



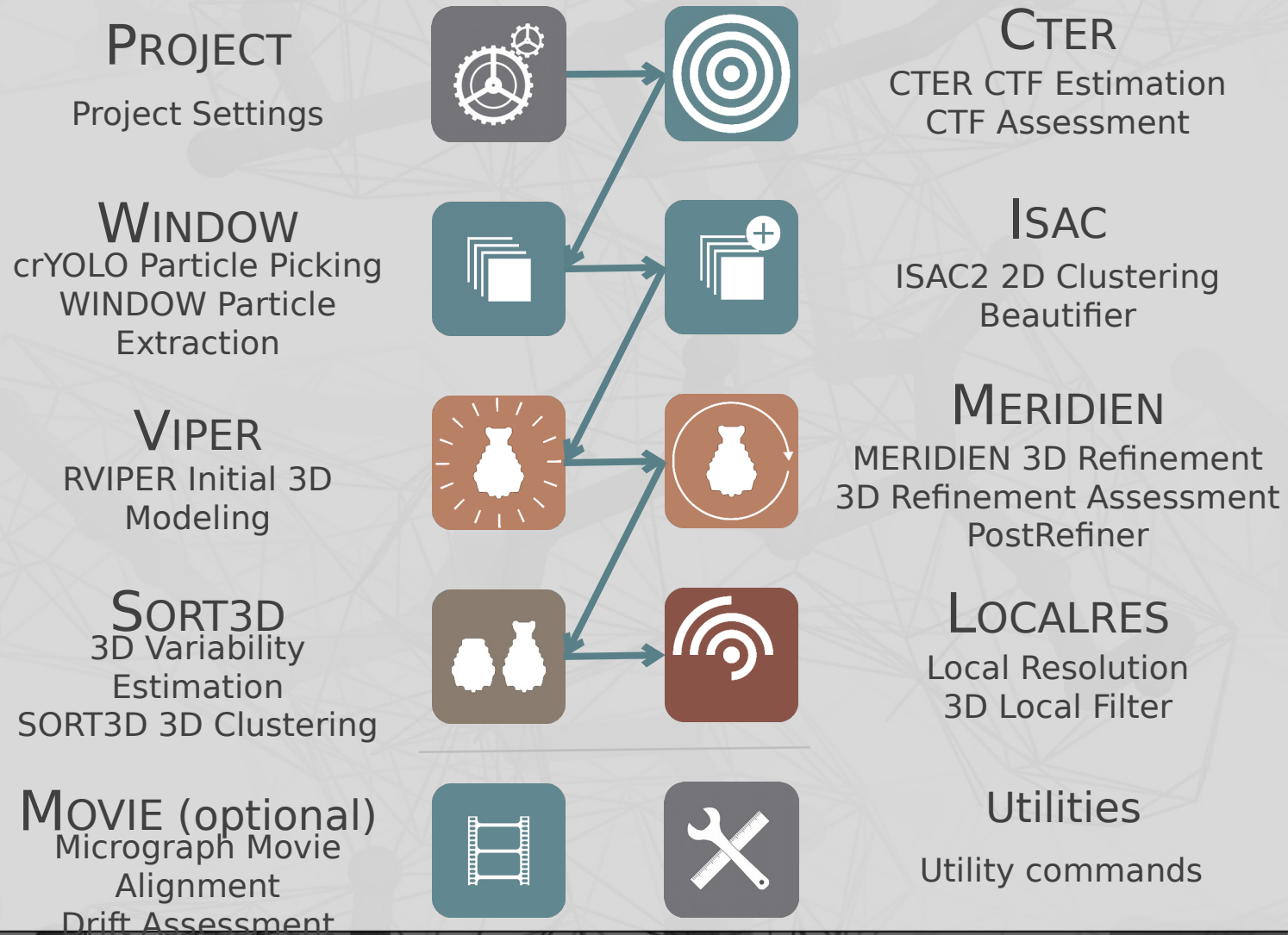
SParx for **H**igh **R**esolution **E**lectron Microscopy

SPHIRE Practical

UTMB Workshop

May 8, 2019

SPHIRE Pipeline



GUI

1. Pipeline Steps Blocks (Icon Buttons)

2. Step Associated Commands (Buttons)

3. Command Associated Parameters (Main & Advanced Tabs)

SPHIRE-GUI Main (Beta Version)

COMMANDS (shift-click for wiki)

- Initial 3D Model - RVIPER
- Change Size of VIPER Model
- Window VIPER Model

ALTERNATIVES (shift-click for wiki)

- Initial 3D Model - VIPER
- PDB File Conversion

UTILITIES (shift-click for wiki)

- Display Data
- Adaptive 3D Mask
- Angular Distribution

Parameters:

- Input images stack: required, Class2D/best.hdf, Select .hdf
- Output directory: required, VIPER
- Target particle radius [Pixels]: 29, 29
- Point-group symmetry: C5, C5
- RVIPER iterations: 10, 10
- Restarting iteration: 0, 0
- MPI processors: 48
- MPI command line template: [empty]
- Submit job to queue:
- Job name: sxrviper
- Submission command: qsub
- Submission script template: msgui_qsub_fillup.sh, Select Template

Buttons: Save parameters, Load parameters, Generate command line, Run command

4. Job Submission (Only in Main Tab)

SPHIRE Pipeline



PROJECT
Project Settings



CTER
CTER CTF Estimation
CTF Assessment

WINDOW
crYOLO Particle Picking
WINDOW Particle
Extraction



ISAC
ISAC2 2D Clustering
Beautifier

VIPER
RVIPER Initial 3D
Modeling



MERIDIEN
MERIDIEN 3D Refinement
3D Refinement Assessment
PostRefiner

SORT3D
3D Variability
Estimation
SORT3D 3D Clustering

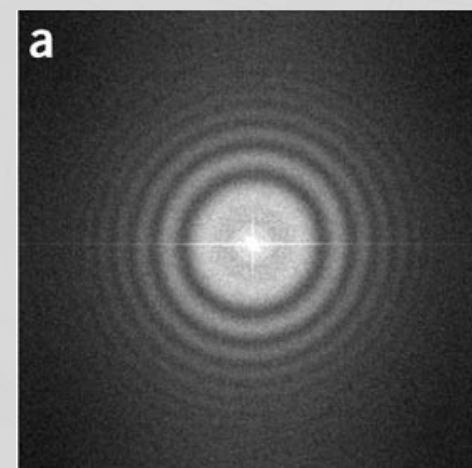


LOCALRES
Local Resolution
3D Local Filter

MOVIE (optional)
Micrograph Movie
Alignment
Drift Assessment



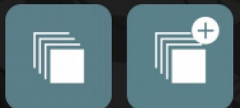
Utilities
Utility commands





COMMANDS (shift-click for wiki)

- [CTF Estimation](#)
- CTF Assessment



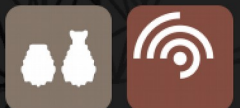
ALTERNATIVES (shift-click for wiki)

- Resample Micrographs



UTILITIES (shift-click for wiki)

- Display Data
- Organize Micrographs/Movies
- Batch Pipeline Execution
- CTF refine (Meridien)
- CTF refine (Stack)
- TransSPHIRE GUI



Main Advanced

sp_cter

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

Input micrograph path pattern	<input type="text" value="required"/>	<input type="text" value="Corrected_sums/corrsum_dw/TcdA1-*_frames.mrc"/>	<input type="button" value="Select MRC micrograph"/>	<input type="button" value="Select any micrograph"/>
Output directory	<input type="text" value="required"/>	<input type="text" value="CTFest"/>		
Micrograph selection file	<input type="text" value="none"/>	<input type="text" value="none"/>	<input type="button" value="Select micrograph list"/>	<input type="button" value="Select any micrograph"/>
Pixel size [Å]	<input type="text" value="1.14"/>	<input type="text" value="1.14"/>		
Microscope spherical aberration (Cs) [mm]	<input type="text" value="2.0"/>	<input type="text" value="2.0"/>		
Microscope voltage [kV]	<input type="text" value="300.0"/>	<input type="text" value="300.0"/>		
Amplitude contrast [%]	<input type="text" value="10.0"/>	<input type="text" value="10.0"/>		
Lowest resolution [Å]	<input type="text" value="-1.0"/>	<input type="text" value="-1.0"/>		
Highest resolution [Å]	<input type="text" value="-1.0"/>	<input type="text" value="-1.0"/>		
MPI processors	<input type="text" value="96"/>			
MPI command line template	<input type="text"/>			
Submit job to queue	<input checked="" type="checkbox"/>			
Job name	<input type="text" value="sp_cter"/>			
Submission command	<input type="text" value="sbatch"/>			
Submission script template	<input type="text" value="tplates/sphire/sphire_defq_ppn16_threads1.sh"/>	<input type="button" value="Select template"/>		



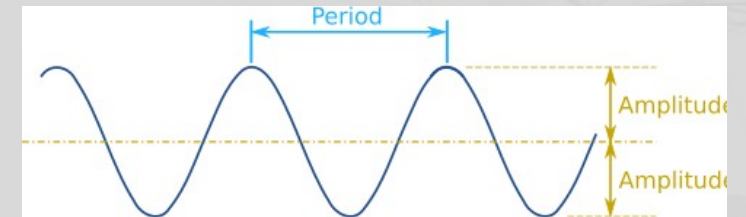


Main Advanced

sp_cter

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

Input micrograph path pattern	<input type="text" value="required"/>	<input type="text" value="Corrected_sums/corrsum_dw/TcdA1-*_frames.mrc"/>	<input type="button" value="Select MRC micrograph"/>	<input type="button" value="Select any micrograph"/>
Output directory	<input type="text" value="required"/>	<input type="text" value="CTFest"/>		
Micrograph selection file	<input type="text" value="none"/>	<input type="text" value="none"/>	<input type="button" value="Select micrograph list"/>	<input type="button" value="Select any micrograph"/>
Pixel size [Å]	<input type="text" value="1.14"/>	<input type="text" value="1.14"/>		
Microscope spherical aberration (Cs) [mm]	<input type="text" value="2.0"/>	<input type="text" value="2.0"/>		
Microscope voltage [kV]	<input type="text" value="300.0"/>	<input type="text" value="300.0"/>		
Amplitude contrast [%]	<input type="text" value="10.0"/>	<input type="text" value="10.0"/>		
Lowest resolution [Å]	<input type="text" value="-1.0"/>	<input type="text" value="-1.0"/>		
Highest resolution [Å]	<input type="text" value="-1.0"/>	<input type="text" value="-1.0"/>		
MPI processors	<input type="text" value="96"/>		<input type="button" value="Save parameters"/>	
MPI command line template	<input type="text"/>		<input type="button" value="Load parameters"/>	
Submit job to queue	<input checked="" type="checkbox"/>			
Job name	<input type="text" value="sp_cter"/>			
Submission command	<input type="text" value="sbatch"/>		<input type="button" value="Generate command line"/>	
			<input type="button" value="Add to pipeline folder"/>	
Submission script template	<input type="text" value="tplates/sphire/sphire_defq_ppn16_threads1.sh"/>	<input type="button" value="Select template"/>	<input type="button" value="Run command"/>	



Max frequency is determined by Nyquist



Main Advanced

sp_cter

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

Input micrograph path pattern	<input type="text" value="required"/>	<input type="text" value="Corrected_sums/corrsum_dw/TcdA1-*_frames.mrc"/>	<input type="button" value="Select MRC micrograph"/>	<input type="button" value="Select any micrograph"/>
Output directory	<input type="text" value="required"/>	<input type="text" value="CTFest"/>		
Micrograph selection file	<input type="text" value="none"/>	<input type="text" value="none"/>	<input type="button" value="Select micrograph list"/>	<input type="button" value="Select any micrograph"/>
Pixel size [Å]	<input type="text" value="1.14"/>	<input type="text" value="1.14"/>		
Microscope spherical aberration (Cs) [mm]	<input type="text" value="2.0"/>	<input type="text" value="2.0"/>		
Microscope voltage [kV]	<input type="text" value="300.0"/>	<input type="text" value="300.0"/>		
Amplitude contrast [%]	<input type="text" value="10.0"/>	<input type="text" value="10.0"/>		
Lowest resolution [Å]	<input type="text" value="-1.0"/>	<input type="text" value="-1.0"/>		
Highest resolution [Å]	<input type="text" value="-1.0"/>	<input type="text" value="-1.0"/>		
MPI processors	<input type="text" value="96"/>		<input type="button" value="Save parameters"/>	
MPI command line template	<input type="text"/>		<input type="button" value="Load parameters"/>	
Submit job to queue	<input checked="" type="checkbox"/>			
Job name	<input type="text" value="sp_cter"/>			
Submission command	<input type="text" value="sbatch"/>		<input type="button" value="Generate command line"/>	
			<input type="button" value="Add to pipeline folder"/>	
Submission script template	<input type="text" value="tplates/sphire/sphire_defq_ppn16_threads1.sh"/>	<input type="button" value="Select template"/>	<input type="button" value="Run command"/>	

Constants of your microscope



Main Advanced

sp_cter

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

Input micrograph path pattern	<input type="text" value="required"/>	<input type="text" value="Corrected_sums/corrsum_dw/TcdA1-*_frames.mrc"/>	<input type="button" value="Select MRC micrograph"/>	<input type="button" value="Select any micrograph"/>
Output directory	<input type="text" value="required"/>	<input type="text" value="CTFest"/>		
Micrograph selection file	<input type="text" value="none"/>	<input type="text" value="none"/>	<input type="button" value="Select micrograph list"/>	<input type="button" value="Select any micrograph"/>
Pixel size [Å]	<input type="text" value="1.14"/>	<input type="text" value="1.14"/>		
Microscope spherical aberration (Cs) [mm]	<input type="text" value="2.0"/>	<input type="text" value="2.0"/>		
Microscope voltage [kV]	<input type="text" value="300.0"/>	<input type="text" value="300.0"/>		
Amplitude contrast [%]	<input type="text" value="10.0"/>	<input type="text" value="10.0"/>		
Lowest resolution [Å]	<input type="text" value="-1.0"/>	<input type="text" value="-1.0"/>		
Highest resolution [Å]	<input type="text" value="-1.0"/>	<input type="text" value="-1.0"/>		
MPI processors	<input type="text" value="96"/>		<input type="button" value="Save parameters"/>	
MPI command line template	<input type="text"/>		<input type="button" value="Load parameters"/>	
Submit job to queue	<input checked="" type="checkbox"/>			
job name	<input type="text" value="sp_cter"/>			
Submission command	<input type="text" value="sbatch"/>		<input type="button" value="Generate command line"/>	
			<input type="button" value="Add to pipeline folder"/>	
Submission script template	<input type="text" value="tplates/sphire/sphire_defq_ppn16_threads1.sh"/>	<input type="button" value="Select template"/>	<input type="button" value="Run command"/>	

Initial Ac value for optimization.



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 [Å]	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 [Å]	-1.0	-1.0		
Highest resolution [Å]	-1.0	-1.0		
MPI processors		96	Save parameters	
MPI command line template			Load parameters	
Submit job to queue	<input checked="" type="checkbox"/>			
Job name		sp_cter		
Submission command		sbatch	Generate command line	
			Add to pipeline folder	
Submission script template		tplates/sphire/sphire_defq_ppn16_threads1.sh	Select template	Run command

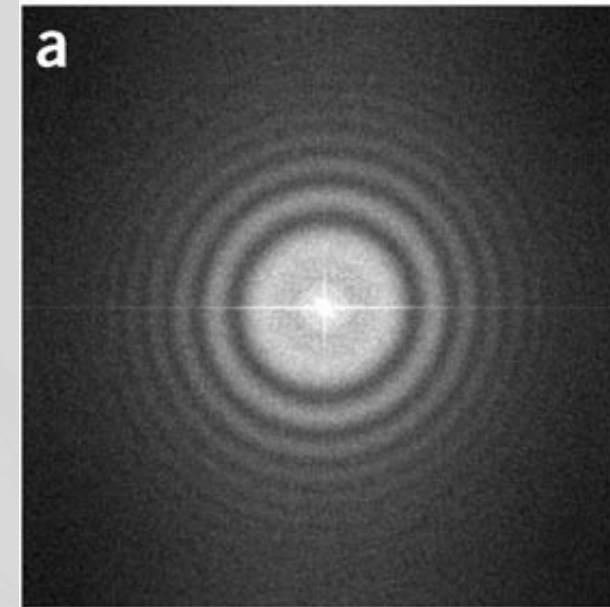
Frequency range during optimization. Fitting is done in this range. CTER estimates it automatically.



Main Advanced

sp_cter Set advanced parameters

CTF window size [Pixels]	<input type="text" value="512"/>	<input type="text" value="512"/>
Number of CTF estimates per micrograph	<input type="text" value="16"/>	<input type="text" value="16"/>
X overlap [%]	<input type="text" value="50"/>	<input type="text" value="50"/>
Y overlap [%]	<input type="text" value="50"/>	<input type="text" value="50"/>
Edge x [pixels]	<input type="text" value="0"/>	<input type="text" value="0"/>
Edge y [pixels]	<input type="text" value="0"/>	<input type="text" value="0"/>
Check consistency of inputs	<input type="text" value="NO"/>	<input type="checkbox"/>
Enable debug mode	<input type="text" value="NO"/>	<input type="checkbox"/>
Volta Phase Plate Dataset	<input type="text" value="NO"/>	<input type="checkbox"/>
Minimum defocus search [um]	<input type="text" value="0.3"/>	<input type="text" value="0.3"/>
Maximum defocus search [um]	<input type="text" value="9.0"/>	<input type="text" value="9.0"/>
Defocus search step [um]	<input type="text" value="0.1"/>	<input type="text" value="0.1"/>
Minimum phase search [degrees]	<input type="text" value="5.0"/>	<input type="text" value="5.0"/>
Maximum phase search [degrees]	<input type="text" value="175.0"/>	<input type="text" value="175.0"/>
Phase search step [degrees]	<input type="text" value="5.0"/>	<input type="text" value="5.0"/>
Use PW spectrum	<input type="text" value="NO"/>	<input type="checkbox"/>
Skip calculation of 2D power spectra	<input type="text" value="YES"/>	<input checked="" type="checkbox"/>



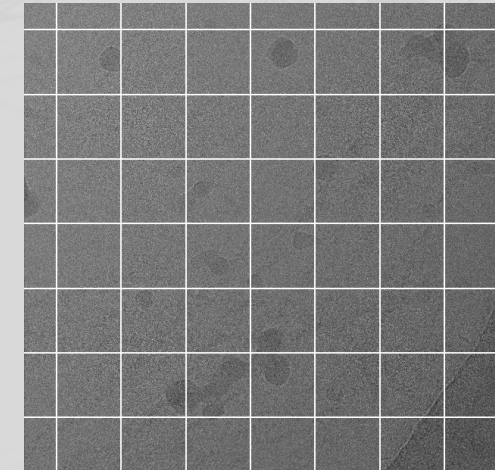
How fine the range between 0 and Nyquist is sampled in the spectra is determined by the **CTF window size**



Main Advanced

sp_cter Set advanced parameters

CTF window size [Pixels]	<input type="text" value="512"/>	<input type="text" value="512"/>
Number of CTF estimates per micrograph	<input type="text" value="16"/>	<input type="text" value="16"/>
X overlap [%]	<input type="text" value="50"/>	<input type="text" value="50"/>
Y overlap [%]	<input type="text" value="50"/>	<input type="text" value="50"/>
Edge x [pixels]	<input type="text" value="0"/>	<input type="text" value="0"/>
Edge y [pixels]	<input type="text" value="0"/>	<input type="text" value="0"/>
Check consistency of inputs	<input type="text" value="NO"/>	<input type="checkbox"/>
Enable debug mode	<input type="text" value="NO"/>	<input type="checkbox"/>
Volta Phase Plate Dataset	<input type="text" value="NO"/>	<input type="checkbox"/>
Minimum defocus search [um]	<input type="text" value="0.3"/>	<input type="text" value="0.3"/>
Maximum defocus search [um]	<input type="text" value="9.0"/>	<input type="text" value="9.0"/>
Defocus search step [um]	<input type="text" value="0.1"/>	<input type="text" value="0.1"/>
Minimum phase search [degrees]	<input type="text" value="5.0"/>	<input type="text" value="5.0"/>
Maximum phase search [degrees]	<input type="text" value="175.0"/>	<input type="text" value="175.0"/>
Phase search step [degrees]	<input type="text" value="5.0"/>	<input type="text" value="5.0"/>
Use PW spectrum	<input type="text" value="NO"/>	<input type="checkbox"/>
Skip calculation of 2D power spectra	<input type="text" value="YES"/>	<input checked="" type="checkbox"/>



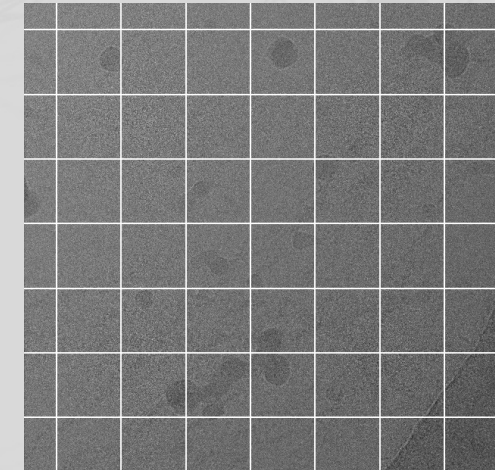
To estimate the error for the estimated parameters CTER does the estimation multiple times for random subsets of the tiles.



Main **Advanced**

sp_cter Set advanced parameters

CTF window size [Pixels]	<input type="text" value="512"/>	<input type="text" value="512"/>
Number of CTF estimates per micrograph	<input type="text" value="16"/>	<input type="text" value="16"/>
X overlap [%]	<input type="text" value="50"/>	<input type="text" value="50"/>
Y overlap [%]	<input type="text" value="50"/>	<input type="text" value="50"/>
Edge x [pixels]	<input type="text" value="0"/>	<input type="text" value="0"/>
Edge y [pixels]	<input type="text" value="0"/>	<input type="text" value="0"/>
Check consistency of inputs	<input type="text" value="NO"/>	<input type="checkbox"/>
Enable debug mode	<input type="text" value="NO"/>	<input type="checkbox"/>
Volta Phase Plate Dataset	<input type="text" value="NO"/>	<input type="checkbox"/>
Minimum defocus search [um]	<input type="text" value="0.3"/>	<input type="text" value="0.3"/>
Maximum defocus search [um]	<input type="text" value="9.0"/>	<input type="text" value="9.0"/>
Defocus search step [um]	<input type="text" value="0.1"/>	<input type="text" value="0.1"/>
Minimum phase search [degrees]	<input type="text" value="5.0"/>	<input type="text" value="5.0"/>
Maximum phase search [degrees]	<input type="text" value="175.0"/>	<input type="text" value="175.0"/>
Phase search step [degrees]	<input type="text" value="5.0"/>	<input type="text" value="5.0"/>
Use PW spectrum	<input type="text" value="NO"/>	<input type="checkbox"/>
Skip calculation of 2D power spectra	<input type="text" value="YES"/>	<input checked="" type="checkbox"/>



The tiles overlap.



