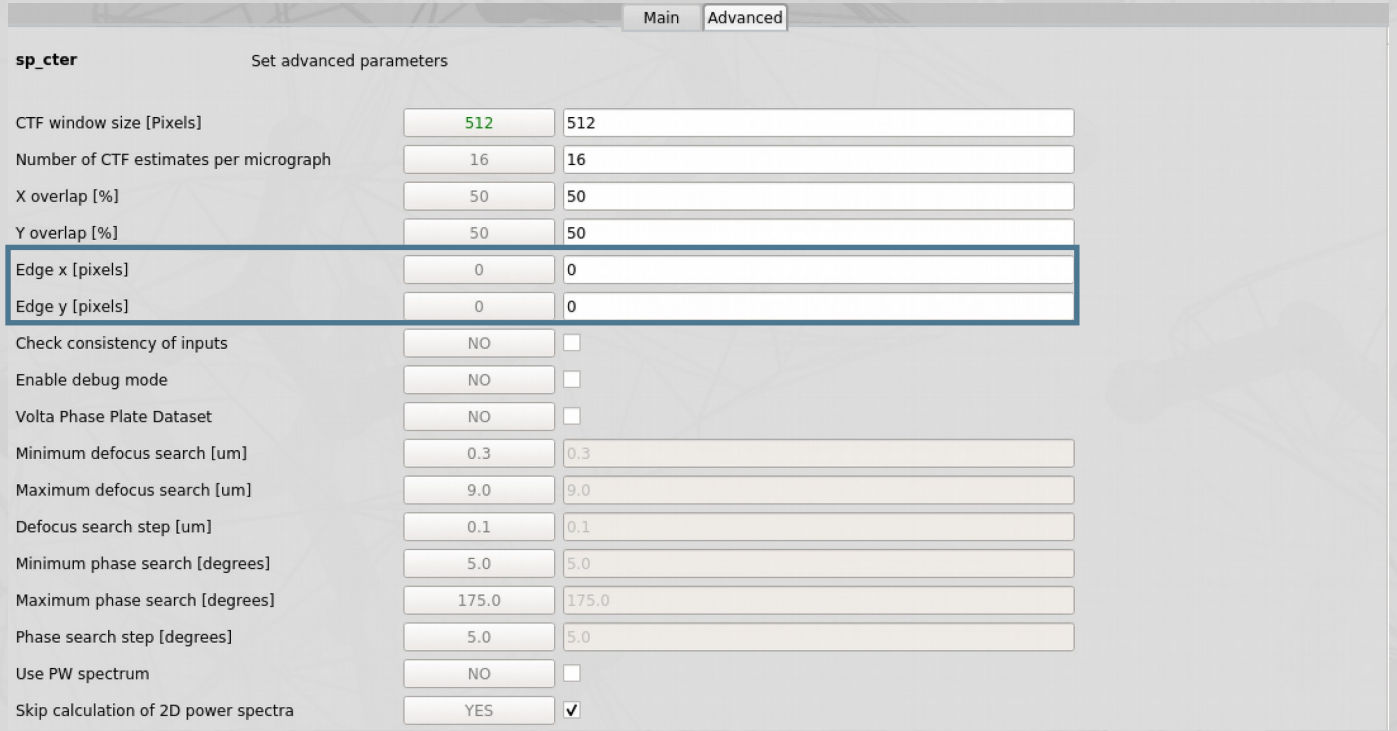
| Minimum phase search [degrees] | 5.0 | 5.0 |
| Maximum phase search [degrees] | 175.0 | 175.0 |
| Phase search step [degrees] | 5.0 | 5.0 |
| Use PW spectrum | NO | ☐ |
| Skip calculation of 2D power spectra | YES | ☑ |



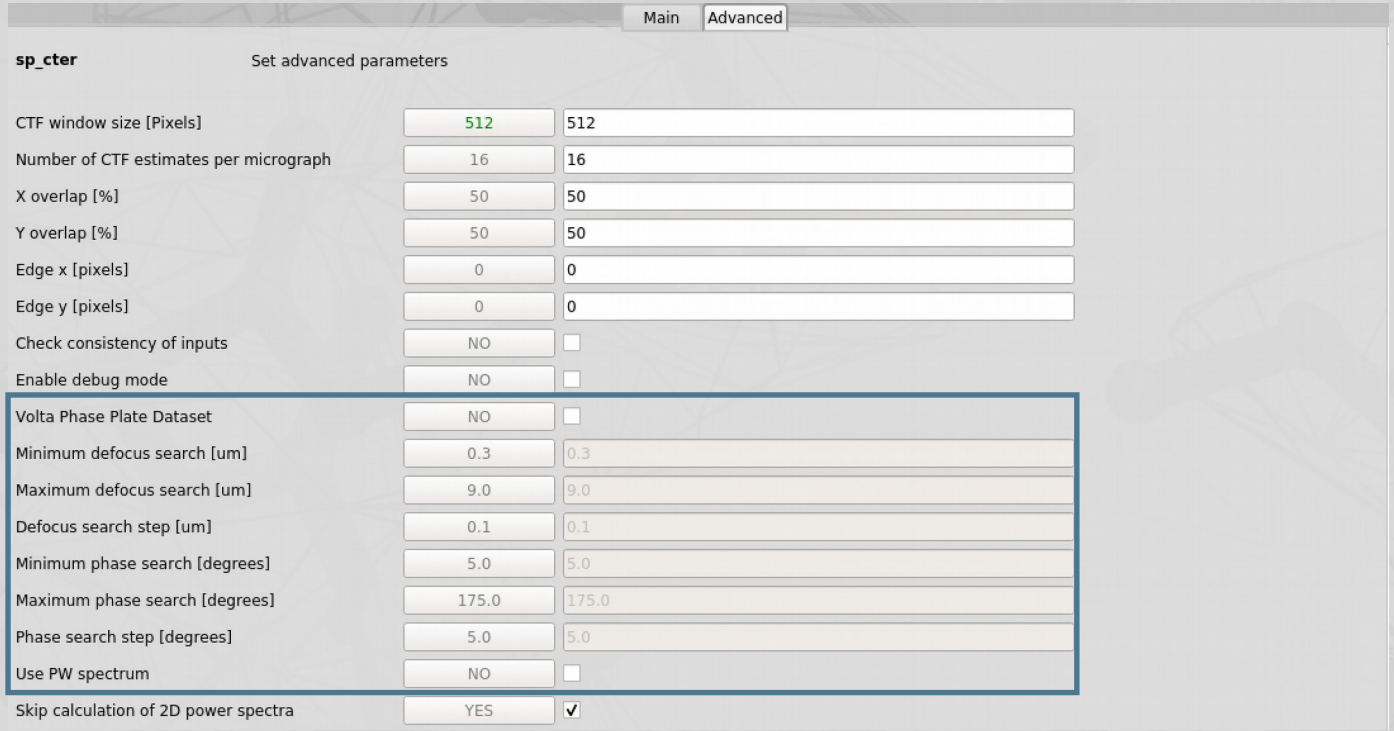
The tiles overlap.

# CTER



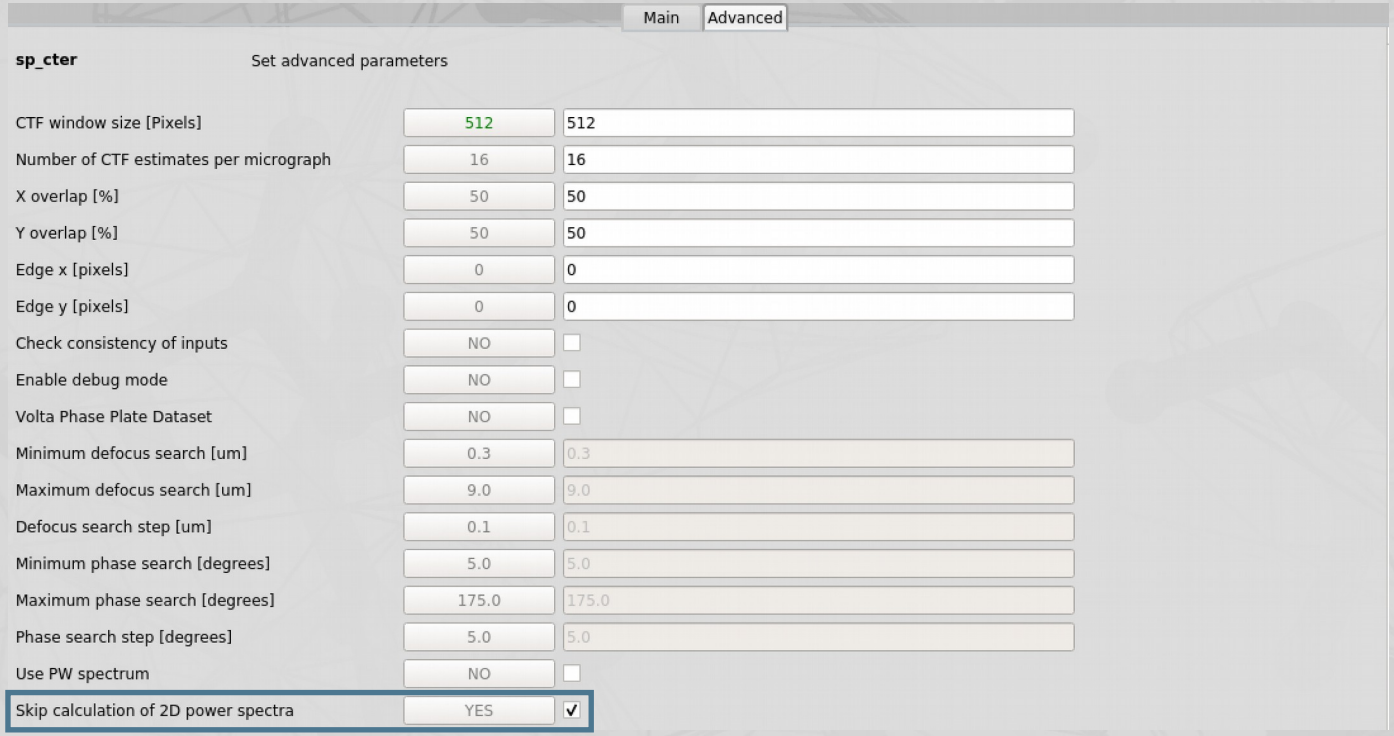

Margin in x- and y- direction of the micrograph to ignore during fitting.

# CTER



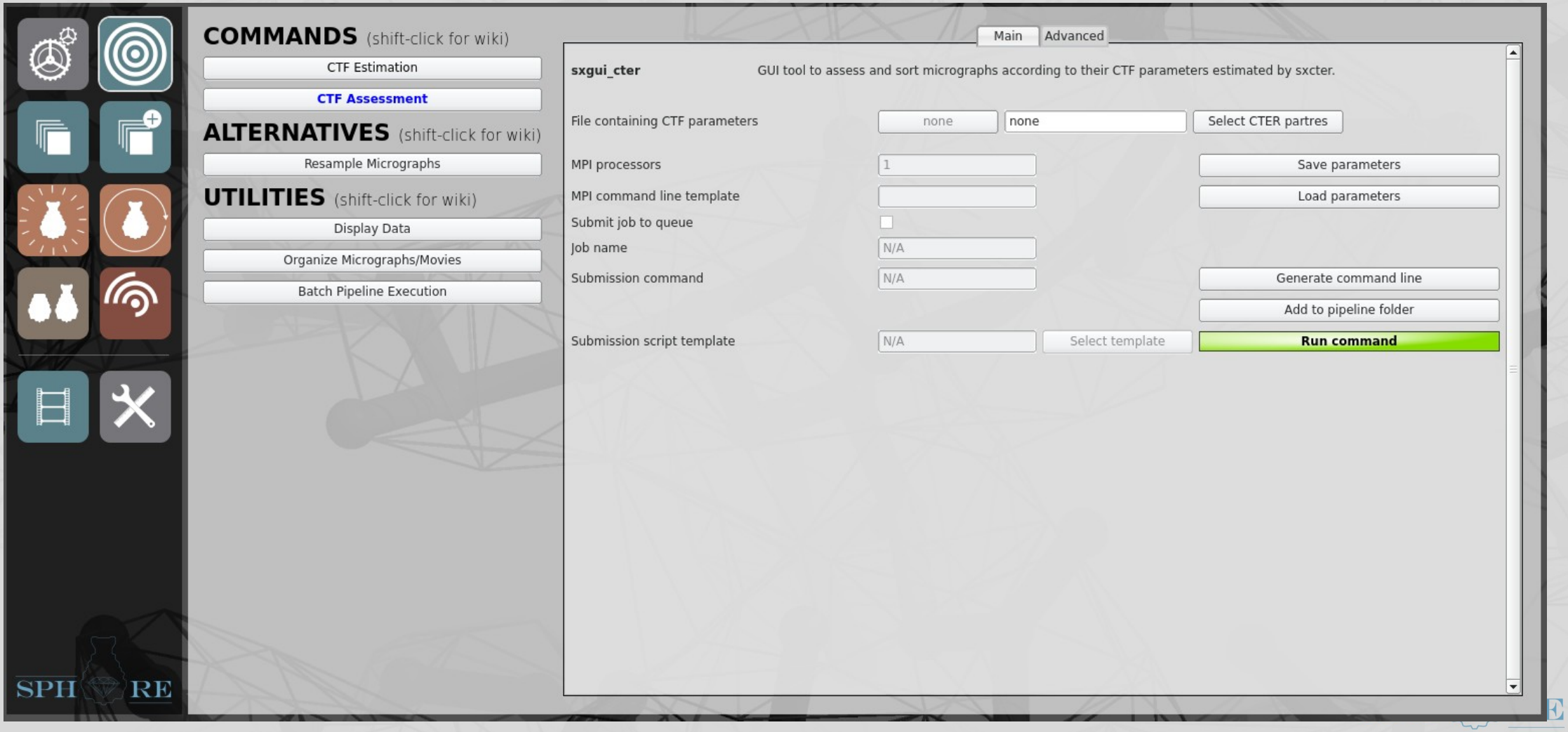

Parameters for phase plate optimization.

# CTER

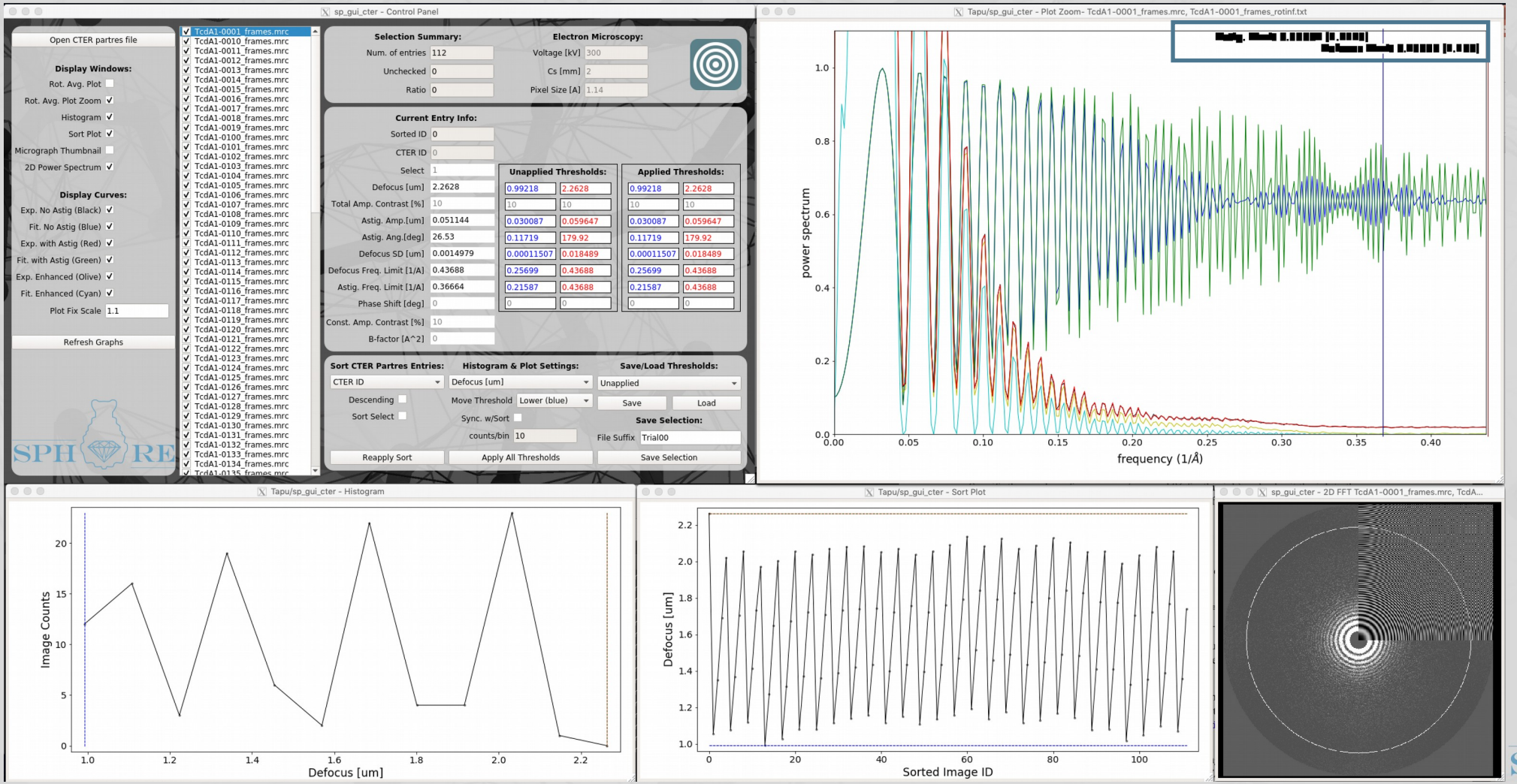



Uncheck this box to write 2D power spectra.

# CTER Assessment



# CTER Assessment




Remote glitch?

# SPHIRE Pipeline

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Combine stacks

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MERIDIEN 3D Refinement
3D Refinement Assessment
PostRefiner

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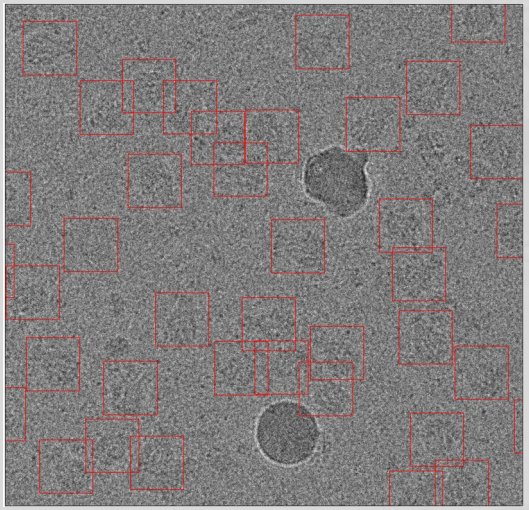
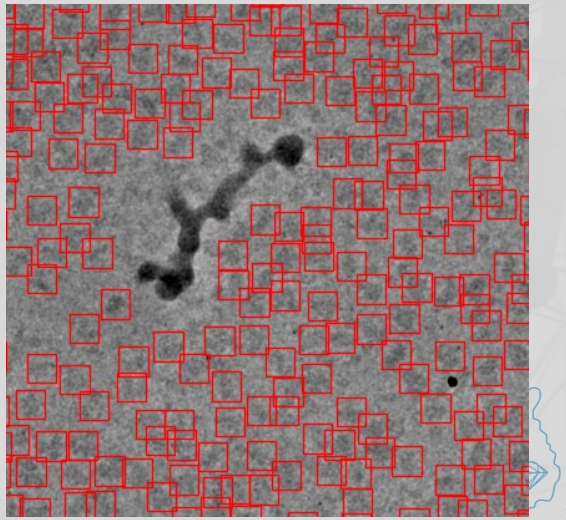
Local Resolution
3D Local Filter

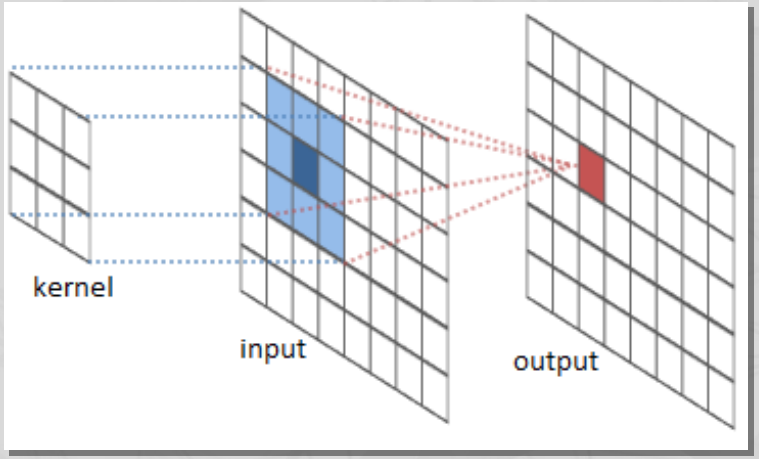
## Utilities
Utility commands





# Convolution

## What is a convolution?



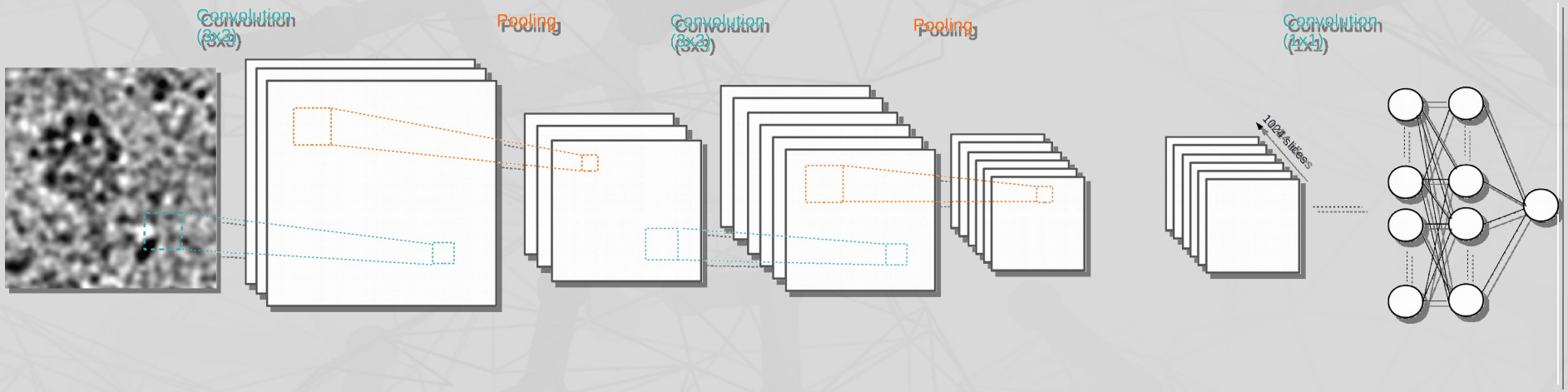

## What does a convolution do?

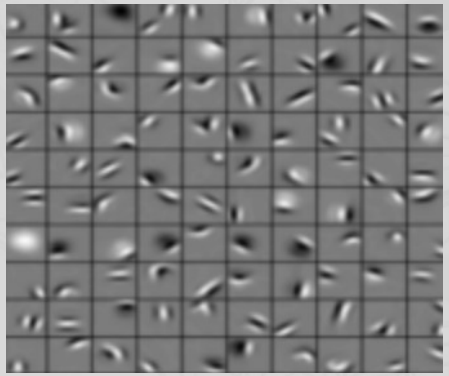

A CNN learns the elements of filter kernels and therefore features.

Trivia: An example of a familiar convolution is the contrast transfer function.

SPHERE

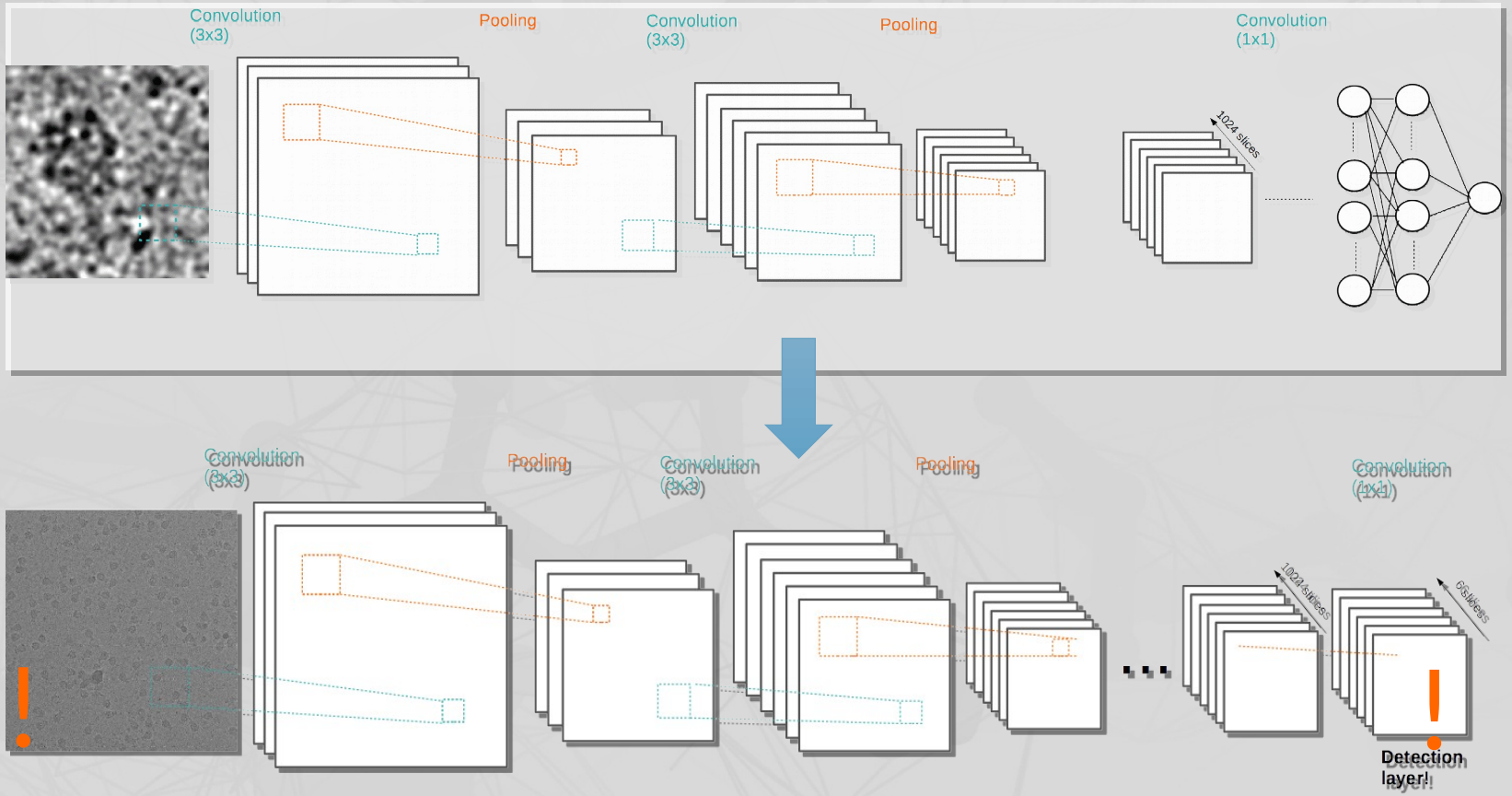
# CNNs: Hierarchies of features



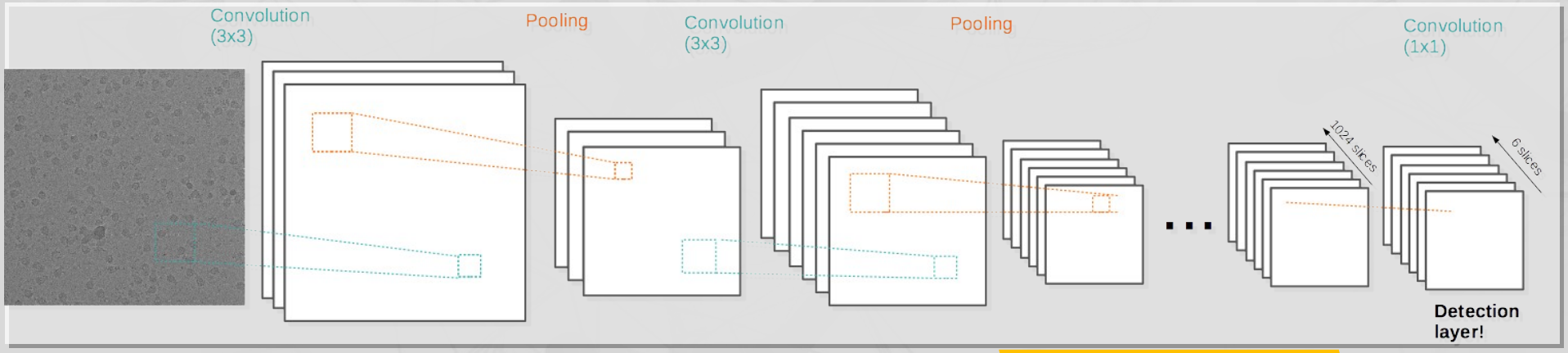
SPHERE

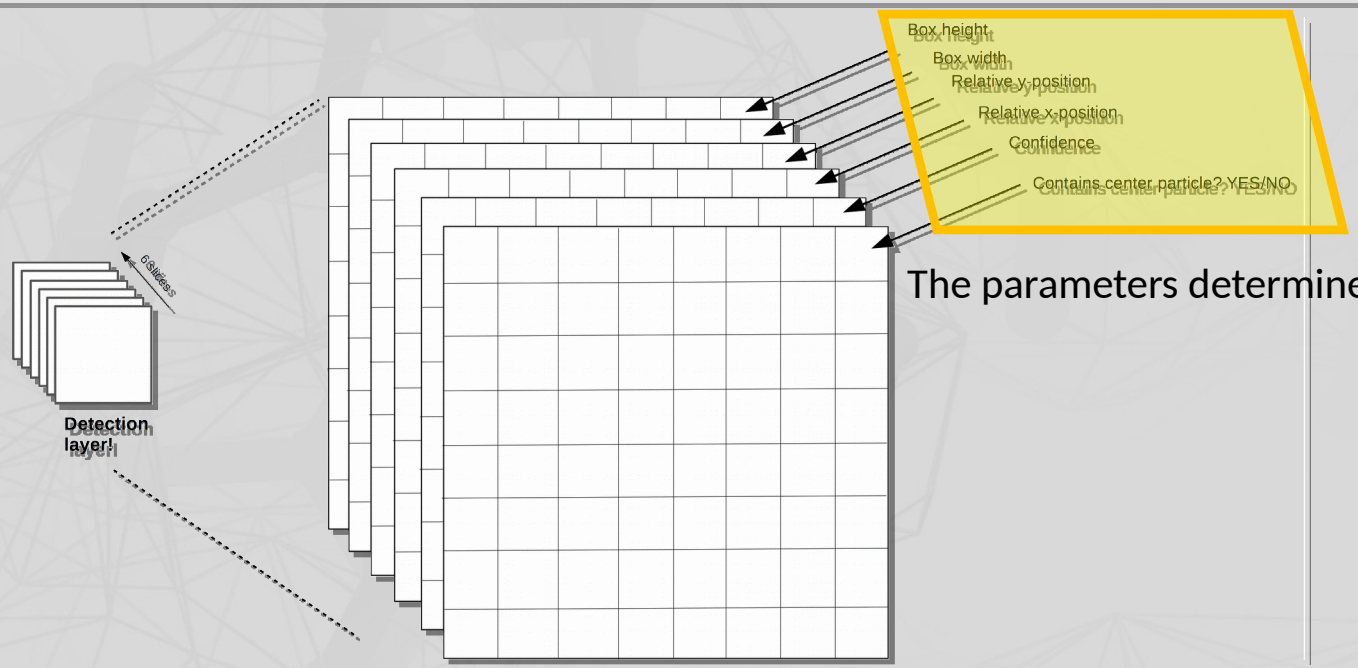
# Method: YOLO




*Redmon, Joseph and Ali Farhadi. "YOLO9000: Better, Faster, Stronger." 2017 IEEE Conference on Computer Vision and Pattern Recognition (CVPR) (2017): 6517-6525.*