Main Advanced

sp_cter Set advanced parameters

CTF window size [Pixels]	<input type="text" value="512"/>	<input type="text" value="512"/>
Number of CTF estimates per micrograph	<input type="text" value="16"/>	<input type="text" value="16"/>
X overlap [%]	<input type="text" value="50"/>	<input type="text" value="50"/>
Y overlap [%]	<input type="text" value="50"/>	<input type="text" value="50"/>
Edge x [pixels]	<input type="text" value="0"/>	<input type="text" value="0"/>
Edge y [pixels]	<input type="text" value="0"/>	<input type="text" value="0"/>
Check consistency of inputs	<input type="text" value="NO"/>	<input type="checkbox"/>
Enable debug mode	<input type="text" value="NO"/>	<input type="checkbox"/>
Volta Phase Plate Dataset	<input type="text" value="NO"/>	<input type="checkbox"/>
Minimum defocus search [um]	<input type="text" value="0.3"/>	<input type="text" value="0.3"/>
Maximum defocus search [um]	<input type="text" value="9.0"/>	<input type="text" value="9.0"/>
Defocus search step [um]	<input type="text" value="0.1"/>	<input type="text" value="0.1"/>
Minimum phase search [degrees]	<input type="text" value="5.0"/>	<input type="text" value="5.0"/>
Maximum phase search [degrees]	<input type="text" value="175.0"/>	<input type="text" value="175.0"/>
Phase search step [degrees]	<input type="text" value="5.0"/>	<input type="text" value="5.0"/>
Use PW spectrum	<input type="text" value="NO"/>	<input type="checkbox"/>
Skip calculation of 2D power spectra	<input type="text" value="YES"/>	<input checked="" type="checkbox"/>

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



Main Advanced

sp_cter

Set advanced parameters

CTF window size [Pixels]	<input type="text" value="512"/>	<input type="text" value="512"/>
Number of CTF estimates per micrograph	<input type="text" value="16"/>	<input type="text" value="16"/>
X overlap [%]	<input type="text" value="50"/>	<input type="text" value="50"/>
Y overlap [%]	<input type="text" value="50"/>	<input type="text" value="50"/>
Edge x [pixels]	<input type="text" value="0"/>	<input type="text" value="0"/>
Edge y [pixels]	<input type="text" value="0"/>	<input type="text" value="0"/>
Check consistency of inputs	<input type="text" value="NO"/>	<input type="checkbox"/>
Enable debug mode	<input type="text" value="NO"/>	<input type="checkbox"/>
Volta Phase Plate Dataset	<input type="text" value="NO"/>	<input type="checkbox"/>
Minimum defocus search [um]	<input type="text" value="0.3"/>	<input type="text" value="0.3"/>
Maximum defocus search [um]	<input type="text" value="9.0"/>	<input type="text" value="9.0"/>
Defocus search step [um]	<input type="text" value="0.1"/>	<input type="text" value="0.1"/>
Minimum phase search [degrees]	<input type="text" value="5.0"/>	<input type="text" value="5.0"/>
Maximum phase search [degrees]	<input type="text" value="175.0"/>	<input type="text" value="175.0"/>
Phase search step [degrees]	<input type="text" value="5.0"/>	<input type="text" value="5.0"/>
Use PW spectrum	<input type="text" value="NO"/>	<input type="checkbox"/>
Skip calculation of 2D power spectra	<input type="text" value="YES"/>	<input checked="" type="checkbox"/>

Parameters for phase plate optimization.



Main Advanced


sp_cter Set advanced parameters

CTF window size [Pixels]	<input type="text" value="512"/>	<input type="text" value="512"/>
Number of CTF estimates per micrograph	<input type="text" value="16"/>	<input type="text" value="16"/>
X overlap [%]	<input type="text" value="50"/>	<input type="text" value="50"/>
Y overlap [%]	<input type="text" value="50"/>	<input type="text" value="50"/>
Edge x [pixels]	<input type="text" value="0"/>	<input type="text" value="0"/>
Edge y [pixels]	<input type="text" value="0"/>	<input type="text" value="0"/>
Check consistency of inputs	<input type="text" value="NO"/>	<input type="checkbox"/>
Enable debug mode	<input type="text" value="NO"/>	<input type="checkbox"/>
Volta Phase Plate Dataset	<input type="text" value="NO"/>	<input type="checkbox"/>
Minimum defocus search [um]	<input type="text" value="0.3"/>	<input type="text" value="0.3"/>
Maximum defocus search [um]	<input type="text" value="9.0"/>	<input type="text" value="9.0"/>
Defocus search step [um]	<input type="text" value="0.1"/>	<input type="text" value="0.1"/>
Minimum phase search [degrees]	<input type="text" value="5.0"/>	<input type="text" value="5.0"/>
Maximum phase search [degrees]	<input type="text" value="175.0"/>	<input type="text" value="175.0"/>
Phase search step [degrees]	<input type="text" value="5.0"/>	<input type="text" value="5.0"/>
Use PW spectrum	<input type="text" value="NO"/>	<input type="checkbox"/>
Skip calculation of 2D power spectra	<input type="text" value="YES"/>	<input checked="" type="checkbox"/>

Uncheck this box to write 2D power spectra.

CTER Assessment





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

sgui_cter GUI tool to assess and sort micrographs according to their CTF parameters estimated by sxcter.

Main | Advanced

File containing CTF parameters	<input type="text" value="none"/>	<input type="text" value="none"/>	<input type="button" value="Select CTER partres"/>
MPI processors	<input type="text" value="1"/>		<input type="button" value="Save parameters"/>
MPI command line template	<input type="text"/>		<input type="button" value="Load parameters"/>
Submit job to queue	<input type="checkbox"/>		
job name	<input type="text" value="N/A"/>		
Submission command	<input type="text" value="N/A"/>		<input type="button" value="Generate command line"/>
Submission script template	<input type="text" value="N/A"/>	<input type="button" value="Select template"/>	<input type="button" value="Run command"/>

SPH RE

CTER Assessment



Open CTER partres file

Display Windows:

- Rot. Avg. Plot
- Rot. Avg. Plot Zoom
- Histogram
- Sort Plot
- Micrograph Thumbnail
- 2D Power Spectrum

Display Curves:

- Exp. No Astig (Black)
- Fit. No Astig (Blue)
- Exp. with Astig (Red)
- Fit. with Astig (Green)
- Exp. Enhanced (Olive)
- Fit. Enhanced (Cyan)

Plot Fix Scale 1.1

Refresh Graphs

SPH RE

sp_gui_cter - Control Panel

Selection Summary:

Num. of entries 112 Voltage [kV] 300
 Unchecked 0 Cs [mm] 2
 Ratio 0 Pixel Size [Å] 1.14

Current Entry Info:

Sorted ID 0
 CTER ID 0
 Select 1

Defocus [um] 2.2628

Total Amp. Contrast [%] 10

Astig. Amp. [um] 0.051144

Astig. Ang. [deg] 26.53

Defocus SD [um] 0.0014979

Defocus Freq. Limit [1/Å] 0.43688

Astig. Freq. Limit [1/Å] 0.36664

Phase Shift [deg] 0

Const. Amp. Contrast [%] 10

B-factor [Å²] 0

Unapplied Thresholds:

0.99218	2.2628
0.030087	0.059647
0.11719	179.92
0.00011507	0.018489
0.25699	0.43688
0.21587	0.43688

Applied Thresholds:

0.99218	2.2628
0.030087	0.059647
0.11719	179.92
0.00011507	0.018489
0.25699	0.43688
0.21587	0.43688

Sort CTER Partres Entries:

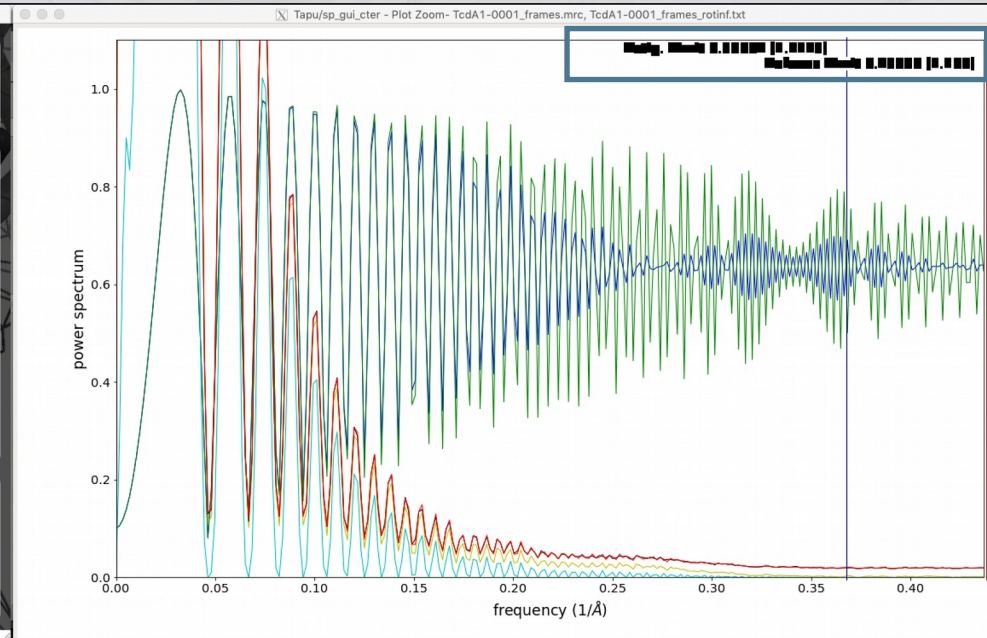
CTER ID Defocus [um] Unapplied

Descending Move Threshold Lower (blue) Save Load

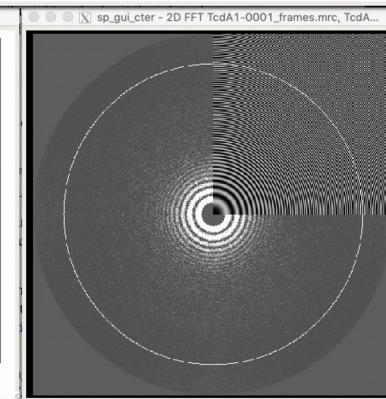
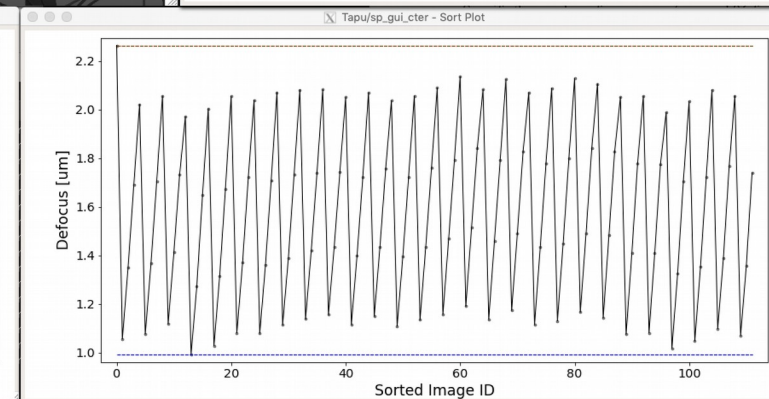
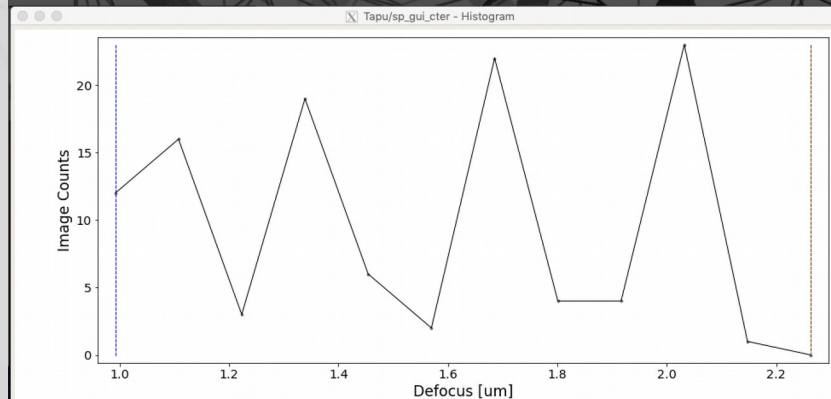
Sort Select Sync. w/Sort Save Selection:

counts/bin 10 File Suffix Trial00

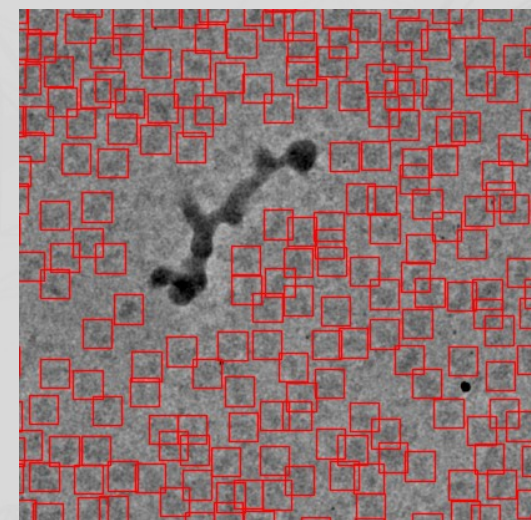
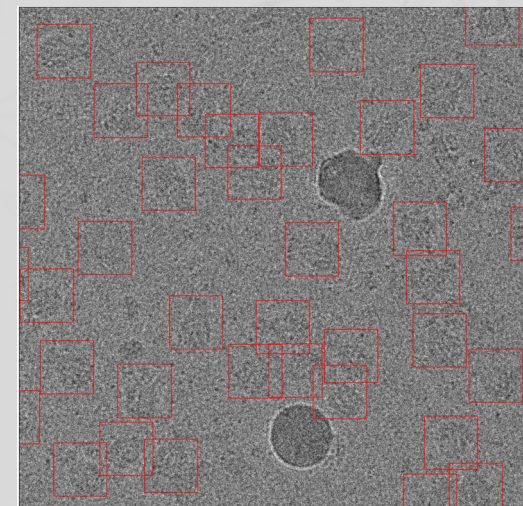
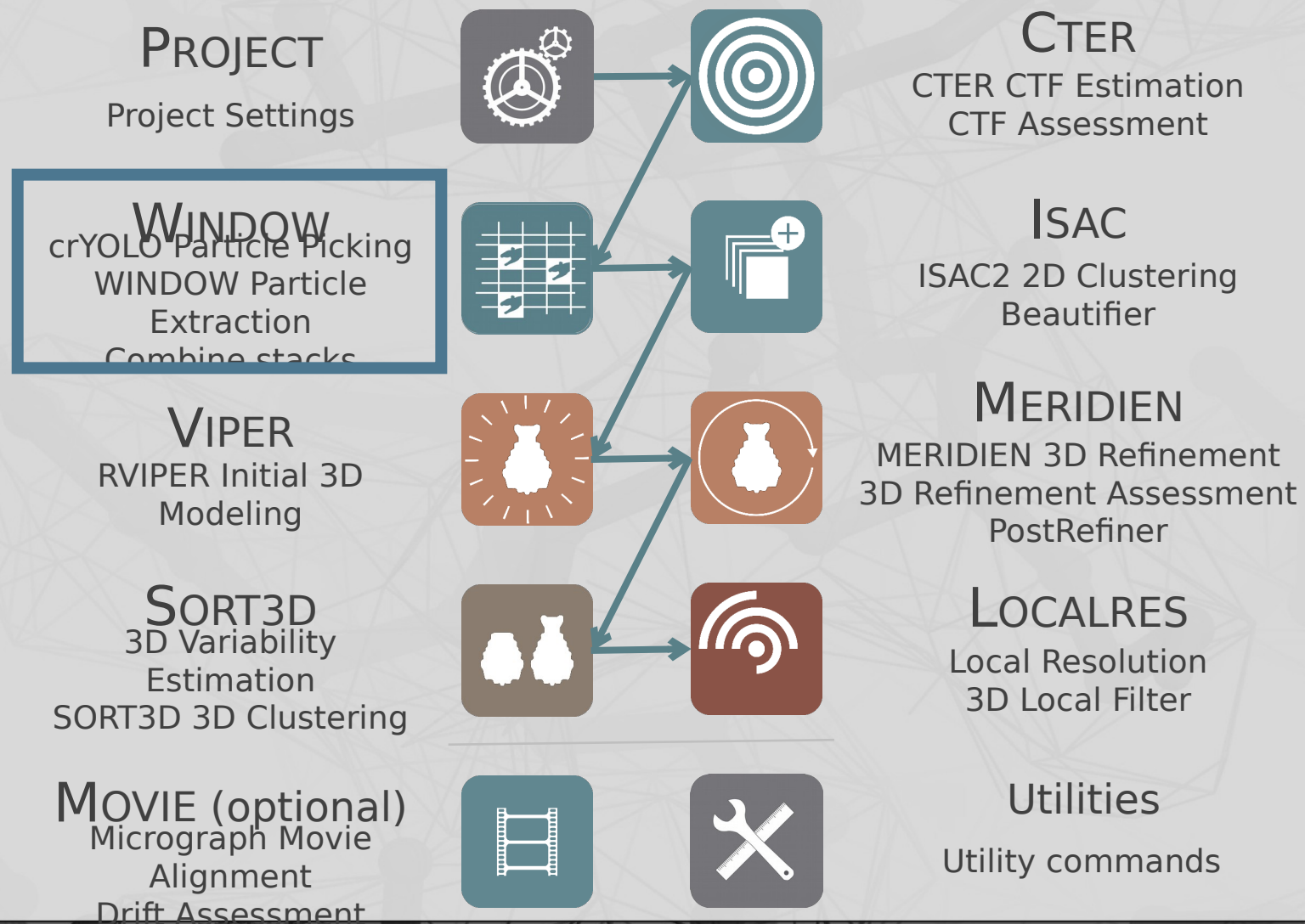
Reapply Sort Apply All Thresholds Save Selection



Remote glitch?



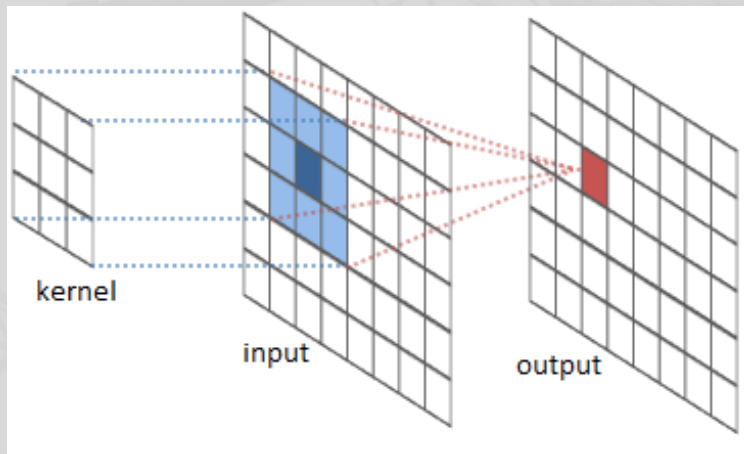
SPHIRE Pipeline



Convolution



What is a convolution?



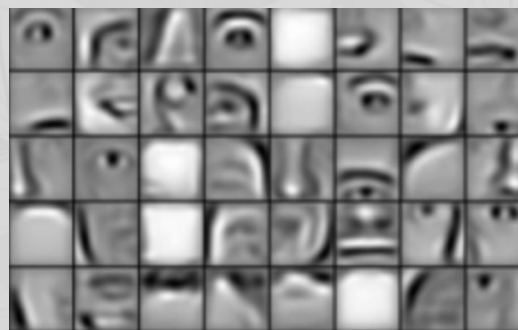
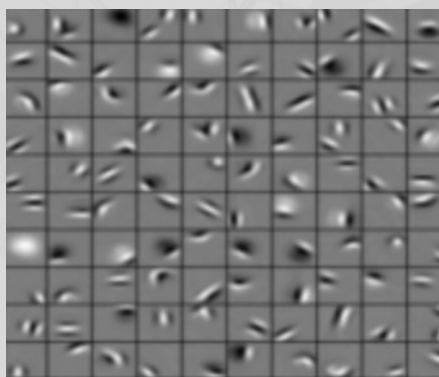
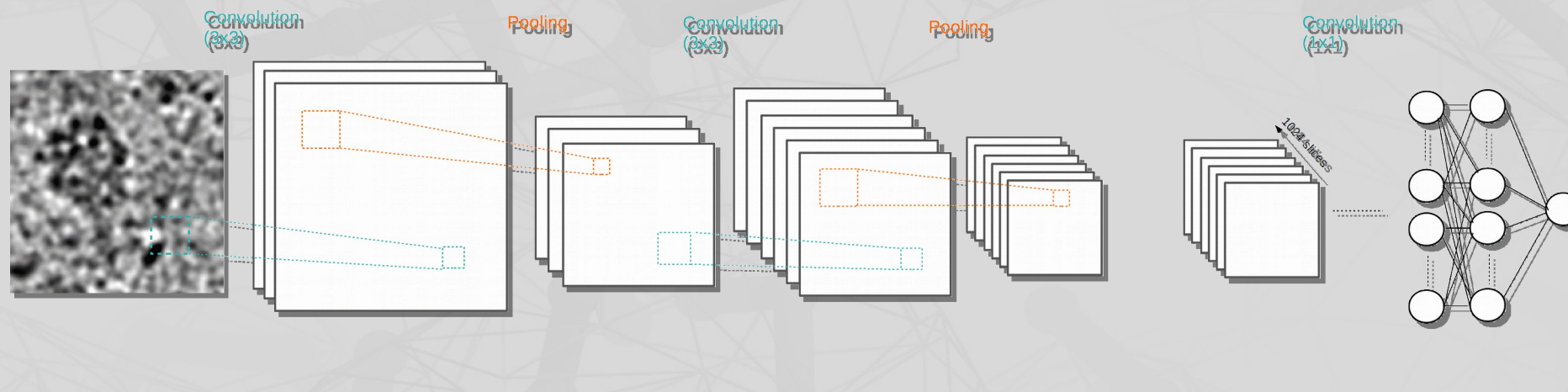
What does a convolution do?



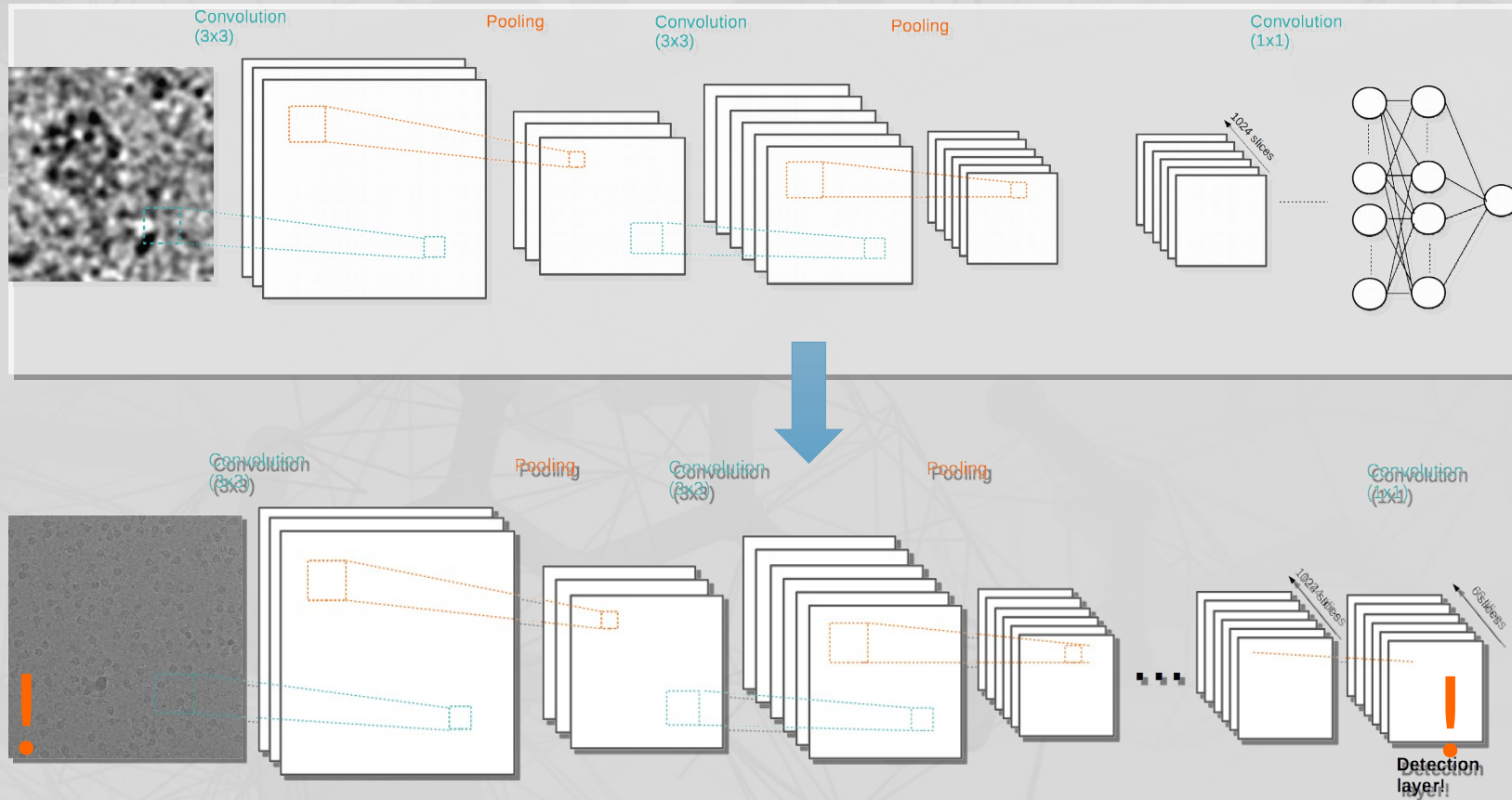
A CNN learns the elements of filter kernels and therefore features.

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

CNNs: Hierarchies of features

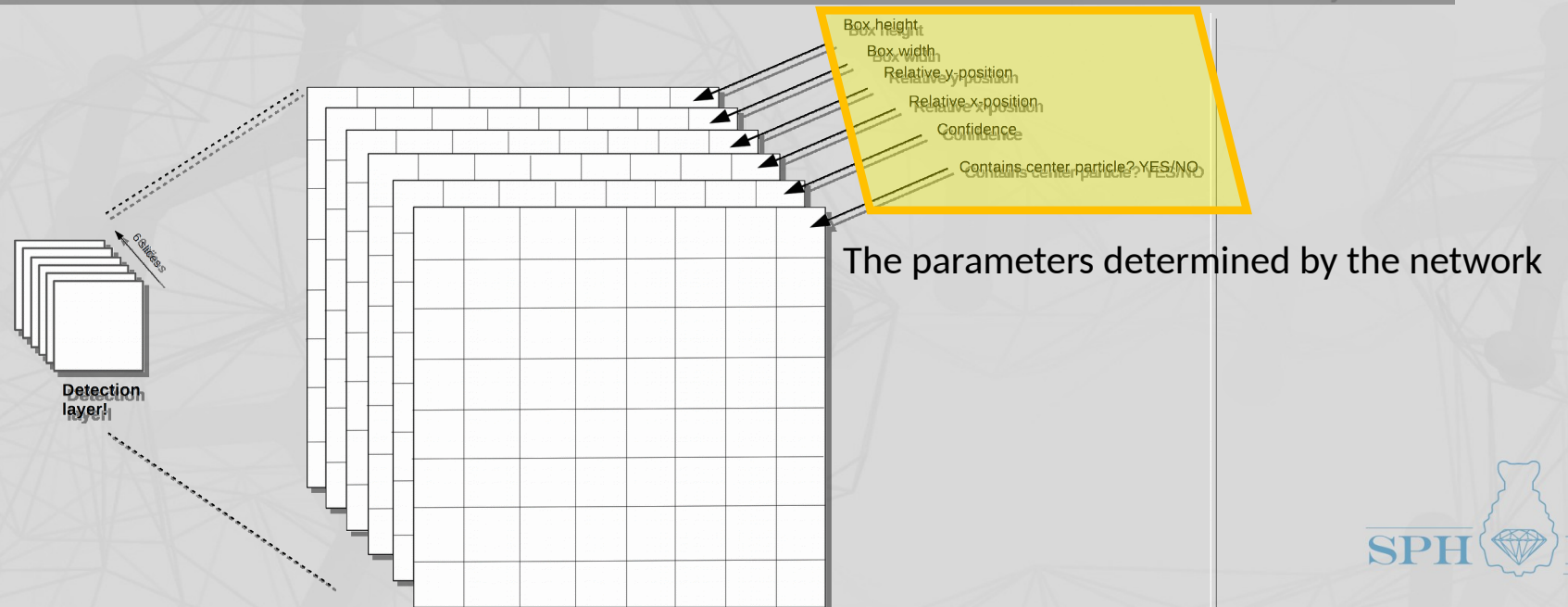
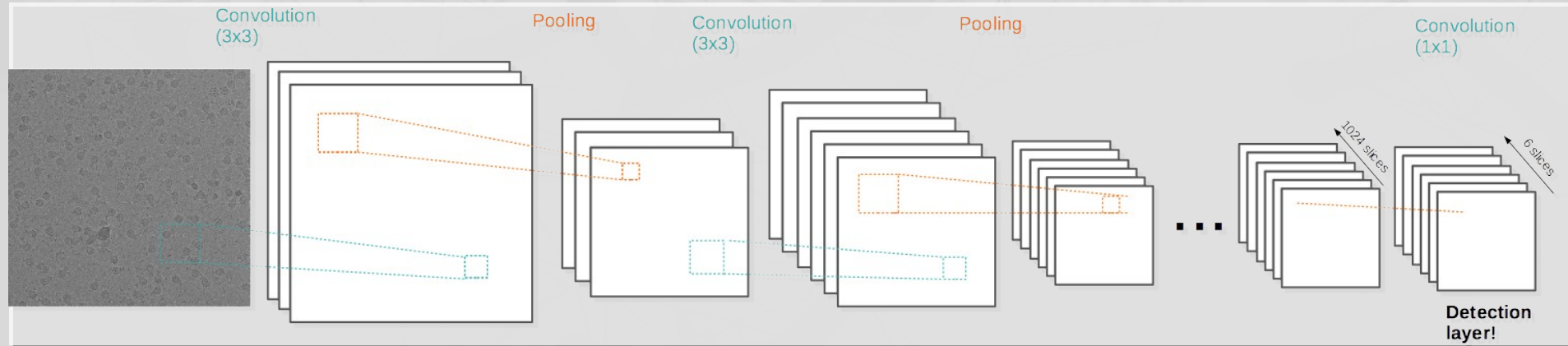


Method: YOLO



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

Method: YOLO





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

sp_cryolo_predict Prediction with crYOLO, a deep learning high accuracy particle picking procedure.

Main Advanced

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	FILES/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	<input type="checkbox"/>		
Job name	sp_cryolo_predict		
Submission command	sbatch		Generate command line
Submission script template	/sphire_defq_ppn16_threads1.sh	Select template	Add to pipeline folder
			Run command



sp_cryolo_predict Prediction with crYOLO, a deep learning high accuracy particle picking procedure.

Main Advanced

crYOLO predict executable	<input type="text" value="none"/>	<input type="text" value="cryolo_1.3_cpu/bin/cryolo_predict.py"/>	<input type="button" value="Select python file"/>
Config file	required	<input type="text" value="DATASET/CRYOLO_FILES/config.json"/>	<input type="button" value="Select JSON file"/>
Image directory	<input type="text" value="none"/>	<input type="text" value="EST06/Corrected_sums/corrsum_dw"/>	<input type="button" value="Select directory"/>
Model path	required	<input type="text" value="FILES/gmodel_phosnet_20190314.h5"/>	<input type="button" value="Select h5 file"/>
Output directory	<input type="text" value="none"/>	<input type="text" value="box_files"/>	
MPI processors	<input type="text" value="1"/>		<input type="button" value="Save parameters"/>
MPI command line template	<input type="text"/>		<input type="button" value="Load parameters"/>
Submit job to queue	<input type="checkbox"/>		
Job name	<input type="text" value="sp_cryolo_predict"/>		
Submission command	<input type="text" value="sbatch"/>		<input type="button" value="Generate command line"/>
			<input type="button" value="Add to pipeline folder"/>
Submission script template	<input type="text" value="/sphire_defq_ppn16_threads1.sh"/>	<input type="button" value="Select template"/>	<input type="button" value="Run command"/>

Path of cryolo_predict executable



Main Advanced

sp_cryolo_predict Prediction with crYOLO, a deep learning high accuracy particle picking procedure.

crYOLO predict executable	<input type="text" value="none"/>	<input type="text" value="cryolo_1.3_cpu/bin/cryolo_predict.py"/>	<input type="button" value="Select python file"/>
Config file	required	<input type="text" value="DATASET/CRYOLO_FILES/config.json"/>	<input type="button" value="Select JSON file"/>
Image directory	<input type="text" value="none"/>	<input type="text" value="EST06/Corrected_sums/corrsum_dw"/>	<input type="button" value="Select directory"/>
Model path	required	<input type="text" value="FILES/gmodel_phosnet_20190314.h5"/>	<input type="button" value="Select h5 file"/>
Output directory	<input type="text" value="none"/>	<input type="text" value="box_files"/>	
MPI processors	<input type="text" value="1"/>		<input type="button" value="Save parameters"/>
MPI command line template	<input type="text"/>		<input type="button" value="Load parameters"/>
Submit job to queue	<input type="checkbox"/>		
Job name	<input type="text" value="sp_cryolo_predict"/>		
Submission command	<input type="text" value="sbatch"/>		<input type="button" value="Generate command line"/>
			<input type="button" value="Add to pipeline folder"/>
Submission script template	<input type="text" value="/sphire_defq_ppn16_threads1.sh"/>	<input type="button" value="Select template"/>	<input type="button" value="Run command"/>

CrYOLO configuration file



sp_cryolo_predict Prediction with crYOLO, a deep learning high accuracy particle picking procedure.

Main Advanced

crYOLO predict executable	<input type="text" value="none"/>	<input type="text" value="cryolo_1.3_cpu/bin/cryolo_predict.py"/>	<input type="button" value="Select python file"/>
Config file	required	<input type="text" value="DATASET/CRYOLO_FILES/config.json"/>	<input type="button" value="Select JSON file"/>
Image directory	<input type="text" value="none"/>	<input type="text" value="/EST06/Corrected_sums/corrsum_dw"/>	<input type="button" value="Select directory"/>
Model path	required	<input type="text" value="FILES/gmodel_phosnet_20190314.h5"/>	<input type="button" value="Select h5 file"/>
Output directory	<input type="text" value="none"/>	<input type="text" value="box_files"/>	
MPI processors	<input type="text" value="1"/>		<input type="button" value="Save parameters"/>
MPI command line template	<input type="text"/>		<input type="button" value="Load parameters"/>
Submit job to queue	<input type="checkbox"/>		
Job name	<input type="text" value="sp_cryolo_predict"/>		
Submission command	<input type="text" value="sbatch"/>		<input type="button" value="Generate command line"/>
			<input type="button" value="Add to pipeline folder"/>
Submission script template	<input type="text" value="/sphire_defq_ppn16_threads1.sh"/>	<input type="button" value="Select template"/>	<input type="button" value="Run command"/>

Micrograph directory



sp_cryolo_predict Prediction with crYOLO, a deep learning high accuracy particle picking procedure.

Main Advanced

crYOLO predict executable	<input type="text" value="none"/>	<input type="text" value="cryolo_1.3_cpu/bin/cryolo_predict.py"/>	<input type="button" value="Select python file"/>
Config file	required	<input type="text" value="DATASET/CRYOLO_FILES/config.json"/>	<input type="button" value="Select JSON file"/>
Image directory	<input type="text" value="none"/>	<input type="text" value="EST06/Corrected_sums/corrsum_dw"/>	<input type="button" value="Select directory"/>
Model path	required	<input type="text" value="FILES/gmodel_phosnet_20190314.h5"/>	<input type="button" value="Select h5 file"/>
Output directory	<input type="text" value="none"/>	<input type="text" value="box_files"/>	
MPI processors	<input type="text" value="1"/>		<input type="button" value="Save parameters"/>
MPI command line template	<input type="text"/>		<input type="button" value="Load parameters"/>
Submit job to queue	<input type="checkbox"/>		
Job name	<input type="text" value="sp_cryolo_predict"/>		
Submission command	<input type="text" value="sbatch"/>		<input type="button" value="Generate command line"/>
			<input type="button" value="Add to pipeline folder"/>
Submission script template	<input type="text" value="/sphire_defq_ppn16_threads1.sh"/>	<input type="button" value="Select template"/>	<input type="button" value="Run command"/>

CrYOLO model file,
containing network weights



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	<input type="checkbox"/>
Filament width [Pixel]	100	100
Minimum number of boxes per filament	6	6
Box distance	352	352
Don't split curved filaments	NO	<input type="checkbox"/>
Don't merge filaments	NO	<input type="checkbox"/>

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

cryolo_boxmanager



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

sp_cryolo_boxmanager Displays boxfiles on images. Allows creation of new training data for crYOLO.

Main Advanced

crYOLO boxmanager executable

Input image directory

MPI processors

MPI command line template

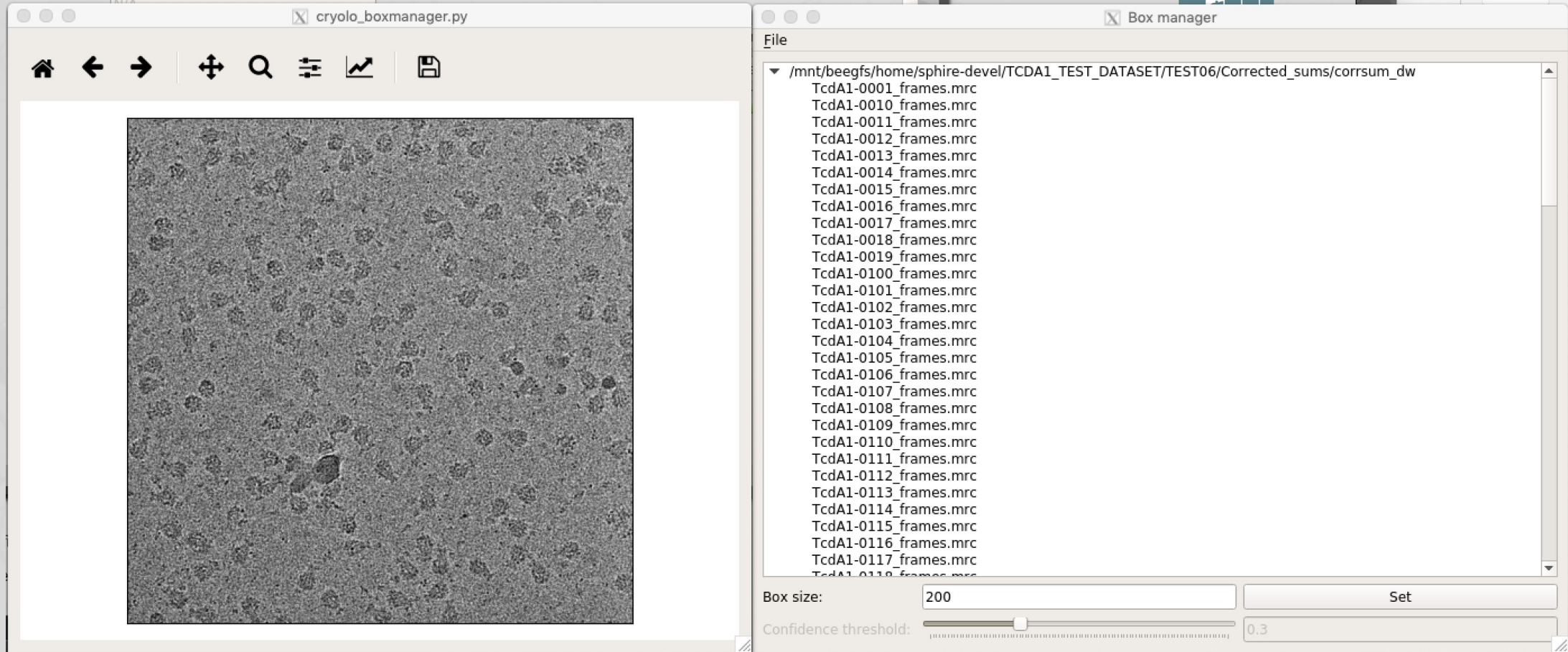
Submit job to queue

job name

Submission command

Submission script template

cryolo_boxmanager



To open coordinates:
File → *Import box files*
Select CBOX



cryolo_boxmanager



The screenshot shows two windows from the cryolo_boxmanager.py application. The left window displays a grayscale micrograph with numerous red square boxes overlaid on it, representing detected particles. The right window, titled "Box manager", shows a file list of mrc files (e.g., TcdA1-0001_frames.mrc to TcdA1-0117_frames.mrc) and controls for "Box size" (set to 205) and "Confidence threshold" (set to 0.3).

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

sp_window

Window particles from micrographs using the particle coordinates.

Input micrograph path pattern	<input type="text" value="Corrected_sums/corrsun_dw/TcdA1-*_frames.mrc"/>	<input type="button" value="required"/>	<input type="button" value="Select MRC micrograph"/>	<input type="button" value="Select any micrograph"/>
Input coordinates path pattern	<input type="text" value="ASET/TEST06/box_files/EMAN/TcdA1-*_frames.box"/>	<input type="button" value="required"/>	<input type="button" value="Select BOX coordinates"/>	<input type="button" value="Select any coordinates"/>
CTF parameters source	<input type="text" value="CTFest/partres.txt"/>	<input type="button" value="required"/>	<input type="button" value="Select CTER partres"/>	
Output directory	<input type="text" value="Particles"/>	<input type="button" value="required"/>		
Micrograph selection file	<input type="text" value="none"/>	<input type="button" value="none"/>	<input type="button" value="Select micrograph list"/>	
Coordinate file format	<input type="text" value="cryolo"/>	<input type="button" value="cryolo"/>		
Particle box size [Pixels]	<input type="text" value="352"/>	<input type="button" value="352"/>		
Invert image contrast	<input checked="" type="checkbox" value="YES"/>	<input type="button" value="YES"/>		
MPI processors	<input type="text" value="48"/>	<input type="button" value="48"/>	<input type="button" value="Save parameters"/>	
MPI command line template	<input type="text" value=""/>	<input type="button" value=""/>	<input type="button" value="Load parameters"/>	
Submit job to queue	<input checked="" type="checkbox"/>	<input type="button" value=""/>		
Job name	<input type="text" value="sp_window"/>	<input type="button" value="sp_window"/>		
Submission command	<input type="text" value="sbatch"/>	<input type="button" value="sbatch"/>	<input type="button" value="Generate command line"/>	
Submission script template	<input type="text" value="templates/sphire/sphire_defq_ppn16_threads1.sh"/>	<input type="button" value="Select template"/>	<input type="button" value="Run command"/>	

Particle extraction



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	<input checked="" type="checkbox"/>		
MPI processors	48		Save parameters	
MPI command line template			Load parameters	
Submit job to queue	<input checked="" type="checkbox"/>			
Job name	sp_window			
Submission command	sbatch		Generate command line	
			Add to pipeline folder	
Submission script template	tplates/sphire/sphire_defq_ppn16_threads1.sh	Select template	Run command	

Micrographs

Particle extraction



sp_window Window particles from micrographs using the particle coordinates.