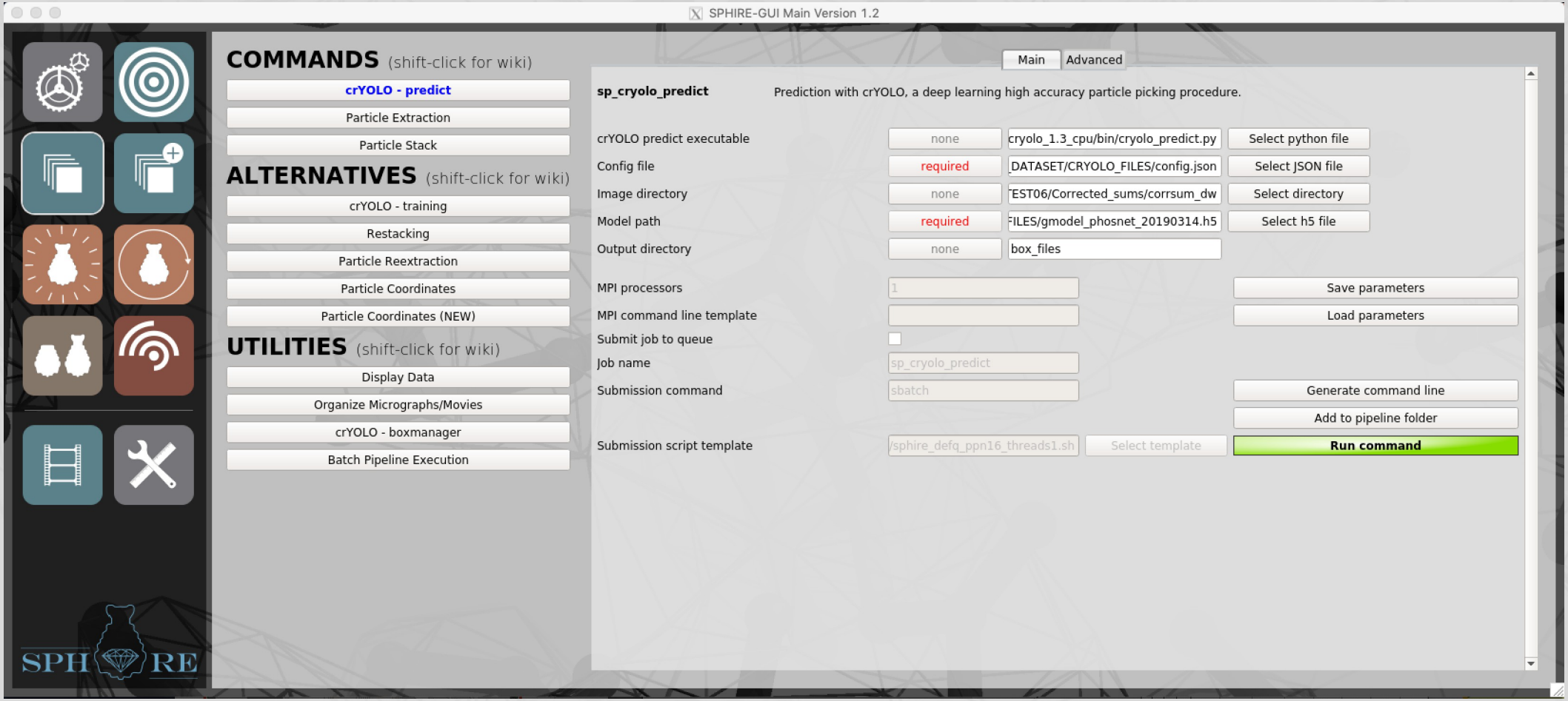
# Method: YOLO



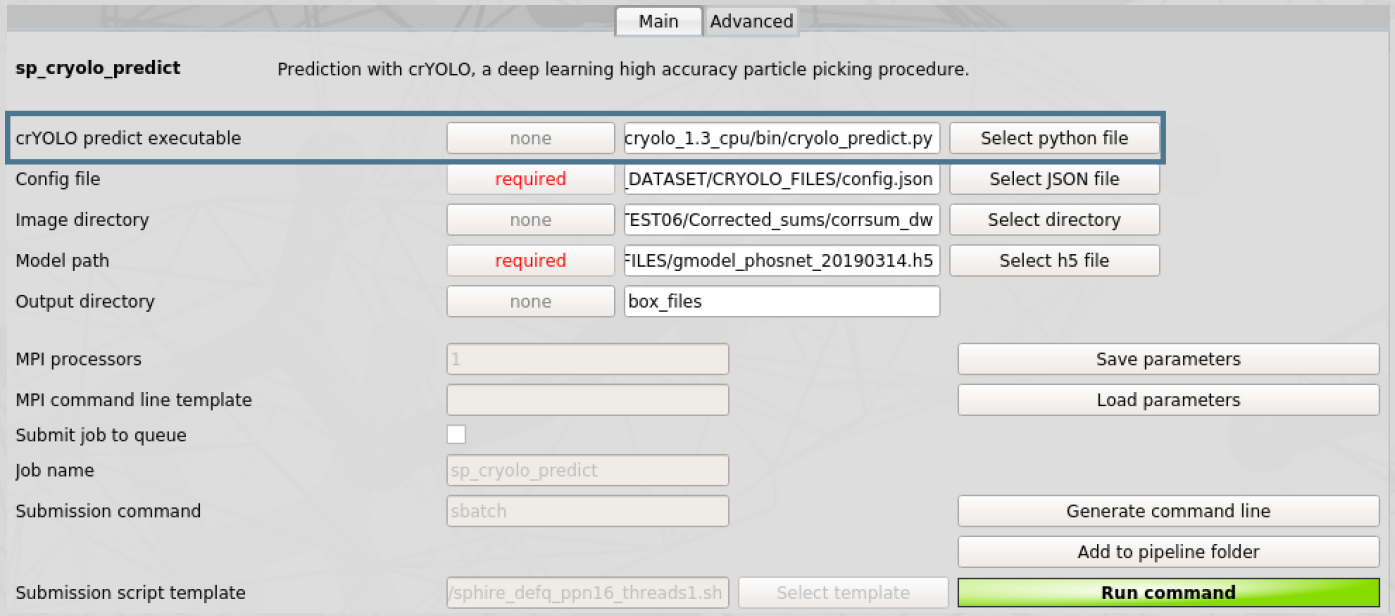




The parameters determined by the network

# CrYOLO

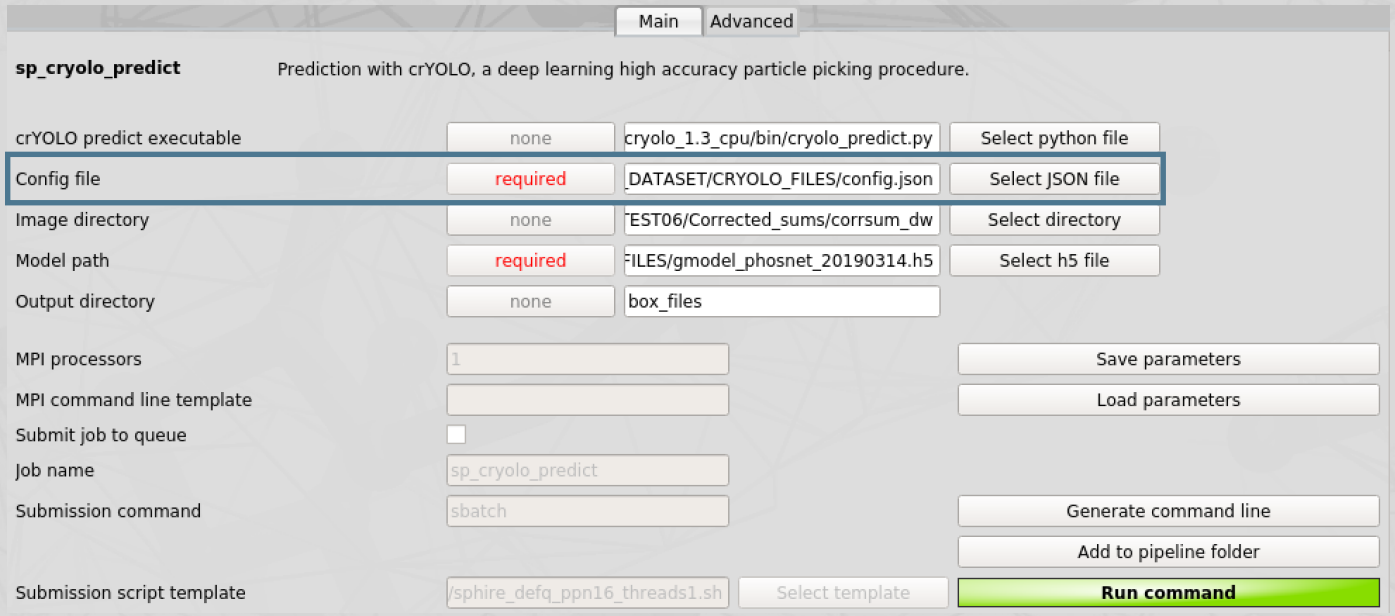

SPHIRE-GUI Main Version 1.2
COMMANDS (shift-click for wiki)
crYOLO - predict
Particle Extraction
Particle Stack
ALTERNATIVES (shift-click for wiki)
crYOLO - training
Restacking
Particle Reextraction
Particle Coordinates
Particle Coordinates (NEW)
UTILITIES (shift-click for wiki)
Display Data
Organize Micrographs/Movies
crYOLO - boxmanager
Batch Pipeline Execution
Main
Advanced
sp_cryolo_predict
Prediction with crYOLO, a deep learning high accuracy particle picking procedure.
crYOLO predict executable
none
cryolo_1.3_cpu/bin/cryolo_predict.py
Select python file
Config file
required
DATASET/CRYOLO_FILES/config.json
Select JSON file
Image directory
none
EST06/Corrected_sums/corrsum_dw
Select directory
Model path
required
ILES/gmodel_phosnet_20190314.h5
Select h5 file
Output directory
none
box_files
MPI processors
1
Save parameters
MPI command line template
Load parameters
Submit job to queue
Job name
sp_cryolo_predict
Submission command
sbatch
Generate command line
Add to pipeline folder
Submission script template
/sphire_defq_ppn16_threads1.sh
Select template
Run command

# CrYOLO



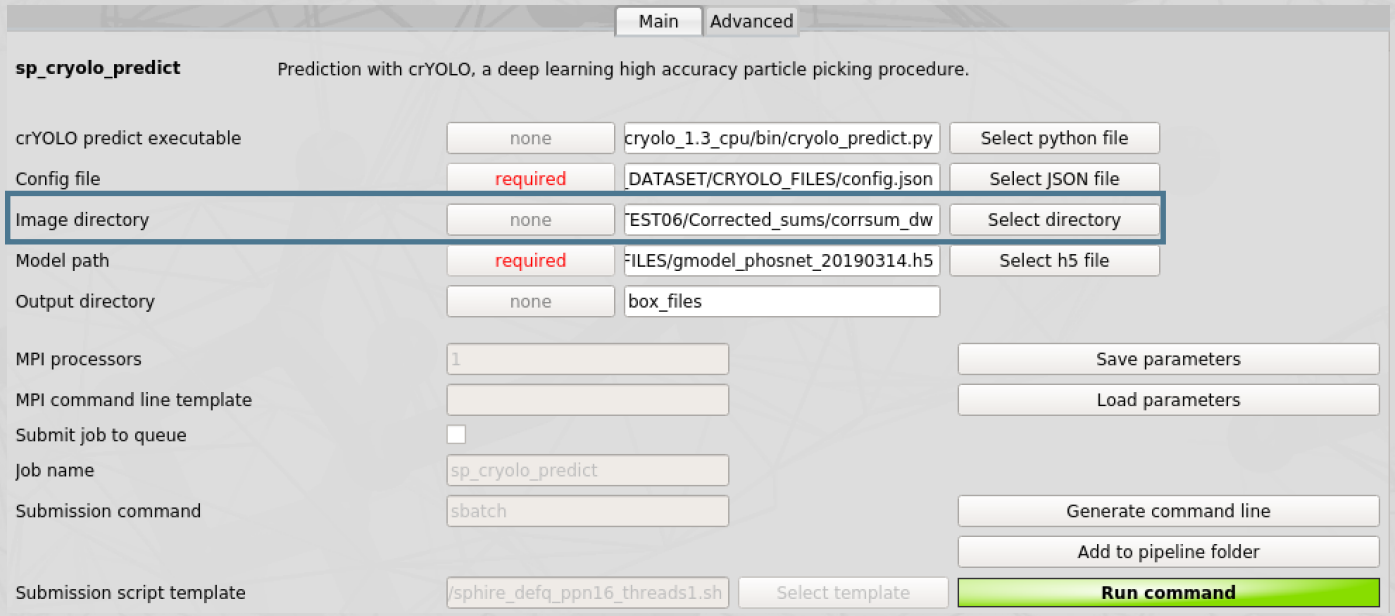

Path of cryolo_predict executable

# CrYOLO



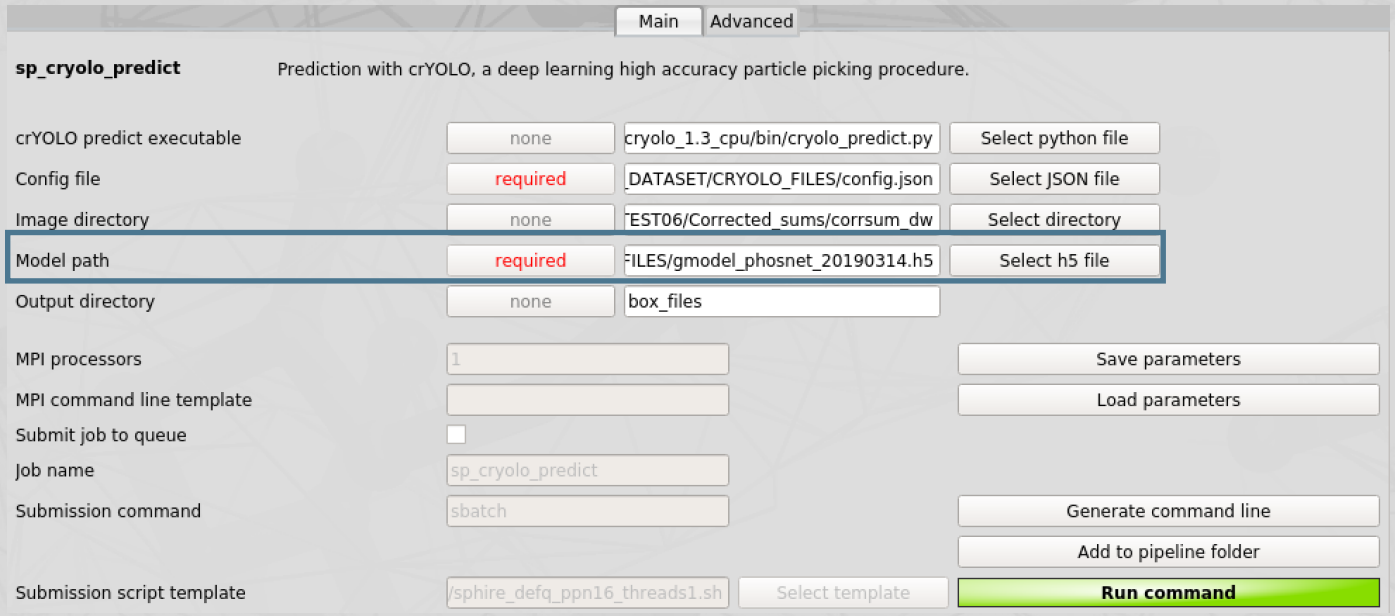

CrYOLO configuration file

# CrYOLO




Micrograph directory

# CrYOLO




CrYOLO model file,
containing network weights

# CrYOLO

Main | Advanced

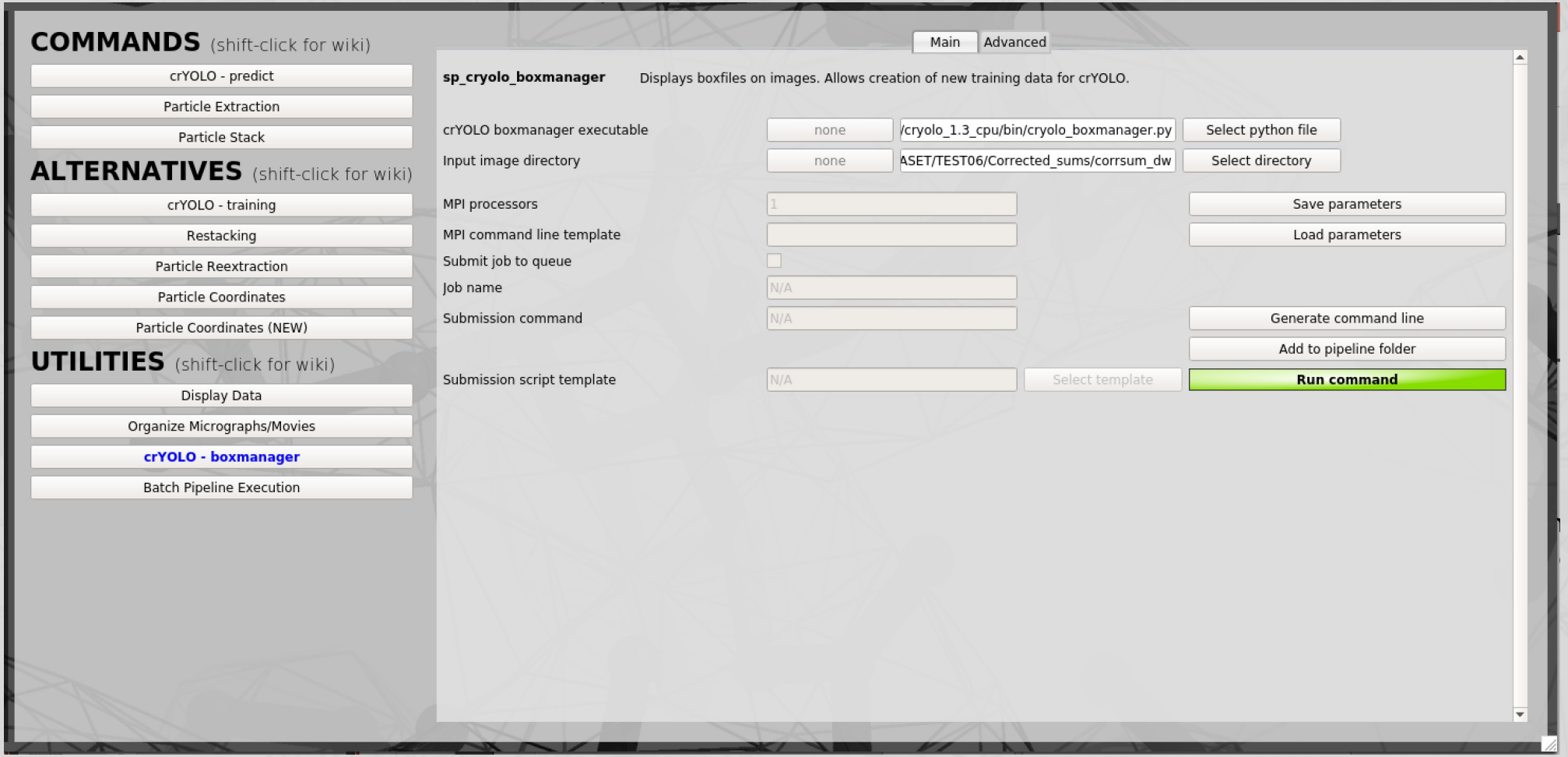
**sp_cryolo_predict** Set advanced parameters

| | | |
|---|---|---|
| Confidence threshold [0-1] | 0.3 | 0.85 |
| GPUs | 0 | 0 |
| GPU memory fraction | 1.0 | 1.0 |
| Number of CPUs | -1 | -1 |
| Filament mode [Yes/No] | NO | ☐ |
| Filament width [Pixel] | 100 | 100 |
| Minimum number of boxes per filament | 6 | 6 |
| Box distance | 352 | 352 |
| Don't split curved filaments | NO | ☐ |
| Don't merge filaments | NO | ☐ |

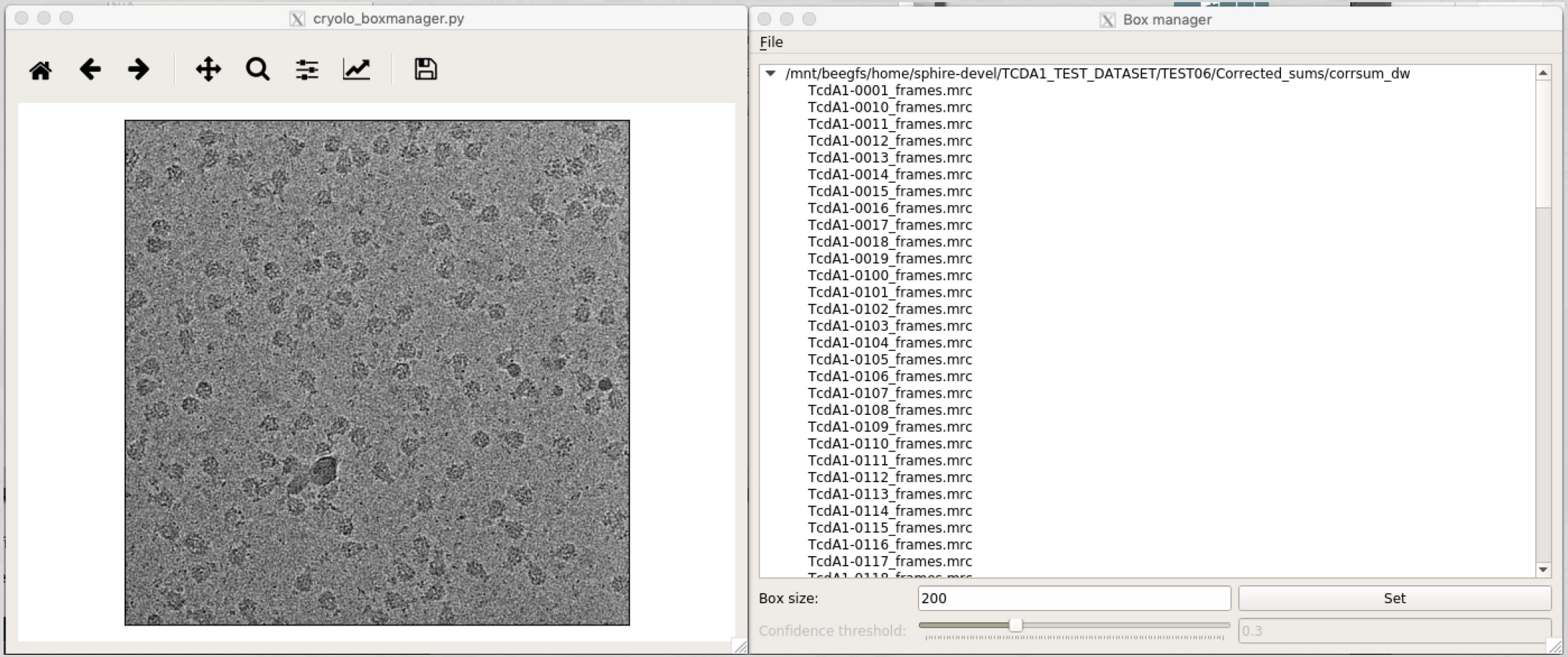
High threshold will be strict.
Low threshold will be permissive.

SPHERE

# cryolo_boxmanager



# cryolo_boxmanager

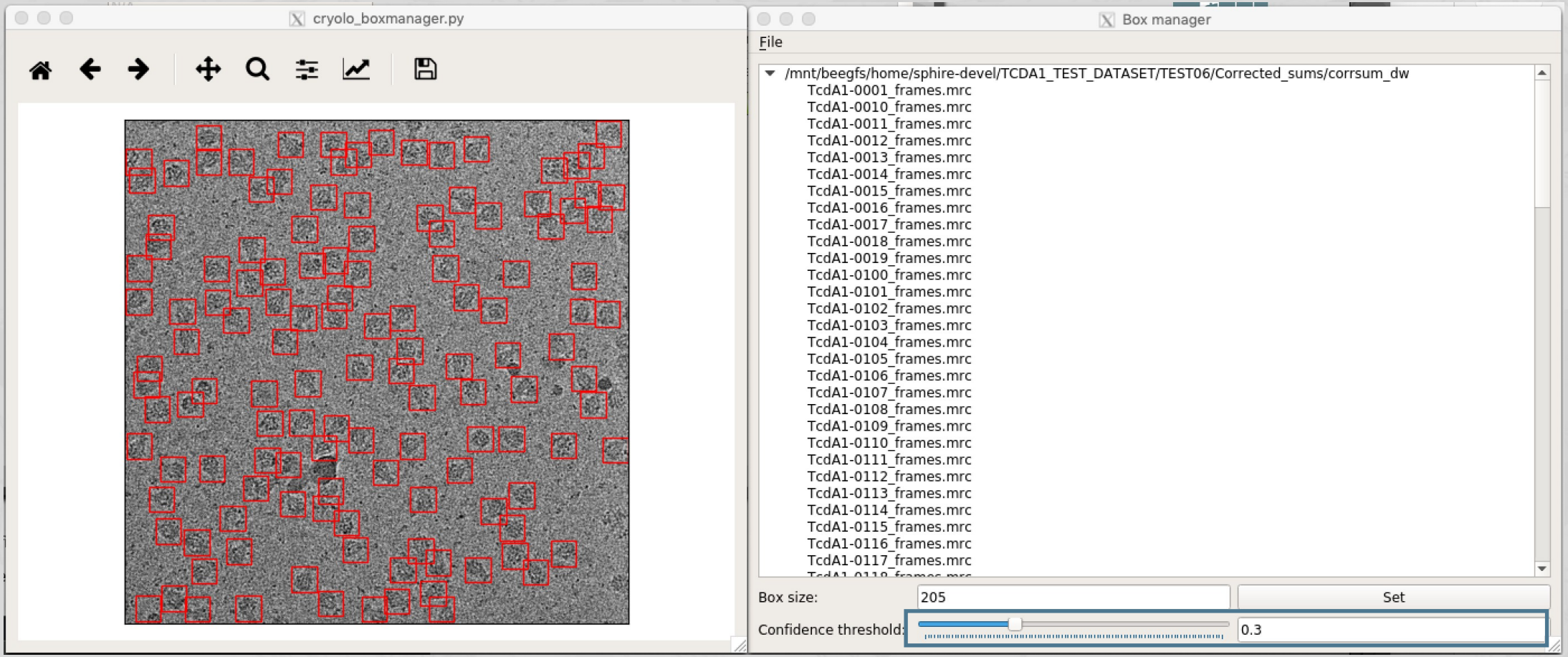



To open coordinates:

*File → Import box files*

Select CBOX

# cryolo_boxmanager




To change threshold, drag slide bar or edit the value in the field.
To write new coordinates: *File → Write box files*

# Particle extraction

**COMMANDS** (shift-click for wiki)

- crYOLO - predict
- **Particle Extraction**
- Particle Stack

**ALTERNATIVES** (shift-click for wiki)

- crYOLO - training
- Restacking
- Particle Reextraction
- Particle Coordinates
- Particle Coordinates (NEW)

**UTILITIES** (shift-click for wiki)

- Display Data
- Organize Micrographs/Movies
- crYOLO - boxmanager
- Batch Pipeline Execution

Main | Advanced

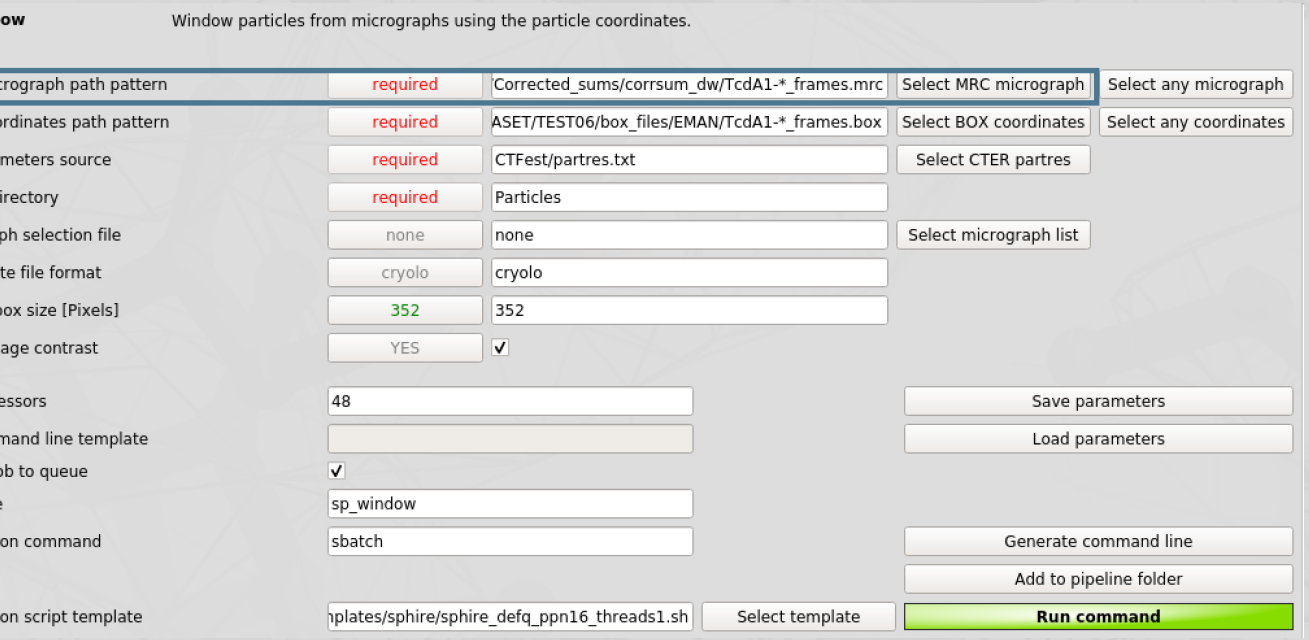
**sp_window** Window particles from micrographs using the particle coordinates.

| | | | | |
|---|---|---|---|---|
| Input micrograph path pattern | required | Corrected_sums/corrsum_dw/TcdA1-*_frames.mrc | Select MRC micrograph | Select any micrograph |
| Input coordinates path pattern | required | ASET/TEST06/box_files/EMAN/TcdA1-*_frames.box | Select BOX coordinates | Select any coordinates |
| CTF parameters source | required | CTFest/partres.txt | Select CTER partres | |
| Output directory | required | Particles | | |
| Micrograph selection file | none | none | Select micrograph list | |
| Coordinate file format | cryolo | cryolo | | |
| Particle box size [Pixels] | 352 | 352 | | |
| Invert image contrast | YES | ☑ | | |

| | | | |
|---|---|---|---|
| MPI processors | 48 | | Save parameters |
| MPI command line template | | | Load parameters |
| Submit job to queue | ☑ | | |
| Job name | sp_window | | |
| Submission command | sbatch | | Generate command line |
| | | | Add to pipeline folder |
| Submission script template | nplates/sphire/sphire_defq_ppn16_threads1.sh | Select template | **Run command** |

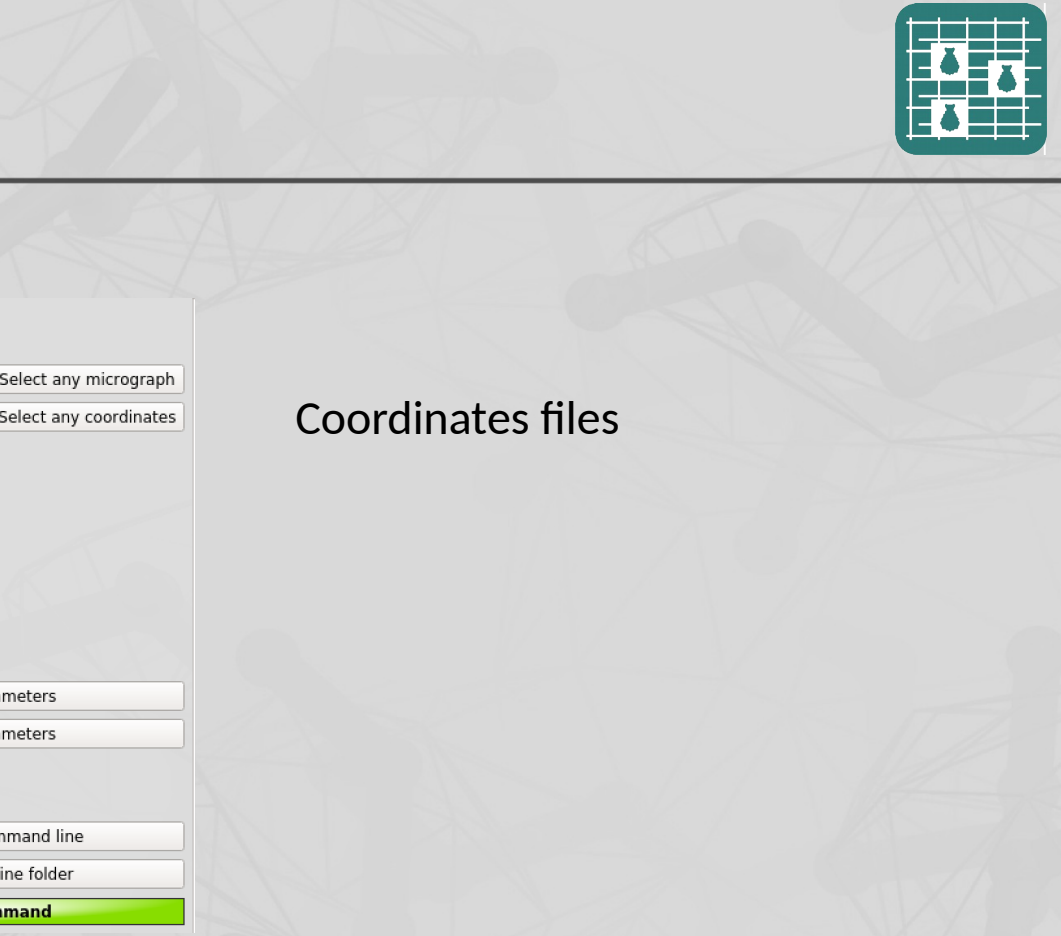
SPHIRE

# Particle extraction



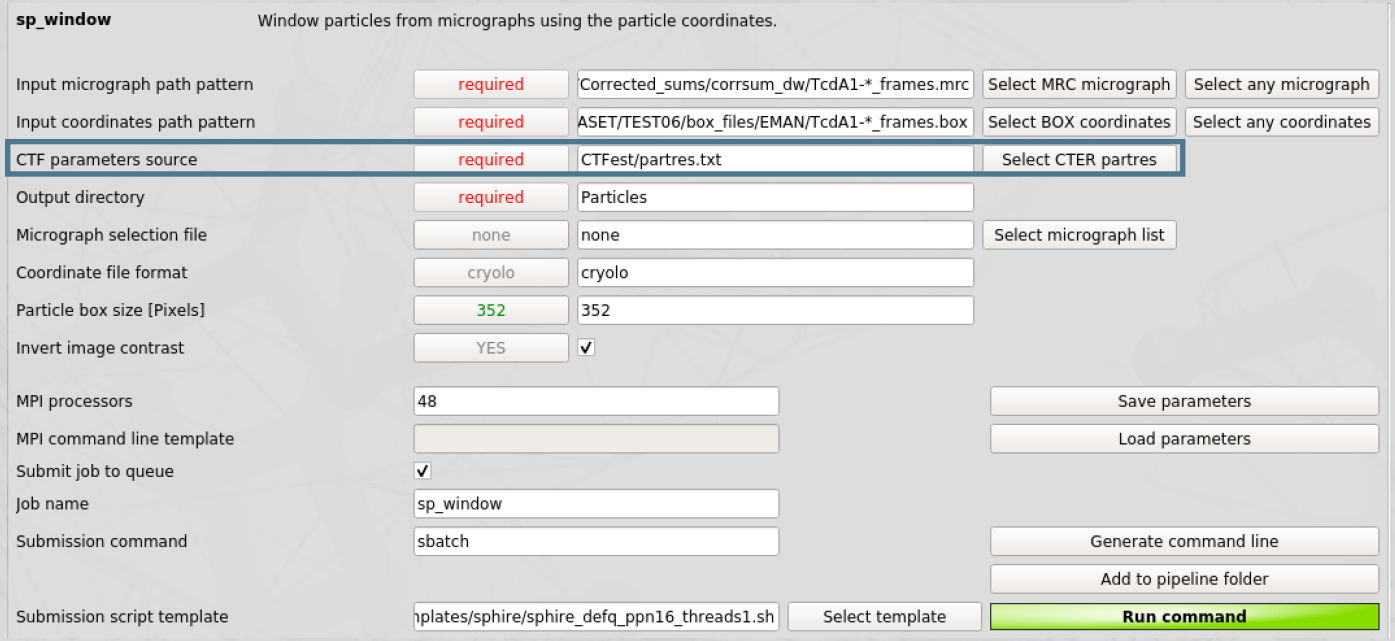

Micrographs

# Particle extraction



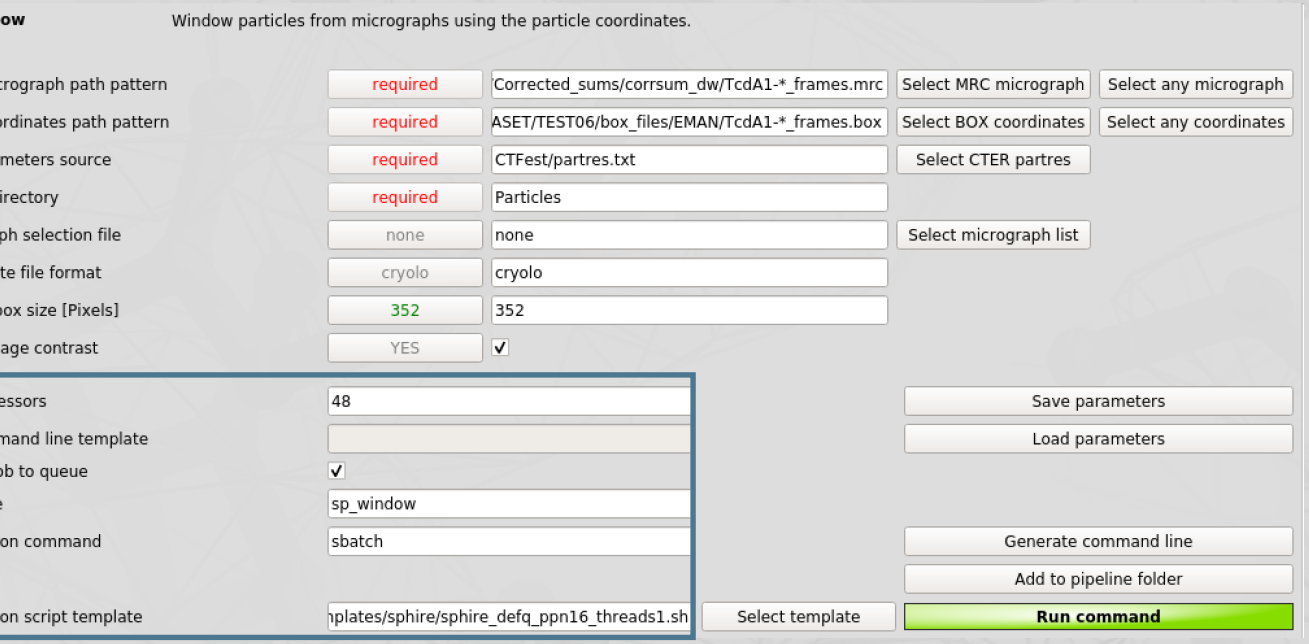

Coordinates files

# Particle extraction




CTF parameter file

# Particle extraction




Cluster settings

# Combine stacks

- Each parallel process will be written to a separate stack.
- Combine into one stack with:

```
e2bdb.py Particles/mpi_proc_* --makevstack=bdb:Particles#data
```

- (We won't actually run this, and will use pre-computed results instead.)