Input micrograph path pattern	<input type="text" value="Corrected_sums/corrsum_dw/TcdA1-*_frames.mrc"/>	<input type="button" value="required"/>	<input type="button" value="Select MRC micrograph"/>	<input type="button" value="Select any micrograph"/>
Input coordinates path pattern	<input type="text" value="ASET/TEST06/box_files/EMAN/TcdA1-*_frames.box"/>	<input type="button" value="required"/>	<input type="button" value="Select BOX coordinates"/>	<input type="button" value="Select any coordinates"/>
CTF parameters source	<input type="text" value="CTFest/partres.txt"/>	<input type="button" value="required"/>	<input type="button" value="Select CTER partres"/>	
Output directory	<input type="text" value="Particles"/>	<input type="button" value="required"/>		
Micrograph selection file	<input type="text" value="none"/>	<input type="button" value="none"/>	<input type="button" value="Select micrograph list"/>	
Coordinate file format	<input type="text" value="cryolo"/>	<input type="button" value="cryolo"/>		
Particle box size [Pixels]	<input type="text" value="352"/>	<input type="button" value="352"/>		
Invert image contrast	<input checked="" type="checkbox" value="YES"/>	<input type="button" value="YES"/>		
MPI processors	<input type="text" value="48"/>	<input type="button" value="48"/>	<input type="button" value="Save parameters"/>	
MPI command line template	<input type="text"/>	<input type="button"/>	<input type="button" value="Load parameters"/>	
Submit job to queue	<input checked="" type="checkbox"/>	<input type="button"/>		
Job name	<input type="text" value="sp_window"/>	<input type="button"/>		
Submission command	<input type="text" value="sbatch"/>	<input type="button"/>	<input type="button" value="Generate command line"/>	
Submission script template	<input type="text" value="tplates/sphire/sphire_defq_ppn16_threads1.sh"/>	<input type="button" value="Select template"/>	<input type="button" value="Run command"/>	

Coordinates files

Particle extraction



sp_window Window particles from micrographs using the particle coordinates.

Input micrograph path pattern	<input type="text" value="required"/>	<input type="text" value="Corrected_sums/corrsum_dw/TcdA1-*_frames.mrc"/>	<input type="button" value="Select MRC micrograph"/>	<input type="button" value="Select any micrograph"/>
Input coordinates path pattern	<input type="text" value="required"/>	<input type="text" value="ASET/TEST06/box_files/EMAN/TcdA1-*_frames.box"/>	<input type="button" value="Select BOX coordinates"/>	<input type="button" value="Select any coordinates"/>
CTF parameters source	<input type="text" value="required"/>	<input type="text" value="CTFest/partres.txt"/>	<input type="button" value="Select CTER partres"/>	
Output directory	<input type="text" value="required"/>	<input type="text" value="Particles"/>		
Micrograph selection file	<input type="text" value="none"/>	<input type="text" value="none"/>	<input type="button" value="Select micrograph list"/>	
Coordinate file format	<input type="text" value="cryolo"/>	<input type="text" value="cryolo"/>		
Particle box size [Pixels]	<input type="text" value="352"/>	<input type="text" value="352"/>		
Invert image contrast	<input type="text" value="YES"/>	<input checked="" type="checkbox"/>		
MPI processors	<input type="text" value="48"/>	<input type="button" value="Save parameters"/>		
MPI command line template	<input type="text"/>	<input type="button" value="Load parameters"/>		
Submit job to queue	<input checked="" type="checkbox"/>			
Job name	<input type="text" value="sp_window"/>			
Submission command	<input type="text" value="sbatch"/>	<input type="button" value="Generate command line"/>		
Submission script template	<input type="text" value="tplates/sphire/sphire_defq_ppn16_threads1.sh"/>	<input type="button" value="Select template"/>	<input type="button" value="Run command"/>	

CTF parameter file

Particle extraction



sp_window Window particles from micrographs using the particle coordinates.

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

MPI processors	<input type="text" value="48"/> 48	Save parameters
MPI command line template	<input type="text"/>	Load parameters
Submit job to queue	<input checked="" type="checkbox"/>	
Job name	<input type="text" value="sp_window"/> sp_window	
Submission command	<input type="text" value="sbatch"/> sbatch	Generate command line
Submission script template	<input type="text" value="templates/sphire/sphire_defq_ppn16_threads1.sh"/> templates/sphire/sphire_defq_ppn16_threads1.sh	Add to pipeline folder

Select template

Cluster settings

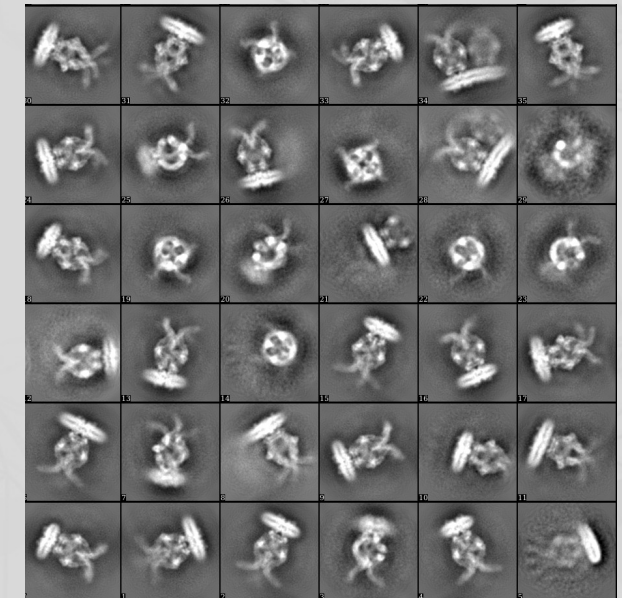
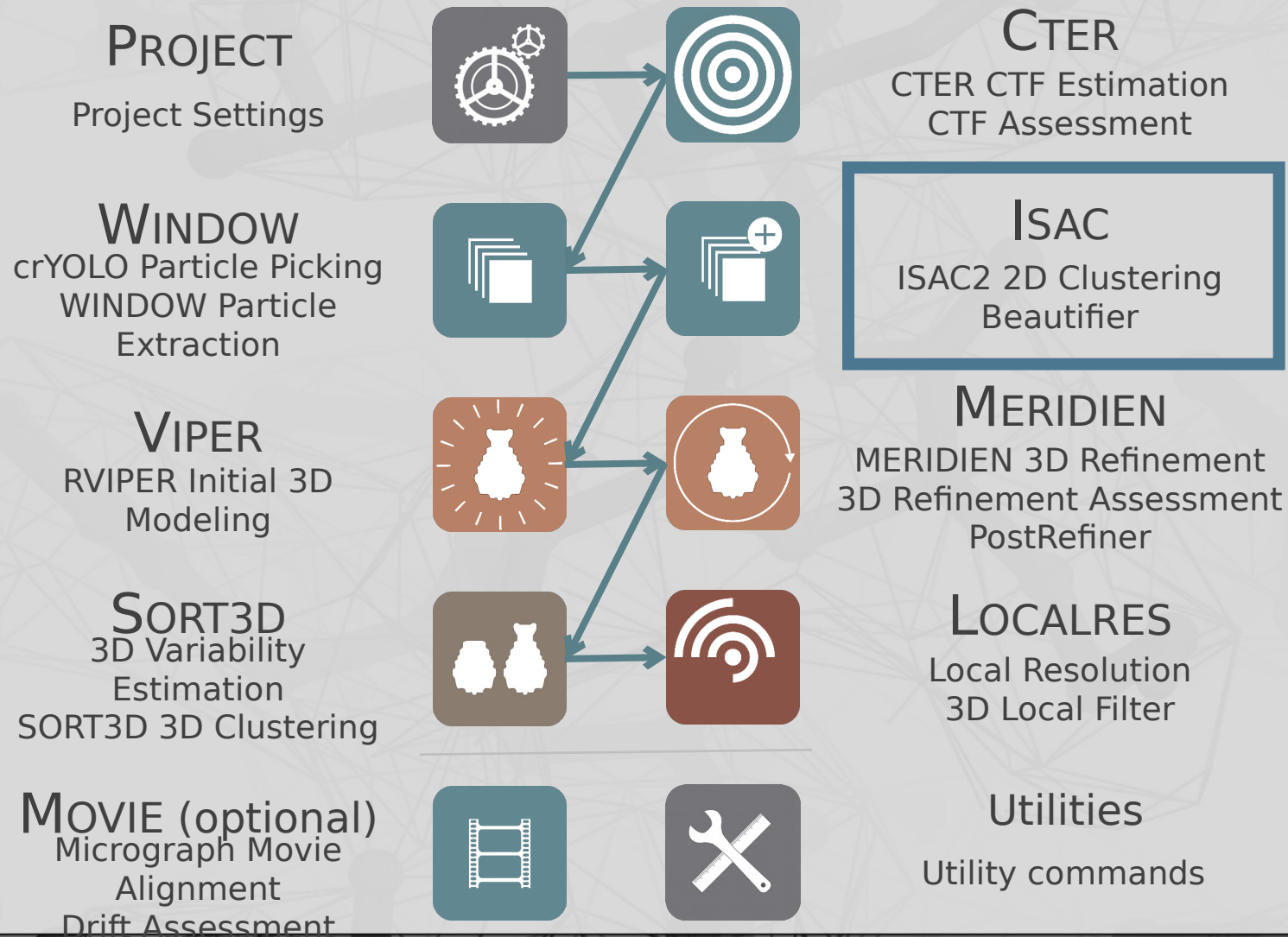
Combine stacks



- 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 Pipeline



Running ISAC



SPHIRE-GUI Main Version 1.2

COMMANDS (shift-click for wiki)

- ISAC2 - 2D Clustering**
- Beautifier
- ISAC2 Stack Subset

UTILITIES (shift-click for wiki)

- Create Virtual Stack
- Separate Into Classes
- Display Data
- Batch Pipeline Execution

sp_isac2 Iterative Stable Alignment and Clustering (ISAC) of a 2D image stack.

Main Advanced

Input image stack	required	level/TCDA1_TEST_DATASET/TEST06/Particles#stack_all	Select BDB image stack
Output directory	required	ISAC	Select directory
Particle radius [Pixels]	145	145	
Images per class	200	100	
CTF phase flipping	NO	<input checked="" type="checkbox"/>	
Phase Plate data	NO	<input type="checkbox"/>	
MPI processors	1		Save parameters
MPI command line template			Load parameters
Submit job to queue	<input type="checkbox"/>		
Job name	sp_isac2		Generate command line
Submission command	sbatch		Add to pipeline folder
Submission script template	jb_templates/sphire/sphire_defq_ppn16_threads1.sh	Select template	Run command

SPHIRE

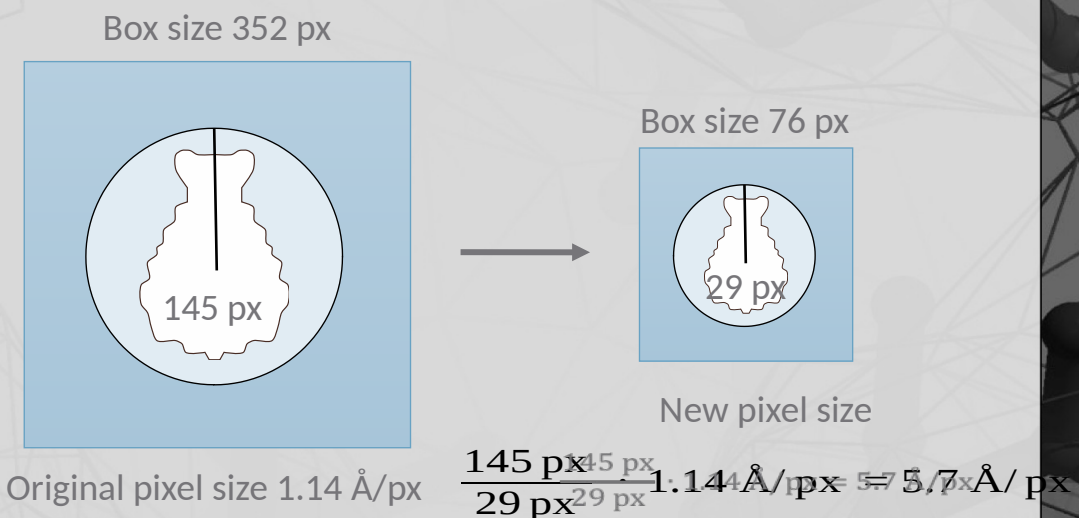
Running ISAC — advanced users



sp_isac2 Set advanced parameters

	Main	Advanced
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	<input checked="" type="checkbox"/>
Restart run	-1	-1
Skip ordered class averages	NO	<input type="checkbox"/>
Filament width [Pixels]	-1	-1
Ignore filament masking (filament use only)	NO	<input type="checkbox"/>

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



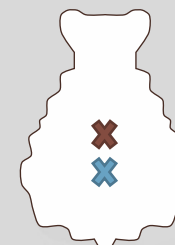
Running ISAC — advanced users



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	<input checked="" type="checkbox"/>
Restart run	-1	-1
Skip ordered class averages	NO	<input type="checkbox"/>
Filament width [Pixels]	-1	-1
Ignore filament masking (filament use only)	NO	<input type="checkbox"/>

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 \neq center of particle
- Use other alignment strategies (ex. MRA) instead

Running ISAC – advanced users

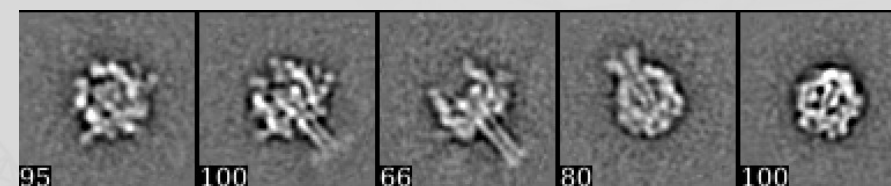


sp_isac2 Set advanced parameters

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	<input checked="" type="checkbox"/>
Restart run	-1	-1
Skip ordered class averages	NO	<input type="checkbox"/>
Filament width [Pixels]	-1	-1
Ignore filament masking (filament use only)	NO	<input type="checkbox"/>

Tunes minimum number of particles per class

$Minimum\ size \leq Members\ per\ class \leq Images\ per\ group$



Adjust if $Images\ per\ group \ll$ or \gg default
Ex. 50 images per group \rightarrow reduce minimum size

Visualization of results

The screenshot shows the SPHIRE-GUI Main Version 1.2 interface. On the left is a sidebar with icons for various functions. The 'UTILITIES' section is expanded, and the 'Display Data' option is highlighted with a red box. The main panel shows the configuration for the 'e2display' utility, which is used to display images, volumes, or 1D plots. The configuration includes input files, MPI processors, and submission options. The 'Run command' button is highlighted in green.

UTILITIES (shift-click for wiki)

- Create Virtual Stack
- Separate Into Classes
- Display Data**
- Batch Pipeline Execution

e2display Displays images, volumes, or 1D plots.

Input files: none | ISAC/ordered_class_averages.hdf | Select any displayables

MPI processors: 1

MPI command line template: [empty]

Submit job to queue:

Job name: N/A

Submission command: N/A

Submission script template: N/A | Select template

Buttons: Save parameters, Load parameters, Generate command line, Add to pipeline folder, Run command

Data assessment



Data assessment

Stack - ordered_class_averages.hdf

Middle click on any class

e2display.py

Save

Snap

App Del Drag Sets

Values font size: 11 Animate

Main Sets

Mag: 1.0

Min: -0.909

Max: 1.198

Brt: 0.058

Cont: 0.774

Gam: 1.0

Click

This window will appear

Data assessment

Stack - ordered_class_averages.hdf

Select classes to delete

e2display.py

Save

Snap

App Del Drag Sets

Values font size: 11 Animate

Main Sets

Mag: 1.0

Min: -0.909

Max: 1.198

Brt: 0.058

Cont: 0.774

Gam: 1.0

Click

This window will appear

Data assessment

Stack - ordered_class_averages.hdf

Select classes to delete

e2display.py

Save

Snap

Save stack

App Del Drag Sets

Values font size: 11 Animate

Main Sets

Mag: 1.0

Min: -0.909

Max: 1.198

Brt: 0.058

Cont: 0.774

Gam: 1.0

Click

This window will appear

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

Beautification



SPHIRE-GUI Main Version 1.2

COMMANDS (shift-click for wiki)

- ISAC2 - 2D Clustering
- Beautifier**
- ISAC2 Stack Subset

UTILITIES (shift-click for wiki)

- Create Virtual Stack
- Separate Into Classes
- Display Data
- Batch Pipeline Execution

sp_compute_isac_avg Perform local 2D alignment of ISAC2 2D clustering results using the original pixel size and full CTF correction.

Main Advanced

Original image stack	required	TASET/TEST06/Particles#stack_all	Select BDB image stack
ISAC2 run directory	required	DA1_TEST_DATASET/TEST06/ISAC	Select directory
Output directory	none	Beautifier	
Pixel size [Å]	1.14	1.14	
Particle radius [Pixels]	145	145	
CTF correction	YES	<input checked="" type="checkbox"/>	
MPI processors	1		Save parameters
MPI command line template			Load parameters
Submit job to queue	<input type="checkbox"/>		
Job name	sp_compute_isac_avg		Generate command line
Submission command	sbatch		Add to pipeline folder
Submission script template	hire_defq_ppn16_threads1.sh	Select template	Run command

SPHIRE

Beautification



sp_compute_isac_avg Perform local 2D alignment of ISAC2 2D clustering results using the original pixel size and full CTF correction.

Main Advanced

Original image stack	required	TASET/TEST06/Particles#stack_all	Select BDB image stack
ISAC2 run directory	required	DA1_TEST_DATASET/TEST06/ISAC	Select directory
Output directory	none	Beautifier	
Pixel size [Å]	1.14	1.14	
Particle radius [Pixels]	145	145	
CTF correction	YES	<input checked="" type="checkbox"/>	
MPI processors	1		Save parameters
MPI command line template			Load parameters
Submit job to queue	<input type="checkbox"/>		
Job name	sp_compute_isac_avg		
Submission command	sbatch		Generate command line
			Add to pipeline folder
Submission script template	hire_defq_ppn16_threads1.sh	Select template	Run command

Stack of all particles

Beautification



Main Advanced

sp_compute_isac_avg Perform local 2D alignment of ISAC2 2D clustering results using the original pixel size and full CTF correction.

Original image stack	required	TASET/TEST06/Particles#stack_all	Select BDB image stack
ISAC2 run directory	required	DA1_TEST_DATASET/TEST06/ISAC	Select directory
Output directory	none	Beautifier	
Pixel size [Å]	1.14	1.14	
Particle radius [Pixels]	145	145	
CTF correction	YES	<input checked="" type="checkbox"/>	
MPI processors	1		Save parameters
MPI command line template			Load parameters
Submit job to queue	<input type="checkbox"/>		
Job name	sp_compute_isac_avg		
Submission command	sbatch		Generate command line
			Add to pipeline folder
Submission script template	hire_defq_ppn16_threads1.sh	Select template	Run command

ISAC directory

Make substack



SPHIRE-GUI Main Version 1.2

COMMANDS (shift-click for wiki)

- ISAC2 - 2D Clustering
- Beautifier
- ISAC2 Stack Subset**

UTILITIES (shift-click for wiki)

- Create Virtual Stack
- Separate Into Classes
- Display Data
- Batch Pipeline Execution

sp_pipe isac_substack

Create a virtual subset stack consisting of particles accounted for by ISAC2 by retrieving particle numbers associated with the ISAC2 or Beautifier class averages. The command also saves a selection file containing the retrieved original image numbers and 2D alignment parameters. In addition, it stores the 2D alignment parameters to the stack header.

Main Advanced

Input bdb image stack	required	TASET/TEST06/Particles#stack_all	Select BDB image stack
ISAC2 or Beautifier run output directory	required	DA1_TEST_DATASET/TEST06/ISAC	Select directory
Output directory	required	Substack	
ISAC2 or Beautifier class averages path	none	T_DATASET/TEST06/ISAC/best.hdf	Select HDF image Select any image
Stack subset basename		isac_substack	isac_substack
MPI processors		1	Save parameters
MPI command line template			Load parameters
Submit job to queue			
Job name		sp_pipe_isac_substack	
Submission command		sbatch	Generate command line
Submission script template		hire_defq_ppn16_threads1.sh	Select template Run command

SPHIRE

Make substack



Main Advanced

sp_pipe isac_substack Create a virtual subset stack consisting of particles accounted for by ISAC2 by retrieving particle numbers associated with the ISAC2 or Beautifier class averages. The command also saves a selection file containing the retrieved original image numbers and 2D alignment parameters. In addition, it stores the 2D alignment parameters to the stack header.

Input bdb image stack	required	TASET/TEST06/Particles#stack_all	Select BDB image stack
ISAC2 or Beautifier run output directory	required	DA1_TEST_DATASET/TEST06/ISAC	Select directory
Output directory	required	Substack	
ISAC2 or Beautifier class averages path	none	T_DATASET/TEST06/ISAC/best.hdf	Select HDF image Select any image
Stack subset basename	isac_substack	isac_substack	
MPI processors	1		Save parameters
MPI command line template			Load parameters
Submit job to queue	<input type="checkbox"/>		
Job name	sp_pipe_isac_substack		
Submission command	sbatch		Generate command line
			Add to pipeline folder
Submission script template	hire_defq_ppn16_threads1.sh	Select template	Run command

Stack of all particles

Make substack



sp_pipe isac_substack Main Advanced

Create a virtual subset stack consisting of particles accounted for by ISAC2 by retrieving particle numbers associated with the ISAC2 or Beautifier class averages. The command also saves a selection file containing the retrieved original image numbers and 2D alignment parameters. In addition, it stores the 2D alignment parameters to the stack header.

Input bdb image stack	required	TASET/TEST06/Particles#stack_all	Select BDB image stack
ISAC2 or Beautifier run output directory	required	DA1_TEST_DATASET/TEST06/ISAC	Select directory
Output directory	required	Substack	
ISAC2 or Beautifier class averages path	none	T_DATASET/TEST06/ISAC/best.hdf	Select HDF image Select any image
Stack subset basename	isac_substack	isac_substack	
MPI processors	1		Save parameters
MPI command line template			Load parameters
Submit job to queue	<input type="checkbox"/>		
Job name	sp_pipe_isac_substack		
Submission command	sbatch		Generate command line
			Add to pipeline folder
Submission script template	hire_defq_ppn16_threads1.sh	Select template	Run command

ISAC directory

Make substack



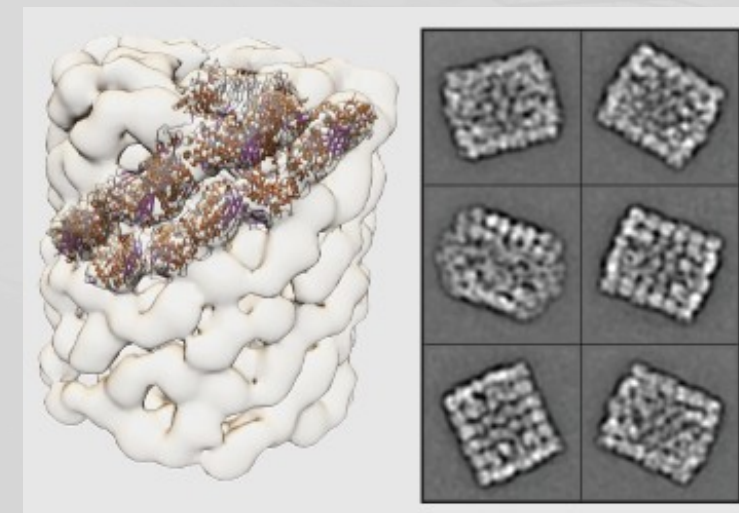
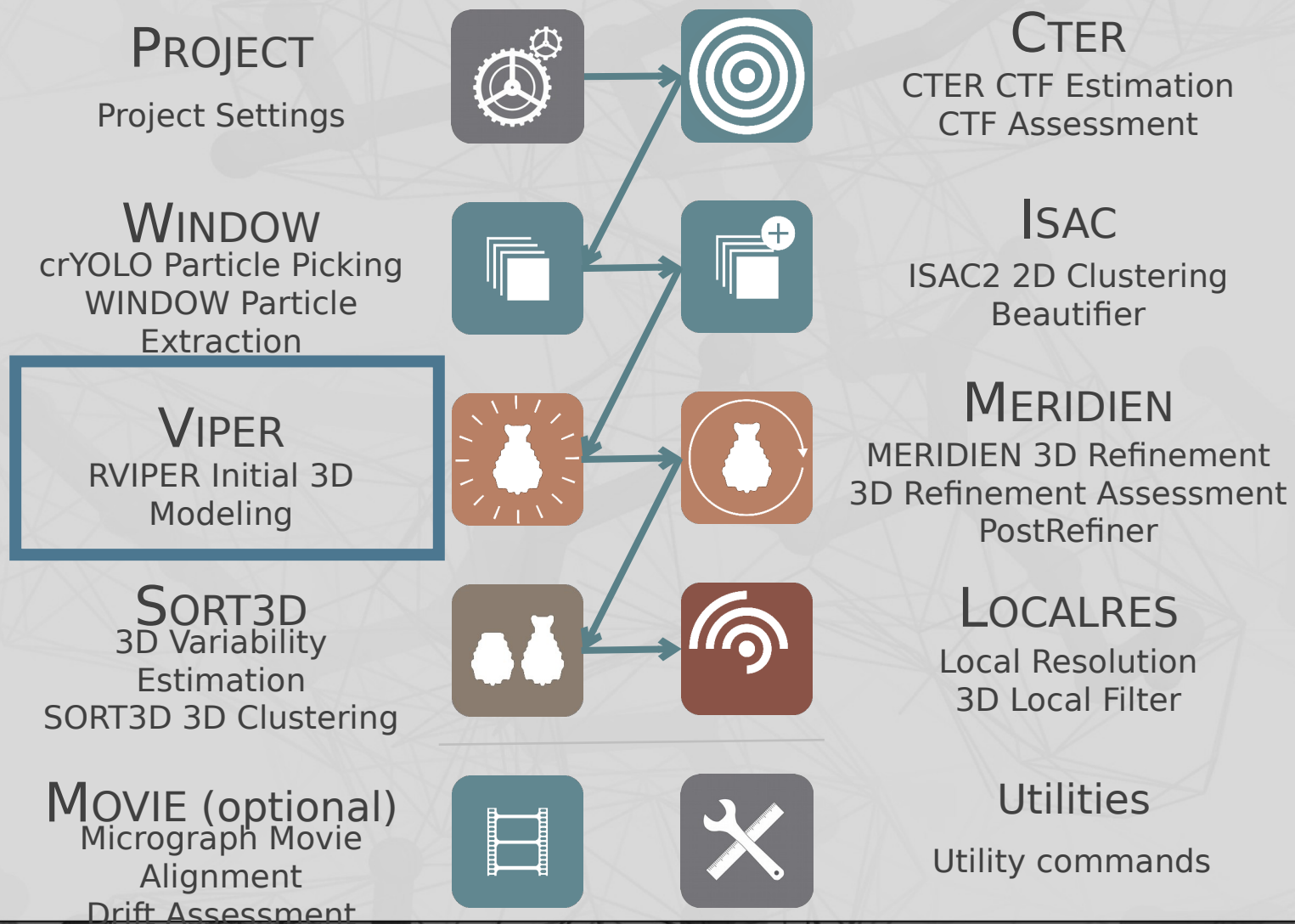
sp_pipe isac_substack Main Advanced

Create a virtual subset stack consisting of particles accounted for by ISAC2 by retrieving particle numbers associated with the ISAC2 or Beautifier class averages. The command also saves a selection file containing the retrieved original image numbers and 2D alignment parameters. In addition, it stores the 2D alignment parameters to the stack header.

Input bdb image stack	required	TASET/TEST06/Particles#stack_all	Select BDB image stack	
ISAC2 or Beautifier run output directory	required	DA1_TEST_DATASET/TEST06/ISAC	Select directory	
Output directory	required	Substack		
ISAC2 or Beautifier class averages path	none	T_DATASET/TEST06/ISAC/best.hdf	Select HDF image	Select any image
Stack subset basename	isac_substack	isac_substack		
MPI processors	1		Save parameters	
MPI command line template			Load parameters	
Submit job to queue	<input type="checkbox"/>			
Job name	sp_pipe_isac_substack			
Submission command	sbatch		Generate command line	
			Add to pipeline folder	
Submission script template	hire_defq_ppn16_threads1.sh	Select template	Run command	

Selected class averages

SPHIRE Pipeline



How to run RVIPER



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

sp_rviper

Reproducible ab initio 3D structure determination. The program determines a validated initial intermediate resolution structure using a subset of class averages produced by ISAC2.

Main | Advanced

Input images stack	required	ASET/TEST06/ISAC/best.hdf	Select HDF image	Select any image
Output directory	required	RVIPER	Select directory	
Particle radius [Pixels]		29		
Point-group symmetry		c5		
MPI processors		1		Save parameters
MPI command line template				Load parameters
Submit job to queue		<input type="checkbox"/>		
Job name		sp_rviper		Generate command line
Submission command		sbatch		Add to pipeline folder
Submission script template		efq_ppn16_threads1.sh	Select template	Run command

SPHIRE



How to run RVIPER



Main Advanced

sp_rviper Reproducible ab initio 3D structure determination. The program determines a validated initial intermediate resolution structure using a subset of class averages produced by ISAC2.

Input images stack	required	ASET/TEST06/ISAC/best.hdf	Select HDF image	Select any image
Output directory	required	RVIPER	Select directory	
Particle radius [Pixels]		29	29	
Point-group symmetry	c5	c5		
MPI processors	1		Save parameters	
MPI command line template			Load parameters	
Submit job to queue	<input type="checkbox"/>			
Job name	sp_rviper			
Submission command	sbatch		Generate command line	
			Add to pipeline folder	
Submission script template	efq_ppn16_threads1.sh	Select template	Run command	

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



Main Advanced

sp_rviper

Reproducible ab initio 3D structure determination. The program determines a validated initial intermediate resolution structure using a subset of class averages produced by ISAC2.

Input images stack	required	ASET/TEST06/ISAC/best.hdf	Select HDF image	Select any image
Output directory	required	RVIPER	Select directory	
Particle radius [Pixels]		29	29	
Point-group symmetry	c5	c5		
MPI processors		1	Save parameters	
MPI command line template			Load parameters	
Submit job to queue		<input type="checkbox"/>		
Job name		sp_rviper		
Submission command		sbatch	Generate command line	
			Add to pipeline folder	
Submission script template		efq_ppn16_threads1.sh	Select template	Run command

Use the radius from ISAC

What to do after running RVIPER



SPHIRE-GUI Main Version 1.2

Main Advanced

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

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

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' /TCDA1_TEST_DATASET/TEST06/ISAC

Output box size [Pixels]: 352 352

Invert handedness: NO

Low-pass filter resolution [A]: -1.0 -1.0

MPI processors: 1


MPI command line template:

Submit job to queue:

Job name: sp_pipe_moon_eliminator

Submission command: sbatch

Submission script template: e/sphire_defq_ppn16_threads1.sh



What to do after running RVIPER



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

Main Advanced

Input volume path	required	RVIPER/main001/average_volume.hdf	Select HDF volume	Select any volume
Output directory	required	VolumeAdjustment		
Output pixel size [Å]	1.14	1.14		
Use molecular mass	NO	<input type="checkbox"/>		
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'	/TCDA1_TEST_DATASET/TEST06/ISAC	Select directory	
Output box size [Pixels]	352	352		
Invert handedness	NO	<input checked="" type="checkbox"/>		
Low-pass filter resolution [Å]	-1.0	-1.0		
MPI processors	1		Save parameters	
MPI command line template			Load parameters	
Submit job to queue	<input type="checkbox"/>			
Job name	sp_pipe_moon_eliminator			
Submission command	sbatch		Generate command line	
Submission script template	e/sphire_defq_ppn16_threads1.sh	Select template	Add to pipeline folder	Run command

From RVIPER

What to do after running RVIPER



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

Main Advanced

Input volume path	required	RVIPER/main001/average_volume.hdf	Select HDF volume	Select any volume
Output directory	required	VolumeAdjustment		
Output pixel size [Å]	1.14	1.14		
Use molecular mass	NO	<input type="checkbox"/>		
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'	/TCDA1_TEST_DATASET/TEST06/ISAC	Select directory	
Output box size [Pixels]	352	352		
Invert handedness	NO	<input checked="" type="checkbox"/>		
Low-pass filter resolution [Å]	-1.0	-1.0		
MPI processors	1		Save parameters	
MPI command line template			Load parameters	
Submit job to queue	<input type="checkbox"/>			
Job name	sp_pipe_moon_eliminator		Generate command line	
Submission command	sbatch		Add to pipeline folder	
Submission script template	e/sphire_defq_ppn16_threads1.sh	Select template	Run command	

Threshold based on molecular weight

Threshold provided by user

What to do after running RVIPER



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

Main Advanced

Input volume path	required	RVIPER/main001/average_volume.hdf	Select HDF volume	Select any volume
Output directory	required	VolumeAdjustment		
Output pixel size [Å]	1.14	1.14		
Use molecular mass	NO	<input type="checkbox"/>		
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'	/TCDA1_TEST_DATASET/TEST06/ISAC	Select directory	
Output box size [Pixels]	352	352		
Invert handedness	NO	<input checked="" type="checkbox"/>		
Low-pass filter resolution [Å]	-1.0	-1.0		
MPI processors	1		Save parameters	
MPI command line template			Load parameters	
Submit job to queue	<input type="checkbox"/>			
Job name	sp_pipe_moon_eliminator		Generate command line	
Submission command	sbatch		Add to pipeline folder	
Submission script template	e/sphire_defq_ppn16_threads1.sh	Select template	Run command	

Downsampling factor from ISAC

What to do after running RVIPER



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

Main Advanced

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	<input type="checkbox"/>		
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'	/TCDA1_TEST_DATASET/TEST06/ISAC	Select directory	
Output box size [Pixels]	352	352		
Invert handedness	NO	<input checked="" type="checkbox"/>		
Low-pass filter resolution [A]	-1.0	-1.0		
MPI processors	1		Save parameters	
MPI command line template			Load parameters	
Submit job to queue	<input type="checkbox"/>			
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	

If known

What to do after running RVIPER



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

Main Advanced

Input volume path	required	RVIPER/main001/average_volume.hdf	Select HDF volume	Select any volume
Output directory	required	VolumeAdjustment		
Output pixel size [Å]	1.14	1.14		
Use molecular mass	NO	<input type="checkbox"/>		
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'	/TCDA1_TEST_DATASET/TEST06/ISAC	Select directory	
Output box size [Pixels]	352	352		
Invert handedness	NO	<input checked="" type="checkbox"/>		
Low-pass filter resolution [Å]	-1.0	-1.0		
MPI processors	1		Save parameters	
MPI command line template			Load parameters	
Submit job to queue	<input type="checkbox"/>			
Job name	sp_pipe_moon_eliminator		Generate command line	
Submission command	sbatch		Add to pipeline folder	
Submission script template	e/sphire_defq_ppn16_threads1.sh	Select template	Run command	

Can also be used to eliminate “moons”



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

sp_mask Mask creation tool for 2D or 3D masks.

Main Advanced

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	<input type="checkbox"/>	
Use molecular mass		NO	<input type="checkbox"/>	
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		<input type="checkbox"/>		
Job name		sp_mask		Generate command line
Submission command		sbatch		Add to pipeline folder
Submission script template		templates/sphire/sphire_defq_ppn16_threads1.sh	Select template	Run command

SPHIRE



Masking



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		
Overwrite outputs	NO	<input type="checkbox"/>		
Use molecular mass	NO	<input type="checkbox"/>		
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	<input type="checkbox"/>			
Job name	sp_mask			
Submission command	sbatch		Generate command line	
Submission script template	emplates/sphire/sphire_defq_ppn16_threads1.sh	Select template	Add to pipeline folder	
			Run command	

Moon-eliminated volume

Masking



Main Advanced

sp_mask Mask creation tool for 2D or 3D masks.

Input image	<input type="text" value="required"/>	<input type="text" value="VolumeAdjustment/vol3d_ref_moon_eliminated.hdf"/>	<input type="button" value="Select HDF volume"/>	<input type="button" value="Select any volume"/>
Output directory	<input type="text" value="required"/>	<input type="text" value="Mask"/>	<input type="button" value="Select directory"/>	
Output prefix	<input type="text" value="sp_mask"/>	<input type="text" value="sp_mask"/>		
Overwrite outputs	<input type="text" value="NO"/>	<input type="checkbox"/>		
Use molecular mass	<input type="text" value="NO"/>	<input type="checkbox"/>		
Molecular mass [kDa]	<input type="text" value="1400"/>	<input type="text" value="1400"/>		
Binarization threshold	<input type="text" value="none"/>	<input type="text" value="9"/>		
Density standard deviation threshold	<input type="text" value="none"/>	<input type="text" value="none"/>		
Number of dilations	<input type="text" value="3"/>	<input type="text" value="3"/>		
Soft-edge width [Pixels]	<input type="text" value="5"/>	<input type="text" value="5"/>		
MPI processors	<input type="text" value="1"/>		<input type="button" value="Save parameters"/>	
MPI command line template	<input type="text"/>		<input type="button" value="Load parameters"/>	
Submit job to queue	<input type="checkbox"/>			
Job name	<input type="text" value="sp_mask"/>			
Submission command	<input type="text" value="sbatch"/>		<input type="button" value="Generate command line"/>	
			<input type="button" value="Add to pipeline folder"/>	
Submission script template	<input type="text" value="emplates/sphire/sphire_defq_ppn16_threads1.sh"/>	<input type="button" value="Select template"/>	<input type="button" value="Run command"/>	

Threshold

Masking



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		
Overwrite outputs	NO	<input type="checkbox"/>		
Use molecular mass	NO	<input type="checkbox"/>		
Molecular mass [kDa]	1400	1400		
Binarization threshold	none	9		
Density standard deviation threshold	none	none		
Number of dilations		3		
Soft-edge width [Pixels]		5		
MPI processors		1	Save parameters	
MPI command line template			Load parameters	
Submit job to queue		<input type="checkbox"/>		
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

Each dilation extends by ~2 voxels

Masking



sp_mask Set advanced parameters

Main **Advanced**

Allow disconnected regions	NO	<input type="checkbox"/>
Fill mask	NO	<input checked="" type="checkbox"/>
Number of erosions	0	0
Soft-edge type	cosine	cosine
Old behaviour	NO	<input type="checkbox"/>
Low pass filter resolution [A]	none	none
Low pass filter falloff [1/Pixel]	0.01	0.01
Pixel size [A/px]	1.14	1.14
Use a second mask	NO	<input type="checkbox"/>
Second mask path	none	none
Second mask shape	none	none
Second - Radius of the mask	none	none
Second - X dimension of the mask	none	none
Second - Y dimension of the mask	none	none
Second - Z dimension of the mask	none	none
Second - Use molecular mass	NO	<input type="checkbox"/>
Second - Molecular mass [kDa]	none	none
Second - Binarization threshold	none	none
Second - Density standard deviation threshold	none	none
Second - Number of dilations	3	3
Second - Number of erosions	0	0
Second - Soft-edge width [Pixels]	5	5
Second - Soft-edge type	cosine	cosine
Second - Old behaviour	NO	<input type="checkbox"/>
Second - Allow disconnected regions	NO	<input type="checkbox"/>
Second - Fill mask	NO	<input type="checkbox"/>
Second - Invert masking	NO	<input type="checkbox"/>

Select HDF volume Select any volume

Fills in empty spaces

Masking



sp_mask Set advanced parameters

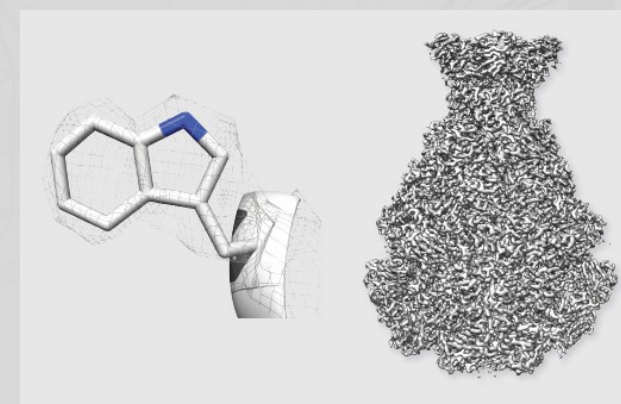
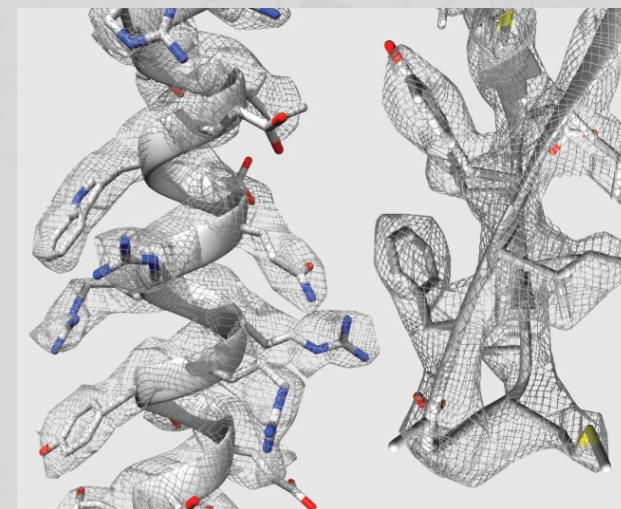
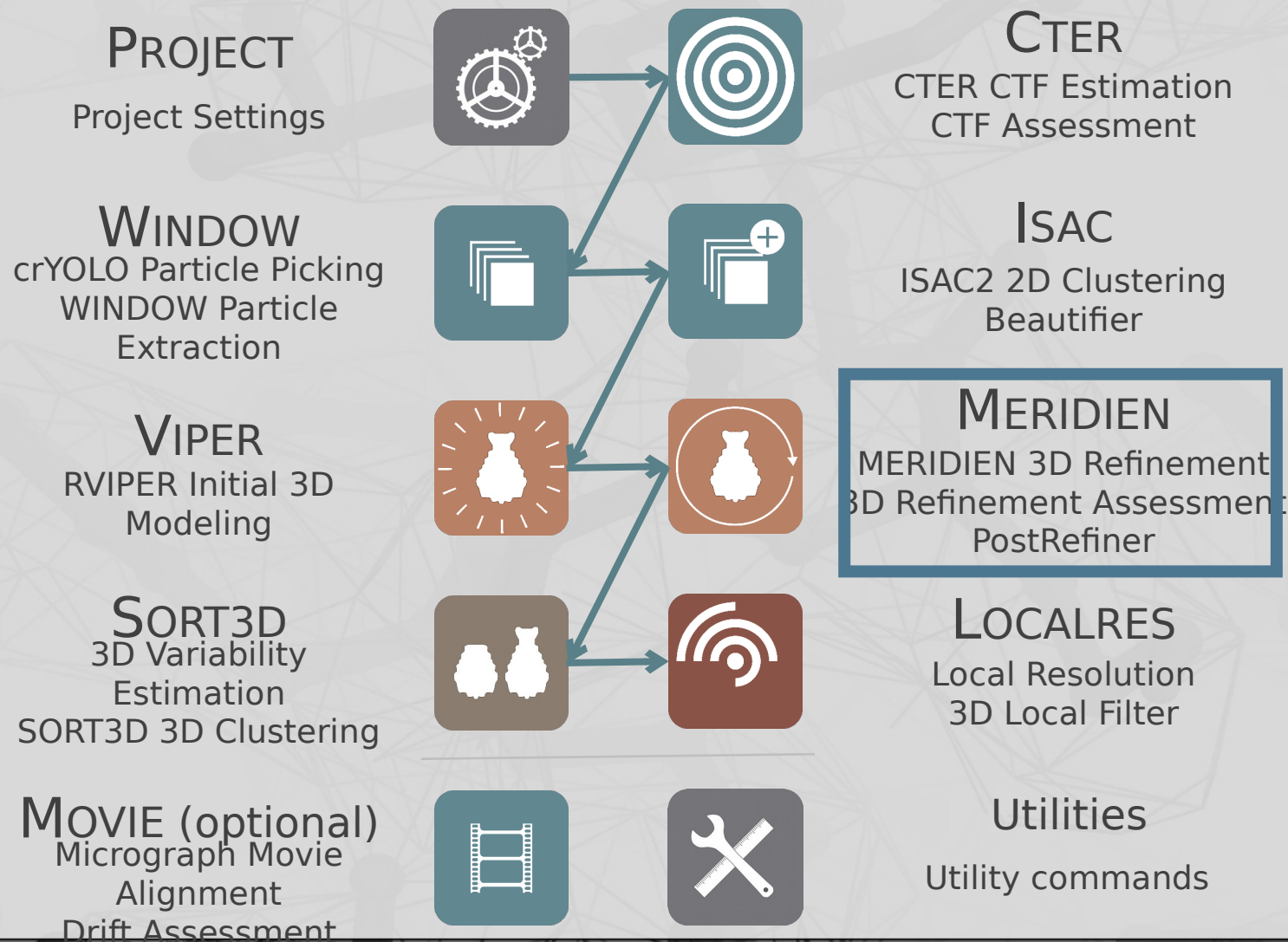
Main **Advanced**

Allow disconnected regions	NO	<input type="checkbox"/>
Fill mask	NO	<input checked="" type="checkbox"/>
Number of erosions	0	0
Soft-edge type	cosine	cosine
Old behaviour	NO	<input type="checkbox"/>
Low pass filter resolution [A]	none	none
Low pass filter falloff [1/Pixel]	0.01	0.01
Pixel size [A/px]	1.14	1.14
Use a second mask	NO	<input type="checkbox"/>
Second mask path	none	none
Second mask shape	none	none
Second - Radius of the mask	none	none
Second - X dimension of the mask	none	none
Second - Y dimension of the mask	none	none
Second - Z dimension of the mask	none	none
Second - Use molecular mass	NO	<input type="checkbox"/>
Second - Molecular mass [kDa]	none	none
Second - Binarization threshold	none	none
Second - Density standard deviation threshold	none	none
Second - Number of dilations	3	3
Second - Number of erosions	0	0
Second - Soft-edge width [Pixels]	5	5
Second - Soft-edge type	cosine	cosine
Second - Old behaviour	NO	<input type="checkbox"/>
Second - Allow disconnected regions	NO	<input type="checkbox"/>
Second - Fill mask	NO	<input type="checkbox"/>
Second - Invert masking	NO	<input type="checkbox"/>

Select HDF volume Select any volume

For helical processing (currently in beta)

SPHIRE Pipeline



Refinement using MERIDIEN



SPHIRE-GUI Main Version 1.2

Main Advanced

COMMANDS (shift-click for wiki)

- 3D Refinement
- 3D Refinement Assessment
- PostRefiner

ALTERNATIVES (shift-click for wiki)

- Import Projection Parameters
- Local Refinement from Stack
- Restart Local Refinement
- 3D Refinement Restart
- Final 3D Reconstruction Only

UTILITIES (shift-click for wiki)

- Display Data
- Volume Adjustment
- Masking
- Angular Distribution
- Balance Angular Distribution
- Batch Pipeline Execution
- CTF refine (Meridien)
- CTF refine (Stack)

sp_meridien (new)

Performs 3D structure refinement using a quasi-Maximum Likelihood approach.