SPHIRE

# SPHIRE Pipeline

## PROJECT
Project Settings

## WINDOW
crYOLO Particle Picking
WINDOW Particle Extraction

## VIPER
RVIPER Initial 3D Modeling

## SORT3D
3D Variability Estimation
SORT3D 3D Clustering

## MOVIE (optional)
Micrograph Movie Alignment
Drift Assessment

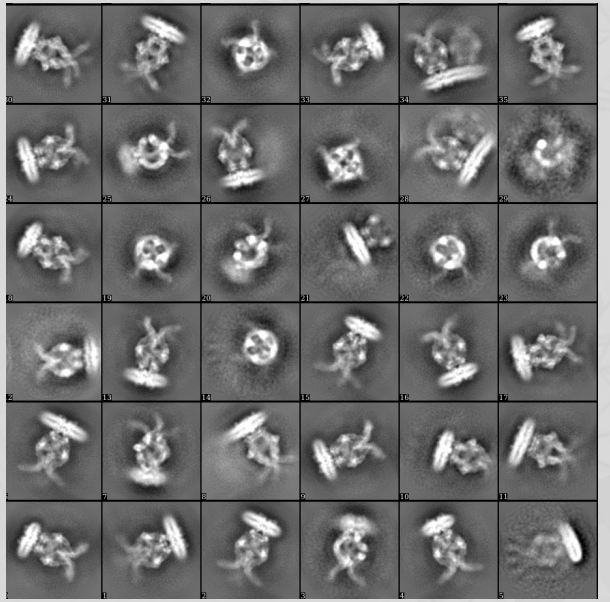
## CTER
CTER CTF Estimation
CTF Assessment

## ISAC
ISAC2 2D Clustering
Beautifier

## MERIDIEN
MERIDIEN 3D Refinement
3D Refinement Assessment
PostRefiner

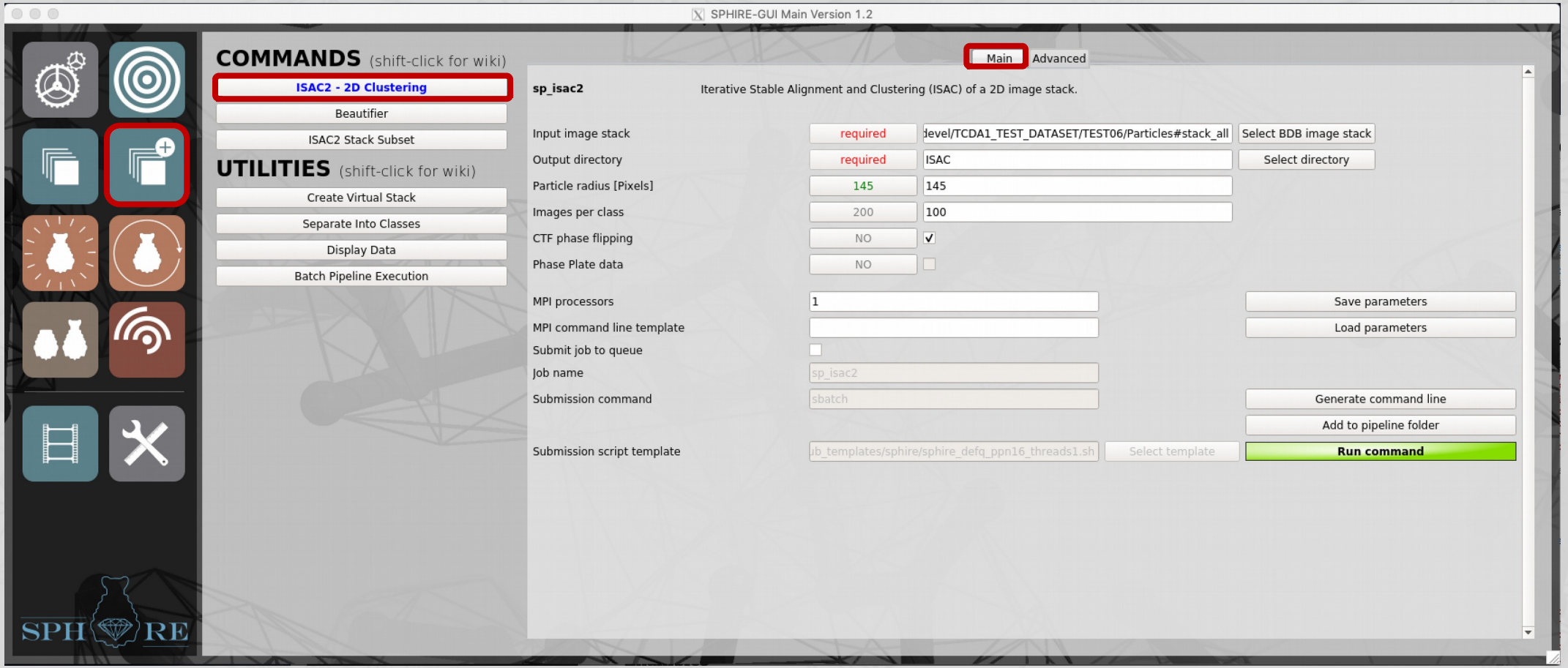
## LOCALRES
Local Resolution
3D Local Filter

## Utilities
Utility commands



SPHIRE

# Running ISAC



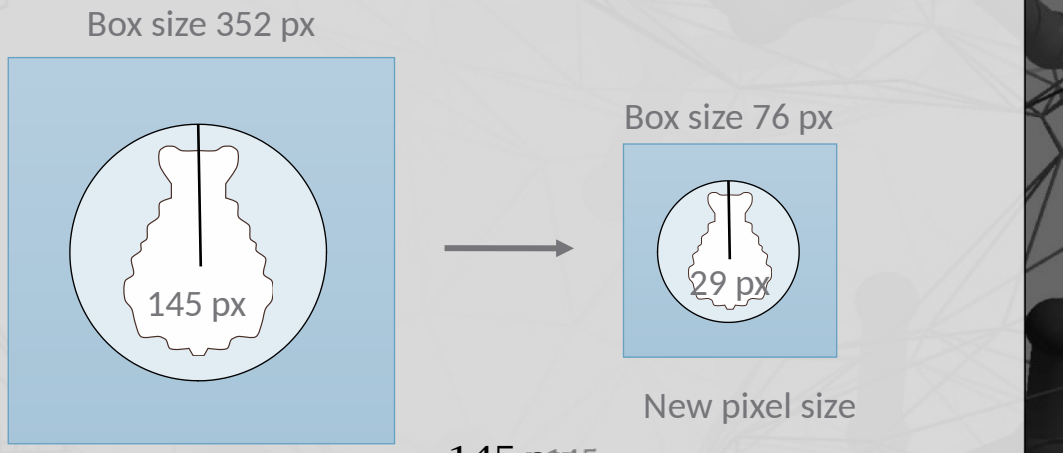
# Running ISAC — advanced users

Main | Advanced

**sp_isac2** Set advanced parameters

| | | |
|---|---|---|
| Translation search range [Pixels] | 1 | 1 |
| Pixel error threshold [Pixels] | 0.7 | 0.7 |
| Target particle radius [Pixels] | 29 | 29 |
| Target particle image size [Pixels] | 76 | 76 |
| Inner ring [Pixels] | 1 | 1 |
| Ring step [Pixels] | 1 | 1 |
| Search step [Pixels] | 1.0 | 1.0 |
| Reference-free alignment iterations | 30 | 30 |
| Centering method | -1 | -1 |
| Discrete angle used for within-group alignment | 90.0 | 90.0 |
| Lowest filter frequency [1/Pixel] | 0.2 | 0.2 |
| Highest filter frequency [1/Pixel] | 0.45 | 0.45 |
| Tangent filter fall-off | 0.2 | 0.2 |
| Maximum generations | 7 | 7 |
| SAC stability check interval | 1 | 1 |
| Number of alignments for stability check | 5 | 5 |
| Minimum size of reproducible classes | 60 | 60 |
| Seed | none | none |
| Do pre-alignment | YES | ☑ |
| Restart run | -1 | -1 |
| Skip ordered class averages | NO | ☐ |
| Filament width [Pixels] | -1 | -1 |
| Ignore filament masking (filament use only) | NO | ☐ |

## Tune rescaling of particles




Only need to be changed in very special cases

- Particles with radius < 29
- Particles that need high resolution information to align ex. small particles in large NanoDisc

# Running ISAC — advanced users

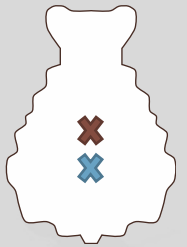
Main | Advanced

**sp_isac2** Set advanced parameters

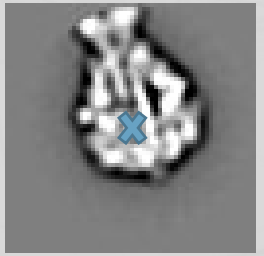
| | | |
|---|---|---|
| Translation search range [Pixels] | 1 | 1 |
| Pixel error threshold [Pixels] | 0.7 | 0.7 |
| Target particle radius [Pixels] | 29 | 29 |
| Target particle image size [Pixels] | 76 | 76 |
| Inner ring [Pixels] | 1 | 1 |
| Ring step [Pixels] | 1 | 1 |
| Search step [Pixels] | 1.0 | 1.0 |
| Reference-free alignment iterations | 30 | 30 |
| Centering method | -1 | -1 |
| Discrete angle used for within-group alignment | 90.0 | 90.0 |
| Lowest filter frequency [1/Pixel] | 0.2 | 0.2 |
| Highest filter frequency [1/Pixel] | 0.45 | 0.45 |
| Tangent filter fall-off | 0.2 | 0.2 |
| Maximum generations | 7 | 7 |
| SAC stability check interval | 1 | 1 |
| Number of alignments for stability check | 5 | 5 |
| Minimum size of reproducible classes | 60 | 60 |
| Seed | none | none |
| Do pre-alignment | YES | ☑ |
| Restart run | -1 | -1 |
| Skip ordered class averages | NO | ☐ |
| Filament width [Pixels] | -1 | -1 |
| Ignore filament masking (filament use only) | NO | ☐ |

Tune initial reference-free 2D alignment

Check performance using e2display.py



Center of particle
Center of mass



Modify/ Uncheck if

- 2D Alignment displaces already well centered particles (ex. Picked with CrYolo)
- Center of mass ≠ center of particle
- Use other alignment strategies (ex. MRA) instead

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# Running ISAC — advanced users

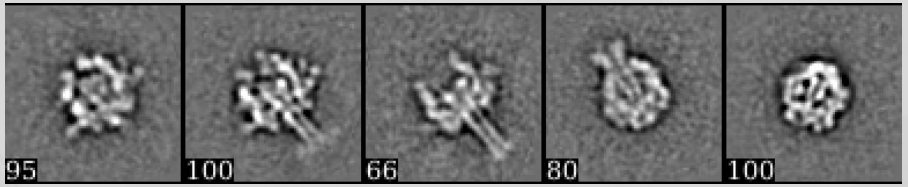
Main | Advanced

**sp_isac2** Set advanced parameters

| | | |
|---|---|---|
| Translation search range [Pixels] | 1 | 1 |
| Pixel error threshold [Pixels] | 0.7 | 0.7 |
| Target particle radius [Pixels] | 29 | 29 |
| Target particle image size [Pixels] | 76 | 76 |
| Inner ring [Pixels] | 1 | 1 |
| Ring step [Pixels] | 1 | 1 |
| Search step [Pixels] | 1.0 | 1.0 |
| Reference-free alignment iterations | 30 | 30 |
| Centering method | -1 | -1 |
| Discrete angle used for within-group alignment | 90.0 | 90.0 |
| Lowest filter frequency [1/Pixel] | 0.2 | 0.2 |
| Highest filter frequency [1/Pixel] | 0.45 | 0.45 |
| Tangent filter fall-off | 0.2 | 0.2 |
| Maximum generations | 7 | 7 |
| SAC stability check interval | 1 | 1 |
| Number of alignments for stability check | 5 | 5 |
| Minimum size of reproducible classes | 60 | 60 |
| Seed | none | none |
| Do pre-alignment | YES | ☑ |
| Restart run | -1 | -1 |
| Skip ordered class averages | NO | ☐ |
| Filament width [Pixels] | -1 | -1 |
| Ignore filament masking (filament use only) | NO | ☐ |

Tunes minimum number of particles per class

*Minimum size* ≤ *Members per class* ≤ *Images per group*




Adjust if Images per group ≪ or ≫ default
Ex. 50 images per group ➜ reduce minimum size

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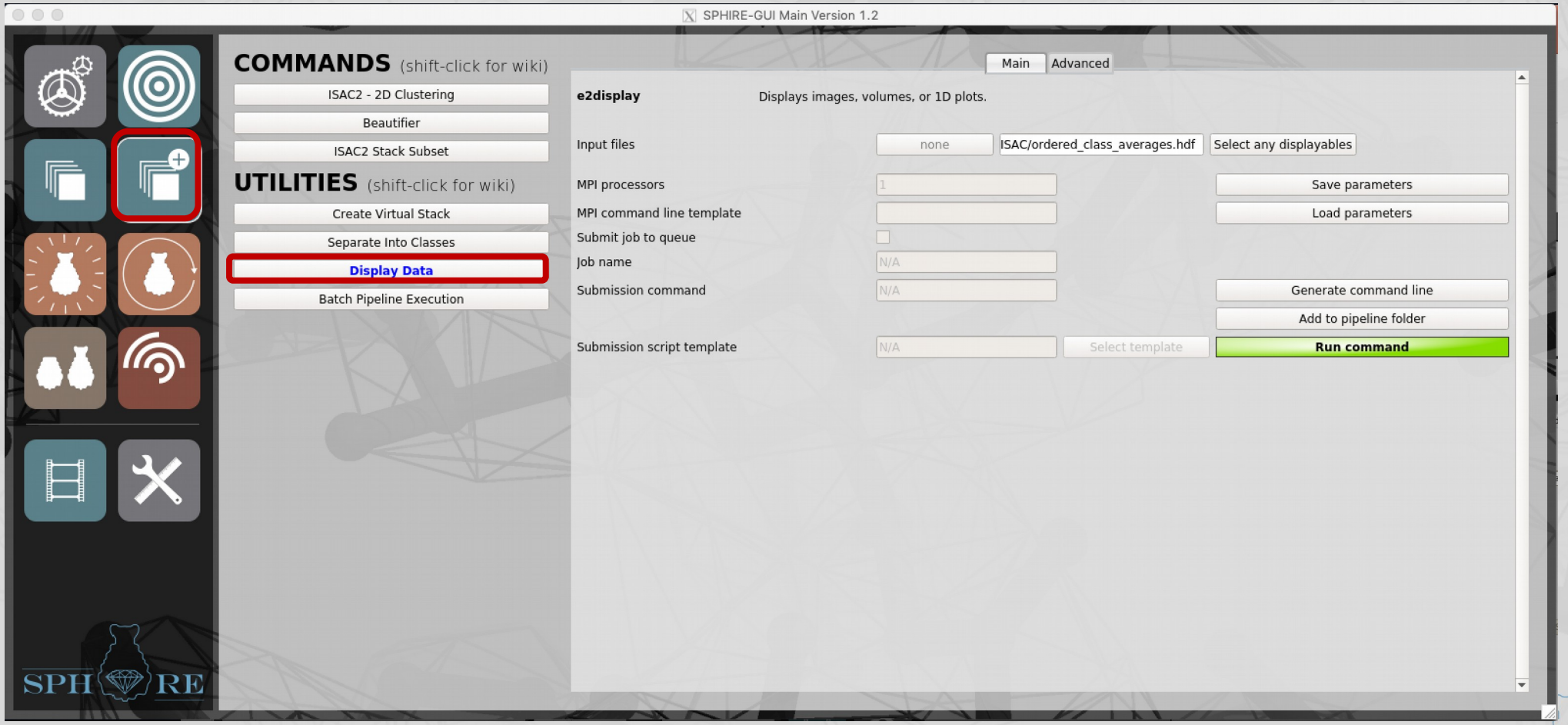
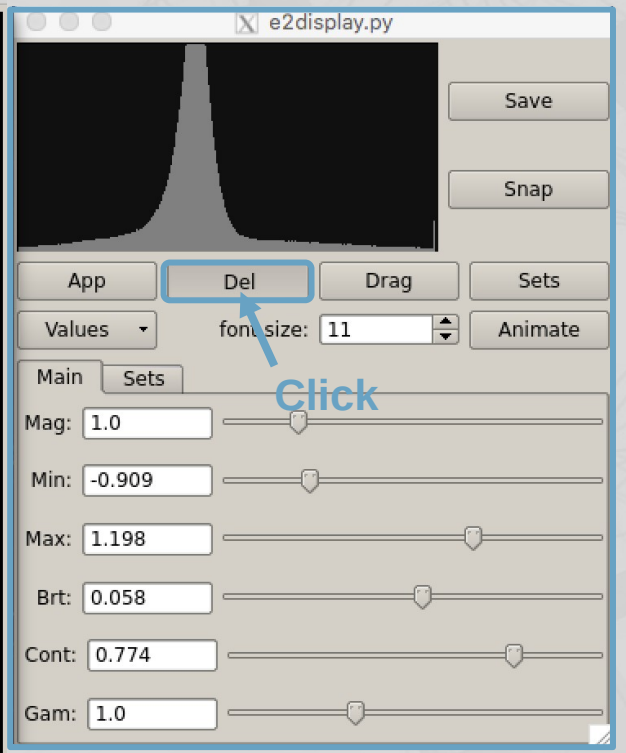
# Visualization of results




SPH RE

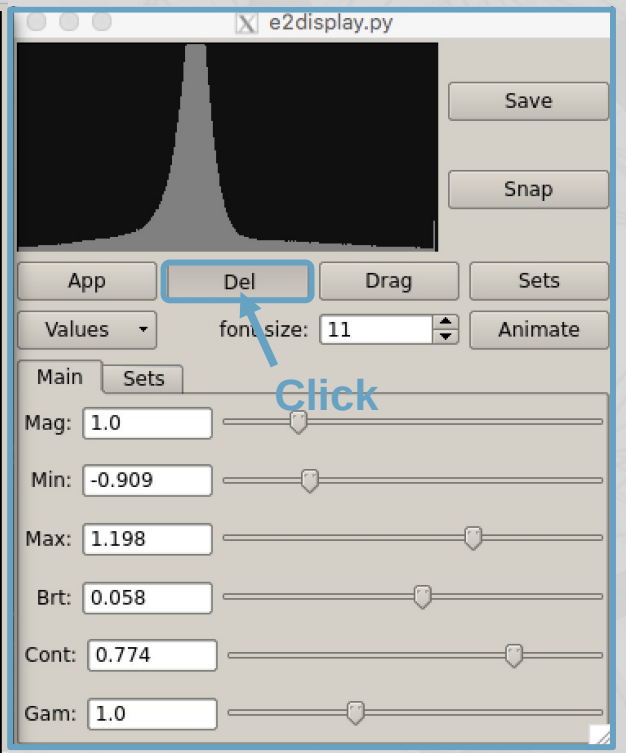
# Data assessment

# Data assessment

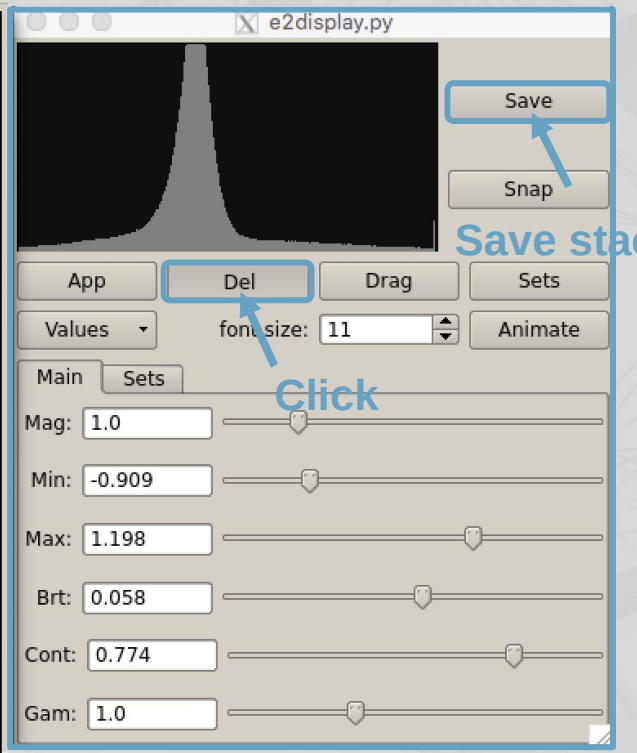

This window will appear

# Data assessment


This window will appear

# Data assessment

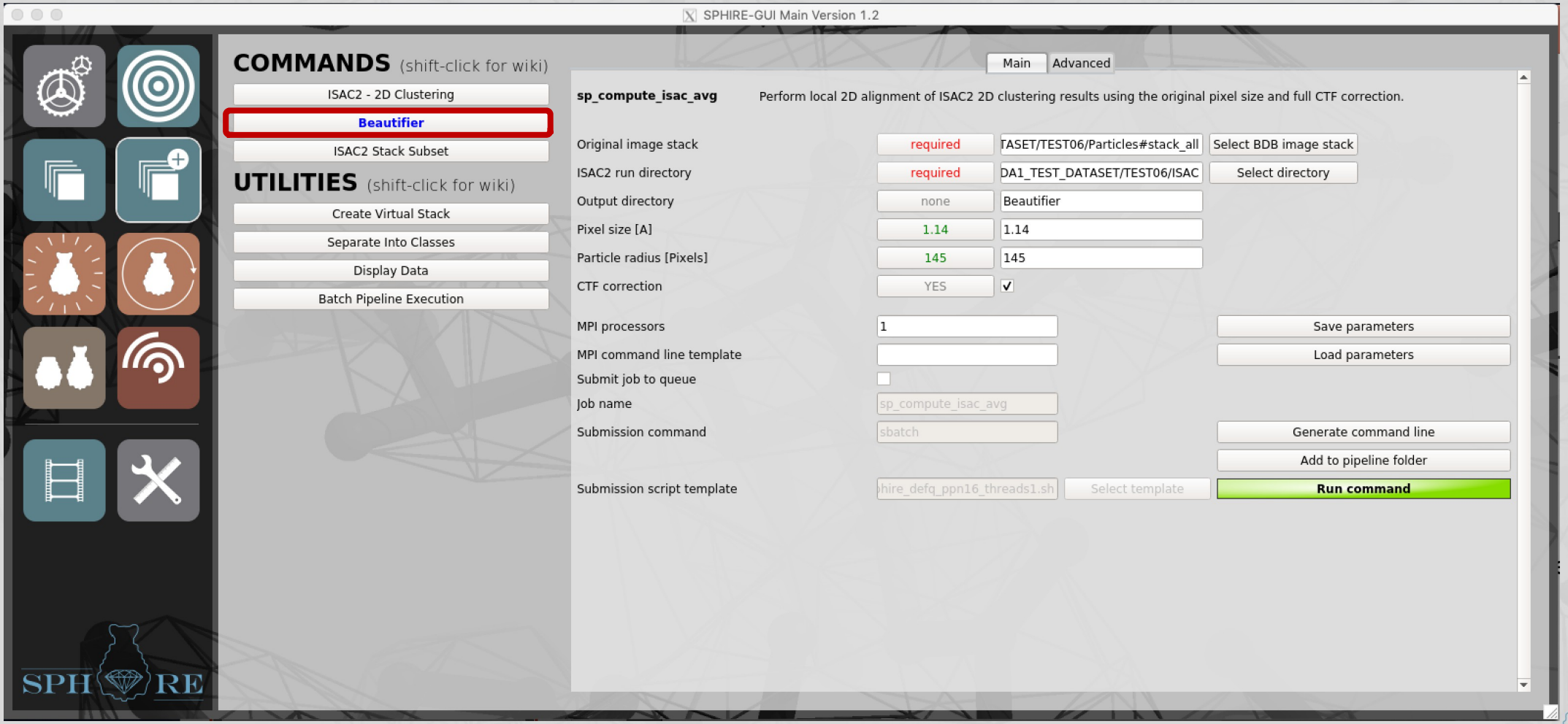




This window will appear

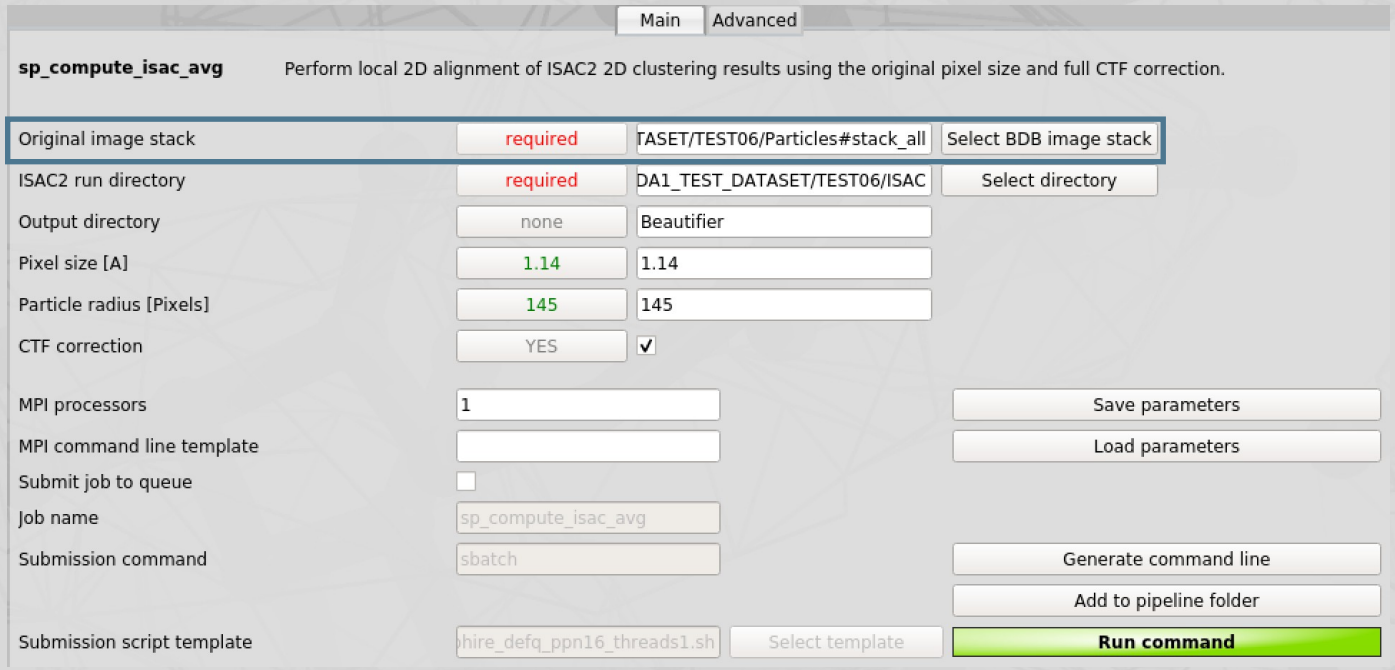
Save stack of good particles as “best.hdf” to be used for further processing.