Input image stack	<input type="text" value="required"/> EST06/Substack#isac_substack	Select BDB image stack
Output directory	<input type="text" value="none"/> Meridien	
Initial 3D reference	<input type="text" value="required"/> vol3d_ref_moon_eliminated.hdf	Select HDF volume <input type="button" value="Select any volume"/>
Read shifts from header	<input type="text" value="YES"/> YES	<input checked="" type="checkbox"/>
Skip the 2D pre-alignment step	<input type="text" value="NO"/> NO	<input type="checkbox"/>
Starting resolution [Å]	<input type="text" value="25.0"/> 25.0	<input type="text" value="25.0"/>
Initial angular sampling step [Degrees]	<input type="text" value="7.5"/> 7.5	<input type="text" value="7.5"/>
Particle radius [Pixels]	<input type="text" value="145"/> 145	<input type="text" value="145"/>
3D mask file	<input type="text" value="none"/> EST06/Mask/sxmask_mask.hdf	Select HDF volume <input type="button" value="Select any volume"/>
Point-group symmetry	<input type="text" value="c5"/> c5	
Memory per node [GB]	<input type="text" value="-1.0"/> -1.0	
MPI processors	<input type="text" value="1"/> 1	<input type="button" value="Save parameters"/>
MPI command line template	<input type="text"/>	<input type="button" value="Load parameters"/>
Submit job to queue	<input type="checkbox"/>	
Job name	<input type="text" value="sp_meridien_new"/>	<input type="button" value="Generate command line"/>
Submission command	<input type="text" value="sbatch"/>	<input type="button" value="Add to pipeline folder"/>
Submission script template	<input type="text" value="re_defq_ppn16_threads1.sh"/> <input type="button" value="Select template"/>	<input type="button" value="Run command"/>



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	<input checked="" type="checkbox"/>	
Skip the 2D pre-alignment step	NO	<input type="checkbox"/>	
Starting resolution [Å]	25.0	25.0	
Initial angular 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	<input type="checkbox"/>		
Job name	sp_meridien_new		
Submission command	sbatch		Generate command line
Submission script template	re_defq_ppn16_threads1.sh	Select template	Run command

Substack (after picking good ISAC classes)

Refinement using MERIDIEN



Main Advanced

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

Input image stack	<input type="text" value="required"/>	<input type="text" value="EST06/Substack#isac_substack"/>	Select BDB image stack
Output directory	<input type="text" value="none"/>	<input type="text" value="Meridien"/>	
Initial 3D reference	<input type="text" value="required"/>	<input type="text" value="vol3d_ref_moon_eliminated.hdf"/>	Select HDF volume Select any volume
Read shifts from header	<input type="text" value="YES"/>	<input checked="" type="checkbox"/>	
Skip the 2D pre-alignment step	<input type="text" value="NO"/>	<input type="checkbox"/>	
Starting resolution [Å]	<input type="text" value="25.0"/>	<input type="text" value="25.0"/>	
Initial angular sampling step [Degrees]	<input type="text" value="7.5"/>	<input type="text" value="7.5"/>	
Particle radius [Pixels]	<input type="text" value="145"/>	<input type="text" value="145"/>	
3D mask file	<input type="text" value="none"/>	<input type="text" value="EST06/Mask/sxmask_mask.hdf"/>	Select HDF volume Select any volume
Point-group symmetry	<input type="text" value="c5"/>	<input type="text" value="c5"/>	
Memory per node [GB]	<input type="text" value="-1.0"/>	<input type="text" value="-1.0"/>	
MPI processors	<input type="text" value="1"/>		<input type="button" value="Save parameters"/>
MPI command line template	<input type="text"/>		<input type="button" value="Load parameters"/>
Submit job to queue	<input type="checkbox"/>		
Job name	<input type="text" value="sp_meridien_new"/>		
Submission command	<input type="text" value="sbatch"/>		<input type="button" value="Generate command line"/>
Submission script template	<input type="text" value="re_defq_ppn16_threads1.sh"/>	<input type="button" value="Select template"/>	<input type="button" value="Run command"/>

Adjusted RVIPER volume

Refinement using MERIDIEN



Main Advanced

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

Input image stack	<input type="text" value="required"/>	<input type="text" value="EST06/Substack#isac_substack"/>	Select BDB image stack
Output directory	<input type="text" value="none"/>	<input type="text" value="Meridien"/>	
Initial 3D reference	<input type="text" value="required"/>	<input type="text" value="vol3d_ref_moon_eliminated.hdf"/>	Select HDF volume Select any volume
Read shifts from header	<input type="text" value="YES"/>	<input checked="" type="checkbox"/>	
Skip the 2D pre-alignment step	<input type="text" value="NO"/>	<input type="checkbox"/>	
Starting resolution [Å]	<input type="text" value="25.0"/>	<input type="text" value="25.0"/>	
Initial angular sampling step [Degrees]	<input type="text" value="7.5"/>	<input type="text" value="7.5"/>	
Particle radius [Pixels]	<input type="text" value="145"/>	<input type="text" value="145"/>	
3D mask file	<input type="text" value="none"/>	<input type="text" value="EST06/Mask/sxmask_mask.hdf"/>	Select HDF volume Select any volume
Point-group symmetry	<input type="text" value="c5"/>	<input type="text" value="c5"/>	
Memory per node [GB]	<input type="text" value="-1.0"/>	<input type="text" value="-1.0"/>	
MPI processors	<input type="text" value="1"/>		<input type="button" value="Save parameters"/>
MPI command line template	<input type="text"/>		<input type="button" value="Load parameters"/>
Submit job to queue	<input type="checkbox"/>		
Job name	<input type="text" value="sp_meridien_new"/>		
Submission command	<input type="text" value="sbatch"/>		<input type="button" value="Generate command line"/>
Submission script template	<input type="text" value="re_defq_ppn16_threads1.sh"/>	<input type="button" value="Select template"/>	<input type="button" value="Run command"/>

To minimize possible model bias

Refinement using MERIDIEN



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

Main Advanced

Input image stack	<input type="text" value="required"/>	<input type="text" value="EST06/Substack#isac_substack"/>	Select BDB image stack
Output directory	<input type="text" value="none"/>	<input type="text" value="Meridien"/>	
Initial 3D reference	<input type="text" value="required"/>	<input type="text" value="vol3d_ref_moon_eliminated.hdf"/>	Select HDF volume <input type="button" value="Select any volume"/>
Read shifts from header	<input type="text" value="YES"/>	<input checked="" type="checkbox"/>	
Skip the 2D pre-alignment step	<input type="text" value="NO"/>	<input type="checkbox"/>	
Starting resolution [Å]	<input type="text" value="25.0"/>	<input type="text" value="25.0"/>	
Initial angular sampling step [Degrees]	<input type="text" value="7.5"/>	<input type="text" value="7.5"/>	
Particle radius [Pixels]	<input type="text" value="145"/>	<input type="text" value="145"/>	
3D mask file	<input type="text" value="none"/>	<input type="text" value="EST06/Mask/sxmask_mask.hdf"/>	Select HDF volume <input type="button" value="Select any volume"/>
Point-group symmetry	<input type="text" value="c5"/>	<input type="text" value="c5"/>	
Memory per node [GB]	<input type="text" value="-1.0"/>	<input type="text" value="-1.0"/>	
MPI processors	<input type="text" value="1"/>		<input type="button" value="Save parameters"/>
MPI command line template	<input type="text"/>		<input type="button" value="Load parameters"/>
Submit job to queue	<input type="checkbox"/>		
Job name	<input type="text" value="sp_meridien_new"/>		
Submission command	<input type="text" value="sbatch"/>		<input type="button" value="Generate command line"/>
Submission script template	<input type="text" value="re_defq_ppn16_threads1.sh"/>	<input type="button" value="Select template"/>	<input type="button" value="Run command"/>

Angular sampling

Refinement using MERIDIEN



Main Advanced

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

Input image stack	<input type="text" value="required"/>	<input type="text" value="EST06/Substack#isac_substack"/>	Select BDB image stack
Output directory	<input type="text" value="none"/>	<input type="text" value="Meridien"/>	
Initial 3D reference	<input type="text" value="required"/>	<input type="text" value="vol3d_ref_moon_eliminated.hdf"/>	Select HDF volume Select any volume
Read shifts from header	<input type="text" value="YES"/>	<input checked="" type="checkbox"/>	
Skip the 2D pre-alignment step	<input type="text" value="NO"/>	<input type="checkbox"/>	
Starting resolution [Å]	<input type="text" value="25.0"/>	<input type="text" value="25.0"/>	
Initial angular sampling step [Degrees]	<input type="text" value="7.5"/>	<input type="text" value="7.5"/>	
Particle radius [Pixels]	<input type="text" value="145"/>	<input type="text" value="145"/>	
3D mask file	<input type="text" value="none"/>	<input type="text" value="EST06/Mask/sxmask_mask.hdf"/>	Select HDF volume Select any volume
Point-group symmetry	<input type="text" value="c5"/>	<input type="text" value="c5"/>	
Memory per node [GB]	<input type="text" value="-1.0"/>	<input type="text" value="-1.0"/>	
MPI processors	<input type="text" value="1"/>		<input type="button" value="Save parameters"/>
MPI command line template	<input type="text"/>		<input type="button" value="Load parameters"/>
Submit job to queue	<input type="checkbox"/>		
Job name	<input type="text" value="sp_meridien_new"/>		
Submission command	<input type="text" value="sbatch"/>		<input type="button" value="Generate command line"/>
Submission script template	<input type="text" value="re_defq_ppn16_threads1.sh"/>	<input type="button" value="Select template"/>	<input type="button" value="Run command"/>

Angular sampling

Refinement using MERIDIEN



Main Advanced

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

Input image stack	<input type="text" value="required"/>	<input type="text" value="EST06/Substack#isac_substack"/>	Select BDB image stack
Output directory	<input type="text" value="none"/>	<input type="text" value="Meridien"/>	
Initial 3D reference	<input type="text" value="required"/>	<input type="text" value="vol3d_ref_moon_eliminated.hdf"/>	Select HDF volume <input type="button" value="Select any volume"/>
Read shifts from header	<input type="text" value="YES"/>	<input checked="" type="checkbox"/>	
Skip the 2D pre-alignment step	<input type="text" value="NO"/>	<input type="checkbox"/>	
Starting resolution [Å]	<input type="text" value="25.0"/>	<input type="text" value="25.0"/>	
Initial angular sampling step [Degrees]	<input type="text" value="7.5"/>	<input type="text" value="7.5"/>	
Particle radius [Pixels]	<input type="text" value="145"/>	<input type="text" value="145"/>	
3D mask file	<input type="text" value="none"/>	<input type="text" value="EST06/Mask/sxmask_mask.hdf"/>	Select HDF volume <input type="button" value="Select any volume"/>
Point-group symmetry	<input type="text" value="c5"/>	<input type="text" value="c5"/>	
Memory per node [GB]	<input type="text" value="-1.0"/>	<input type="text" value="-1.0"/>	
MPI processors	<input type="text" value="1"/>		<input type="button" value="Save parameters"/>
MPI command line template	<input type="text"/>		<input type="button" value="Load parameters"/>
Submit job to queue	<input type="checkbox"/>		
Job name	<input type="text" value="sp_meridien_new"/>		
Submission command	<input type="text" value="sbatch"/>		<input type="button" value="Generate command line"/>
			<input type="button" value="Add to pipeline folder"/>
Submission script template	<input type="text" value="re_defq_ppn16_threads1.sh"/>	<input type="button" value="Select template"/>	<input type="button" value="Run command"/>

May need to adjust empirically

Advanced parameters in MERIDIEN



sp_meridien (new) Set advanced parameters

Main Advanced

Centering method	-1	-1
Target particle radius [Pixels]	29	29
Search range [Pixels]	5.0	5.0
Search step size [Pixels]	1.0	1.0
Theta min [degree]	-1	-1
Theta max [degree]	-1	-1
Even angle method	S	S
Angular neighborhood	-1.0	-1.0
Shake	0.5	0.5
Keep data in memory	YES	<input checked="" type="checkbox"/>
Correlation peaks to be included [%]	99.9	99.9
Apply image norm correction	YES	<input checked="" type="checkbox"/>
Group name for chunks	ptcl_source_image	ptcl_source_image
Limit of improvements	1	1
Convergence criterion A value	0.75	0.75
Reference preparation function	do_volume_mask	do_volume_mask
Python script for user function	none	none <input type="button" value="Select Python script"/>
Logic function	ai_spa	ai_spa
Python script for user function	none	none <input type="button" value="Select Python script"/>
Outlier group ID	none	none
Filament width [px]	-1.0	-1.0
Helical rise [A]	none	none
Plot angular distribution	NO	<input type="checkbox"/>
Main000 folder	none	none <input type="button" value="Select directory"/>

Like in ISAC, images are internally downsampled

Advanced parameters in MERIDIEN



sp_meridien (new) Set advanced parameters

Main Advanced

Centering method	-1	-1
Target particle radius [Pixels]	29	29
Search range [Pixels]	5.0	5.0
Search step size [Pixels]	1.0	1.0
Theta min [degree]	-1	-1
Theta max [degree]	-1	-1
Even angle method	S	S
Angular neighborhood	-1.0	-1.0
Shake	0.5	0.5
Keep data in memory	YES	<input checked="" type="checkbox"/>
Correlation peaks to be included [%]	99.9	99.9
Apply image norm correction	YES	<input checked="" type="checkbox"/>
Group name for chunks	ptcl_source_image	ptcl_source_image
Limit of improvements	1	1
Convergence criterion A value	0.75	0.75
Reference preparation function	do_volume_mask	do_volume_mask
Python script for user function	none	none <input type="button" value="Select Python script"/>
Logic function	ai_spa	ai_spa
Python script for user function	none	none <input type="button" value="Select Python script"/>
Outlier group ID	none	none
Filament width [px]	-1.0	-1.0
Helical rise [A]	none	none
Plot angular distribution	NO	<input type="checkbox"/>
Main000 folder	none	none <input type="button" value="Select directory"/>

Tradeoff between speed and #iterations

Advanced parameters in MERIDIEN



Main **Advanced**

sp_meridien (new) Set advanced parameters

Centering method	<input type="text" value="-1"/>	<input type="text" value="-1"/>
Target particle radius [Pixels]	<input type="text" value="29"/>	<input type="text" value="29"/>
Search range [Pixels]	<input type="text" value="5.0"/>	<input type="text" value="5.0"/>
Search step size [Pixels]	<input type="text" value="1.0"/>	<input type="text" value="1.0"/>
Theta min [degree]	<input type="text" value="-1"/>	<input type="text" value="-1"/>
Theta max [degree]	<input type="text" value="-1"/>	<input type="text" value="-1"/>
Even angle method	<input type="text" value="S"/>	<input type="text" value="S"/>
Angular neighborhood	<input type="text" value="-1.0"/>	<input type="text" value="-1.0"/>
Shake	<input type="text" value="0.5"/>	<input type="text" value="0.5"/>
Keep data in memory	<input type="text" value="YES"/>	<input checked="" type="checkbox"/>
Correlation peaks to be included [%]	<input type="text" value="99.9"/>	<input type="text" value="99.9"/>
Apply image norm correction	<input type="text" value="YES"/>	<input checked="" type="checkbox"/>
Group name for chunks	<input type="text" value="ptcl_source_image"/>	<input type="text" value="ptcl_source_image"/>
Limit of improvements	<input type="text" value="1"/>	<input type="text" value="1"/>
Convergence criterion A value	<input type="text" value="0.75"/>	<input type="text" value="0.75"/>
Reference preparation function	<input type="text" value="do_volume_mask"/>	<input type="text" value="do_volume_mask"/>
Python script for user function	<input type="text" value="none"/>	<input type="text" value="none"/> <input type="button" value="Select Python script"/>
Logic function	<input type="text" value="ai_spa"/>	<input type="text" value="ai_spa"/>
Python script for user function	<input type="text" value="none"/>	<input type="text" value="none"/> <input type="button" value="Select Python script"/>
Outlier group ID	<input type="text" value="none"/>	<input type="text" value="none"/>
Filament width [px]	<input type="text" value="-1.0"/>	<input type="text" value="-1.0"/>
Helical rise [A]	<input type="text" value="none"/>	<input type="text" value="none"/>
Plot angular distribution	<input type="text" value="NO"/>	<input type="checkbox"/>
Main000 folder	<input type="text" value="none"/>	<input type="text" value="none"/> <input type="button" value="Select directory"/>

For helical reconstruction (beta)

Advanced parameters in MERIDIEN



sp_meridien (new) Set advanced parameters

Main Advanced

Centering method	-1	-1
Target particle radius [Pixels]	29	29
Search range [Pixels]	5.0	5.0
Search step size [Pixels]	1.0	1.0
Theta min [degree]	-1	-1
Theta max [degree]	-1	-1
Even angle method	S	S
Angular neighborhood	-1.0	-1.0
Shake	0.5	0.5
Keep data in memory	YES	<input checked="" type="checkbox"/>
Correlation peaks to be included [%]	99.9	99.9
Apply image norm correction	YES	<input checked="" type="checkbox"/>
Group name for chunks	ptcl_source_image	ptcl_source_image
Limit of improvements	1	1
Convergence criterion A value	0.75	0.75
Reference preparation function	do_volume_mask	do_volume_mask
Python script for user function	none	none <input type="button" value="Select Python script"/>
Logic function	ai_spa	ai_spa
Python script for user function	none	none <input type="button" value="Select Python script"/>
Outlier group ID	none	none
Filament width [px]	-1.0	-1.0
Helical rise [A]	none	none
Plot angular distribution	NO	<input type="checkbox"/>
Main000 folder	none	none <input type="button" value="Select directory"/>

Angular search restricted near current orientation

Advanced parameters in MERIDIEN



sp_meridien (new) Set advanced parameters

Centering method	-1	-1
Target particle radius [Pixels]	29	29
Search range [Pixels]	5.0	5.0
Search step size [Pixels]	1.0	1.0
Theta min [degree]	-1	-1
Theta max [degree]	-1	-1
Even angle method	S	S
Angular neighborhood	-1.0	-1.0
Shake	0.5	0.5
Keep data in memory	YES	<input checked="" type="checkbox"/>
Correlation peaks to be included [%]	99.9	99.9
Apply image norm correction	YES	<input checked="" type="checkbox"/>
Group name for chunks	ptcl_source_image	ptcl_source_image
Limit of improvements	1	1
Convergence criterion A value	0.75	0.75
Reference preparation function	do_volume_mask	do_volume_mask
Python script for user function	none	none <input type="button" value="Select Python script"/>
Logic function	ai_spa	ai_spa
Python script for user function	none	none <input type="button" value="Select Python script"/>
Outlier group ID	none	none
Filament width [px]	-1.0	-1.0
Helical rise [A]	none	none
Plot angular distribution	NO	<input type="checkbox"/>
Main000 folder	none	none <input type="button" value="Select directory"/>

Maximum likelihood “smear” value
0.0 → projection-matching

Advanced parameters in MERIDIEN



sp_meridien (new) Set advanced parameters

Main Advanced

Centering method	-1	-1	
Target particle radius [Pixels]	29	29	
Search range [Pixels]	5.0	5.0	
Search step size [Pixels]	1.0	1.0	
Theta min [degree]	-1	-1	
Theta max [degree]	-1	-1	
Even angle method	S	S	
Angular neighborhood	-1.0	-1.0	
Shake	0.5	0.5	
Keep data in memory	YES	<input checked="" type="checkbox"/>	
Correlation peaks to be included [%]	99.9	99.9	
Apply image norm correction	YES	<input checked="" type="checkbox"/>	
Group name for chunks	ptcl_source_image	ptcl_source_image	
Limit of improvements	1	1	
Convergence criterion A value	0.75	0.75	
Reference preparation function	do_volume_mask	do_volume_mask	
Python script for user function	none	none	Select Python script
Logic function	ai_spa	ai_spa	
Python script for user function	none	none	Select Python script
Outlier group ID	none	none	
Filament width [px]	-1.0	-1.0	
Helical rise [A]	none	none	
Plot angular distribution	NO	<input type="checkbox"/>	
Main000 folder	none	none	Select directory

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

Refinement assessment



SPHIRE-GUI Main Version 1.2

Meridien

File Plot

/mnt/beegfs/home/sphire-devel/TCDA1_TEST_DATASET/TEST06/Meridien

Resolution curves

The screenshot displays the SPHIRE-GUI interface. On the left is a sidebar with icons for various tools. The main area is divided into three sections: 'COMMANDS', 'ALTERNATIVES', and 'UTILITIES'. The 'COMMANDS' section is active, showing '3D Refinement Assessment' selected. The 'ALTERNATIVES' section includes options like 'Import Projection Parameters' and 'Local Refinement from Stack'. The 'UTILITIES' section includes 'Display Data', 'Volume Adjustment', and 'Masking'. The 'sp_gui_meridien' configuration panel is open, showing fields for 'MPI processors' (1), 'MPI command line template', 'Submit job to queue' (checkbox), 'job name' (N/A), 'Submission command' (N/A), and 'Submission script template' (N/A). Buttons for 'Save parameters', 'Load parameters', 'Generate command line', 'Add to pipeline folder', and 'Run command' are visible. The 'Resolution curves' plot shows Resolution [Å] on the y-axis (5 to 25) and Runs on the x-axis (0 to 30). Two series are plotted: 'Resolution FSC 0.143' (blue line with 'x' markers) and 'Resolution FSC 0.5' (orange line with 'x' markers). The blue series starts at 25 Å and drops to approximately 4 Å by run 15. The orange series starts at 25 Å and drops to approximately 8 Å by run 10.