SPHIRE

# Beautification

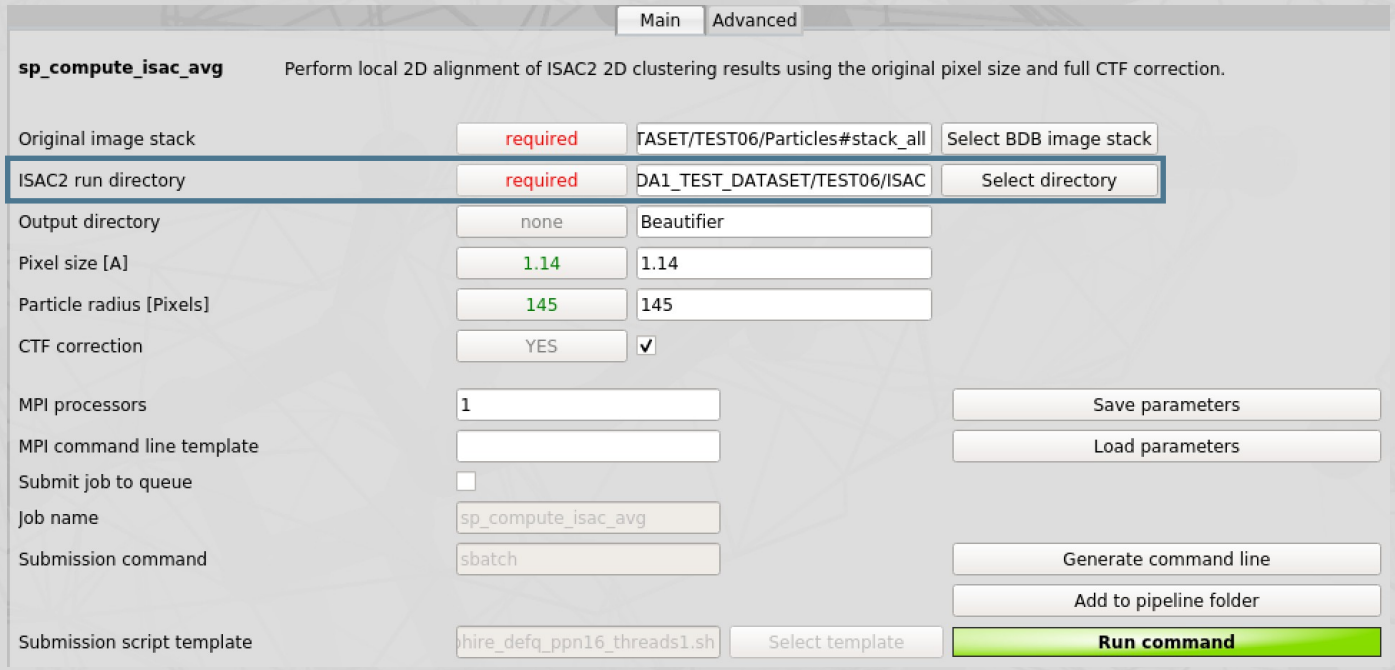


# Beautification



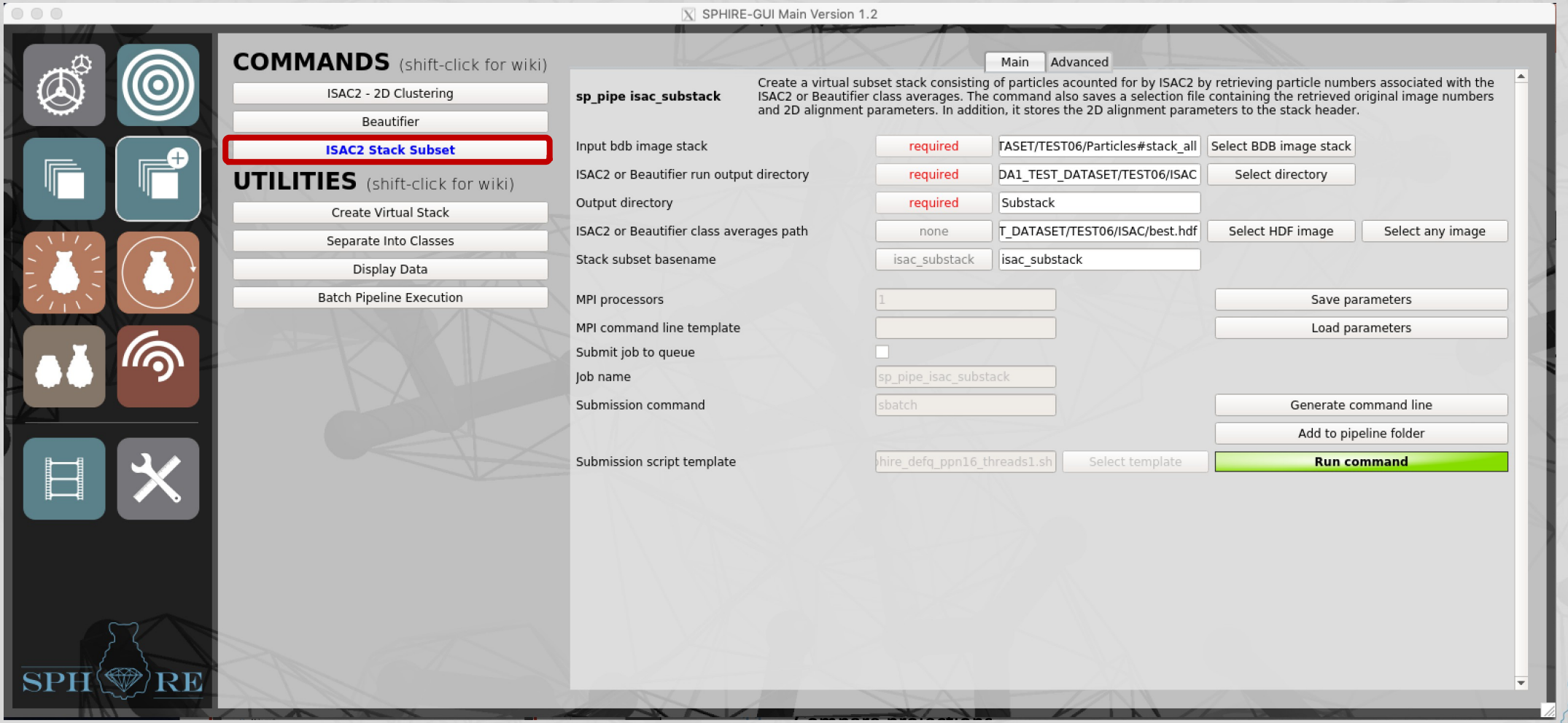

Stack of all particles

# Beautification

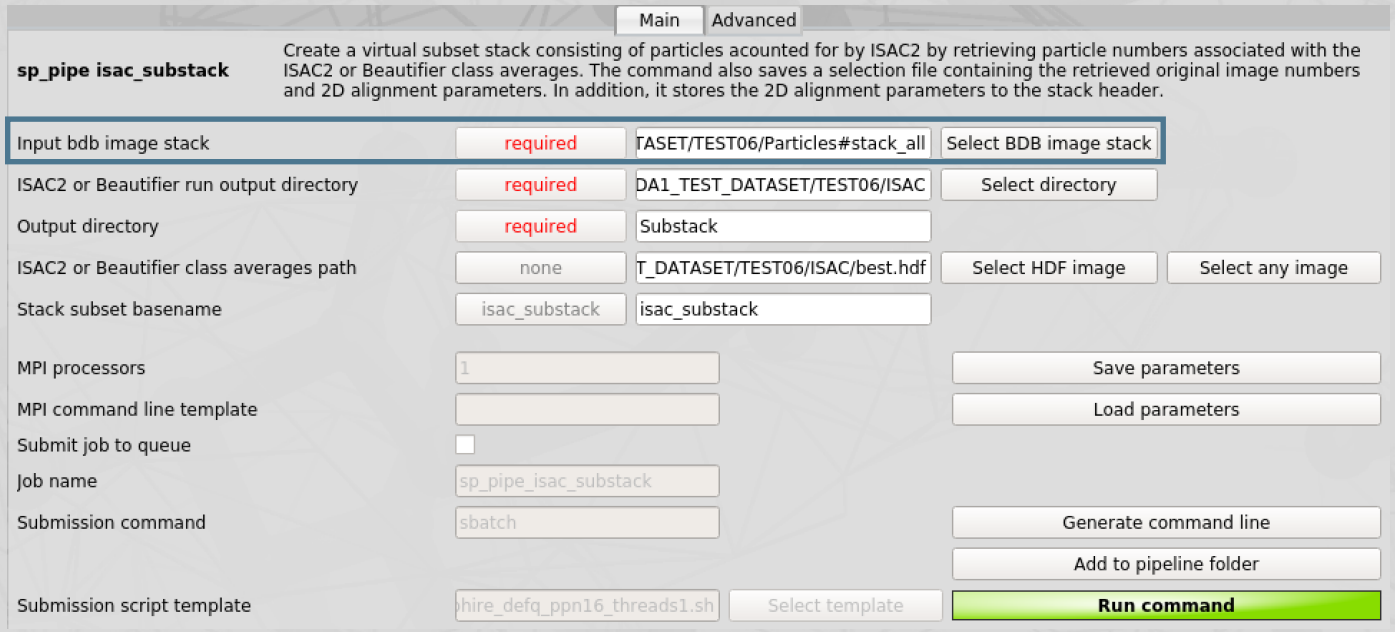



ISAC directory

# Make substack

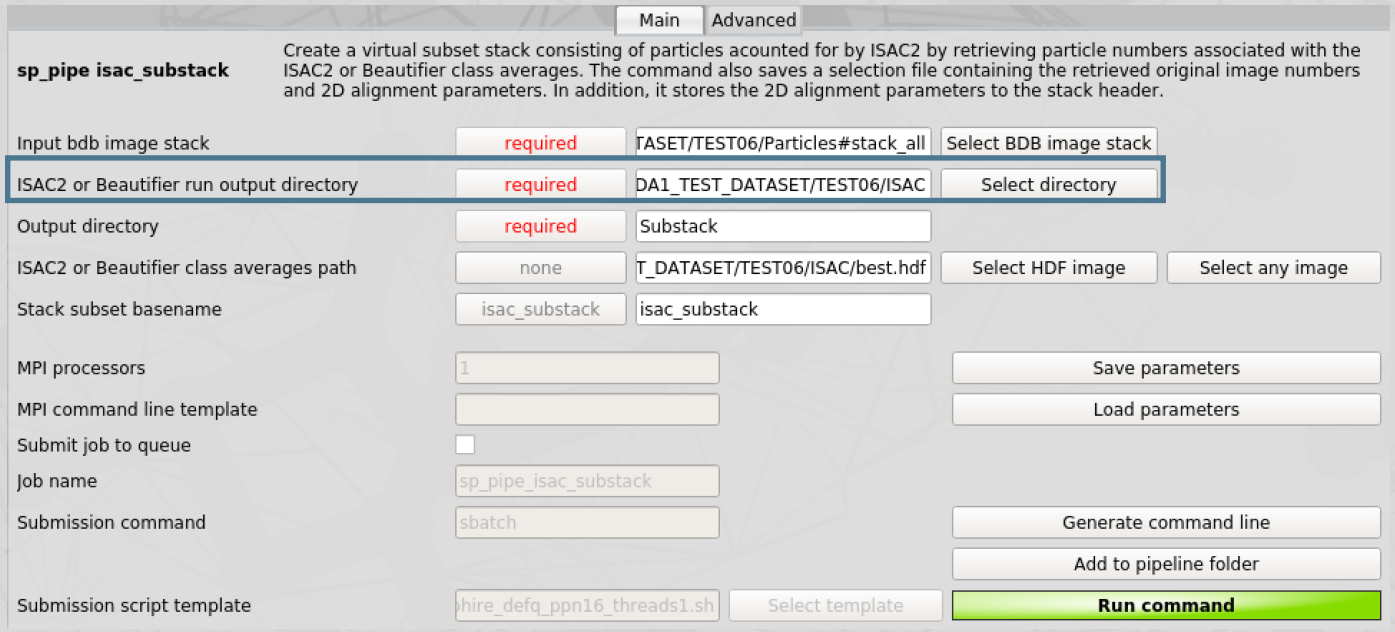


# Make substack



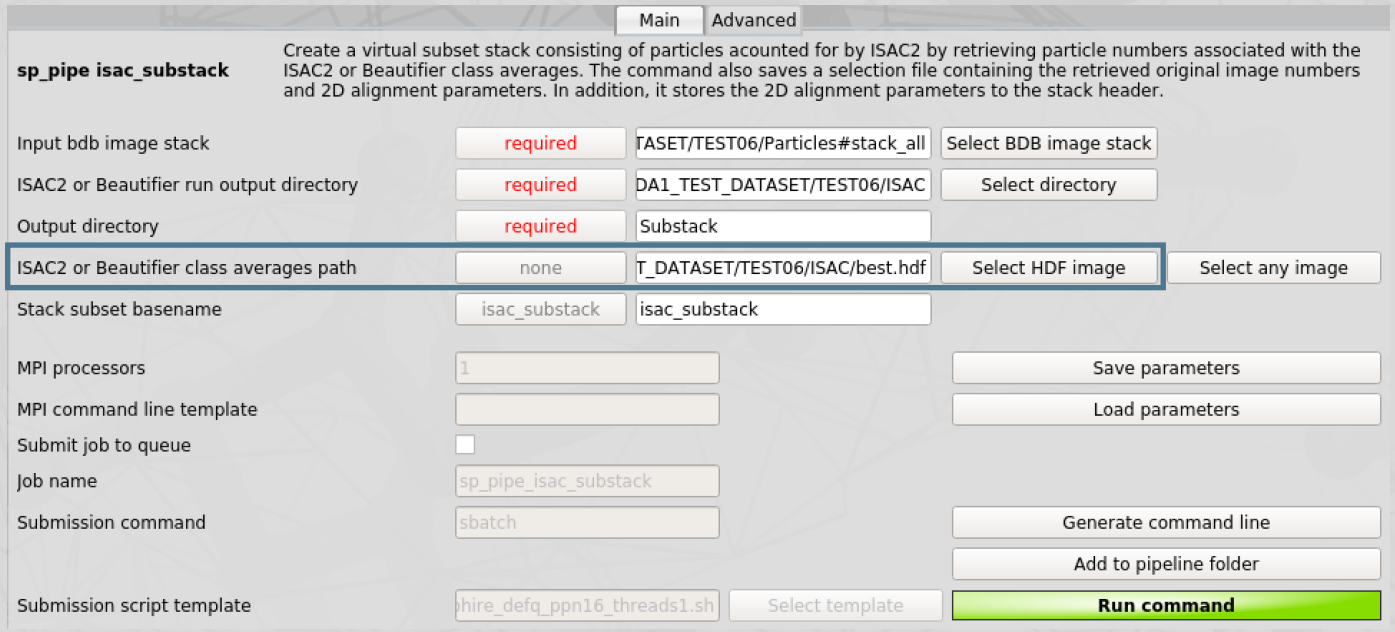

Stack of all particles

# Make substack




ISAC directory

# Make substack




Selected class averages

# SPHIRE Pipeline

**PROJECT**
Project Settings

**WINDOW**
crYOLO Particle Picking
WINDOW Particle Extraction

**VIPER**
RVIPER Initial 3D Modeling

**SORT3D**
3D Variability Estimation
SORT3D 3D Clustering

**MOVIE (optional)**
Micrograph Movie Alignment
Drift Assessment

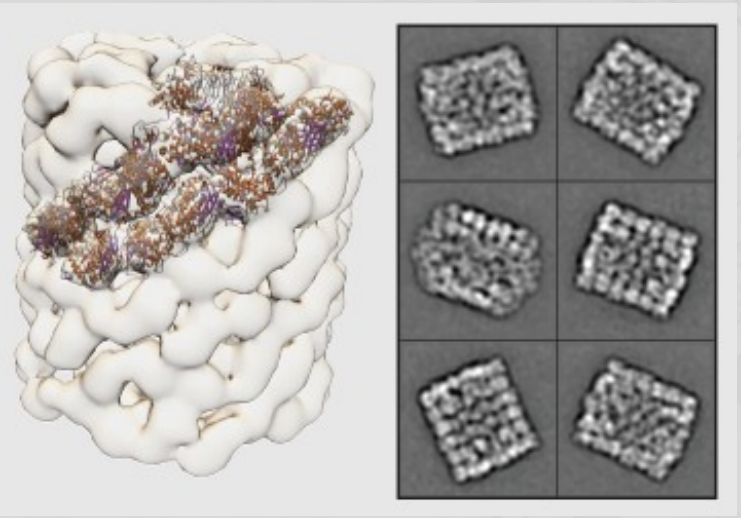
**CTER**
CTER CTF Estimation
CTF Assessment

**ISAC**
ISAC2 2D Clustering
Beautifier

**MERIDIEN**
MERIDIEN 3D Refinement
3D Refinement Assessment
PostRefiner

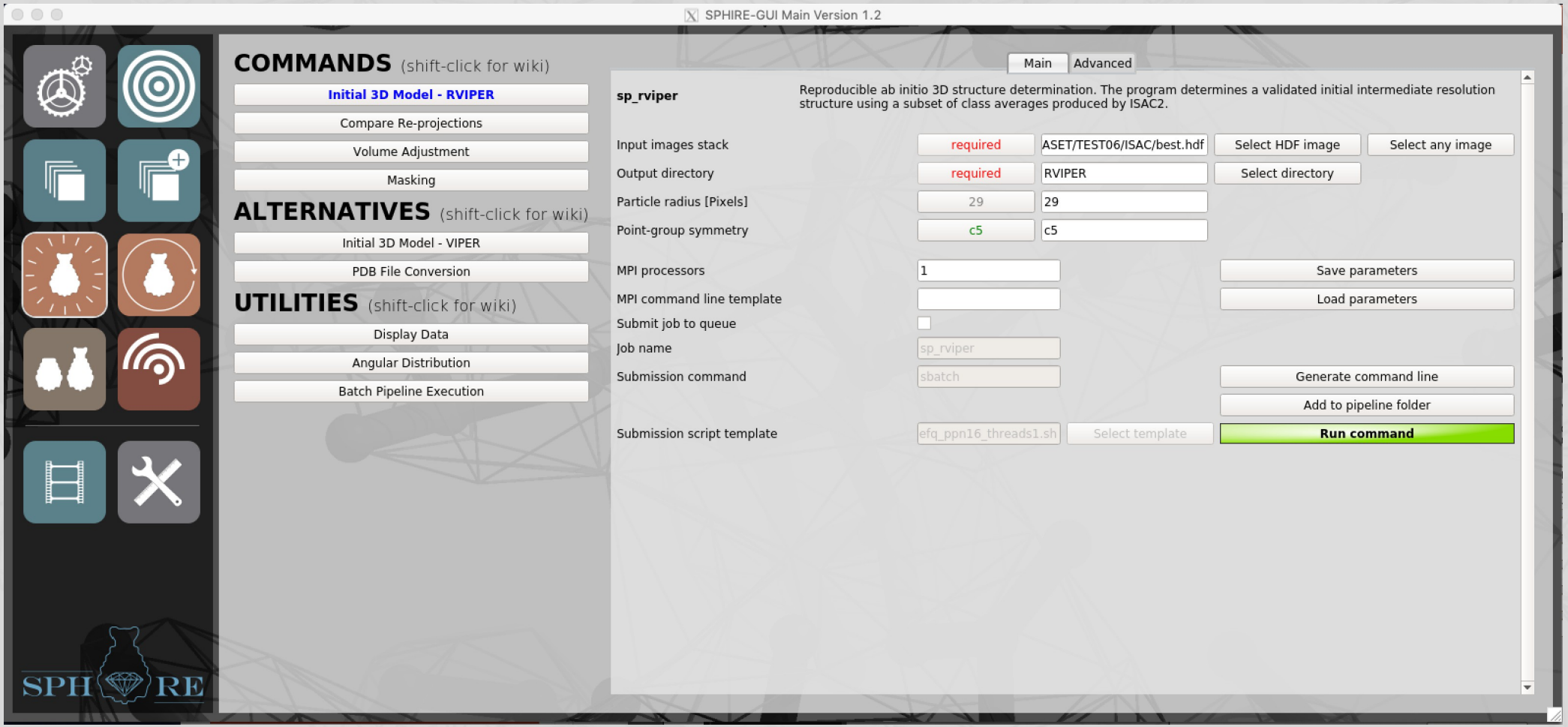
**LOCALRES**
Local Resolution
3D Local Filter

**Utilities**
Utility commands

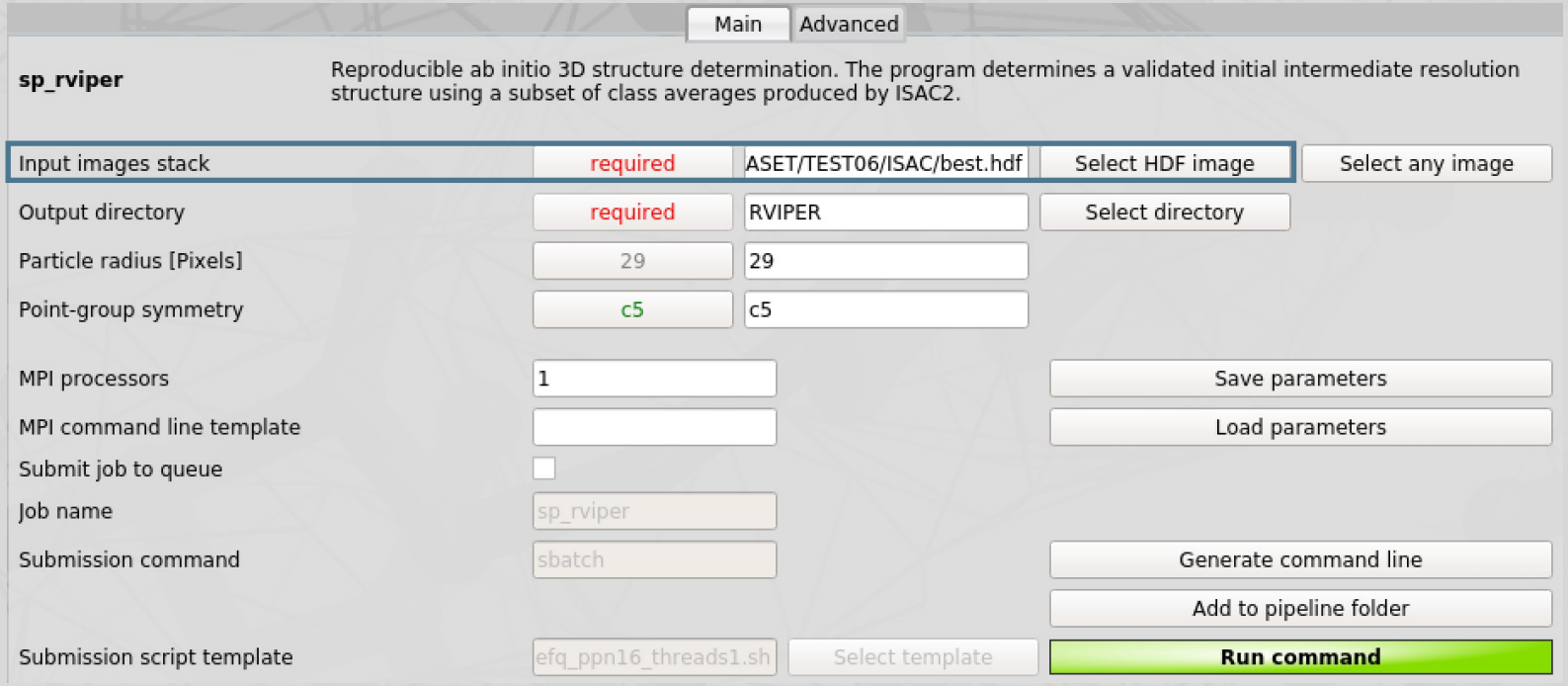


SPHIRE

# How to run RVIPER



# How to run RVIPER




Stack of all particles

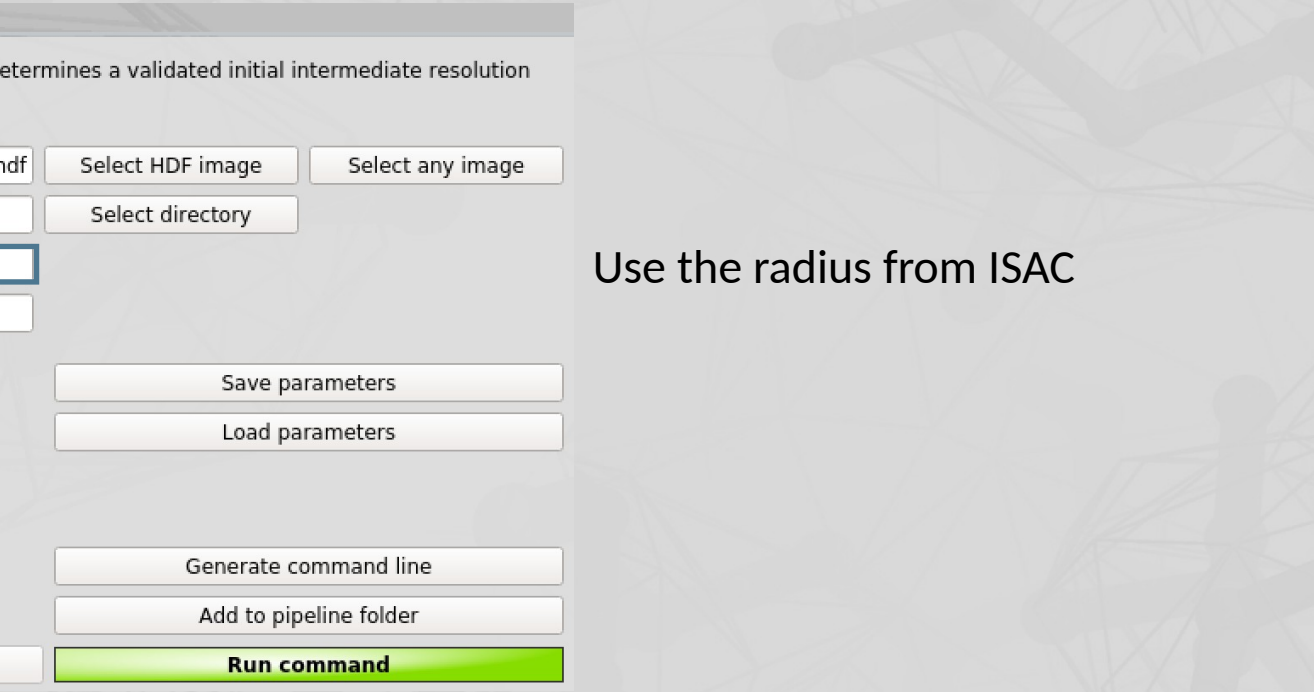
← Remember the radius if using ISAC classes

← Satellite densities can be masked using this.

← How many reproducibility runs.

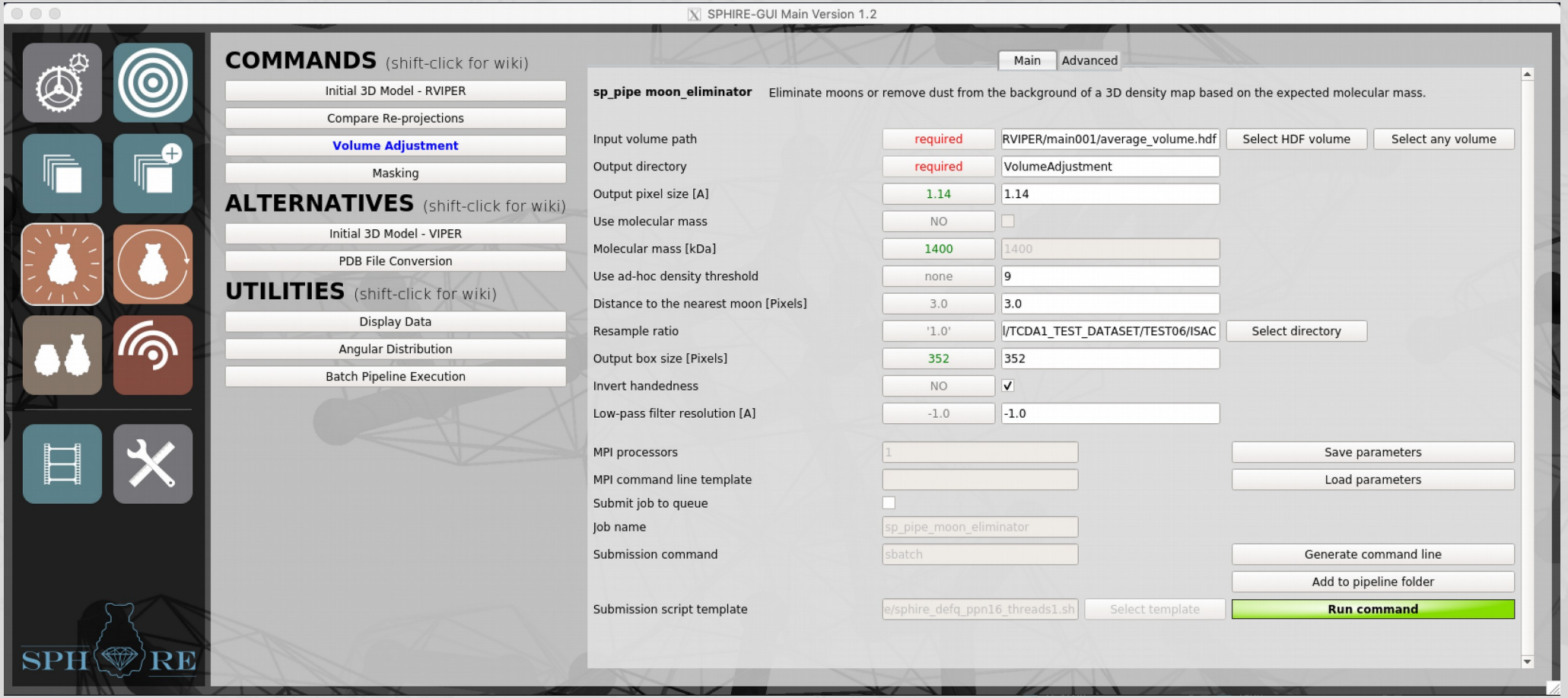
**Tip:** RVIPER is usually run using directly the small class averages from ISAC, but it can be useful to try it with full-sized, beautified classes.

# How to run RVIPER




Use the radius from ISAC

# What to do after running RVIPER



# What to do after running RVIPER

Main | Advanced

**sp_pipe moon_eliminator** Eliminate moons or remove dust from the background of a 3D density map based on the expected molecular mass.

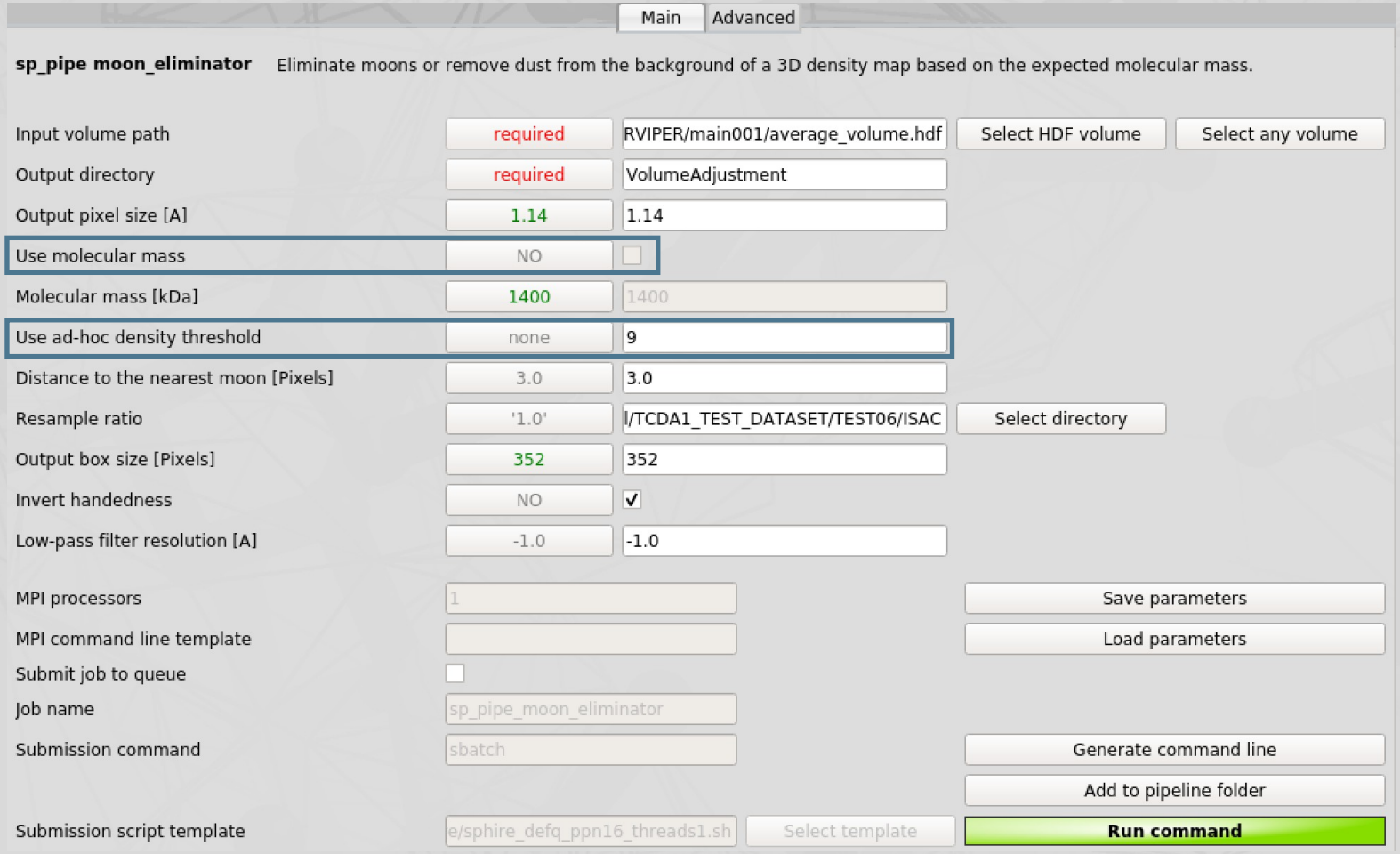
| | | | | |
|---|---|---|---|---|
| Input volume path | required | RVIPER/main001/average_volume.hdf | Select HDF volume | Select any volume |
| Output directory | required | VolumeAdjustment | | |
| Output pixel size [A] | 1.14 | 1.14 | | |
| Use molecular mass | NO | ☐ | | |
| Molecular mass [kDa] | 1400 | 1400 | | |
| Use ad-hoc density threshold | none | 9 | | |
| Distance to the nearest moon [Pixels] | 3.0 | 3.0 | | |
| Resample ratio | '1.0' | l/TCDA1_TEST_DATASET/TEST06/ISAC | Select directory | |
| Output box size [Pixels] | 352 | 352 | | |
| Invert handedness | NO | ☑ | | |
| Low-pass filter resolution [A] | -1.0 | -1.0 | | |

| | | | |
|---|---|---|---|
| MPI processors | 1 | | Save parameters |
| MPI command line template | | | Load parameters |
| Submit job to queue | ☐ | | |
| Job name | sp_pipe_moon_eliminator | | |
| Submission command | sbatch | | Generate command line |
| | | | Add to pipeline folder |
| Submission script template | e/sphire_defq_ppn16_threads1.sh | Select template | **Run command** |

From RVIPER

SPHIRE

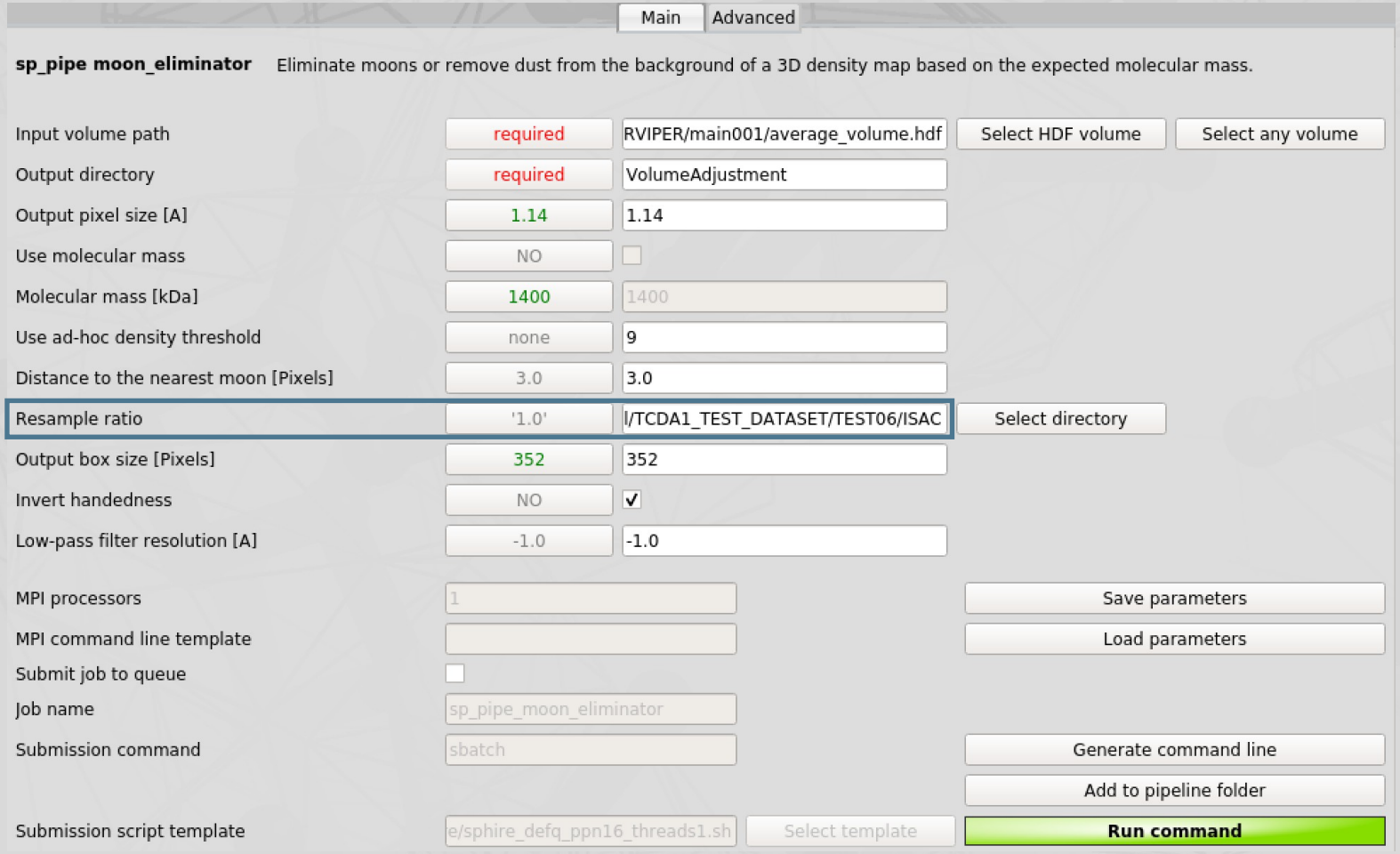
# What to do after running RVIPER




Threshold based on molecular weight

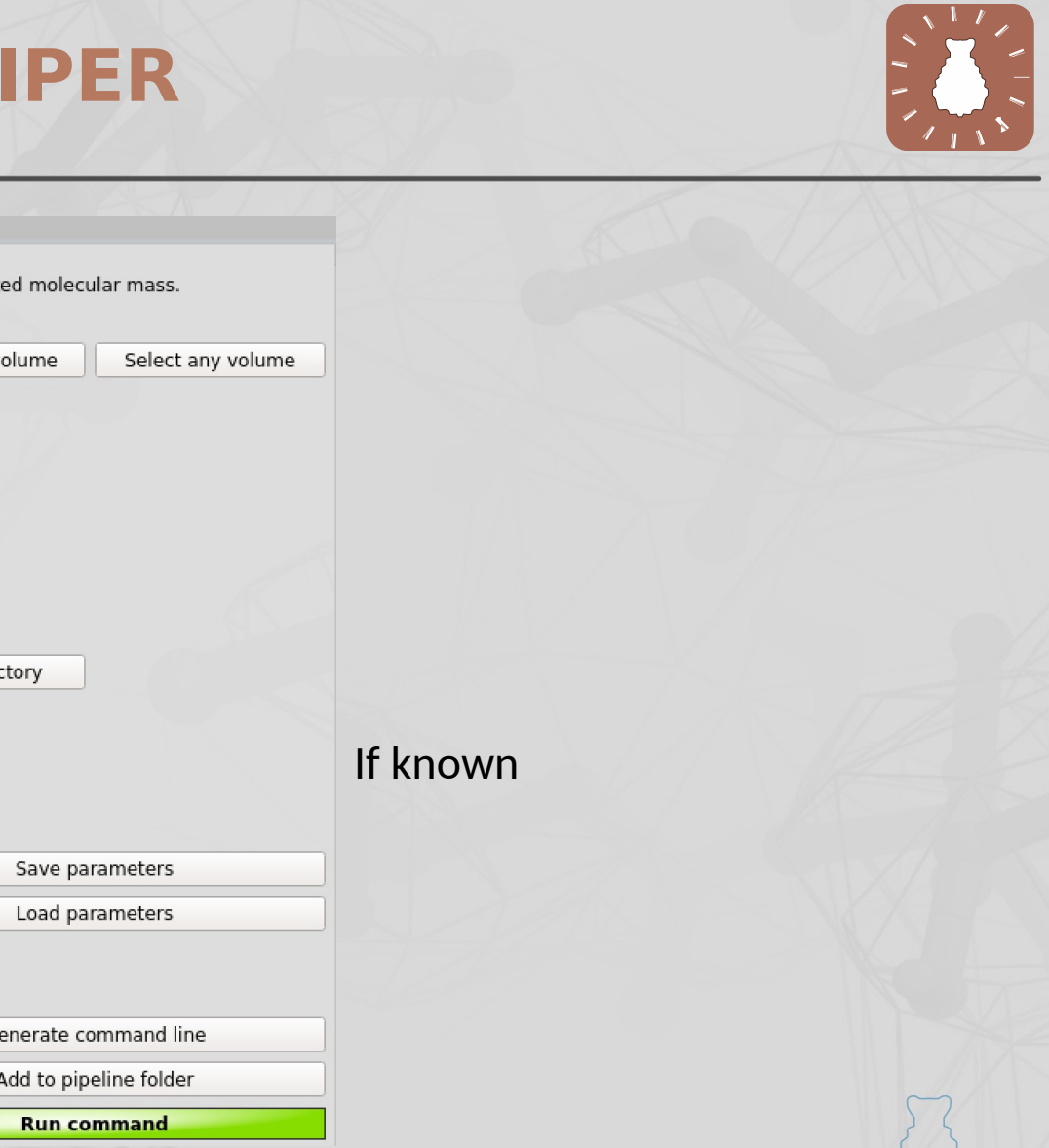
Threshold provided by user

# What to do after running RVIPER




Downsampling factor from ISAC

# What to do after running RVIPER




If known

# What to do after running RVIPER

Main | Advanced

**sp_pipe moon_eliminator** Eliminate moons or remove dust from the background of a 3D density map based on the expected molecular mass.