Runs	Resolution FSC 0.143 [Å]	Resolution FSC 0.5 [Å]
1	25	25
2	15	12
3	9	11
4	8.5	11
5	8.5	11
6	8.5	11
7	8.5	11
8	7	8.5
9	7	8.5
10	7	8.5
11	7	8.5
12	7	8.5
13	7	8.5
14	7	8.5
15	4.5	8.5
16	4.5	8.5
17	4.5	8.5
18	4.5	8.5
19	4.5	8.5
20	4.5	8.5
21	4.5	8.5
22	4.5	8.5
23	4.5	8.5
24	4.5	8.5
25	4.5	8.5
26	4.5	8.5
27	4.5	8.5
28	4.5	8.5
29	4.5	8.5
30	4.5	8.5



X SPHIRE-GUI Main Version 1.2

COMMANDS (shift-click for wiki)

- 3D Refinement
- 3D Refinement Assessment
- PostRefiner
- Import Projection Parameters
- Local Refinement from Stack
- Restart Local Refinement
- 3D Refinement Restart
- Final 3D Reconstruction Only

ALTERNATIVES (shift-click for wiki)

- Display Data
- Volume Adjustment
- Masking
- Angular Distribution
- Balance Angular Distribution
- Batch Pipeline Execution
- CTF refine (Meridien)
- CTF refine (Stack)

UTILITIES (shift-click for wiki)

- 3D Refinement
- 3D Refinement Assessment
- PostRefiner
- Import Projection Parameters
- Local Refinement from Stack
- Restart Local Refinement
- 3D Refinement Restart
- Final 3D Reconstruction Only
- Display Data
- Volume Adjustment
- Masking
- Angular Distribution
- Balance Angular Distribution
- Batch Pipeline Execution
- CTF refine (Meridien)
- CTF refine (Stack)

sp_process --combinemaps (halfset maps)

Post-refine halfset volumes

First unfiltered halfset map

Second unfiltered halfset map

Output directory

Pixel size [Å]

3D mask file

Apply adaptive mask

Binarization threshold

Soft-edge width [Pixels]

Dilation width [Pixels]

MTF file

B-factor enhancement

Low-pass filter frequency [Å]

MPI processors

MPI command line template

Submit job to queue

Job name

Submission command

Submission script template

Main Advanced

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.

<input type="checkbox"/>	locked	<input checked="" type="checkbox"/>			
required	Meridien/vol_0_unfil_027.hdf	<input type="button" value="Select HDF volume"/>	<input type="button" value="Select any volume"/>		
required	Meridien/vol_1_unfil_027.hdf	<input type="button" value="Select HDF volume"/>	<input type="button" value="Select any volume"/>		
required	Sharpening				
1.14	1.14				
none	none	<input type="button" value="Select HDF volume"/>	<input type="button" value="Select any volume"/>		
NO	<input checked="" type="checkbox"/>				
-9999.0	0.02				
5	5				
3	3				
none	none	<input type="button" value="Select MTF data"/>			
0.0	0.0				
0.0	0.0				
1	<input type="button" value="Save parameters"/>				
<input type="text" value=""/>		<input type="button" value="Load parameters"/>			
<input type="checkbox"/>					
<input type="text" value="binemaps_halfset_maps"/>		<input type="button" value="Generate command line"/>			
<input type="text" value="sbatch"/>		<input type="button" value="Add to pipeline folder"/>			
<input type="text" value="defq_ppn16_threads1.sh"/>		<input type="button" value="Select template"/>	<input type="button" value="Run command"/>		

86

PostRefiner



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.

Main **Advanced**

Post-refine halfset volumes locked

First unfiltered halfset map **required** Meridien/vol_0_unfil_027.hdf

Second unfiltered halfset map **required** Meridien/vol_1_unfil_027.hdf

Output directory **required** Sharpening

Pixel size [A] 1.14 1.14

3D mask file none none

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

B-factor enhancement 0.0 0.0

Low-pass filter frequency [A] 0.0 0.0

MPI processors 1

MPI command line template

Submit job to queue

Job name binemaps_halfset_maps

Submission command sbatch

Submission script template defq_ppn16_threads1.sh

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



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	<input type="checkbox"/> locked	<input checked="" type="checkbox"/>
First unfiltered halfset map	<input type="text" value="required"/> Meridien/vol_0_unfil_027.hdf	<input type="button" value="Select HDF volume"/> <input type="button" value="Select any volume"/>
Second unfiltered halfset map	<input type="text" value="required"/> Meridien/vol_1_unfil_027.hdf	<input type="button" value="Select HDF volume"/> <input type="button" value="Select any volume"/>
Output directory	<input type="text" value="required"/> Sharpening	
Pixel size [Å]	<input type="text" value="1.14"/> 1.14	
3D mask file	<input type="text" value="none"/> none	<input type="button" value="Select HDF volume"/> <input type="button" value="Select any volume"/>
Apply adaptive mask	<input type="text" value="NO"/> NO	<input checked="" type="checkbox"/>
Binarization threshold	<input type="text" value="-9999.0"/> -9999.0	<input type="text" value="0.02"/> 0.02
Soft-edge width [Pixels]	<input type="text" value="5"/> 5	
Dilation width [Pixels]	<input type="text" value="3"/> 3	
MTF file	<input type="text" value="none"/> none	<input type="button" value="Select MTF data"/>
B-factor enhancement	<input type="text" value="0.0"/> 0.0	
Low-pass filter frequency [Å]	<input type="text" value="0.0"/> 0.0	
MPI processors	<input type="text" value="1"/> 1	<input type="button" value="Save parameters"/>
MPI command line template	<input type="text"/>	<input type="button" value="Load parameters"/>
Submit job to queue	<input type="checkbox"/>	
Job name	<input type="text" value="binemaps_halfset_maps"/>	
Submission command	<input type="text" value="sbatch"/>	<input type="button" value="Generate command line"/>
		<input type="button" value="Add to pipeline folder"/>
Submission script template	<input type="text" value="defq_ppn16_threads1.sh"/> defq_ppn16_threads1.sh	<input type="button" value="Select template"/> <input type="button" value="Run command"/>

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	<input type="checkbox"/> locked	<input checked="" type="checkbox"/>
First unfiltered halfset map	<input type="text" value="required"/> Meridien/vol_0_unfil_027.hdf	<input type="button" value="Select HDF volume"/> <input type="button" value="Select any volume"/>
Second unfiltered halfset map	<input type="text" value="required"/> Meridien/vol_1_unfil_027.hdf	<input type="button" value="Select HDF volume"/> <input type="button" value="Select any volume"/>
Output directory	<input type="text" value="required"/> Sharpening	
Pixel size [Å]	<input type="text" value="1.14"/> 1.14	
3D mask file	<input type="text" value="none"/> none	<input type="button" value="Select HDF volume"/> <input type="button" value="Select any volume"/>
Apply adaptive mask	<input type="checkbox"/> NO	<input checked="" type="checkbox"/>
Binarization threshold	<input type="text" value="-9999.0"/> -9999.0	<input type="text" value="0.02"/> 0.02
Soft-edge width [Pixels]	<input type="text" value="5"/> 5	
Dilation width [Pixels]	<input type="text" value="3"/> 3	
MTF file	<input type="text" value="none"/> none	<input type="button" value="Select MTF data"/>
B-factor enhancement	<input type="text" value="0.0"/> 0.0	
Low-pass filter frequency [Å]	<input type="text" value="0.0"/> 0.0	
MPI processors	<input type="text" value="1"/> 1	<input type="button" value="Save parameters"/>
MPI command line template	<input type="text"/>	<input type="button" value="Load parameters"/>
Submit job to queue	<input type="checkbox"/>	
Job name	<input type="text" value="binemaps_halfset_maps"/>	
Submission command	<input type="text" value="sbatch"/>	<input type="button" value="Generate command line"/>
		<input type="button" value="Add to pipeline folder"/>
Submission script template	<input type="text" value="defq_ppn16_threads1.sh"/> <input type="button" value="Select template"/>	<input type="button" value="Run command"/>

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



Angular Distribution



SPHIRE-GUI Main Version 1.2

COMMANDS (shift-click for wiki)

- 3D Refinement
- 3D Refinement Assessment
- PostRefiner

ALTERNATIVES (shift-click for wiki)

- Import Projection Parameters
- Local Refinement from Stack
- Restart Local Refinement
- 3D Refinement Restart
- Final 3D Reconstruction Only

UTILITIES (shift-click for wiki)

- Display Data
- Volume Adjustment
- Masking
- Angular Distribution**
- Balance Angular Distribution
- Batch Pipeline Execution
- CTF refine (Meridien)
- CTF refine (Stack)

Main Advanced

sp_pipe angular_distribution Generate a chimera .build file for the visual representation of the resulting projection parameters.

Projection parameters Meridien/final_params_027.txt

Output directory SET/TEST06/Meridien/ang_dist

File prefix ang_dist

Point-group symmetry c5

MPI processors

MPI command line template

Submit job to queue

Job name

Submission command

Submission script template



Angular Distribution



sp_pipe angular_distribution Generate a chimera .bild file for the visual representation of the resulting projection parameters.

Main Advanced

Projection parameters	required	Meridien/final_params_027.txt	Select parameters text
Output directory	required	SET/TEST06/Meridien/ang_dist	
File prefix	none	ang_dist	
Point-group symmetry	c5	c5	
MPI processors		1	Save parameters
MPI command line template			Load parameters
Submit job to queue		<input type="checkbox"/>	
Job name		_pipe_angular_distribution	
Submission command		sbatch	Generate command line
Submission script template		e_defq_ppn16_threads1.sh	Add to pipeline folder
		Select template	Run command

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`

SPHIRE-GUI Main Version 1.2

COMMANDS (shift-click for wiki)

- 3D Refinement
- 3D Refinement Assessment
- PostRefiner

ALTERNATIVES (shift-click for wiki)

- Import Projection Parameters
- Local Refinement from Stack
- Restart Local Refinement
- 3D Refinement Restart
- Final 3D Reconstruction Only

UTILITIES (shift-click for wiki)

- Display Data
- Volume Adjustment
- Masking
- Angular Distribution
- Balance Angular Distribution
- Batch Pipeline Execution
- CTF refine (Meridien)**
- CTF refine (Stack)

sp_ctf_refine meridien Refine the defocus per particle

Input stack path: Select BDB image stack Select any image stack

Output directory: required

Meridien directory: required Select directory

Path to mask: none Select HDF volume Select any volume

MPI processors:

MPI command line template:

Submit job to queue:

Job name:

Submission command:

Submission script template: Select template

Save parameters

Load parameters

Generate command line

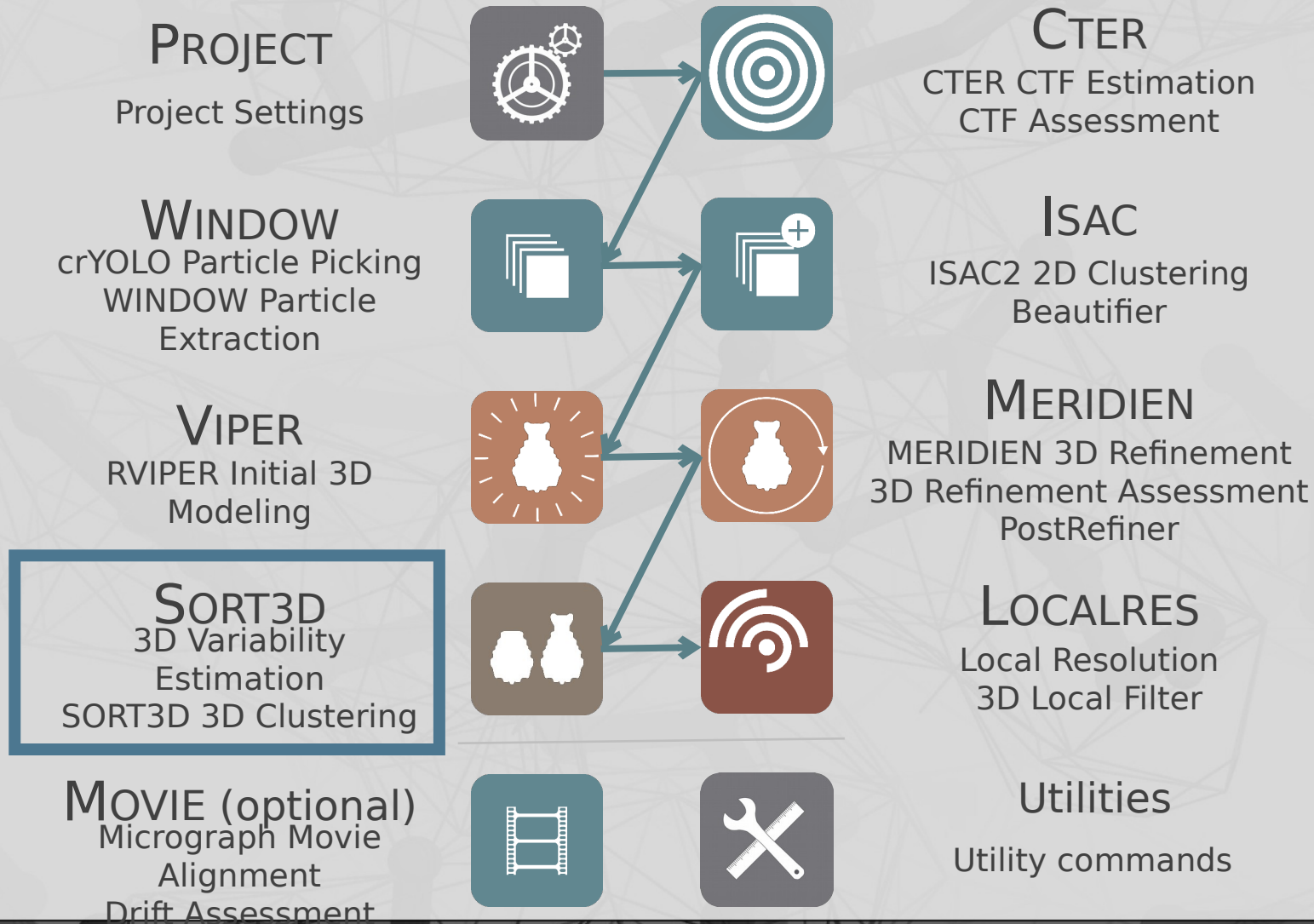
Add to pipeline folder

Run command

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



SPHIRE Pipeline



3D Variability Preprocess



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

3D Variability Preprocess

Prepare input stack for handling symmetry. Please skip this preparation step if the structure is asymmetrical (i.e. c1), since it is required only when the structure has internal symmetry. Notice this step can be run with only one CPU and there is no MPI version for it.

Main Advanced

Symmetrise input stack: locked

Input image stack: required Substack#isac_substack Select BDB image stack

Output directory: required Var_prep

Point-group symmetry: c5 c5

MPI processors: 1

MPI command line template:

Submit job to queue:

Job name: riability_symmetrize

Submission command: sbatch

Submission script template: _ppn16_threads1.sh Select template

Save parameters

Load parameters

Generate command line

Add to pipeline folder

Run command

3D Variability Preprocess



Main Advanced

sp_3dvariability --symmetrize Prepare input stack for handling symmetry. Please skip this preparation step if the structure is asymmetrical (i.e. c1), since it is required only when the structure has internal symmetry. Notice this step can be run with only one CPU and there is no MPI version for it.

Symmetrise input stack	<input type="checkbox"/> locked	<input checked="" type="checkbox"/>	
Input image stack	<input type="text" value="required"/>	<input type="text" value="Substack#isac_substack"/>	Select BDB image stack
Output directory	<input type="text" value="required"/>	<input type="text" value="Var_prep"/>	
Point-group symmetry	<input type="text" value="c5"/>	<input type="text" value="c5"/>	
MPI processors	<input type="text" value="1"/>		<input type="button" value="Save parameters"/>
MPI command line template	<input type="text"/>		<input type="button" value="Load parameters"/>
Submit job to queue	<input type="checkbox"/>		
Job name	<input type="text" value="variability_symmetrize"/>		
Submission command	<input type="text" value="sbatch"/>		<input type="button" value="Generate command line"/>
Submission script template	<input type="text" value="ppn16_threads1.sh"/>	<input type="button" value="Select template"/>	<input type="button" value="Run command"/>

Images from good ISAC classes

3D Variability Preprocess



Main Advanced

sp_3dvariability --symmetrize Prepare input stack for handling symmetry. Please skip this preparation step if the structure is asymmetrical (i.e. c1), since it is required only when the structure has internal symmetry. Notice this step can be run with only one CPU and there is no MPI version for it.

Symmetrise input stack	<input type="checkbox"/> locked	<input checked="" type="checkbox"/>
Input image stack	<input type="text" value="required"/> Substack#isac_substack	Select BDB image stack
Output directory	<input type="text" value="required"/> Var_prep	
Point-group symmetry	<input type="text" value="c5"/> c5	
MPI processors	<input type="text" value="1"/>	<input type="button" value="Save parameters"/>
MPI command line template	<input type="text"/>	<input type="button" value="Load parameters"/>
Submit job to queue	<input type="checkbox"/>	
Job name	<input type="text" value="variability_symmetrize"/>	
Submission command	<input type="text" value="sbatch"/>	<input type="button" value="Generate command line"/>
Submission script template	<input type="text" value="ppn16_threads1.sh"/> Select template	<input type="button" value="Run command"/>

Data set will be multiplied by the symmetry

3D Variability Estimation



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

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

Main Advanced

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	<input checked="" type="checkbox"/>	
Python overhead memory		0.5	5
MPI processors		1	
MPI command line template			
Submit job to queue		<input type="checkbox"/>	
Job name		sp_3dvariability	
Submission command		sbatch	
Submission script template		_ppn16_threads1.sh	Select template

Save parameters

Load parameters

Generate command line

Add to pipeline folder

Run command

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	<input checked="" type="checkbox"/>	
Python overhead memory	0.5	5	
MPI processors	1		Save parameters
MPI command line template			Load parameters
Submit job to queue	<input type="checkbox"/>		
Job name	sp_3dvariability		
Submission command	sbatch		Generate command line
			Add to pipeline folder
Submission script template	_ppn16_threads1.sh	Select template	Run command

Symmetry-multiplied stack

3D Variability Estimation



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

Main Advanced

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	<input checked="" type="checkbox"/>	
Python overhead memory	0.5	5	
MPI processors	1		Save parameters
MPI command line template			Load parameters
Submit job to queue	<input type="checkbox"/>		
Job name	sp_3dvariability		Generate command line
Submission command	sbatch		Add to pipeline folder
Submission script template	_ppn16_threads1.sh	Select template	Run command

Memory per CPU (GB, test empirically)



SPHIRE-GUI Main Version 1.2

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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	<input type="button" value="Select directory"/>
Output directory	none	Sort3D	<input type="button" value="Select directory"/>
Iteration ID of 3D refinement for importing data	-1	-1	
3D mask	none	/Mask/sxmask_mask.hdf	<input type="button" value="Select HDF volume"/> <input type="button" value="Select any volume"/>
Focus 3D mask	none	none	<input type="button" value="Select HDF volume"/> <input type="button" value="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	<input type="checkbox"/>	
Swap percentage [%]	1.0	1.0	
Memory per node [GB]	-1.0	120	
MPI processors	<input type="text" value="1"/>		<input type="button" value="Save parameters"/>
MPI command line template	<input type="text"/>		<input type="button" value="Load parameters"/>
Submit job to queue	<input type="checkbox"/>		
Job name	<input type="text" value="depth_refinement_dir"/>		<input type="button" value="Generate command line"/>
Submission command	<input type="text" value="sbatch"/>		<input type="button" value="Add to pipeline folder"/>
Submission script template	<input type="text" value="t_ppn16_threads1.sh"/> <input type="button" value="Select template"/>		<input style="background-color: #90EE90;" type="button" value="Run command"/>

100

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	<input type="checkbox"/>	
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	<input type="checkbox"/>		
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