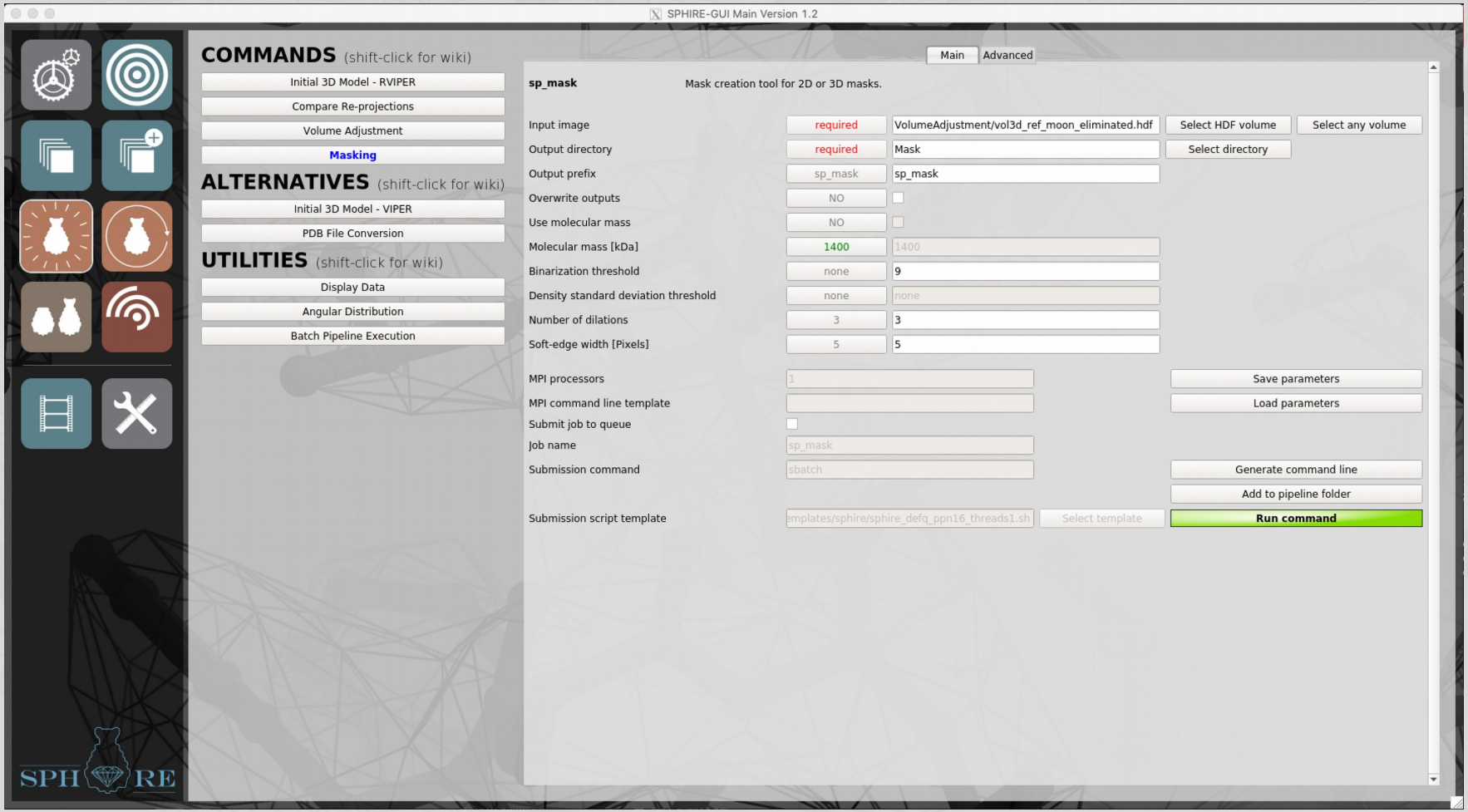
| | | | | |
|---|---|---|---|---|
| Input volume path | required | RVIPER/main001/average_volume.hdf | Select HDF volume | Select any volume |
| Output directory | required | VolumeAdjustment | | |
| Output pixel size [A] | 1.14 | 1.14 | | |
| Use molecular mass | NO | ☐ | | |
| Molecular mass [kDa] | 1400 | 1400 | | |
| Use ad-hoc density threshold | none | 9 | | |
| Distance to the nearest moon [Pixels] | 3.0 | 3.0 | | |
| Resample ratio | '1.0' | l/TCDA1_TEST_DATASET/TEST06/ISAC | Select directory | |
| Output box size [Pixels] | 352 | 352 | | |
| Invert handedness | NO | ☑ | | |
| Low-pass filter resolution [A] | -1.0 | -1.0 | | |

| | | | |
|---|---|---|---|
| MPI processors | 1 | | Save parameters |
| MPI command line template | | | Load parameters |
| Submit job to queue | ☐ | | |
| Job name | sp_pipe_moon_eliminator | | |
| Submission command | sbatch | | Generate command line |
| | | | Add to pipeline folder |
| Submission script template | e/sphire_defq_ppn16_threads1.sh | Select template | **Run command** |

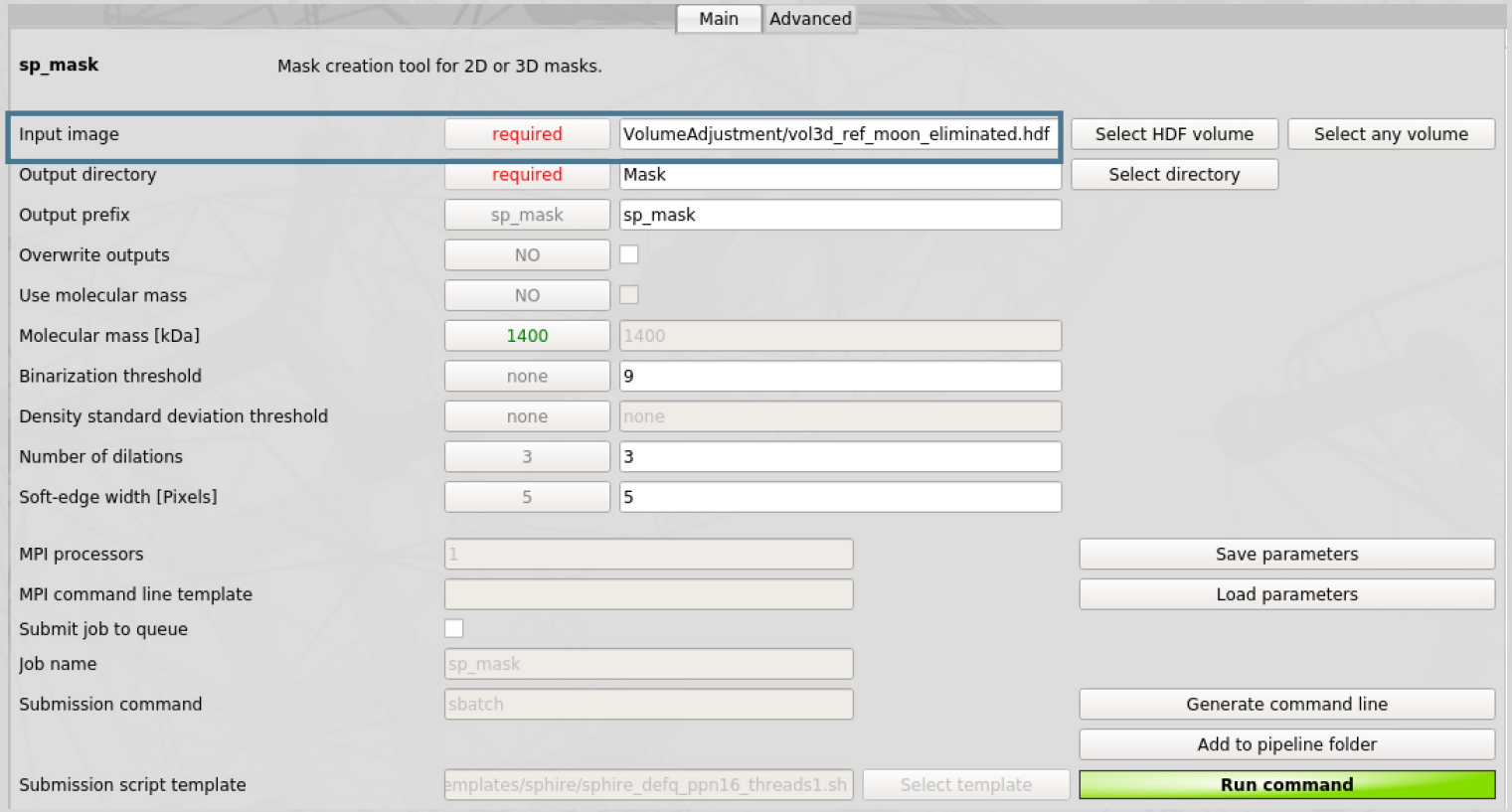
Can also be used to eliminate “moons”

SPHIRE

# Masking

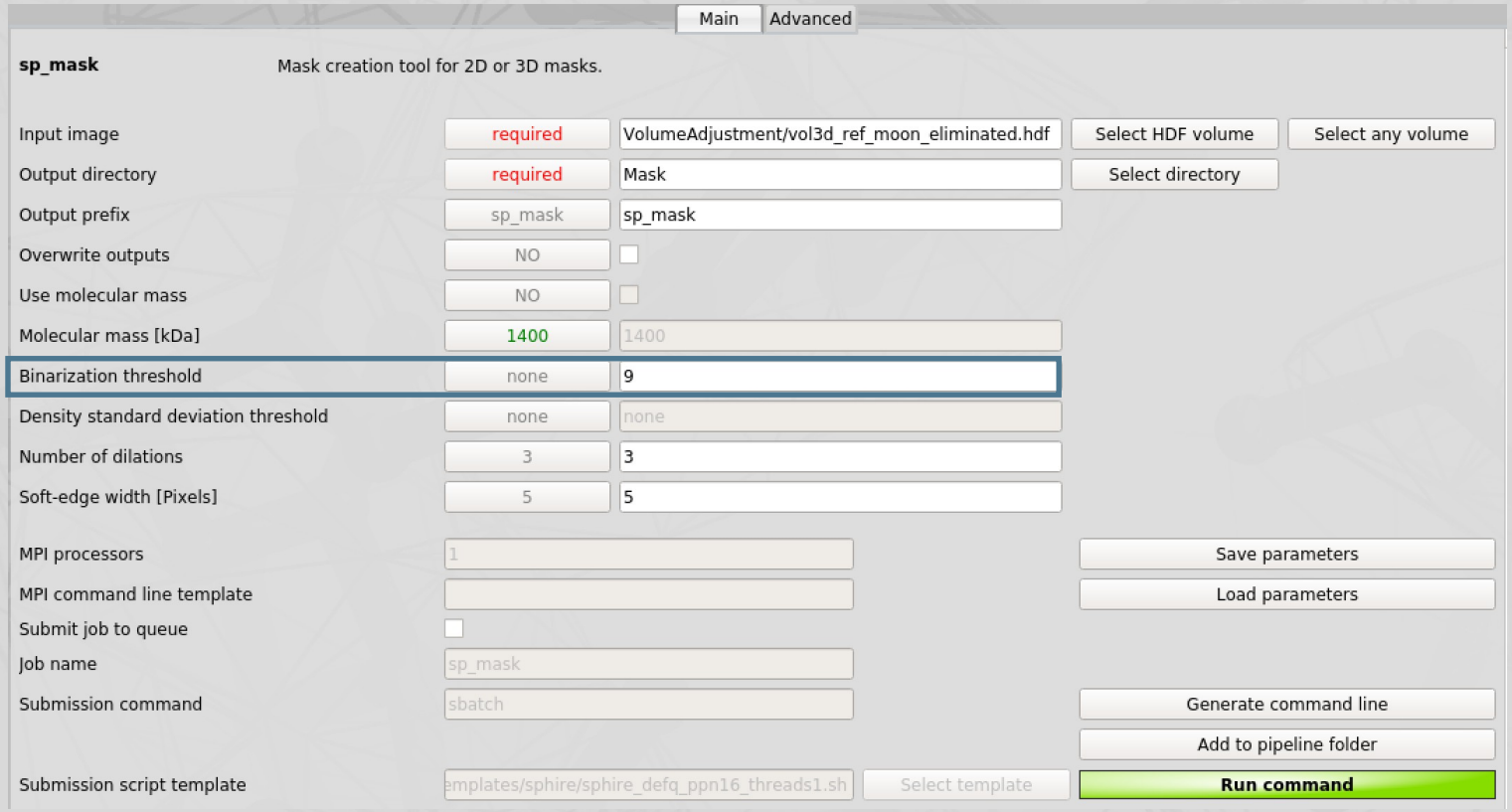

SPHIRE-GUI Main Version 1.2
COMMANDS (shift-click for wiki)
Initial 3D Model - RVIPER
Compare Re-projections
Volume Adjustment
Masking
ALTERNATIVES (shift-click for wiki)
Initial 3D Model - VIPER
PDB File Conversion
UTILITIES (shift-click for wiki)
Display Data
Angular Distribution
Batch Pipeline Execution
Main
Advanced
sp_mask
Mask creation tool for 2D or 3D masks.
Input image
required
VolumeAdjustment/vol3d_ref_moon_eliminated.hdf
Select HDF volume
Select any volume
Output directory
required
Mask
Select directory
Output prefix
sp_mask
sp_mask
Overwrite outputs
NO
Use molecular mass
NO
Molecular mass [kDa]
1400
1400
Binarization threshold
none
9
Density standard deviation threshold
none
none
Number of dilations
3
3
Soft-edge width [Pixels]
5
5
MPI processors
1
Save parameters
MPI command line template
Load parameters
Submit job to queue
Job name
sp_mask
Submission command
sbatch
Generate command line
Add to pipeline folder
Submission script template
emplates/sphire/sphire_defq_ppn16_threads1.sh
Select template
Run command
SPH RE

# Masking



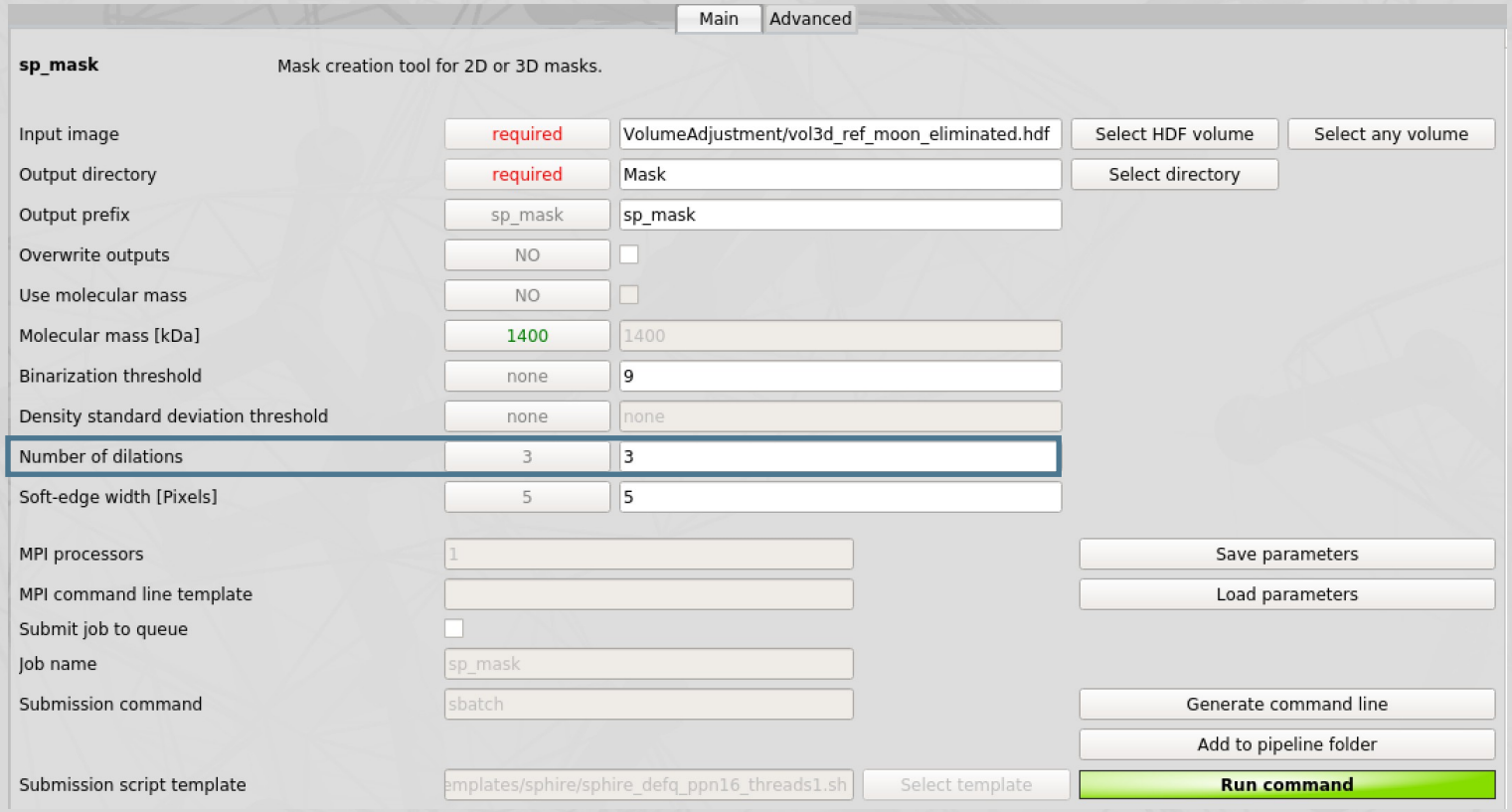

Moon-eliminated volume

# Masking



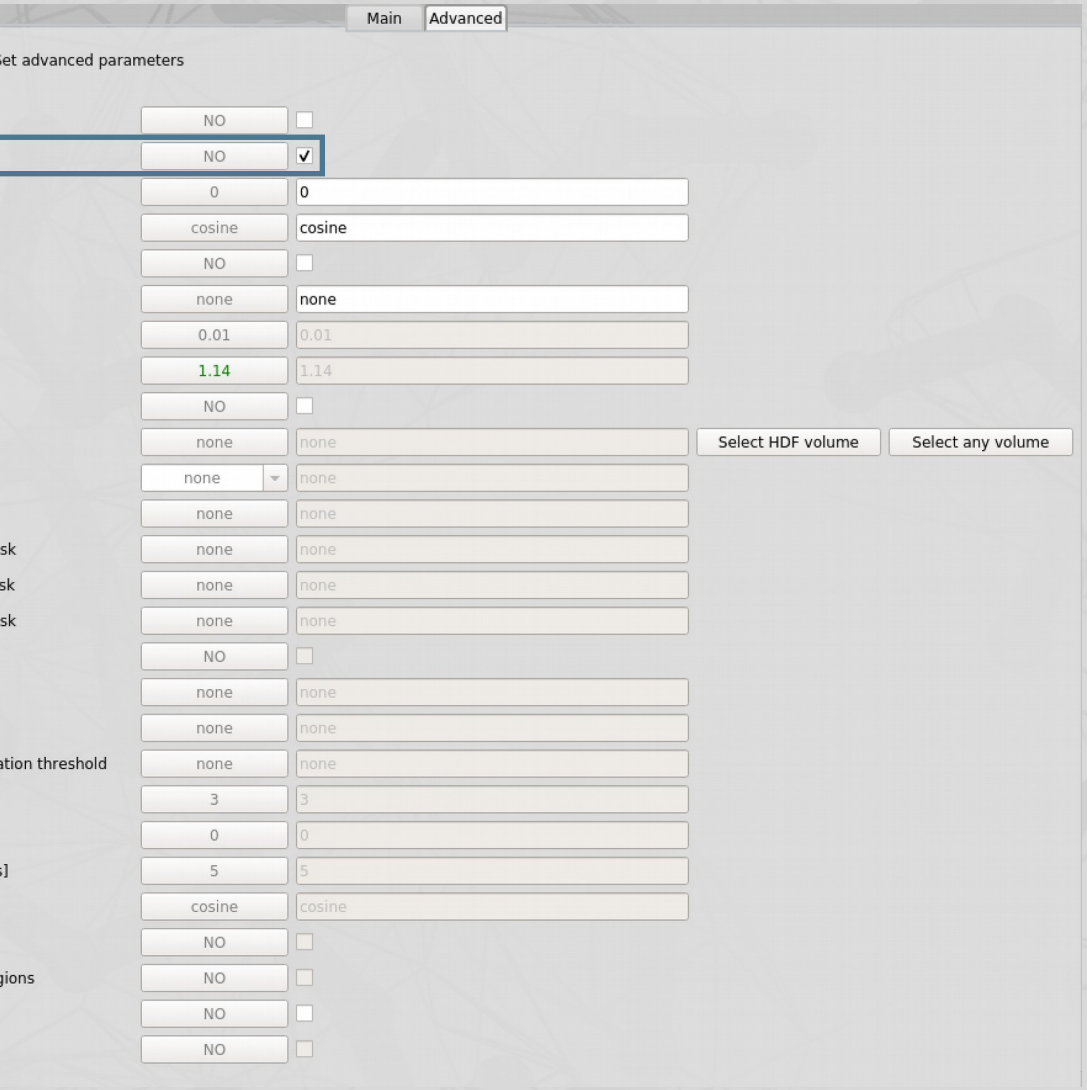

Threshold

# Masking



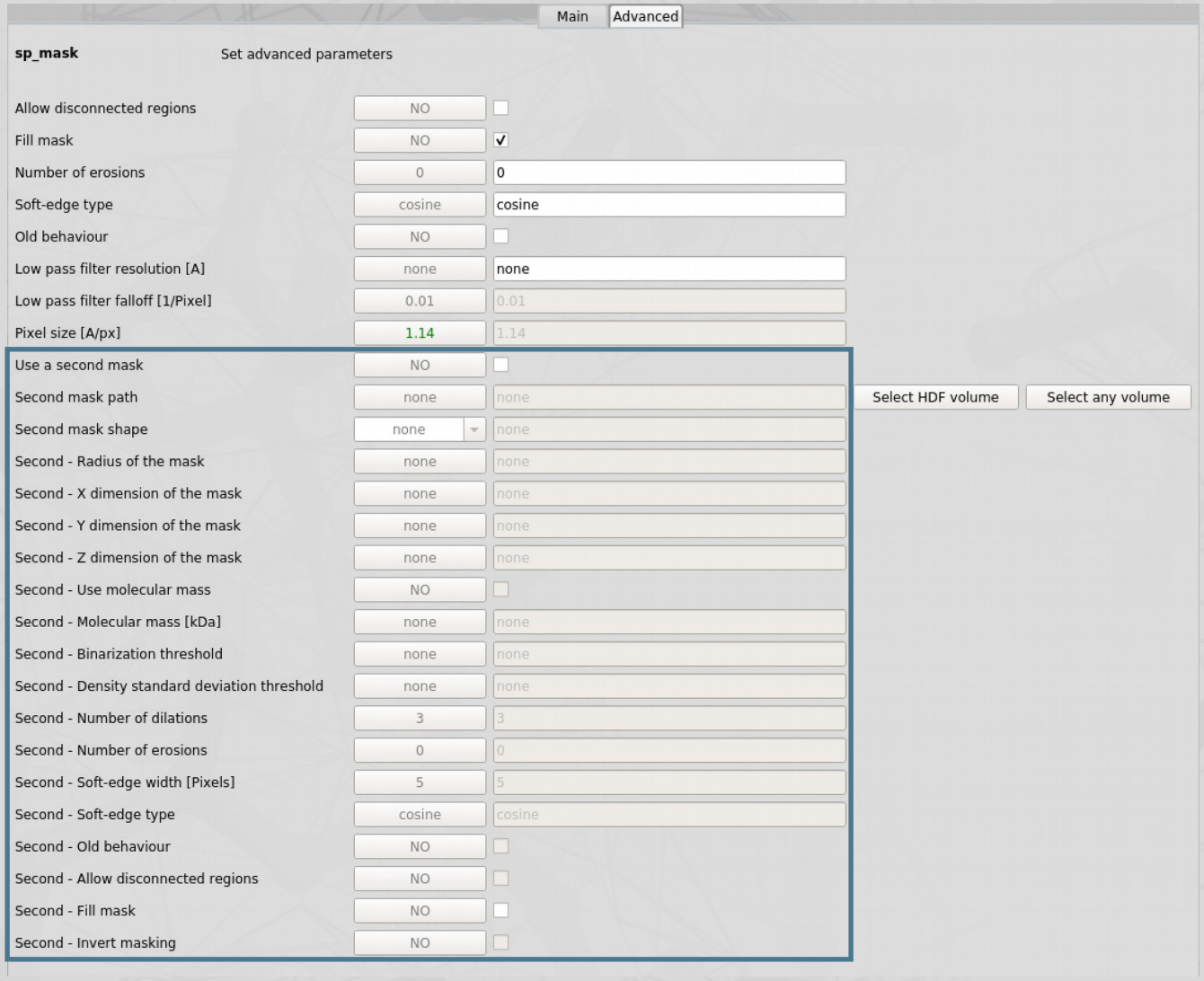

Each dilation extends by ~2 voxels

# Masking




Fills in empty spaces

# Masking




For helical processing (currently in beta)

# SPHIRE Pipeline

## PROJECT
Project Settings

## WINDOW
crYOLO Particle Picking
WINDOW Particle Extraction

## VIPER
RVIPER Initial 3D Modeling

## SORT3D
3D Variability Estimation
SORT3D 3D Clustering

## MOVIE (optional)
Micrograph Movie Alignment
Drift Assessment

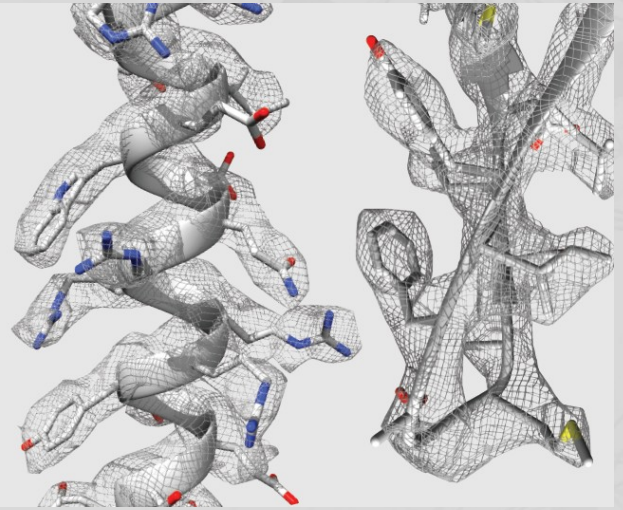
## CTER
CTER CTF Estimation
CTF Assessment

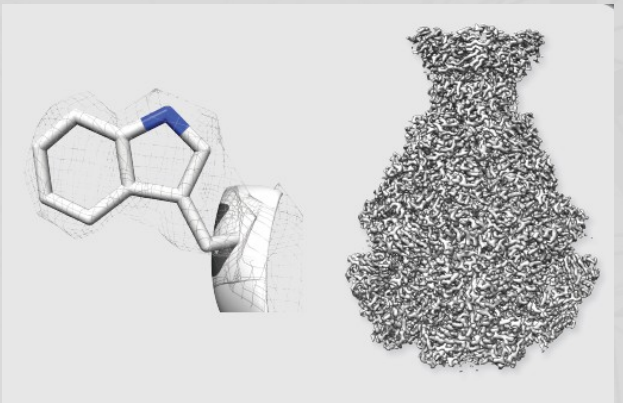
## ISAC
ISAC2 2D Clustering
Beautifier

## MERIDIEN
MERIDIEN 3D Refinement
3D Refinement Assessment
PostRefiner

## LOCALRES
Local Resolution
3D Local Filter

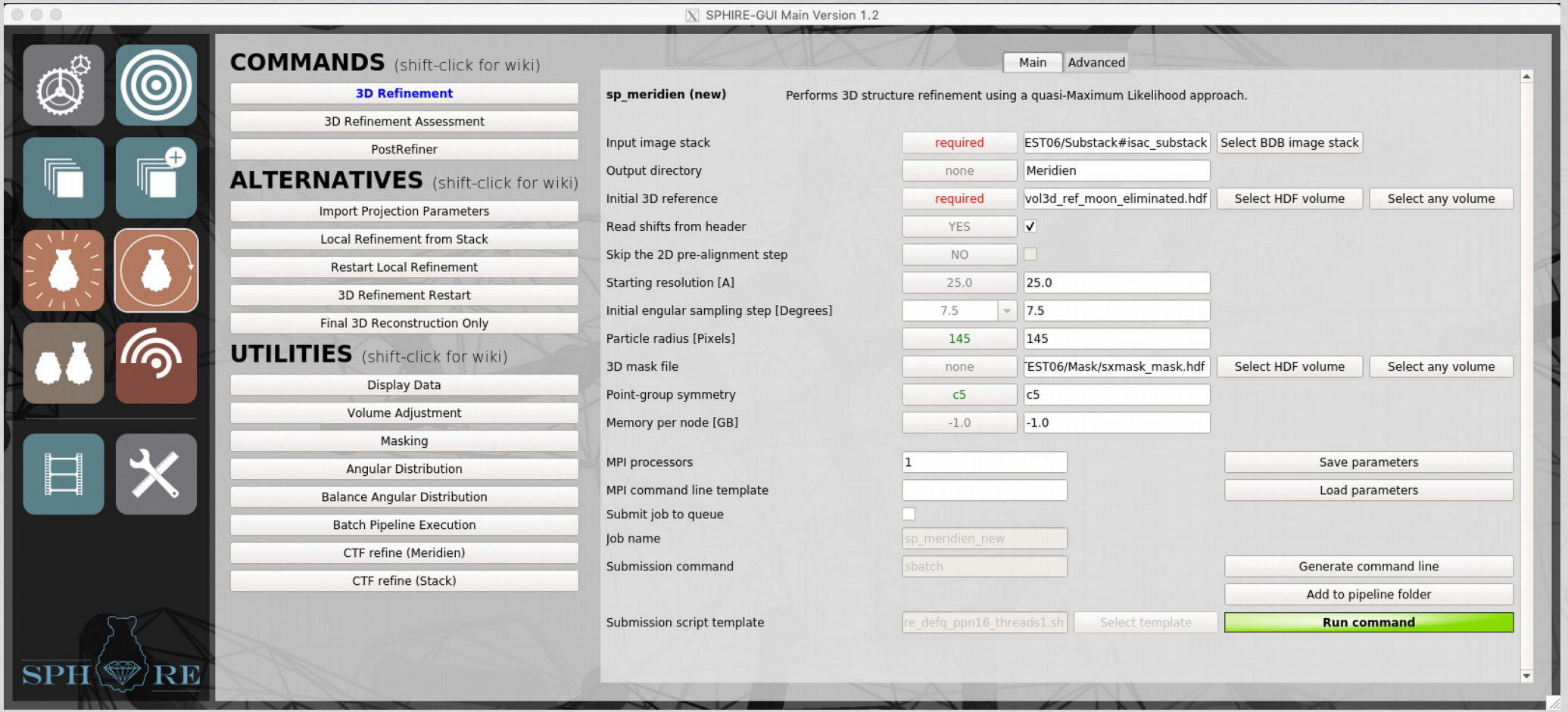
## Utilities
Utility commands





SPHIRE

# Refinement using MERIDIEN



# Refinement using MERIDIEN

Main | Advanced

**sp_meridien (new)** Performs 3D structure refinement using a quasi-Maximum Likelihood approach.

| | | | | |
|---|---|---|---|---|
| Input image stack | required | EST06/Substack#isac_substack | Select BDB image stack | |
| Output directory | none | Meridien | | |
| Initial 3D reference | required | vol3d_ref_moon_eliminated.hdf | Select HDF volume | Select any volume |
| Read shifts from header | YES | ☑ | | |
| Skip the 2D pre-alignment step | NO | ☐ | | |
| Starting resolution [A] | 25.0 | 25.0 | | |
| Initial engular sampling step [Degrees] | 7.5 | 7.5 | | |
| Particle radius [Pixels] | 145 | 145 | | |
| 3D mask file | none | EST06/Mask/sxmask_mask.hdf | Select HDF volume | Select any volume |
| Point-group symmetry | c5 | c5 | | |
| Memory per node [GB] | -1.0 | -1.0 | | |

| | | | |
|---|---|---|---|
| MPI processors | 1 | | Save parameters |
| MPI command line template | | | Load parameters |
| Submit job to queue | ☐ | | |
| Job name | sp_meridien_new | | |
| Submission command | sbatch | | Generate command line |
| | | | Add to pipeline folder |
| Submission script template | re_defq_ppn16_threads1.sh | Select template | **Run command** |

Substack (after picking good ISAC classes)

SPHIRE

# Refinement using MERIDIEN

Main | Advanced

**sp_meridien (new)** Performs 3D structure refinement using a quasi-Maximum Likelihood approach.

| | | | | |
|---|---|---|---|---|
| Input image stack | required | EST06/Substack#isac_substack | Select BDB image stack | |
| Output directory | none | Meridien | | |
| Initial 3D reference | required | vol3d_ref_moon_eliminated.hdf | Select HDF volume | Select any volume |
| Read shifts from header | YES | ☑ | | |
| Skip the 2D pre-alignment step | NO | ☐ | | |
| Starting resolution [A] | 25.0 | 25.0 | | |
| Initial engular sampling step [Degrees] | 7.5 | 7.5 | | |
| Particle radius [Pixels] | 145 | 145 | | |
| 3D mask file | none | EST06/Mask/sxmask_mask.hdf | Select HDF volume | Select any volume |
| Point-group symmetry | c5 | c5 | | |
| Memory per node [GB] | -1.0 | -1.0 | | |

| | | | |
|---|---|---|---|
| MPI processors | 1 | Save parameters | |
| MPI command line template | | Load parameters | |
| Submit job to queue | ☐ | | |
| Job name | sp_meridien_new | | |
| Submission command | sbatch | Generate command line | |
| | | Add to pipeline folder | |
| Submission script template | re_defq_ppn16_threads1.sh | Select template | **Run command** |