Sort3D



Main Advanced

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

Meriden refinement directory	<input type="text" value="required"/>	<input type="text" value="ATASET/TEST06/Meriden"/>	<input type="button" value="Select directory"/>
Output directory	<input type="text" value="none"/>	<input type="text" value="Sort3D"/>	<input type="button" value="Select directory"/>
Iteration ID of 3D refinement for importing data	<input type="text" value="-1"/>	<input type="text" value="-1"/>	
3D mask	<input type="text" value="none"/>	<input type="text" value="/Mask/sxmask_mask.hdf"/>	<input type="button" value="Select HDF volume"/> <input type="button" value="Select any volume"/>
Focus 3D mask	<input type="text" value="none"/>	<input type="text" value="none"/>	<input type="button" value="Select HDF volume"/> <input type="button" value="Select any volume"/>
Estimated particle radius [Pixels]	<input type="text" value="145"/>	<input type="text" value="145"/>	
Point-group symmetry	<input type="text" value="c5"/>	<input type="text" value="c5"/>	
Number of images per group	<input type="text" value="1000"/>	<input type="text" value="4500"/>	
Group splitting rate	<input type="text" value="1"/>	<input type="text" value="1"/>	
Minimum size of reproducible class	<input type="text" value="-1"/>	<input type="text" value="-1"/>	
Swap flag	<input type="text" value="NO"/>	<input type="checkbox"/>	
Swap percentage [%]	<input type="text" value="1.0"/>	<input type="text" value="1.0"/>	
Memory per node [GB]	<input type="text" value="-1.0"/>	<input type="text" value="120"/>	
MPI processors	<input type="text" value="1"/>		<input type="button" value="Save parameters"/>
MPI command line template	<input type="text"/>		<input type="button" value="Load parameters"/>
Submit job to queue	<input type="checkbox"/>		
Job name	<input type="text" value="depth_refinement_dir"/>		
Submission command	<input type="text" value="sbatch"/>		<input type="button" value="Generate command line"/>
			<input type="button" value="Add to pipeline folder"/>
Submission script template	<input type="text" value="q_ppn16_threads1.sh"/>	<input type="button" value="Select template"/>	<input type="button" value="Run command"/>

Mask file



Sort3D



Main Advanced

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

Meriden refinement directory	<input type="text" value="required"/>	<input type="text" value="ATASET/TEST06/Meriden"/>	<input type="button" value="Select directory"/>
Output directory	<input type="text" value="none"/>	<input type="text" value="Sort3D"/>	<input type="button" value="Select directory"/>
Iteration ID of 3D refinement for importing data	<input type="text" value="-1"/>	<input type="text" value="-1"/>	
3D mask	<input type="text" value="none"/>	<input type="text" value="/Mask/sxmask_mask.hdf"/>	<input type="button" value="Select HDF volume"/> <input type="button" value="Select any volume"/>
Focus 3D mask	<input type="text" value="none"/>	<input type="text" value="none"/>	<input type="button" value="Select HDF volume"/> <input type="button" value="Select any volume"/>
Estimated particle radius [Pixels]	<input type="text" value="145"/>	<input type="text" value="145"/>	
Point-group symmetry	<input type="text" value="c5"/>	<input type="text" value="c5"/>	
Number of images per group	<input type="text" value="1000"/>	<input type="text" value="4500"/>	
Group splitting rate	<input type="text" value="1"/>	<input type="text" value="1"/>	
Minimum size of reproducible class	<input type="text" value="-1"/>	<input type="text" value="-1"/>	
Swap flag	<input type="text" value="NO"/>	<input type="checkbox"/>	
Swap percentage [%]	<input type="text" value="1.0"/>	<input type="text" value="1.0"/>	
Memory per node [GB]	<input type="text" value="-1.0"/>	<input type="text" value="120"/>	
MPI processors	<input type="text" value="1"/>		<input type="button" value="Save parameters"/>
MPI command line template	<input type="text"/>		<input type="button" value="Load parameters"/>
Submit job to queue	<input type="checkbox"/>		
Job name	<input type="text" value="depth_refinement_dir"/>		
Submission command	<input type="text" value="sbatch"/>		<input type="button" value="Generate command line"/>
			<input type="button" value="Add to pipeline folder"/>
Submission script template	<input type="text" value="q_ppn16_threads1.sh"/>	<input type="button" value="Select template"/>	<input type="button" value="Run command"/>

Optional focused mask



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	<input type="text" value="required"/>	<input type="text" value="ATASET/TEST06/Meridien"/>	<input type="button" value="Select directory"/>
Output directory	<input type="text" value="none"/>	<input type="text" value="Sort3D"/>	<input type="button" value="Select directory"/>
Iteration ID of 3D refinement for importing data	<input type="text" value="-1"/>	<input type="text" value="-1"/>	
3D mask	<input type="text" value="none"/>	<input type="text" value="/Mask/sxmask_mask.hdf"/>	<input type="button" value="Select HDF volume"/> <input type="button" value="Select any volume"/>
Focus 3D mask	<input type="text" value="none"/>	<input type="text" value="none"/>	<input type="button" value="Select HDF volume"/> <input type="button" value="Select any volume"/>
Estimated particle radius [Pixels]	<input type="text" value="145"/>	<input type="text" value="145"/>	
Point-group symmetry	<input type="text" value="c5"/>	<input type="text" value="c5"/>	
Number of images per group	<input type="text" value="1000"/>	<input type="text" value="4500"/>	
Group splitting rate	<input type="text" value="1"/>	<input type="text" value="1"/>	
Minimum size of reproducible class	<input type="text" value="-1"/>	<input type="text" value="-1"/>	
Swap flag	<input type="text" value="NO"/>	<input type="checkbox"/>	
Swap percentage [%]	<input type="text" value="1.0"/>	<input type="text" value="1.0"/>	
Memory per node [GB]	<input type="text" value="-1.0"/>	<input type="text" value="120"/>	
MPI processors	<input type="text" value="1"/>		<input type="button" value="Save parameters"/>
MPI command line template	<input type="text"/>		<input type="button" value="Load parameters"/>
Submit job to queue	<input type="checkbox"/>		
Job name	<input type="text" value="depth_refinement_dir"/>		
Submission command	<input type="text" value="sbatch"/>		<input type="button" value="Generate command line"/>
			<input type="button" value="Add to pipeline folder"/>
Submission script template	<input type="text" value="q_ppn16_threads1.sh"/>	<input type="button" value="Select template"/>	<input type="button" value="Run command"/>

Number of images/class (approximate)



Sort3D



Main Advanced

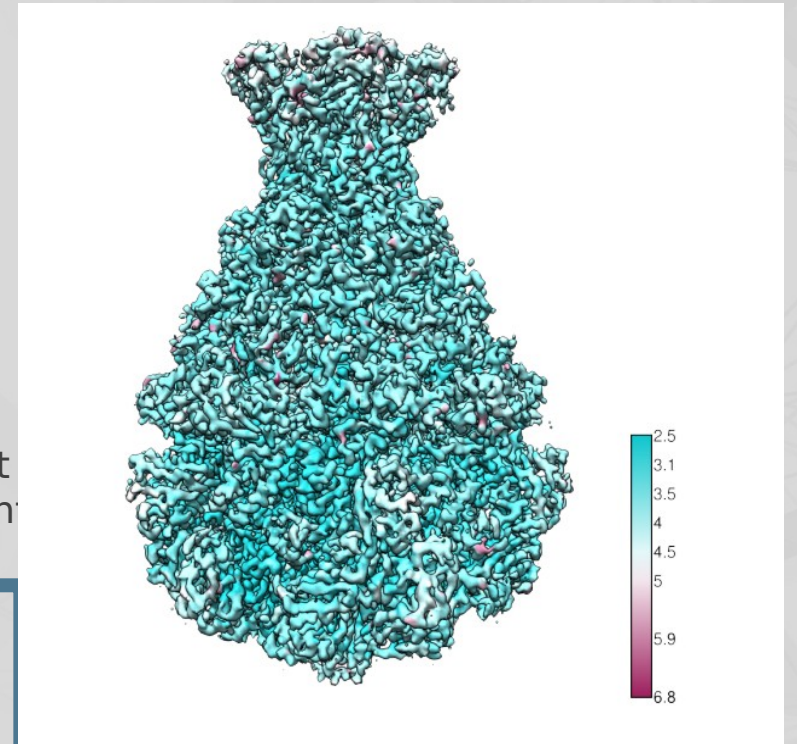
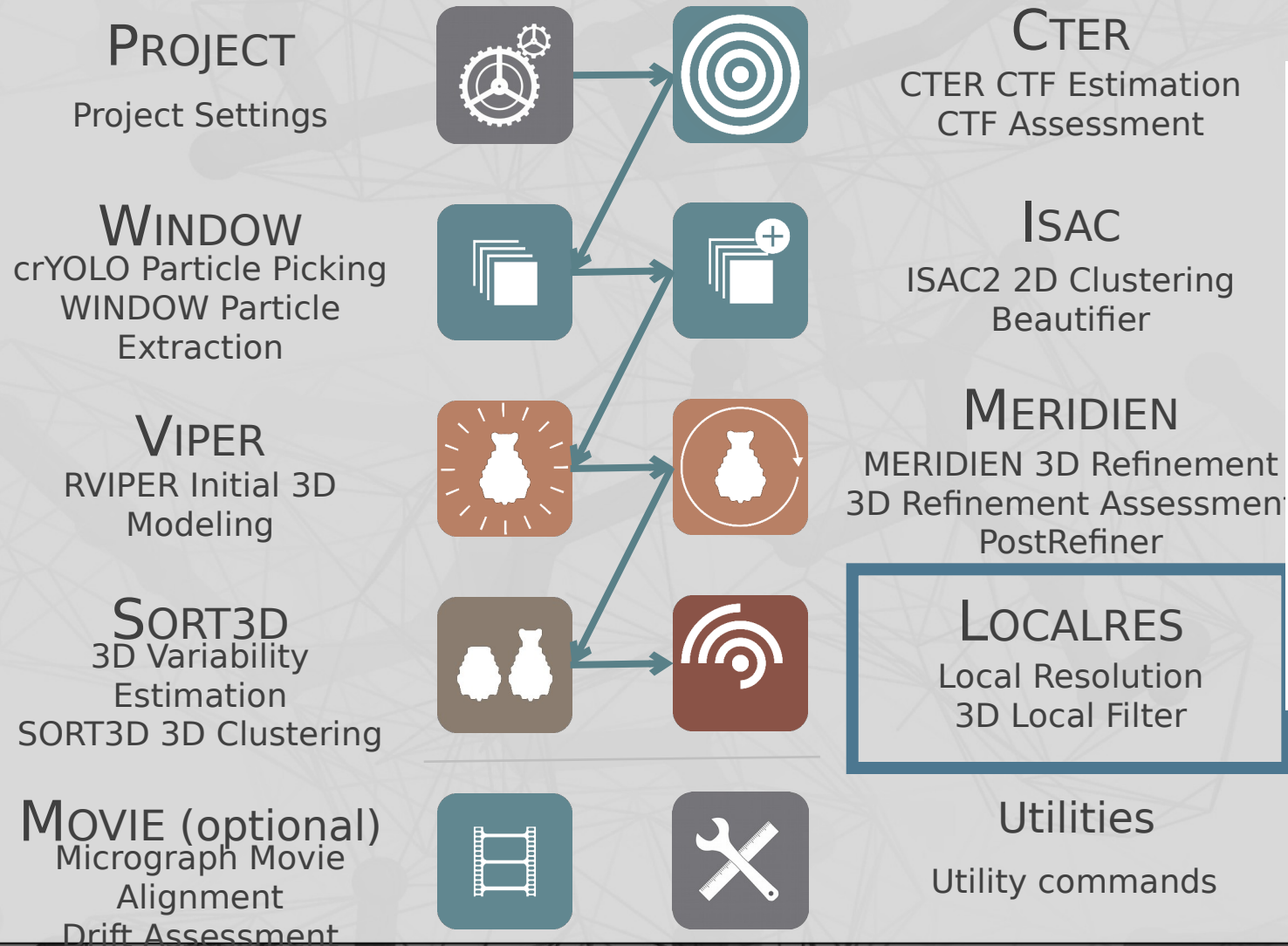
sp_sort3d_depth --refinement_dir Initialize from a given iteration of meridien run using the associated parameters, i.e., full set of orientation parameters per image, including orientation probabilities, normalizations and so on.

Meridien refinement directory	<input type="text" value="required"/>	<input type="text" value="ATASET/TEST06/Meridien"/>	<input type="button" value="Select directory"/>
Output directory	<input type="text" value="none"/>	<input type="text" value="Sort3D"/>	<input type="button" value="Select directory"/>
Iteration ID of 3D refinement for importing data	<input type="text" value="-1"/>	<input type="text" value="-1"/>	
3D mask	<input type="text" value="none"/>	<input type="text" value="/Mask/sxmask_mask.hdf"/>	<input type="button" value="Select HDF volume"/> <input type="button" value="Select any volume"/>
Focus 3D mask	<input type="text" value="none"/>	<input type="text" value="none"/>	<input type="button" value="Select HDF volume"/> <input type="button" value="Select any volume"/>
Estimated particle radius [Pixels]	<input type="text" value="145"/>	<input type="text" value="145"/>	
Point-group symmetry	<input type="text" value="c5"/>	<input type="text" value="c5"/>	
Number of images per group	<input type="text" value="1000"/>	<input type="text" value="4500"/>	
Group splitting rate	<input type="text" value="1"/>	<input type="text" value="1"/>	
Minimum size of reproducible class	<input type="text" value="-1"/>	<input type="text" value="-1"/>	
Swap flag	<input type="text" value="NO"/>	<input type="checkbox"/>	
Swap percentage [%]	<input type="text" value="1.0"/>	<input type="text" value="1.0"/>	
Memory per node [GB]	<input type="text" value="-1.0"/>	<input type="text" value="120"/>	
MPI processors	<input type="text" value="1"/>		<input type="button" value="Save parameters"/>
MPI command line template	<input type="text"/>		<input type="button" value="Load parameters"/>
Submit job to queue	<input type="checkbox"/>		
Job name	<input type="text" value="depth_refinement_dir"/>		
Submission command	<input type="text" value="sbatch"/>		<input type="button" value="Generate command line"/>
			<input type="button" value="Add to pipeline folder"/>
Submission script template	<input type="text" value="q_ppn16_threads1.sh"/>	<input type="button" value="Select template"/>	<input type="button" value="Run command"/>

Memory (GB) per node



SPHIRE Pipeline



Local Resolution



SPHIRE-GUI Main Version 1.2_rc4

COMMANDS (shift-click for w.wiki)

- Local Resolution
- 3D Local Filter

UTILITIES (shift-click for wiki)

- Display Data
- Volume Adjustment
- Adaptive 3D Mask
- Angular Distribution
- Batch Pipeline Execution

Local Resolution

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_0_unfil_023.hdf [Select HDF volume] [Select any volume]

Output volume: required localres.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 [Å]: 1.14 1.14

MPI processors: 1 [Save parameters]

MPI command line template: [Load parameters]

Submit job to queue:

Job name: sxlocres [Generate command line]

Submission command: qsub -V [Add to pipeline folder]

Submission script template: [qsub_templates/msgui_qsub_template_ppn24_env.sh] [Select template] [Run command]

Local Resolution

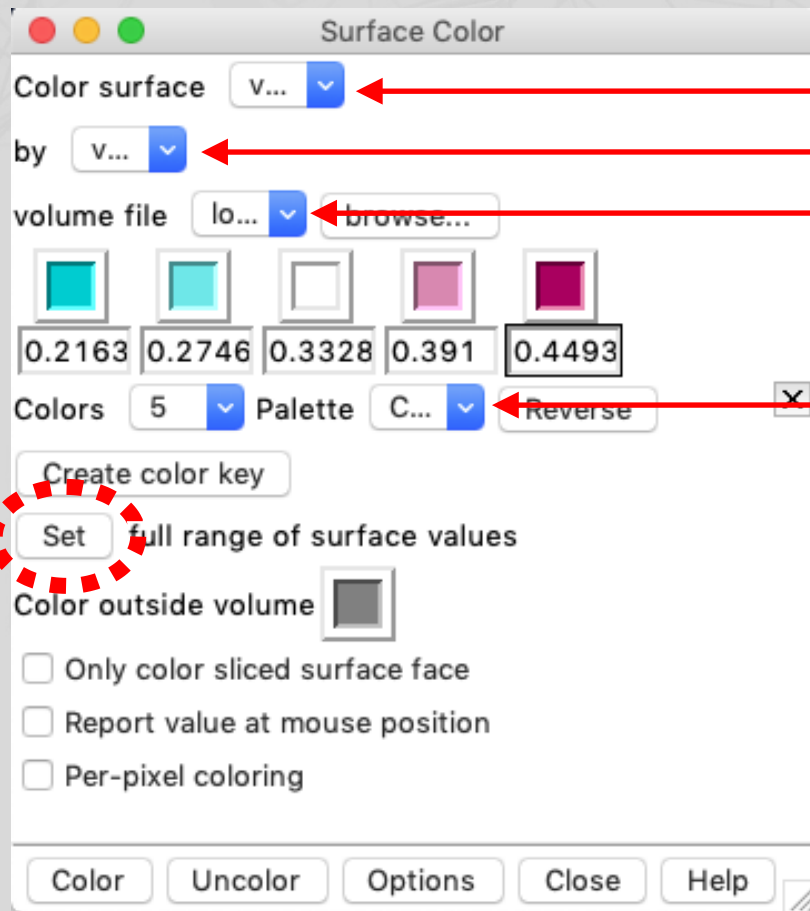


sxlocres

Compute local resolution of a map.

First half-map	required	<input type="text" value="Refine3D/vol_0_unfil_023.hdf"/>	<input type="button" value="Select HDF volume"/>	<input type="button" value="Select any volume"/>
Second half-map	required	<input type="text" value="Refine3D/vol_1_unfil_023.hdf"/>	<input type="button" value="Select HDF volume"/>	<input type="button" value="Select any volume"/>
Output volume	required	<input type="text" value="localres1_4.hdf"/>		
3D mask	none	<input type="text" value="Sharpening/vol_adaptive_mask.hdf"/>	<input type="button" value="Select HDF volume"/>	<input type="button" value="Select any volume"/>
Mask radius [Pixels]	145	<input type="text" value="145"/>		
Window size [Pixels]	7	<input type="text" value="7"/>		
Fourier shell step size [Pixels]	1.0	<input type="text" value="2"/>		
Local resolution criterion	0.143	<input type="text" value="0.143"/>		
FSC output file	no curve	<input type="text" value="no curve"/>		
Save Angstrom local resolution	NO	<input checked="" type="checkbox"/>		
Pixel size of half-maps [A]	1.14	<input type="text" value="1.14"/>		
MPI processors	<input type="text" value="96"/>		<input type="button" value="Save parameters"/>	
MPI command line template	<input type="text"/>		<input type="button" value="Load parameters"/>	
Submit job to queue	<input checked="" type="checkbox"/>			
job name	<input type="text" value="sxlocres"/>			
Submission command	<input type="text" value="qsub -V"/>		<input type="button" value="Generate command line"/>	
			<input type="button" value="Add to pipeline folder"/>	
Submission script template	<input type="text" value="qsub_templates/msgui_qsub_template_ppn24_env.sh"/>	<input type="button" value="Select template"/>	<input type="button" value="Run command"/>	

Local Resolution: Visualization



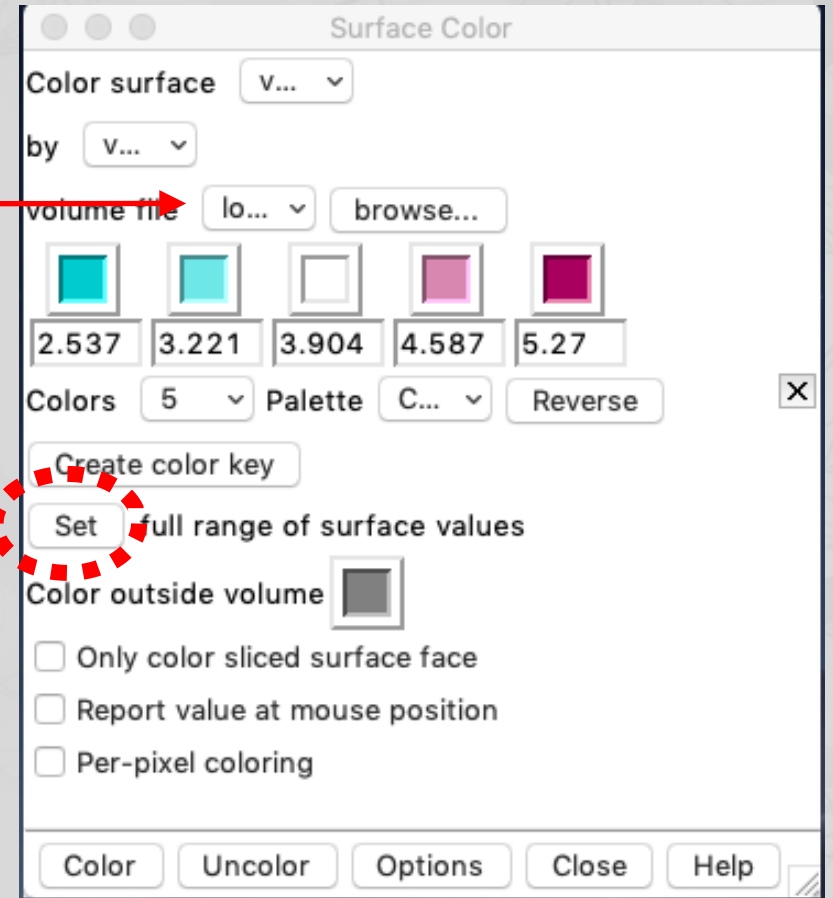
vol_combined.hdf

by: volume data value

localres.hdf

localres_ang.hdf

Palette: Cyan-Maroon



Local Filter



SPHIRE-GUI Main Version 1.2_rc4

COMMANDS (shift-click for wiki)

- Local Resolution
- 3D Local Filter**

UTILITIES (shift-click for wiki)

- Display Data
- Volume Adjustment
- Adaptive 3D Mask
- Angular Distribution
- Batch Pipeline Execution

3D Local Filter

Main | Advanced

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	<input type="checkbox"/>			
Job name	sxfilterlocal		Generate command line	
Submission command	qsub		Add to pipeline folder	
Submission script template	msgui_qsub.sh	Select template	Run command	

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	<input type="checkbox"/>			
Job name		sxfilterlocal		
Submission command		qsub	Generate command line	
			Add to pipeline folder	
Submission script template		msgui_qsub.sh	Select template	Run command

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