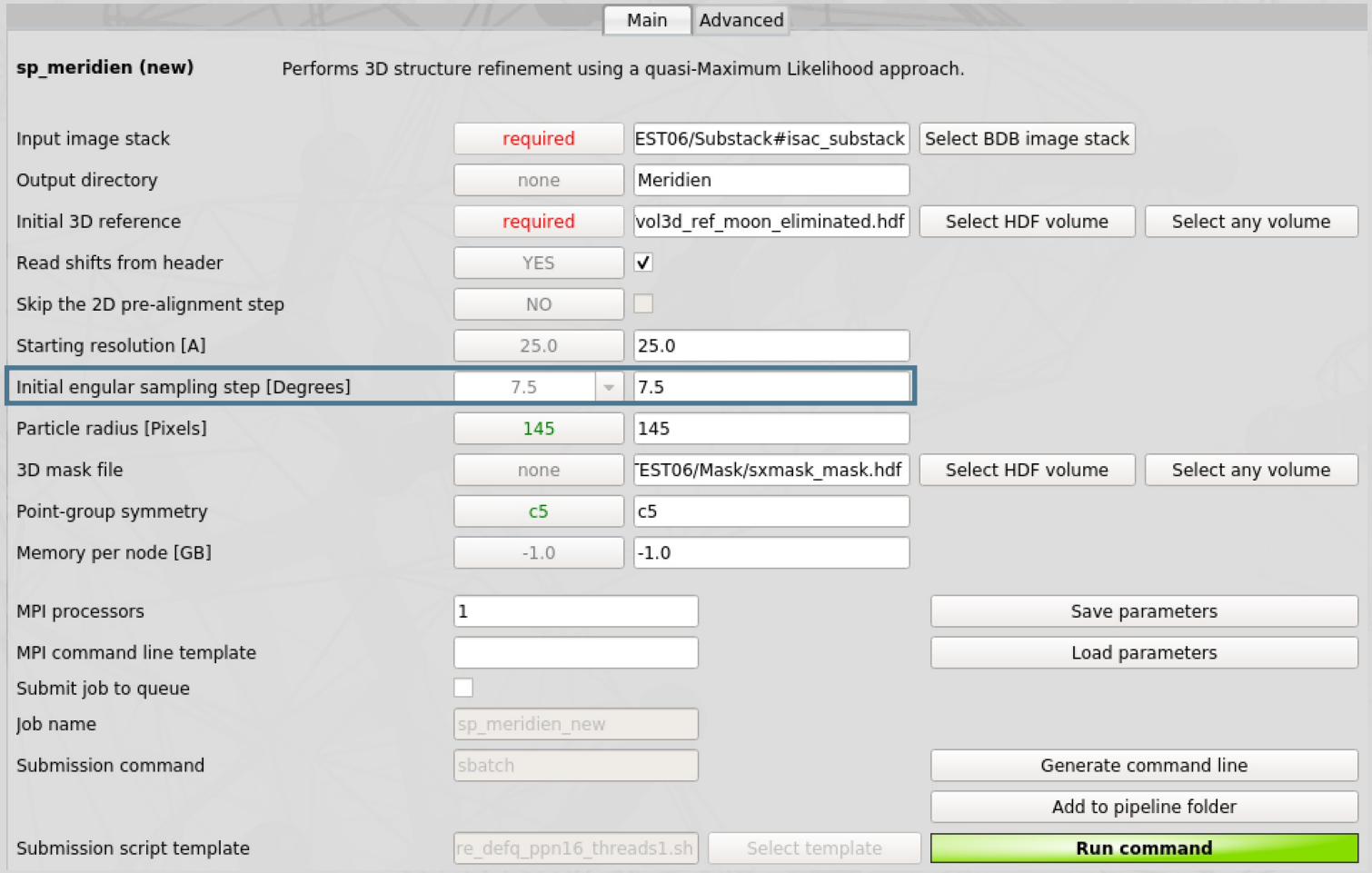
Adjusted RVIPER volume

SPHIRE

# Refinement using MERIDIEN

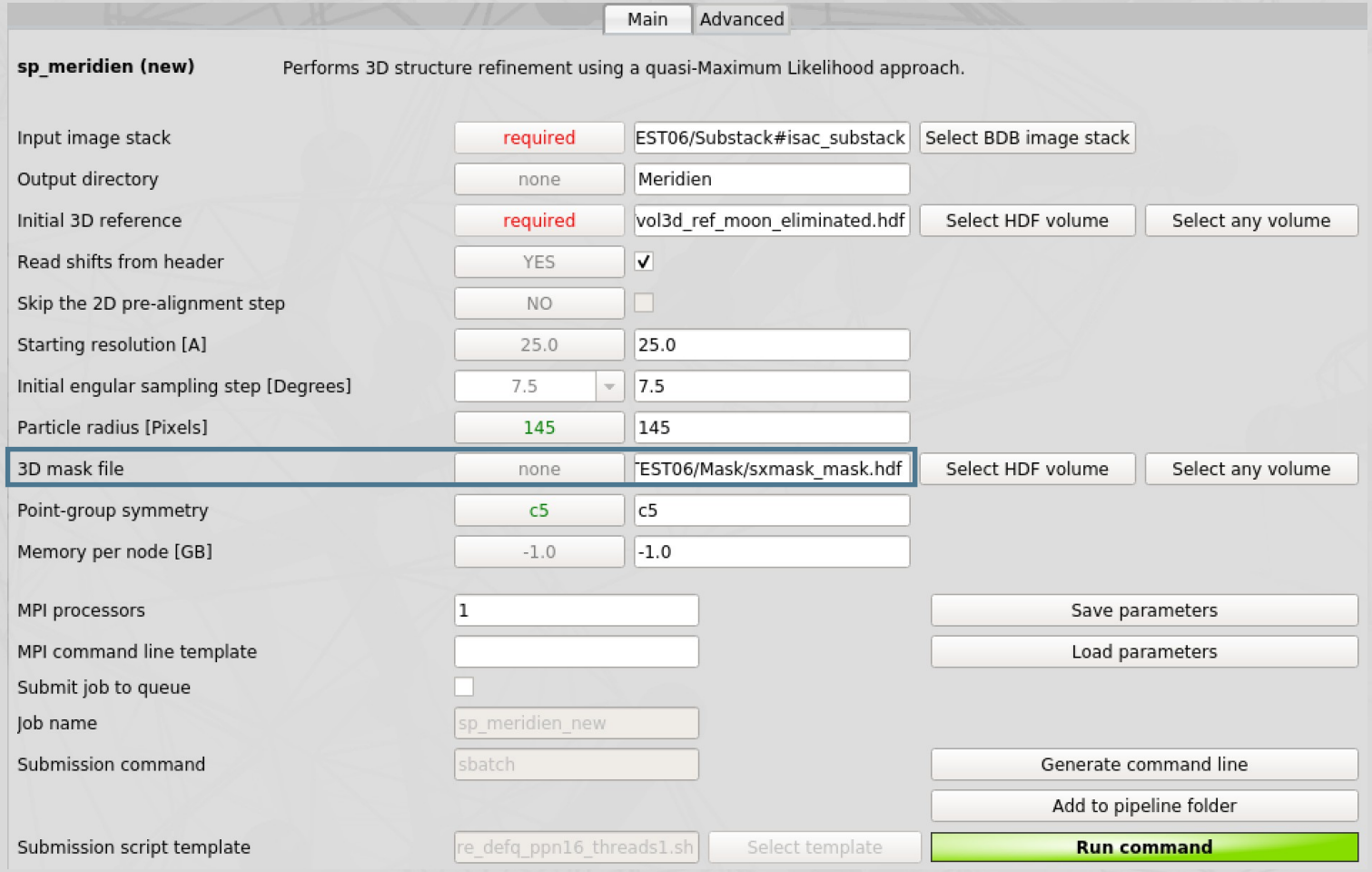

To minimize possible model bias

# Refinement using MERIDIEN




Angular sampling

# Refinement using MERIDIEN




Angular sampling

# Refinement using MERIDIEN

Main | Advanced

**sp_meridien (new)** Performs 3D structure refinement using a quasi-Maximum Likelihood approach.

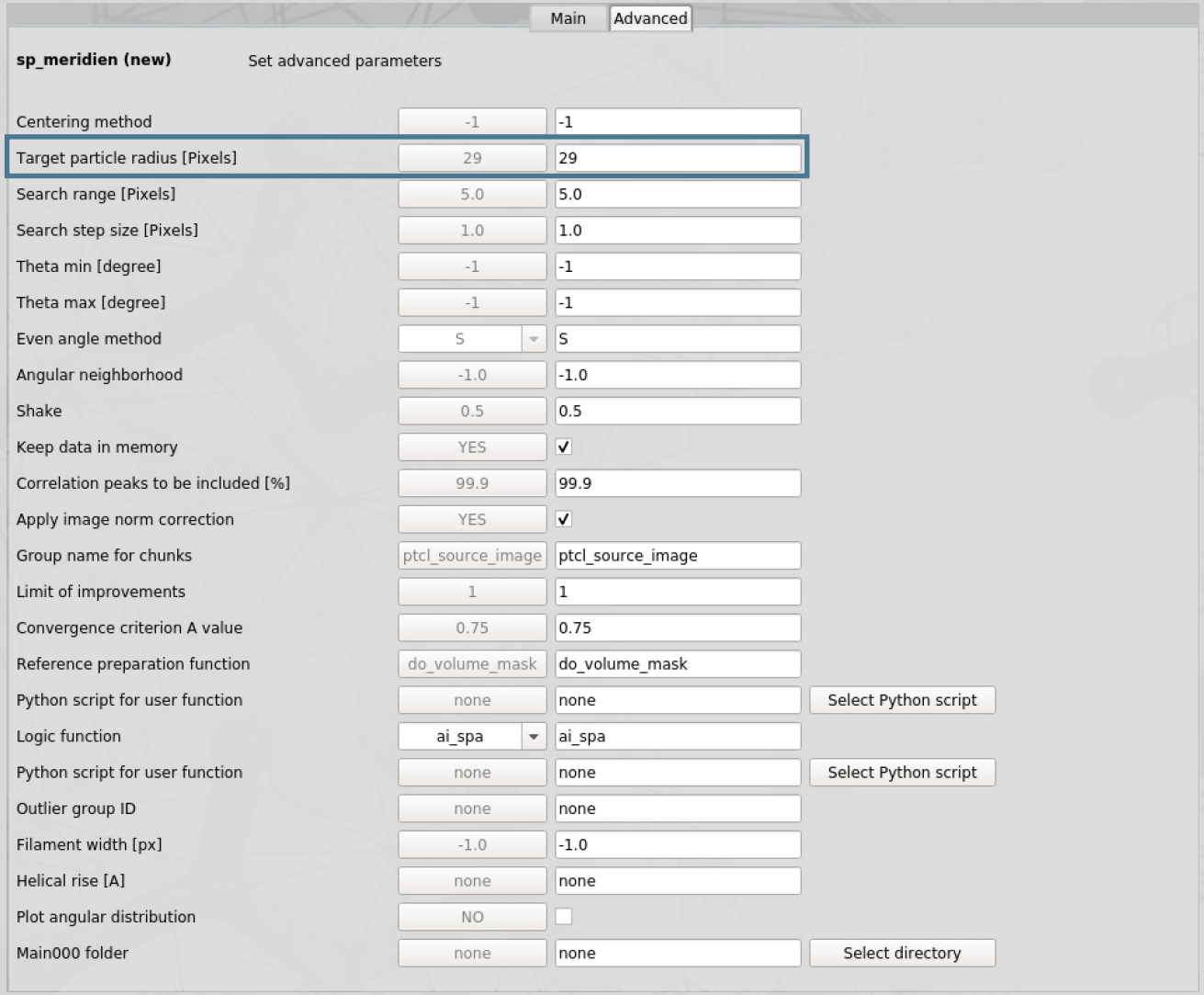
| | | | | |
|---|---|---|---|---|
| Input image stack | required | EST06/Substack#isac_substack | Select BDB image stack | |
| Output directory | none | Meridien | | |
| Initial 3D reference | required | vol3d_ref_moon_eliminated.hdf | Select HDF volume | Select any volume |
| Read shifts from header | YES | ☑ | | |
| Skip the 2D pre-alignment step | NO | ☐ | | |
| Starting resolution [A] | 25.0 | 25.0 | | |
| Initial engular sampling step [Degrees] | 7.5 | 7.5 | | |
| Particle radius [Pixels] | 145 | 145 | | |
| 3D mask file | none | EST06/Mask/sxmask_mask.hdf | Select HDF volume | Select any volume |
| Point-group symmetry | c5 | c5 | | |
| Memory per node [GB] | -1.0 | -1.0 | | |

| | | | |
|---|---|---|---|
| MPI processors | 1 | | Save parameters |
| MPI command line template | | | Load parameters |
| Submit job to queue | ☐ | | |
| Job name | sp_meridien_new | | |
| Submission command | sbatch | | Generate command line |
| | | | Add to pipeline folder |
| Submission script template | re_defq_ppn16_threads1.sh | Select template | **Run command** |

May need to adjust empirically

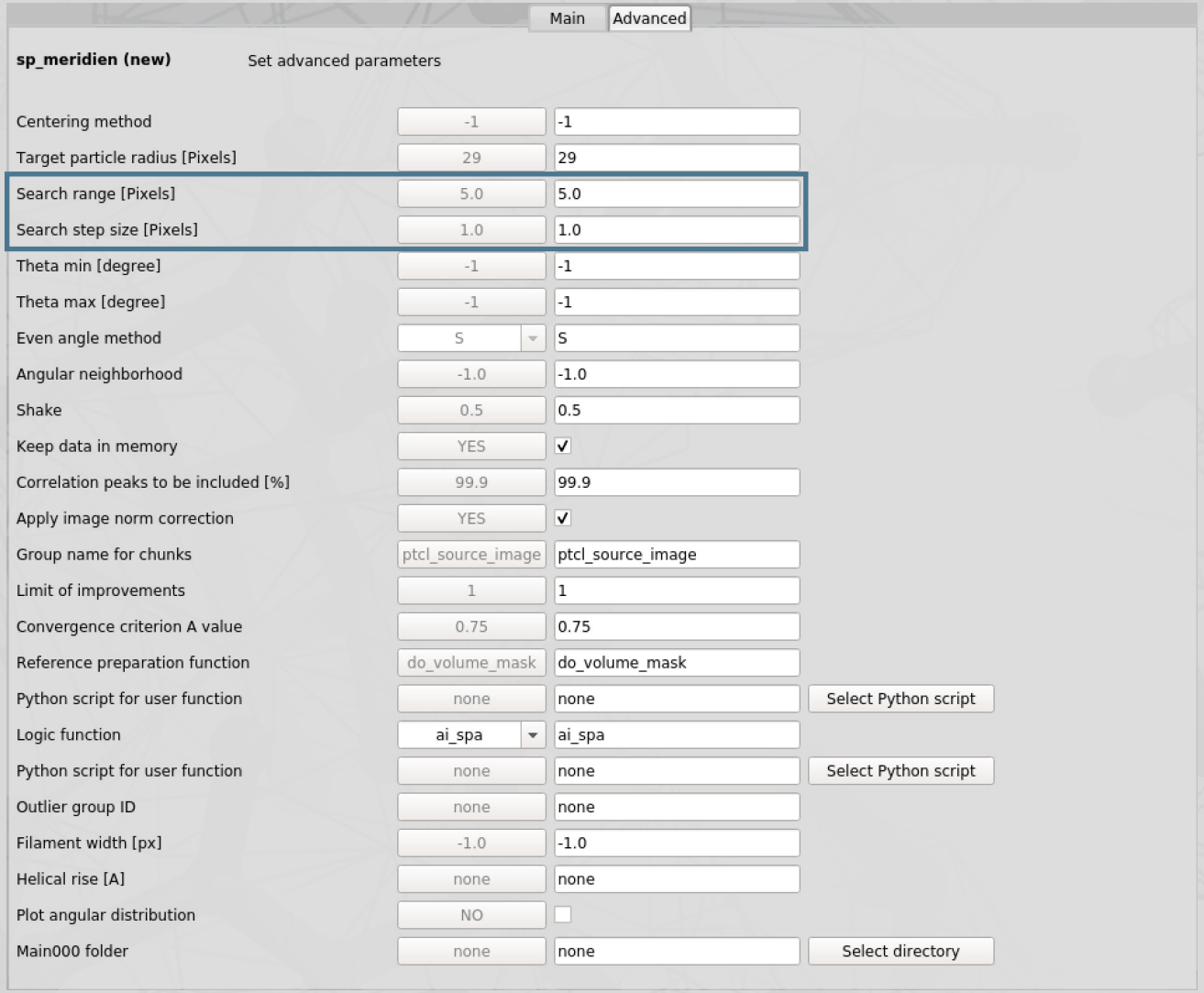
SPHIRE

# Advanced parameters in MERIDIEN



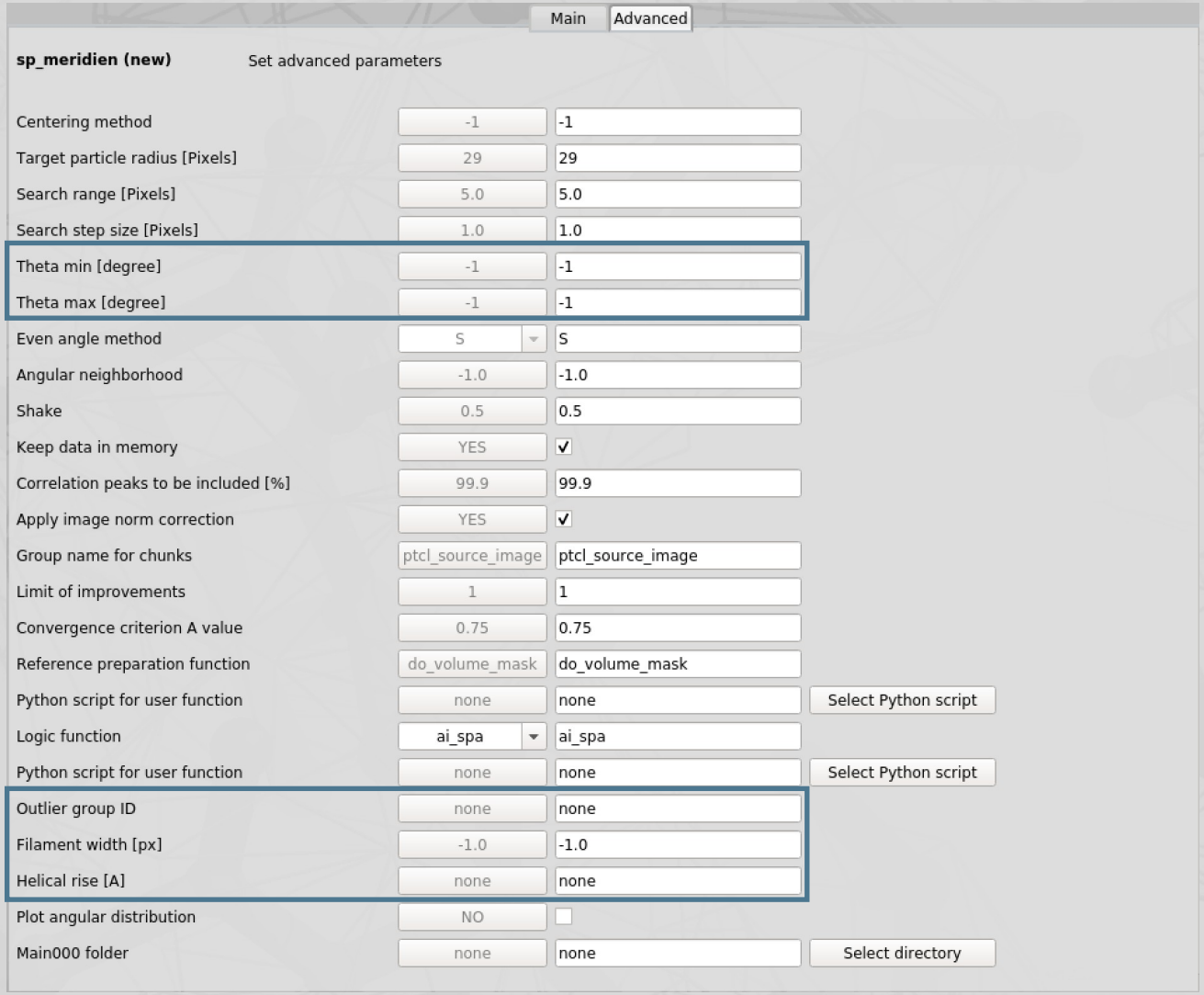

Like in ISAC, images are internally downsampled

# Advanced parameters in MERIDIEN



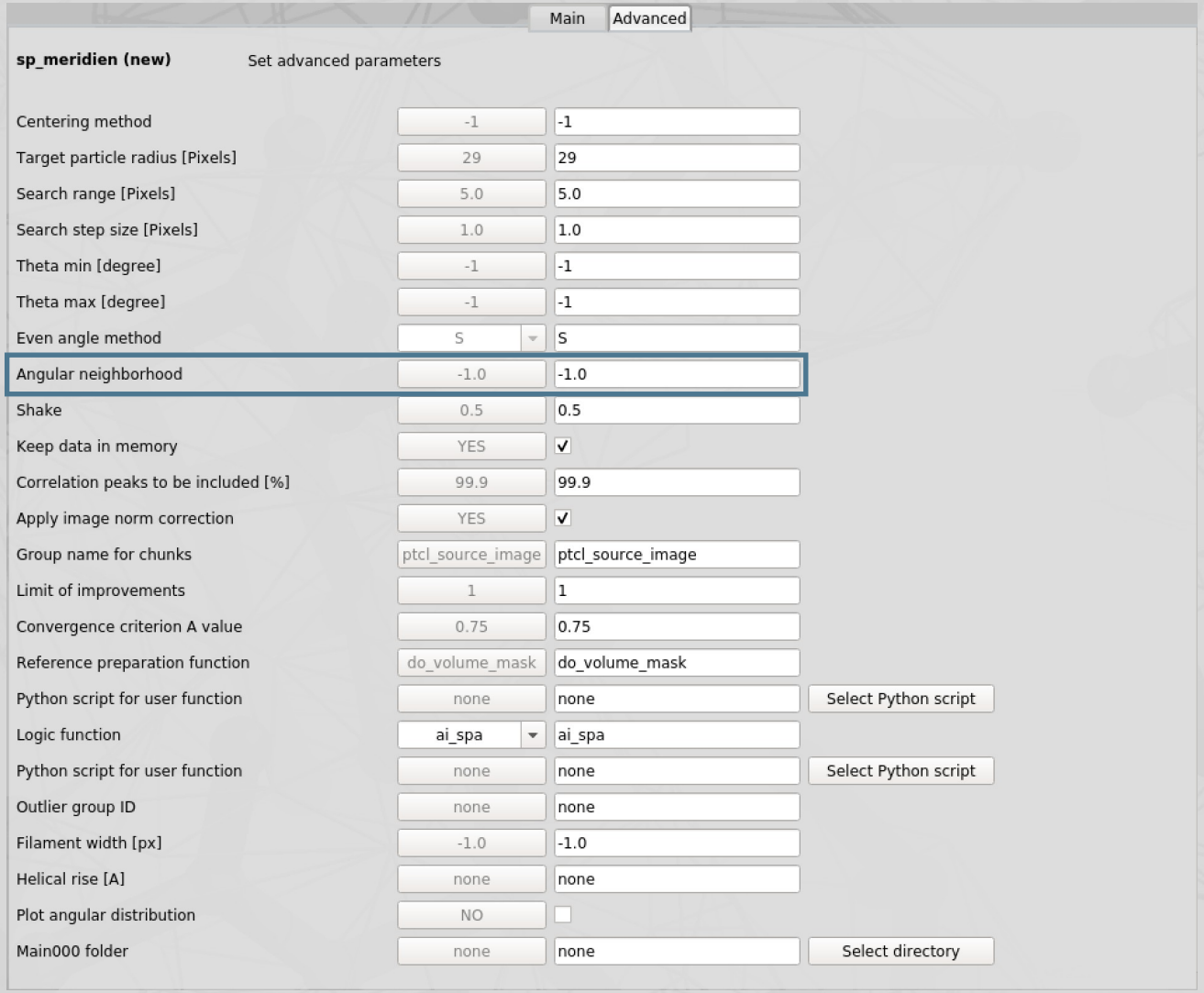

Tradeoff between speed and #iterations

# Advanced parameters in MERIDIEN



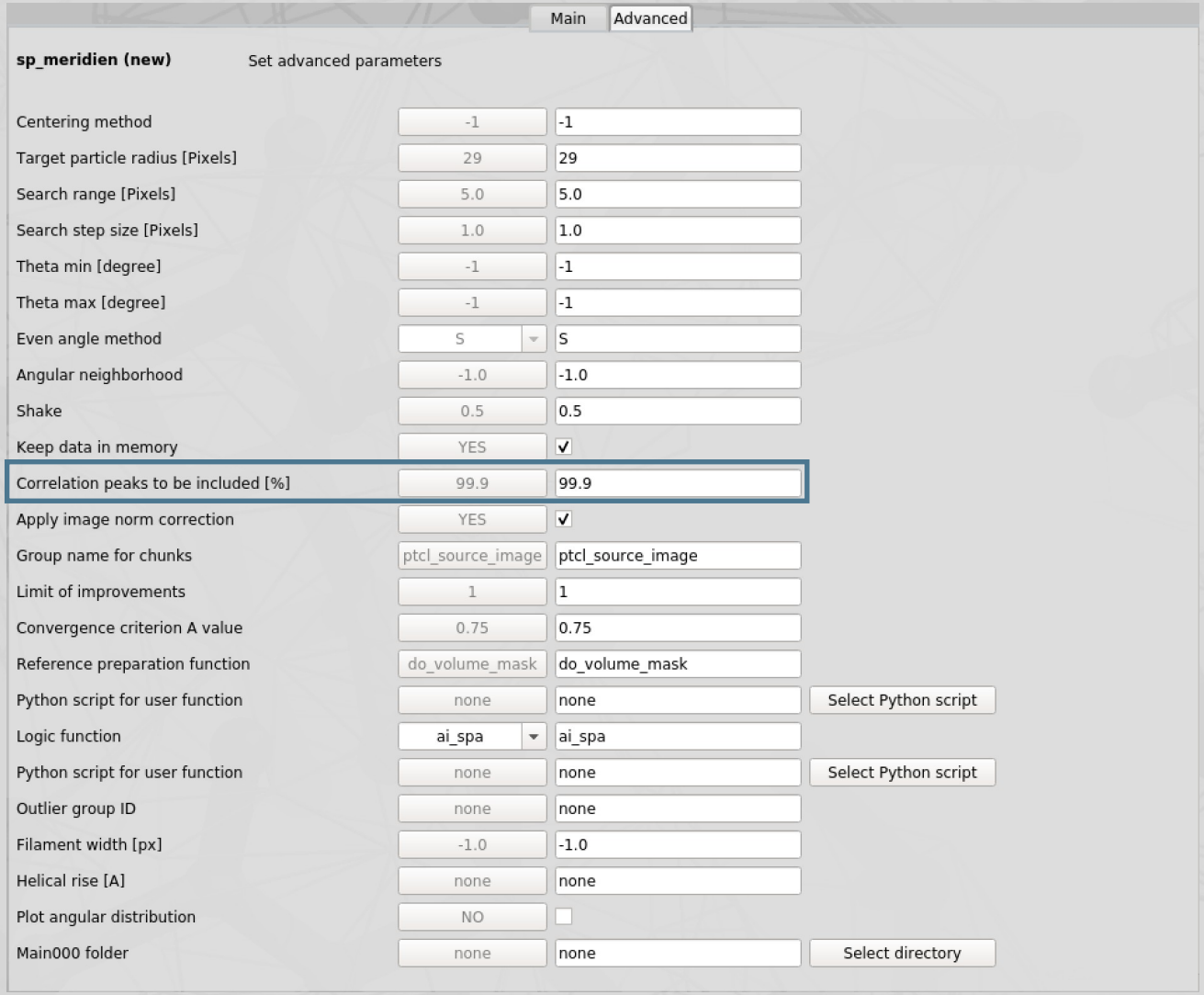

For helical reconstruction (beta)

# Advanced parameters in MERIDIEN



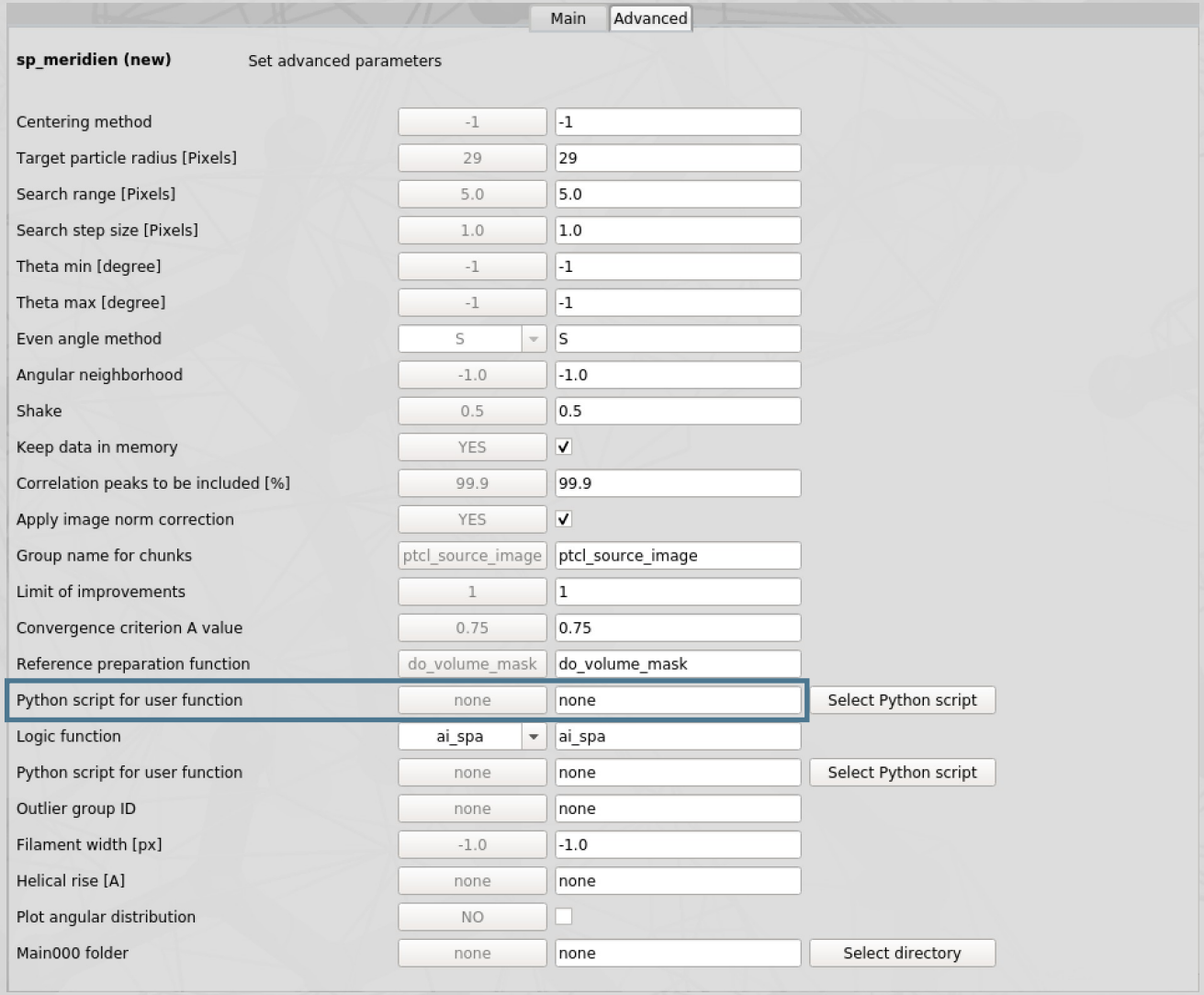

Angular search restricted near current orientation

# Advanced parameters in MERIDIEN



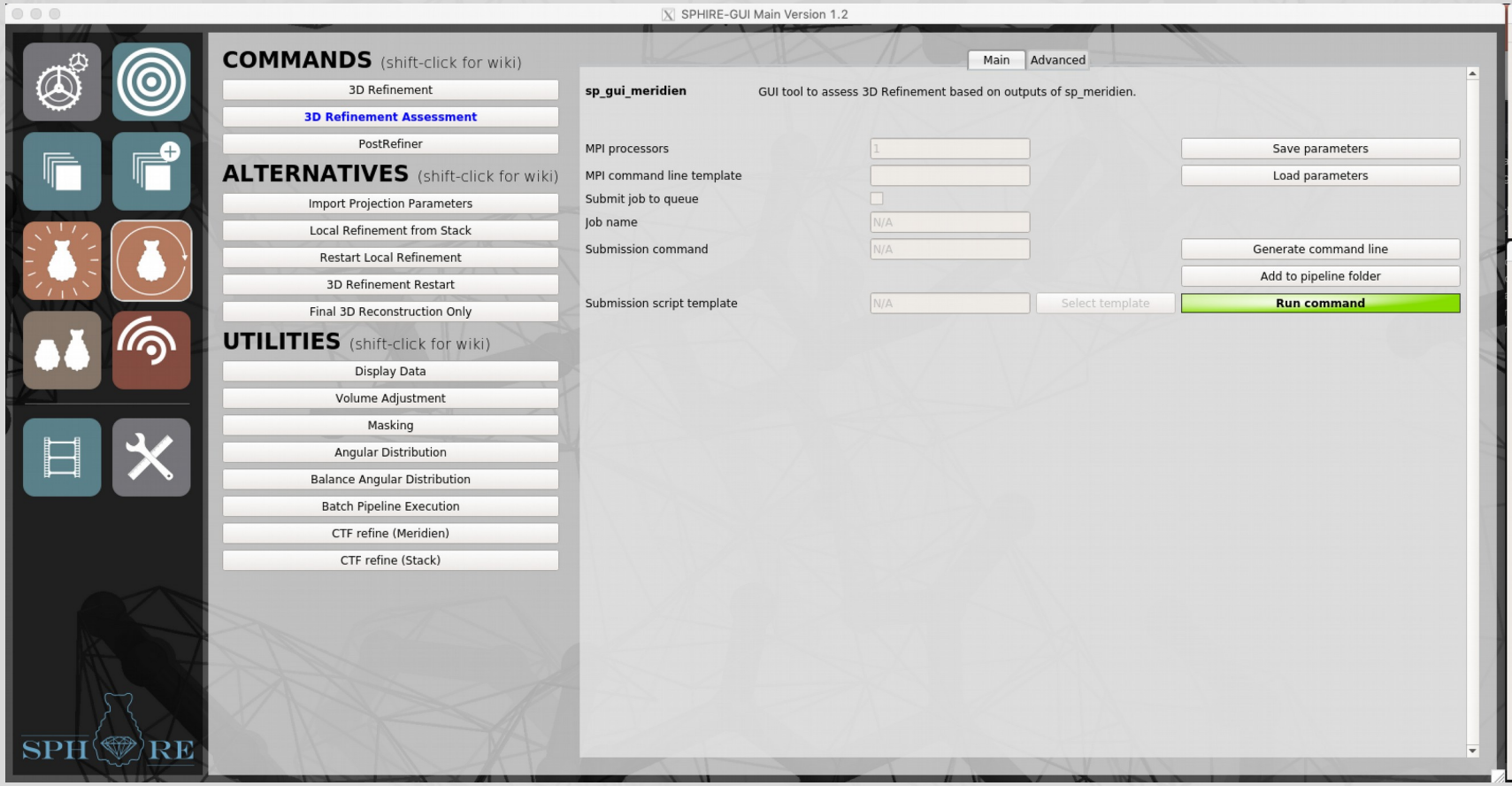

Maximum likelihood “smear” value
0.0 → projection-matching

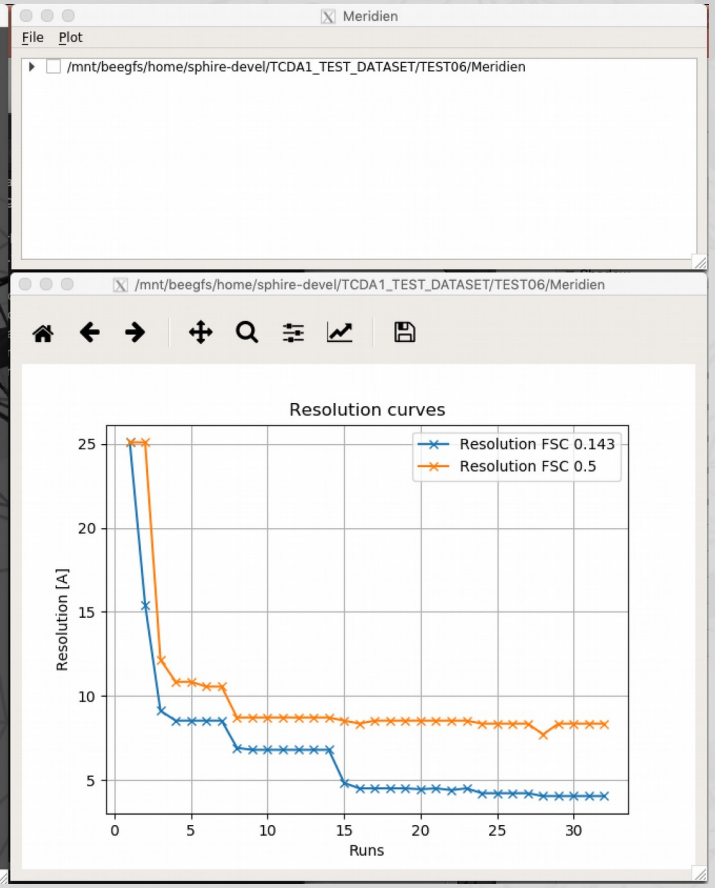
# Advanced parameters in MERIDIEN




Customized script for special cases, e.g., mixed symmetry

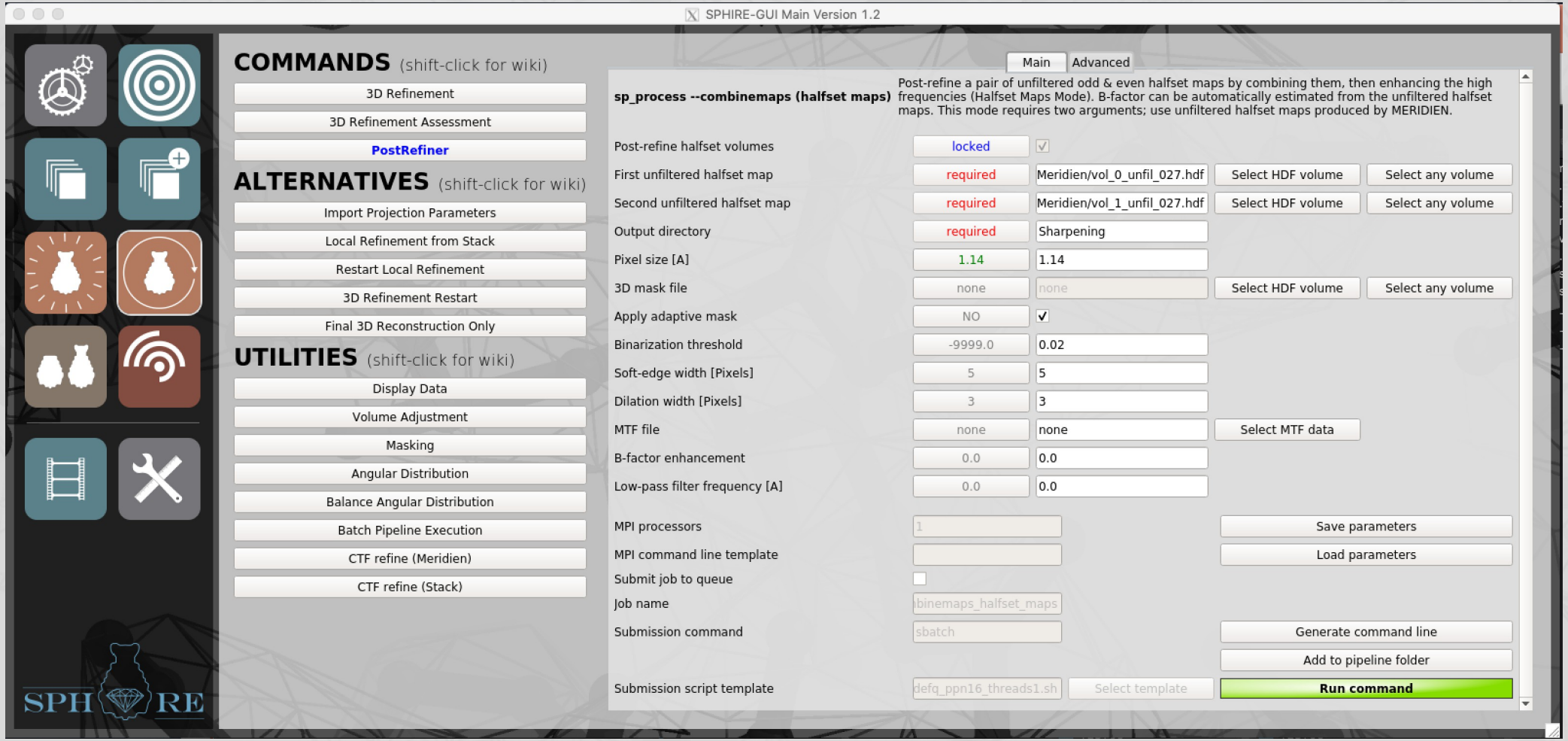
# Refinement assessment




SPHIRE

# PostRefiner



# PostRefiner

Main | Advanced

**sp_process --combinemaps (halfset maps)** Post-refine a pair of unfiltered odd & even halfset maps by combining them, then enhancing the high frequencies (Halfset Maps Mode). B-factor can be automatically estimated from the unfiltered halfset maps. This mode requires two arguments; use unfiltered halfset maps produced by MERIDIEN.

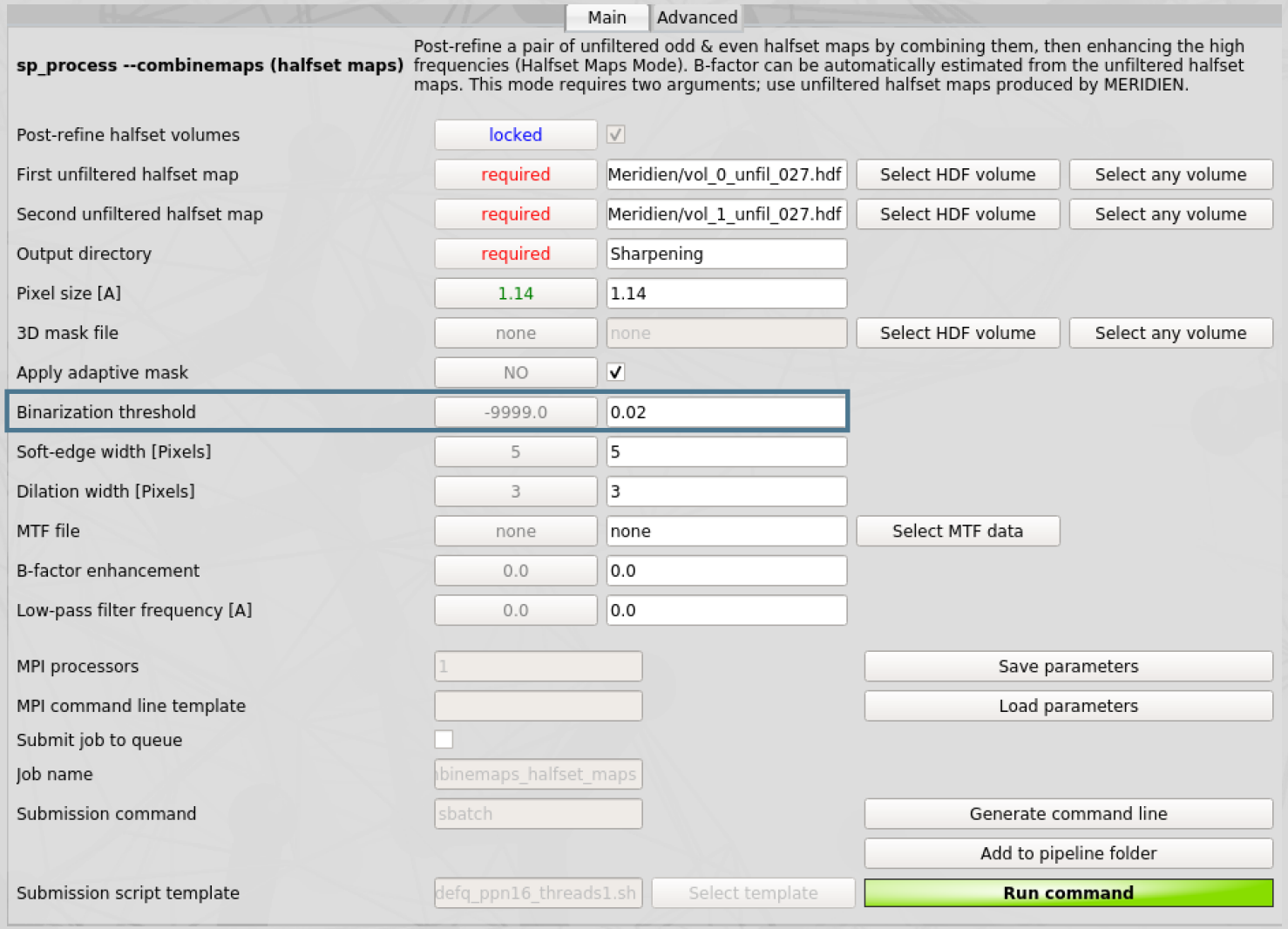
| | | | | |
|---|---|---|---|---|
| Post-refine halfset volumes | locked | ☑ | | |
| First unfiltered halfset map | required | Meridien/vol_0_unfil_027.hdf | Select HDF volume | Select any volume |
| Second unfiltered halfset map | required | Meridien/vol_1_unfil_027.hdf | Select HDF volume | Select any volume |
| Output directory | required | Sharpening | | |
| Pixel size [A] | 1.14 | 1.14 | | |
| 3D mask file | none | none | Select HDF volume | Select any volume |
| Apply adaptive mask | NO | ☑ | | |
| Binarization threshold | -9999.0 | 0.02 | | |
| Soft-edge width [Pixels] | 5 | 5 | | |
| Dilation width [Pixels] | 3 | 3 | | |
| MTF file | none | none | Select MTF data | |
| B-factor enhancement | 0.0 | 0.0 | | |
| Low-pass filter frequency [A] | 0.0 | 0.0 | | |

| | | | |
|---|---|---|---|
| MPI processors | 1 | | Save parameters |
| MPI command line template | | | Load parameters |
| Submit job to queue | ☐ | | |
| Job name | binemaps_halfset_maps | | |
| Submission command | sbatch | | Generate command line |
| | | | Add to pipeline folder |
| Submission script template | defq_ppn16_threads1.sh | Select template | **Run command** |

Input volumes (if you copy & paste, don't forget to change second filename!)

SPHERE

# PostRefiner




Threshold for masking

# PostRefiner

Main | Advanced

**sp_process --combinemaps (halfset maps)** Post-refine a pair of unfiltered odd & even halfset maps by combining them, then enhancing the high frequencies (Halfset Maps Mode). B-factor can be automatically estimated from the unfiltered halfset maps. This mode requires two arguments; use unfiltered halfset maps produced by MERIDIEN.

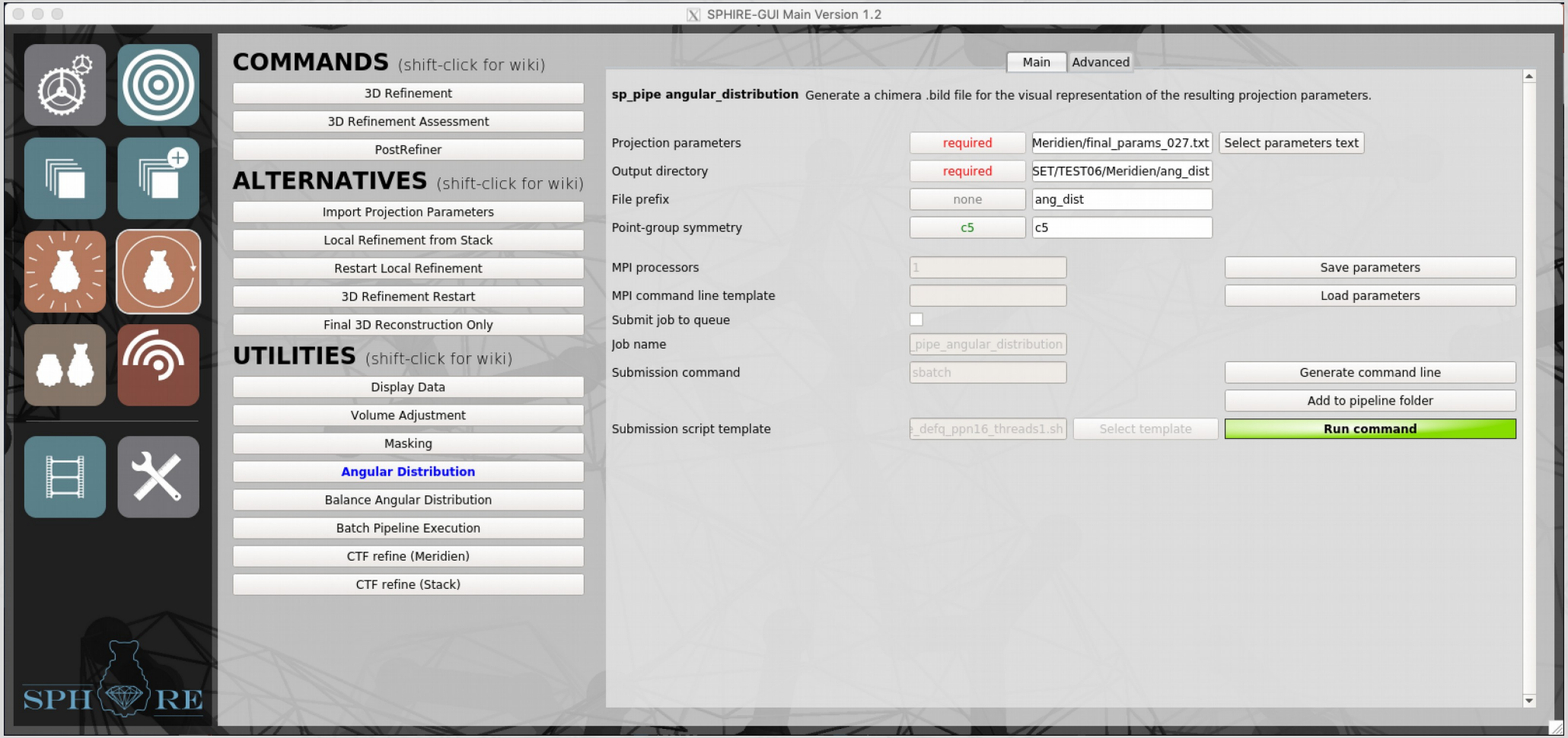
| | | | | |
|---|---|---|---|---|
| Post-refine halfset volumes | locked | ☑ | | |
| First unfiltered halfset map | required | Meridien/vol_0_unfil_027.hdf | Select HDF volume | Select any volume |
| Second unfiltered halfset map | required | Meridien/vol_1_unfil_027.hdf | Select HDF volume | Select any volume |
| Output directory | required | Sharpening | | |
| Pixel size [A] | 1.14 | 1.14 | | |
| 3D mask file | none | none | Select HDF volume | Select any volume |
| Apply adaptive mask | NO | ☑ | | |
| Binarization threshold | -9999.0 | 0.02 | | |
| Soft-edge width [Pixels] | 5 | 5 | | |
| Dilation width [Pixels] | 3 | 3 | | |
| MTF file | none | none | Select MTF data | |
| B-factor enhancement | 0.0 | 0.0 | | |
| Low-pass filter frequency [A] | 0.0 | 0.0 | | |

| | | | |
|---|---|---|---|
| MPI processors | 1 | | Save parameters |
| MPI command line template | | | Load parameters |
| Submit job to queue | ☐ | | |
| Job name | binemaps_halfset_maps | | |
| Submission command | sbatch | | Generate command line |
| | | | Add to pipeline folder |
| Submission script template | defq_ppn16_threads1.sh | Select template | **Run command** |

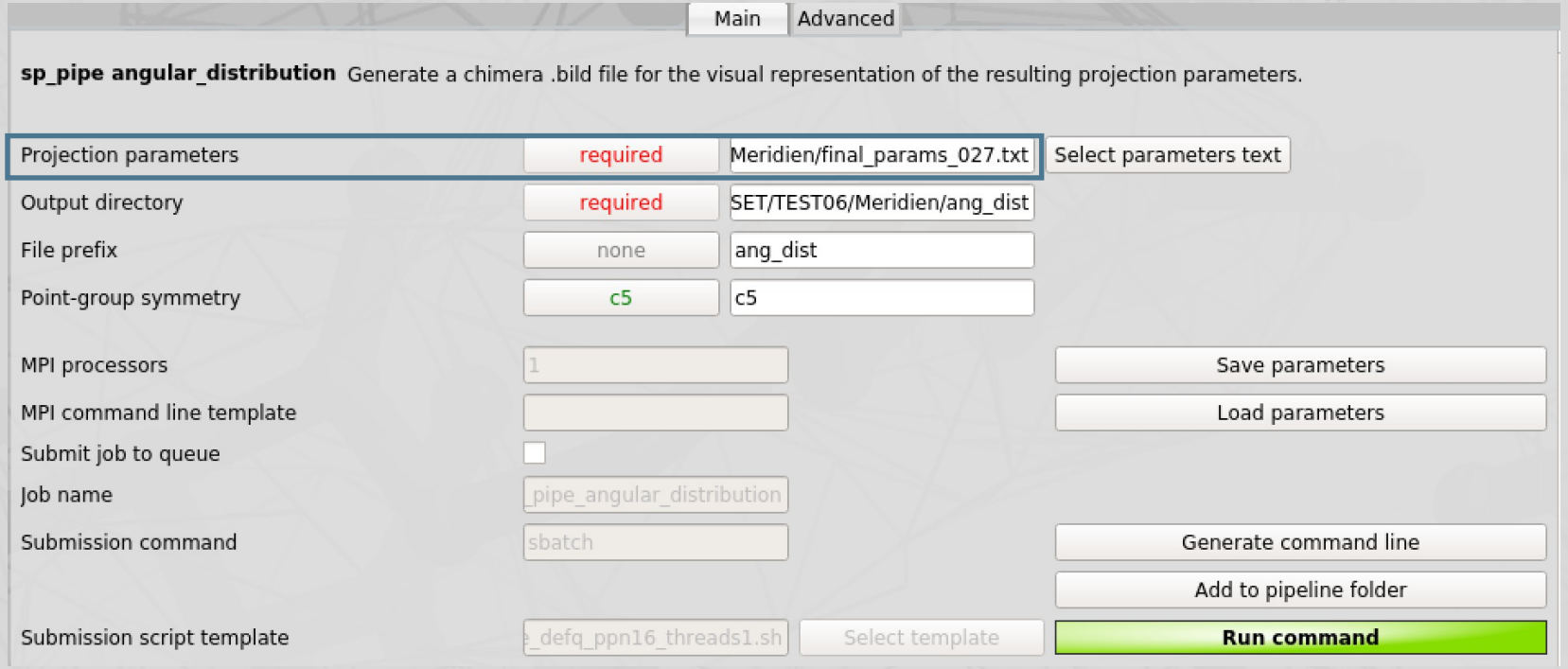
Cutoff for low-pass filter
0.0 → Automatic
-1 → No filtration

SPHERE

# Angular Distribution



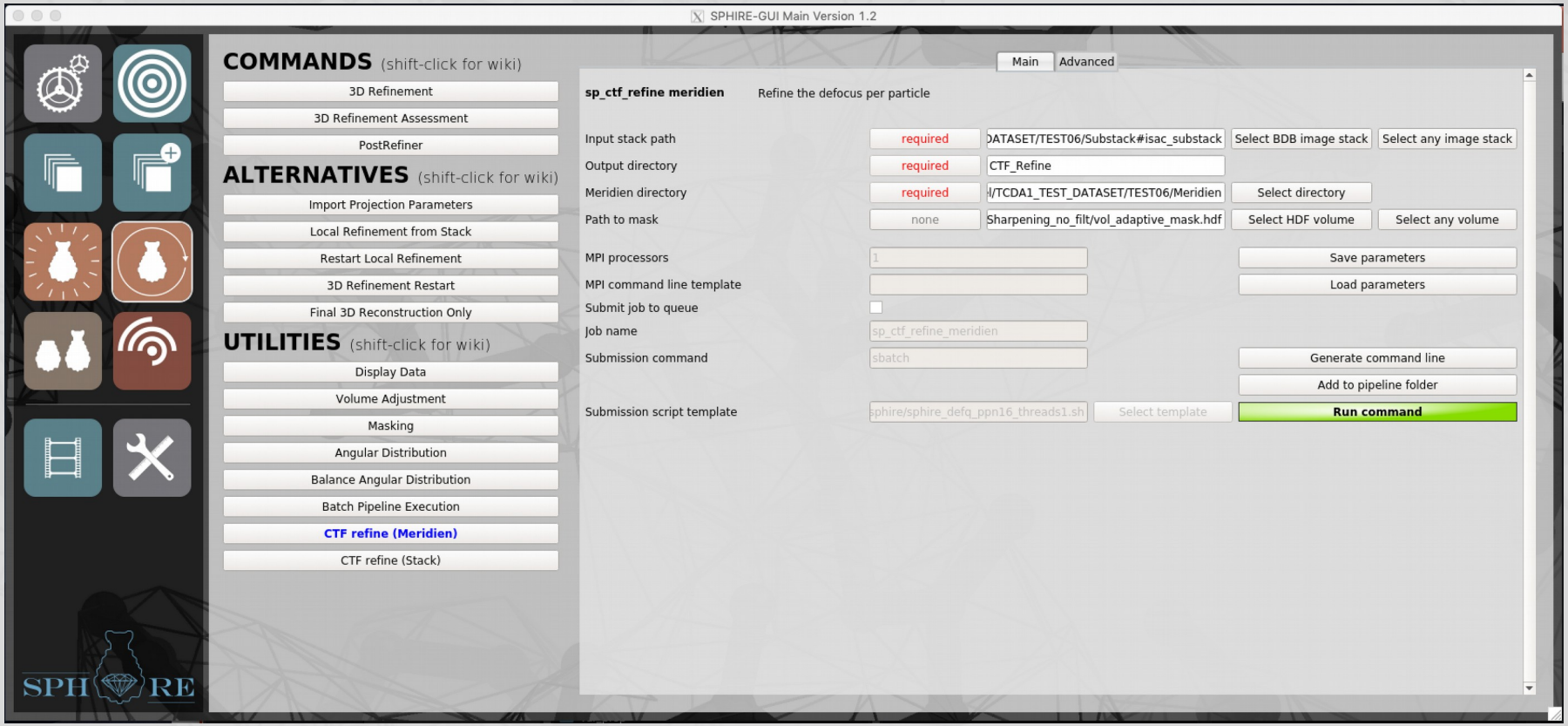
# Angular Distribution




Refinement parameters (“best” iteration’s values will be in top-level Meridien directory)

# CTF Refinement (simplified)

- sp_header.py bdb:Substack#isac_substack' --params='xform.projection' --import=Meridien/final_params_027.txt




Next, run MERIDIEN with new stack, sharpen, etc.

# SPHIRE Pipeline

**PROJECT**
Project Settings

**WINDOW**
crYOLO Particle Picking
WINDOW Particle Extraction

**VIPER**
RVIPER Initial 3D Modeling

**SORT3D**
3D Variability Estimation
SORT3D 3D Clustering

**MOVIE (optional)**
Micrograph Movie Alignment
Drift Assessment

**CTER**
CTER CTF Estimation
CTF Assessment

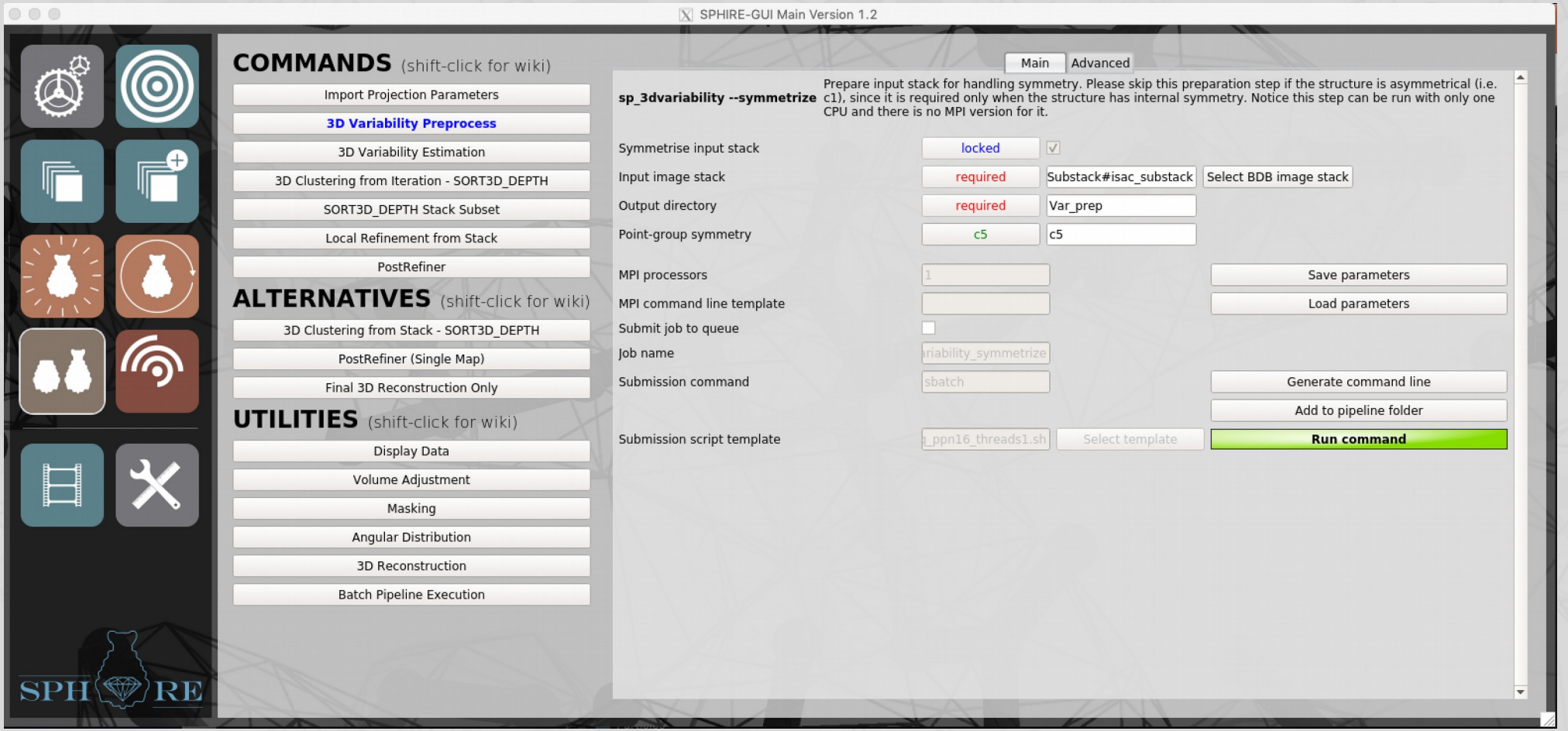
**ISAC**
ISAC2 2D Clustering
Beautifier

**MERIDIEN**
MERIDIEN 3D Refinement
3D Refinement Assessment
PostRefiner

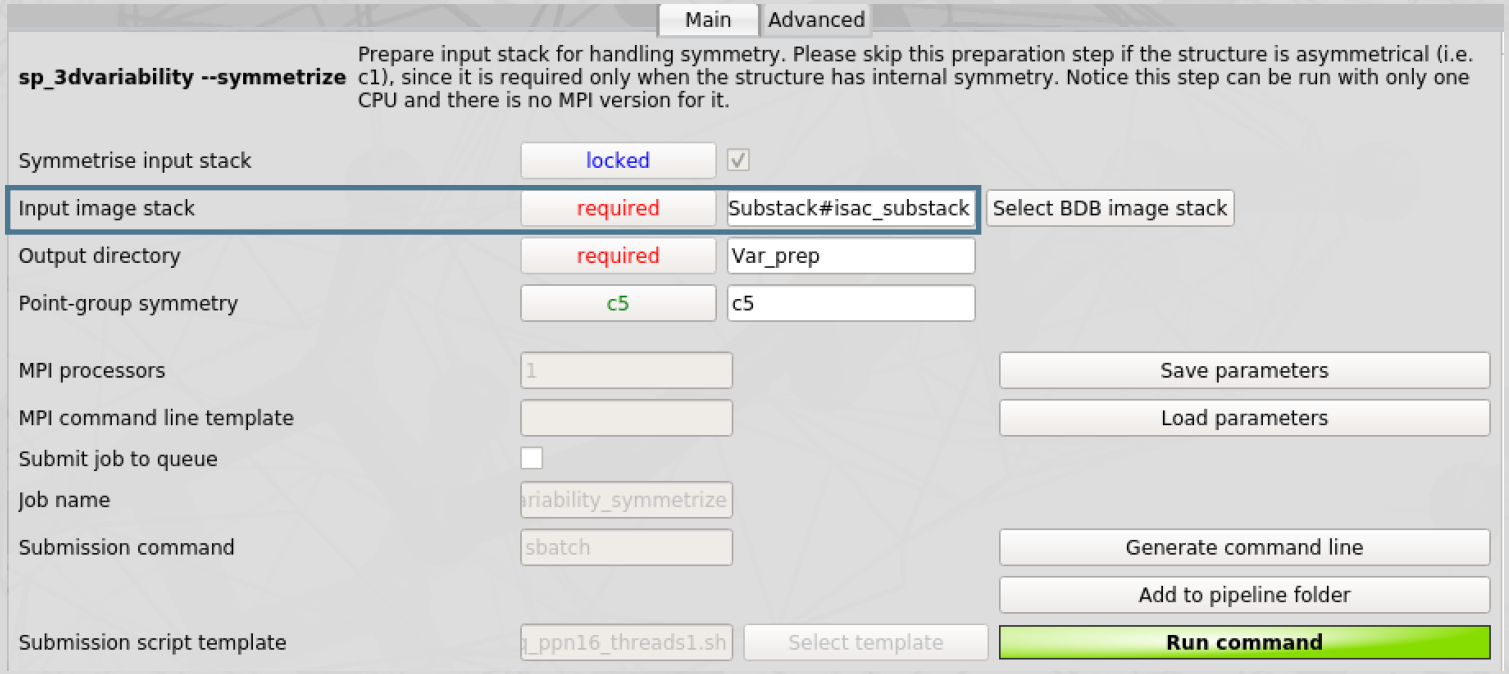
**LOCALRES**
Local Resolution
3D Local Filter

**Utilities**
Utility commands

# 3D Variability **Preprocess**

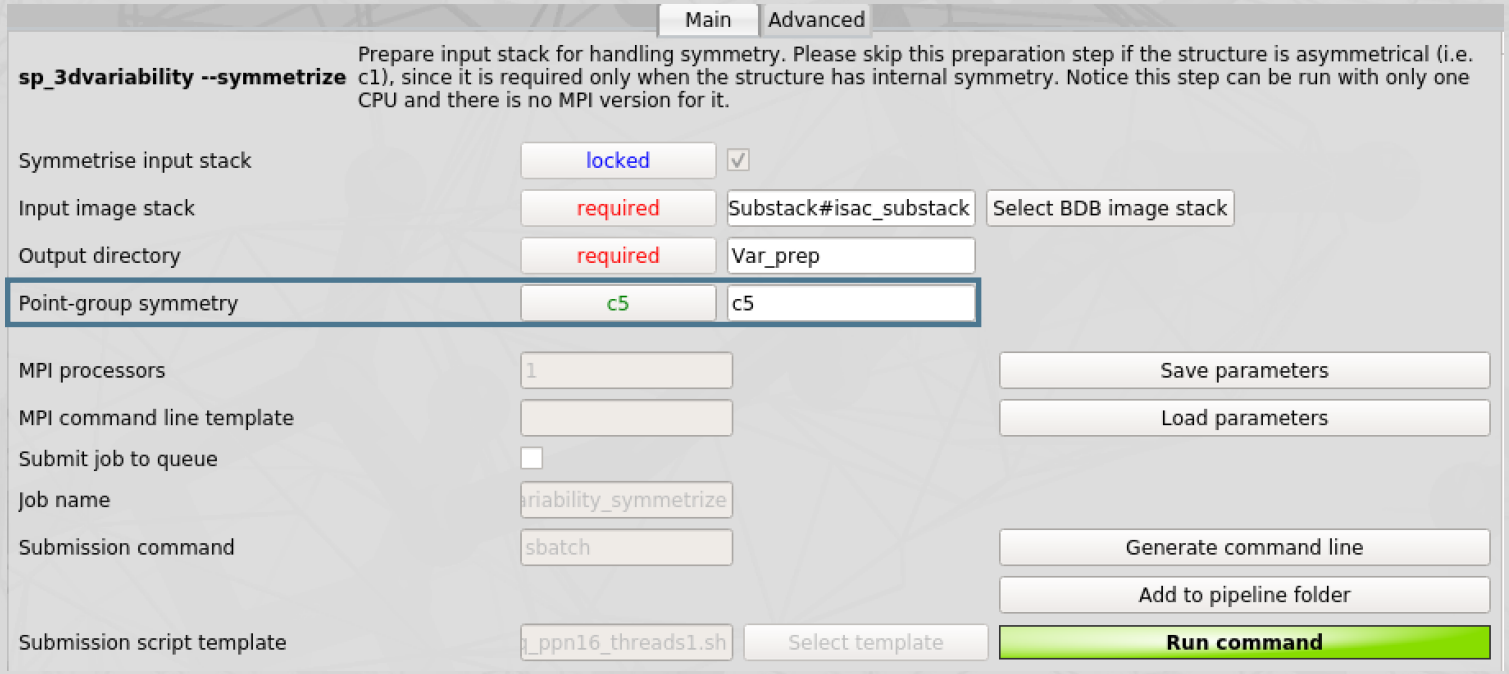


# 3D Variability **Preprocess**



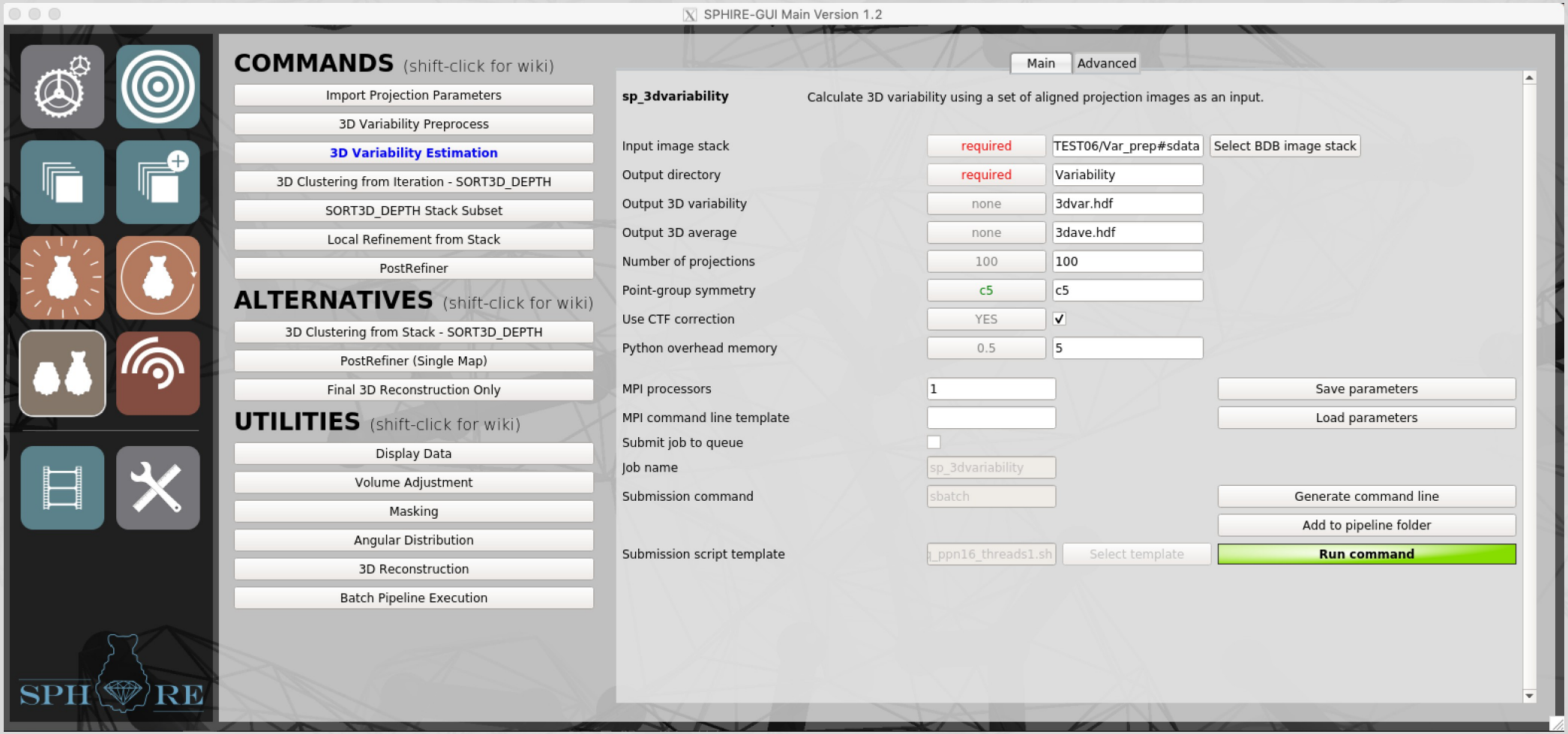

Images from good ISAC classes

# 3D Variability **Preprocess**




Data set will be multiplied by the symmetry

# 3D Variability Estimation



# 3D Variability **Estimation**

Main | Advanced

**sp_3dvariability** Calculate 3D variability using a set of aligned projection images as an input.

| | | | |
|---|---|---|---|
| Input image stack | required | TEST06/Var_prep#sdata | Select BDB image stack |
| Output directory | required | Variability | |
| Output 3D variability | none | 3dvar.hdf | |
| Output 3D average | none | 3dave.hdf | |
| Number of projections | 100 | 100 | |
| Point-group symmetry | c5 | c5 | |
| Use CTF correction | YES | ☑ | |
| Python overhead memory | 0.5 | 5 | |

| | | | |
|---|---|---|---|
| MPI processors | 1 | | Save parameters |
| MPI command line template | | | Load parameters |
| Submit job to queue | ☐ | | |
| Job name | sp_3dvariability | | |
| Submission command | sbatch | | Generate command line |
| | | | Add to pipeline folder |
| Submission script template | l_ppn16_threads1.sh | Select template | **Run command** |

Symmetry-multiplied stack

SPHIRE

# 3D Variability **Estimation**

Main | Advanced

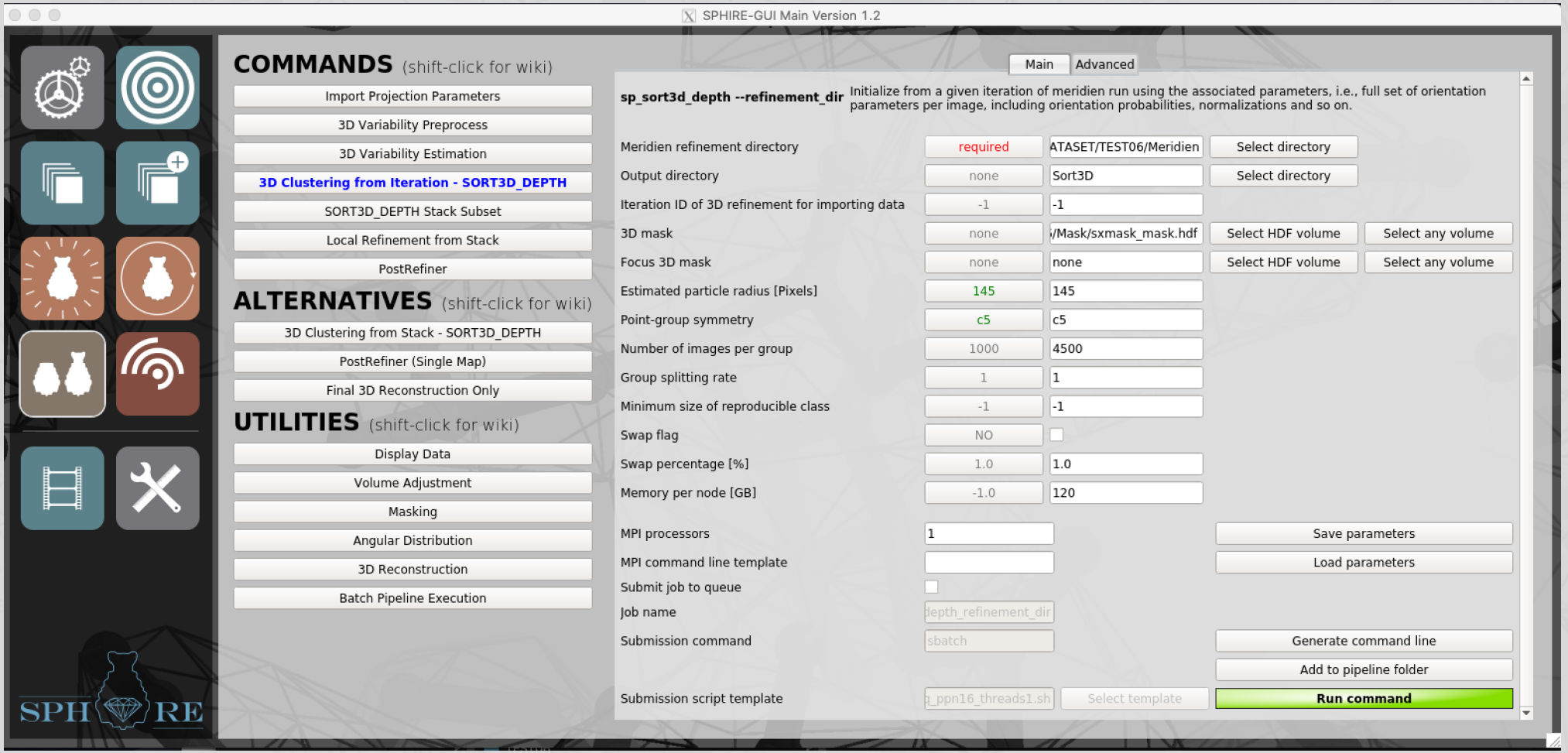
**sp_3dvariability** Calculate 3D variability using a set of aligned projection images as an input.

| | | | |
|---|---|---|---|
| Input image stack | required | TEST06/Var_prep#sdata | Select BDB image stack |
| Output directory | required | Variability | |
| Output 3D variability | none | 3dvar.hdf | |
| Output 3D average | none | 3dave.hdf | |
| Number of projections | 100 | 100 | |
| Point-group symmetry | c5 | c5 | |
| Use CTF correction | YES | ✓ | |
| Python overhead memory | 0.5 | 5 | |
| MPI processors | 1 | | Save parameters |
| MPI command line template | | | Load parameters |
| Submit job to queue | ☐ | | |
| Job name | sp_3dvariability | | |
| Submission command | sbatch | | Generate command line |
| | | | Add to pipeline folder |
| Submission script template | ı_ppn16_threads1.sh | Select template | **Run command** |

Memory per CPU (GB, test empirically)

SPHIRE

# Sort3D


SPHIRE-GUI Main Version 1.2
COMMANDS (shift-click for wiki)
Import Projection Parameters
3D Variability Preprocess
3D Variability Estimation
3D Clustering from Iteration - SORT3D_DEPTH
SORT3D_DEPTH Stack Subset
Local Refinement from Stack
PostRefiner
ALTERNATIVES (shift-click for wiki)
3D Clustering from Stack - SORT3D_DEPTH
PostRefiner (Single Map)
Final 3D Reconstruction Only
UTILITIES (shift-click for wiki)
Display Data
Volume Adjustment
Masking
Angular Distribution
3D Reconstruction
Batch Pipeline Execution
Main
Advanced
sp_sort3d_depth --refinement_dir
Initialize from a given iteration of meridien run using the associated parameters, i.e., full set of orientation parameters per image, including orientation probabilities, normalizations and so on.
Meridien refinement directory
required
ATASET/TEST06/Meridien
Select directory
Output directory
none
Sort3D
Select directory
Iteration ID of 3D refinement for importing data
-1
-1
3D mask
none
/Mask/sxmask_mask.hdf
Select HDF volume
Select any volume
Focus 3D mask
none
none
Select HDF volume
Select any volume
Estimated particle radius [Pixels]
145
145
Point-group symmetry
c5
c5
Number of images per group
1000
4500
Group splitting rate
1
1
Minimum size of reproducible class
-1
-1
Swap flag
NO
Swap percentage [%]
1.0
1.0
Memory per node [GB]
-1.0
120
MPI processors
1
Save parameters
MPI command line template
Load parameters
Submit job to queue
Job name
depth_refinement_dir
Submission command
sbatch
Generate command line
Add to pipeline folder
Submission script template
_ppn16_threads1.sh
Select template
Run command

# Sort3D

Main | Advanced

**sp_sort3d_depth --refinement_dir** Initialize from a given iteration of meridien run using the associated parameters, i.e., full set of orientation parameters per image, including orientation probabilities, normalizations and so on.

| | | | | |
|---|---|---|---|---|
| Meridien refinement directory | required | ATASET/TEST06/Meridien | Select directory | |
| Output directory | none | Sort3D | Select directory | |
| Iteration ID of 3D refinement for importing data | -1 | -1 | | |
| 3D mask | none | /Mask/sxmask_mask.hdf | Select HDF volume | Select any volume |
| Focus 3D mask | none | none | Select HDF volume | Select any volume |
| Estimated particle radius [Pixels] | 145 | 145 | | |
| Point-group symmetry | c5 | c5 | | |
| Number of images per group | 1000 | 4500 | | |
| Group splitting rate | 1 | 1 | | |
| Minimum size of reproducible class | -1 | -1 | | |
| Swap flag | NO | ☐ | | |
| Swap percentage [%] | 1.0 | 1.0 | | |
| Memory per node [GB] | -1.0 | 120 | | |

| | | | |
|---|---|---|---|
| MPI processors | 1 | | Save parameters |
| MPI command line template | | | Load parameters |
| Submit job to queue | ☐ | | |
| Job name | depth_refinement_dir | | |
| Submission command | sbatch | | Generate command line |
| | | | Add to pipeline folder |
| Submission script template | q_ppn16_threads1.sh | Select template | **Run command** |

Refinement directory

SPHIRE

# Sort3D

Main | Advanced

**sp_sort3d_depth --refinement_dir** Initialize from a given iteration of meridien run using the associated parameters, i.e., full set of orientation parameters per image, including orientation probabilities, normalizations and so on.

| | | | | |
|---|---|---|---|---|
| Meridien refinement directory | required | ATASET/TEST06/Meridien | Select directory | |
| Output directory | none | Sort3D | Select directory | |
| Iteration ID of 3D refinement for importing data | -1 | -1 | | |
| 3D mask | none | /Mask/sxmask_mask.hdf | Select HDF volume | Select any volume |
| Focus 3D mask | none | none | Select HDF volume | Select any volume |
| Estimated particle radius [Pixels] | 145 | 145 | | |
| Point-group symmetry | c5 | c5 | | |
| Number of images per group | 1000 | 4500 | | |
| Group splitting rate | 1 | 1 | | |
| Minimum size of reproducible class | -1 | -1 | | |
| Swap flag | NO | ☐ | | |
| Swap percentage [%] | 1.0 | 1.0 | | |
| Memory per node [GB] | -1.0 | 120 | | |

| | | | |
|---|---|---|---|
| MPI processors | 1 | | Save parameters |
| MPI command line template | | | Load parameters |
| Submit job to queue | ☐ | | |
| Job name | depth_refinement_dir | | |
| Submission command | sbatch | | Generate command line |
| | | | Add to pipeline folder |
| Submission script template | q_ppn16_threads1.sh | Select template | **Run command** |

Mask file

# Sort3D

Main | Advanced

**sp_sort3d_depth --refinement_dir** Initialize from a given iteration of meridien run using the associated parameters, i.e., full set of orientation parameters per image, including orientation probabilities, normalizations and so on.

| | | | | |
|---|---|---|---|---|
| Meridien refinement directory | required | ATASET/TEST06/Meridien | Select directory | |
| Output directory | none | Sort3D | Select directory | |
| Iteration ID of 3D refinement for importing data | -1 | -1 | | |
| 3D mask | none | /Mask/sxmask_mask.hdf | Select HDF volume | Select any volume |
| Focus 3D mask | none | none | Select HDF volume | Select any volume |
| Estimated particle radius [Pixels] | 145 | 145 | | |
| Point-group symmetry | c5 | c5 | | |
| Number of images per group | 1000 | 4500 | | |
| Group splitting rate | 1 | 1 | | |
| Minimum size of reproducible class | -1 | -1 | | |
| Swap flag | NO | ☐ | | |
| Swap percentage [%] | 1.0 | 1.0 | | |
| Memory per node [GB] | -1.0 | 120 | | |

| | | | |
|---|---|---|---|
| MPI processors | 1 | | Save parameters |
| MPI command line template | | | Load parameters |
| Submit job to queue | ☐ | | |
| Job name | depth_refinement_dir | | |
| Submission command | sbatch | | Generate command line |
| | | | Add to pipeline folder |
| Submission script template | q_ppn16_threads1.sh | Select template | **Run command** |

Optional focused mask

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# Sort3D

Main | Advanced

**sp_sort3d_depth --refinement_dir** Initialize from a given iteration of meridien run using the associated parameters, i.e., full set of orientation parameters per image, including orientation probabilities, normalizations and so on.

| | | | | |
|---|---|---|---|---|
| Meridien refinement directory | required | ATASET/TEST06/Meridien | Select directory | |
| Output directory | none | Sort3D | Select directory | |
| Iteration ID of 3D refinement for importing data | -1 | -1 | | |
| 3D mask | none | /Mask/sxmask_mask.hdf | Select HDF volume | Select any volume |
| Focus 3D mask | none | none | Select HDF volume | Select any volume |
| Estimated particle radius [Pixels] | 145 | 145 | | |
| Point-group symmetry | c5 | c5 | | |
| Number of images per group | 1000 | 4500 | | |
| Group splitting rate | 1 | 1 | | |
| Minimum size of reproducible class | -1 | -1 | | |
| Swap flag | NO | ☐ | | |
| Swap percentage [%] | 1.0 | 1.0 | | |
| Memory per node [GB] | -1.0 | 120 | | |

| | | | |
|---|---|---|---|
| MPI processors | 1 | | Save parameters |
| MPI command line template | | | Load parameters |
| Submit job to queue | ☐ | | |
| Job name | depth_refinement_dir | | |
| Submission command | sbatch | | Generate command line |
| | | | Add to pipeline folder |
| Submission script template | q_ppn16_threads1.sh | Select template | **Run command** |

Number of images/class (approximate)

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# Sort3D

Main | Advanced

**sp_sort3d_depth --refinement_dir** Initialize from a given iteration of meridien run using the associated parameters, i.e., full set of orientation parameters per image, including orientation probabilities, normalizations and so on.

| | | | | |
|---|---|---|---|---|
| Meridien refinement directory | required | ATASET/TEST06/Meridien | Select directory | |
| Output directory | none | Sort3D | Select directory | |
| Iteration ID of 3D refinement for importing data | -1 | -1 | | |
| 3D mask | none | /Mask/sxmask_mask.hdf | Select HDF volume | Select any volume |
| Focus 3D mask | none | none | Select HDF volume | Select any volume |
| Estimated particle radius [Pixels] | 145 | 145 | | |
| Point-group symmetry | c5 | c5 | | |
| Number of images per group | 1000 | 4500 | | |
| Group splitting rate | 1 | 1 | | |
| Minimum size of reproducible class | -1 | -1 | | |
| Swap flag | NO | ☐ | | |
| Swap percentage [%] | 1.0 | 1.0 | | |
| Memory per node [GB] | -1.0 | 120 | | |

| | | | |
|---|---|---|---|
| MPI processors | 1 | | Save parameters |
| MPI command line template | | | Load parameters |
| Submit job to queue | ☐ | | |
| Job name | depth_refinement_dir | | |
| Submission command | sbatch | | Generate command line |
| | | | Add to pipeline folder |
| Submission script template | q_ppn16_threads1.sh | Select template | **Run command** |

Memory (GB) per node

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# SPHIRE Pipeline

## PROJECT
Project Settings

## WINDOW
crYOLO Particle Picking
WINDOW Particle Extraction

## VIPER
RVIPER Initial 3D Modeling

## SORT3D
3D Variability Estimation
SORT3D 3D Clustering

## MOVIE (optional)
Micrograph Movie Alignment
Drift Assessment

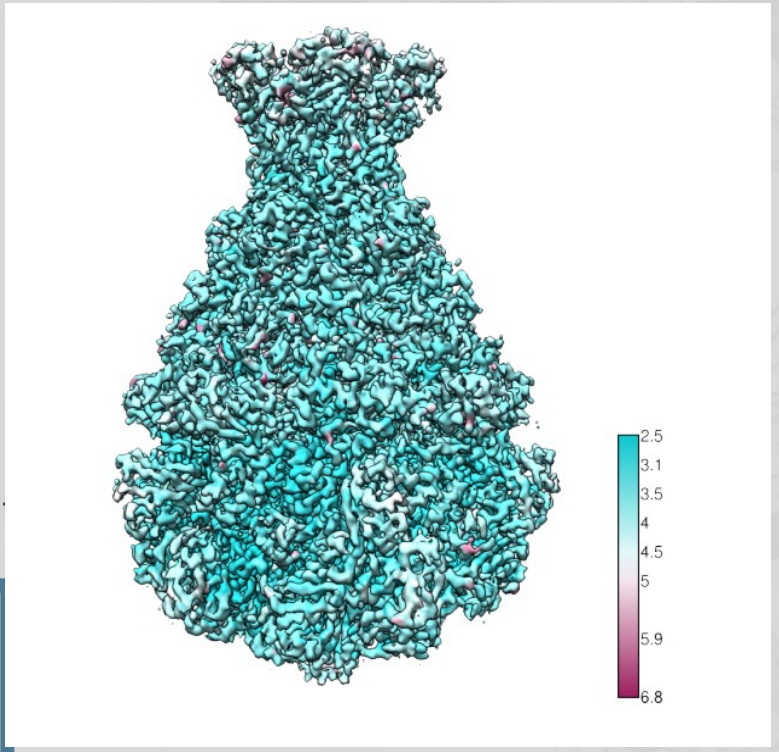
## CTER
CTER CTF Estimation
CTF Assessment

## ISAC
ISAC2 2D Clustering
Beautifier

## MERIDIEN
MERIDIEN 3D Refinement
3D Refinement Assessment
PostRefiner

## LOCALRES
Local Resolution
3D Local Filter

## Utilities
Utility commands

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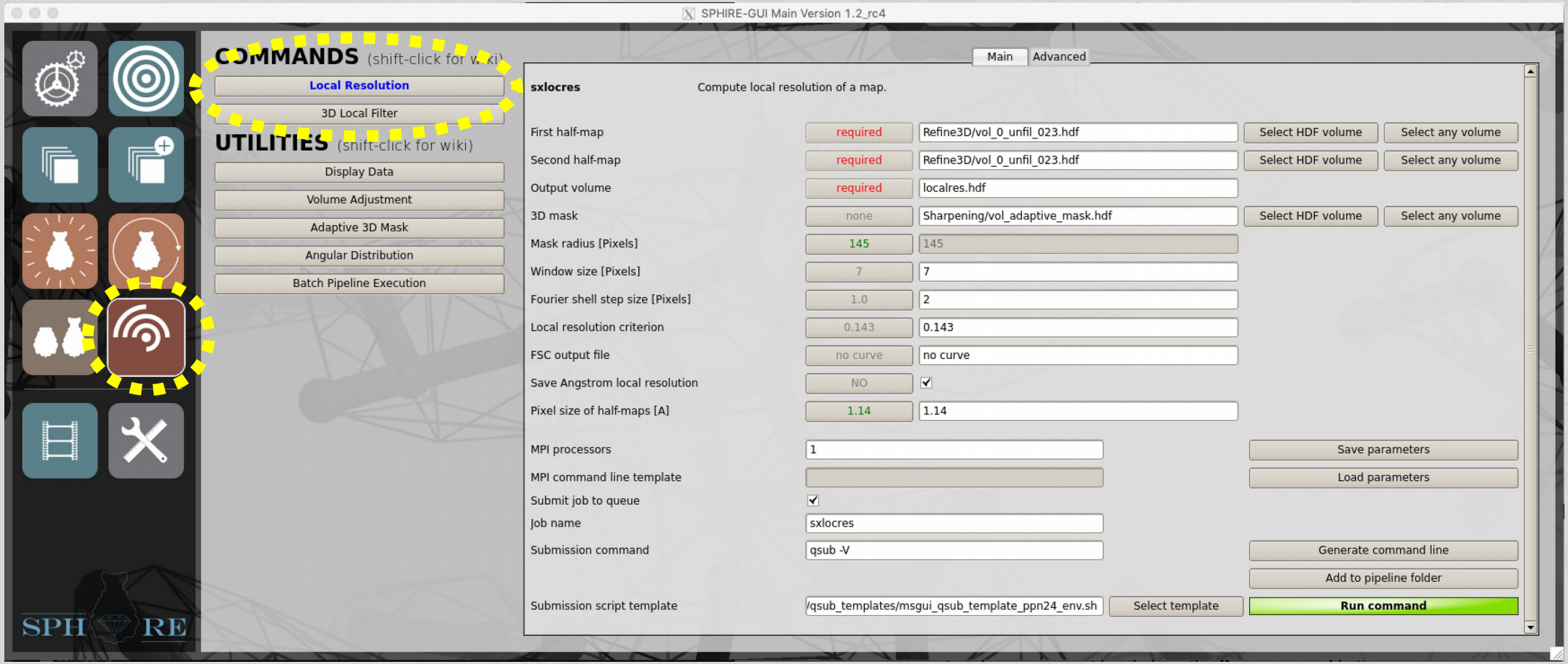
# Local Resolution

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# Local Resolution

**sxlocres** Compute local resolution of a map.

| | | | | |
|---|---|---|---|---|
| First half-map | required | Refine3D/vol_0_unfil_023.hdf | Select HDF volume | Select any volume |
| Second half-map | required | Refine3D/vol_1_unfil_023.hdf | Select HDF volume | Select any volume |
| Output volume | required | localres1_4.hdf | | |
| 3D mask | none | Sharpening/vol_adaptive_mask.hdf | Select HDF volume | Select any volume |
| Mask radius [Pixels] | 145 | 145 | | |
| Window size [Pixels] | 7 | 7 | | |
| Fourier shell step size [Pixels] | 1.0 | 2 | | |
| Local resolution criterion | 0.143 | 0.143 | | |
| FSC output file | no curve | no curve | | |
| Save Angstrom local resolution | NO | ☑ | | |
| Pixel size of half-maps [A] | 1.14 | 1.14 | | |

| | | | |
|---|---|---|---|
| MPI processors | 96 | | Save parameters |
| MPI command line template | | | Load parameters |
| Submit job to queue | ☑ | | |
| Job name | sxlocres | | |
| Submission command | qsub -V | | Generate command line |
| | | | Add to pipeline folder |
| Submission script template | qsub_templates/msgui_qsub_template_ppn24_env.sh | Select template | **Run command** |

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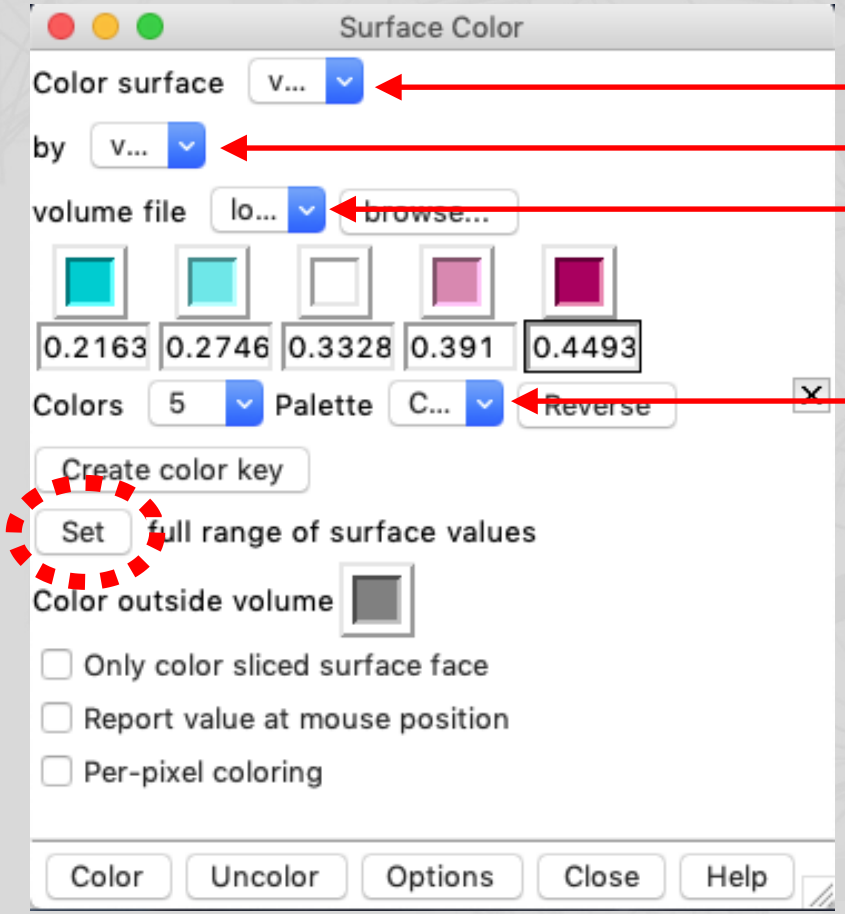
# Local Resolution: Visualization



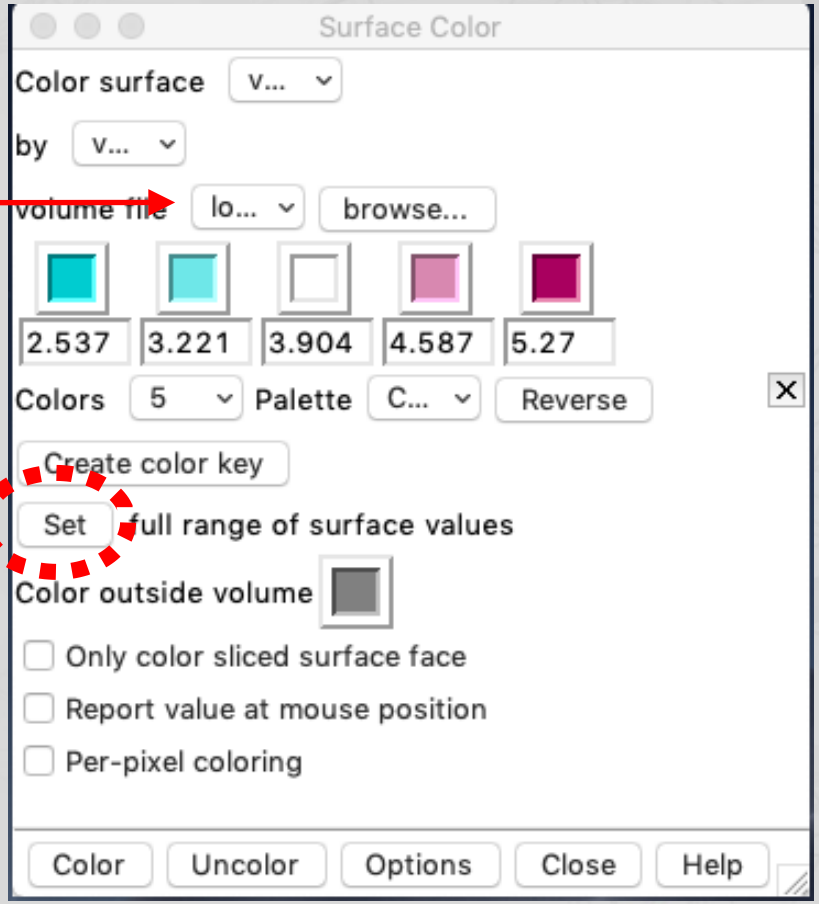

vol_combined.hdf

by: volume data value

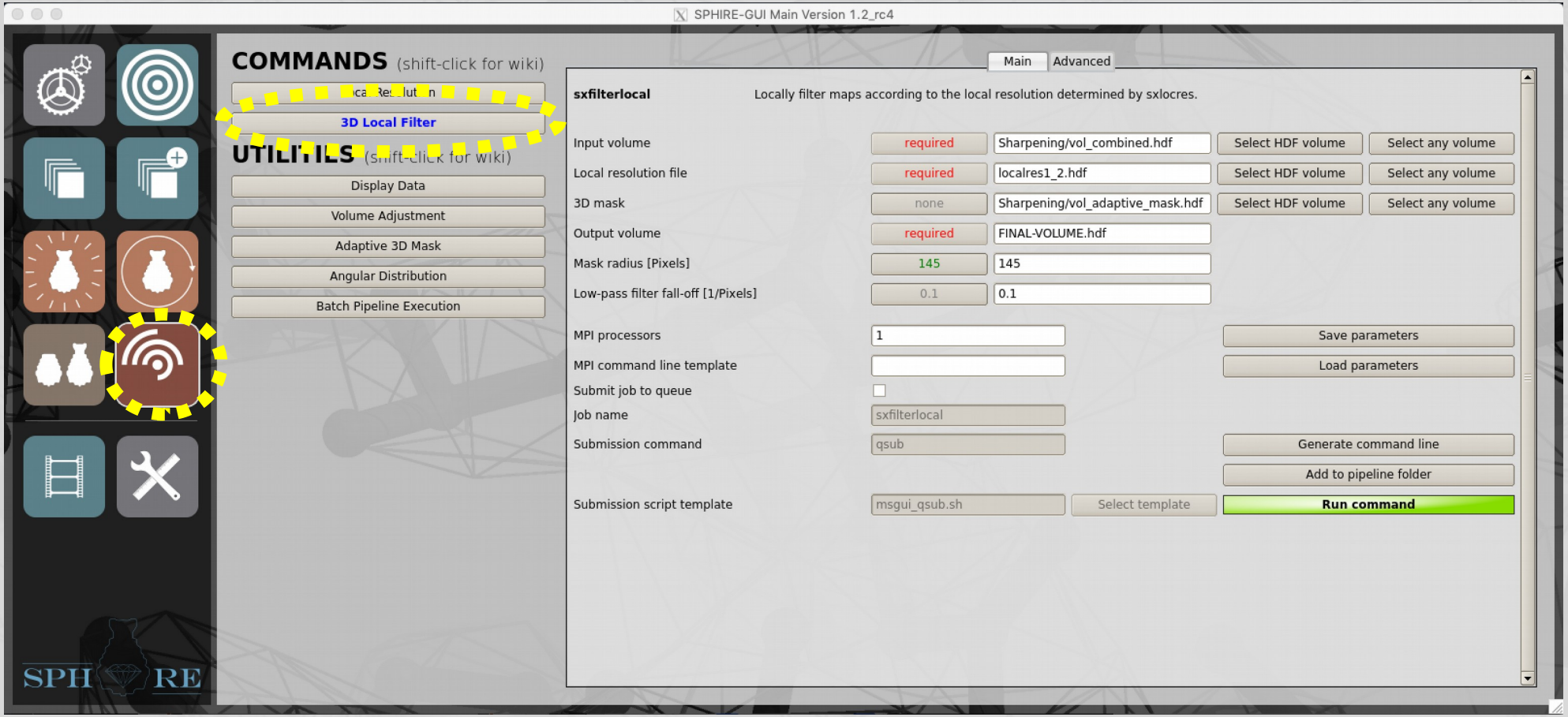
localres.hdf

Palette: Cyan-Maroon

localres_ang.hdf



# Local Filter



# Local Filter

**sxfilterlocal** Locally filter maps according to the local resolution determined by sxlocres.

| | | | | |
|---|---|---|---|---|
| Input volume | required | Sharpening/vol_combined.hdf | Select HDF volume | Select any volume |
| Local resolution file | required | localres1_2.hdf | Select HDF volume | Select any volume |
| 3D mask | none | Sharpening/vol_adaptive_mask.hdf | Select HDF volume | Select any volume |
| Output volume | required | FINAL-VOLUME.hdf | | |
| Mask radius [Pixels] | 145 | 145 | | |
| Low-pass filter fall-off [1/Pixels] | 0.1 | 0.1 | | |

| | | | |
|---|---|---|---|
| MPI processors | 1 | | Save parameters |
| MPI command line template | | | Load parameters |
| Submit job to queue | ☐ | | |
| Job name | sxfilterlocal | | |
| Submission command | qsub | | Generate command line |
| | | | Add to pipeline folder |
| Submission script template | msgui_qsub.sh | Select template | **Run command** |

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*You have now processed your first data set using SPHIRE!*