SEMC Appion workshop series

PARTII: SINGLE PARTICLE ANALYSIS



September 19, 2019



New York Structural Biology Center













Schedule September 19, 2019 10am-2pm

A practical workshop that continues the singleparticle analysis workflow from the Part 1 workshop. The topics include Appion advanced features, moving into commonly used packages and validation methods.

- Overview of SPA processing and web tools
- Advanced Appion features
- Photo
- - RELION
- cryoSPARC
- [if time allows] • 3. Validation & additional tools - FSC & 3D-FSC / local res & ResMap / other methods

 - 4. Closing [Q&A with SEMC staff]

• 1. Workshop intro

2. Going from Appion to other packages



What is Appion?



The overall mission of NRAMM is to develop, test and apply technology for automating and streamlining cryo-electron microscopy (cryoEM) for structural biology.







Appion is a "pipeline" for processing and analysis of EM images.





Automated image acquisition

Automated image processing



Simons Electron Microscopy Center

Acknowledgements

Microscopy Center at the New York Structural Biology Center CEM.

- Some of this work was performed at the Simons Electron
- that is supported by a grant from the Simons Foundation
- (Grant Number: SF349247) with additional support from: NIH S10 OD019994-01, the Agouron Institute (Grant Number: F00316), NIH S10 RR029300-01, NIH S10 RR017291-01, NYSTAR, and NIH C06 RR017528-01-



SEMC operations team

Directors





Staff













EMG@nysbc.org







SEMC Appion development team



Swapnil Bahktar



Shaker Krit



Anchi Cheng





Alex Noble

Tomography



Туре	Frequency	
feedback	yearly	Gene
knowledge	yearly	SEM
computation	quarterly	SEMC
access	monthly	SEMC Ne
troubleshooting	weekly	User P
instrumentation	daily	Advanc 1-1 train

SEMC Training

Title

Description

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State of the center

IC EM Course

Theory behind EM Graduate level course

Appion workshop

Data processing workshops

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roject Discussion Meetings

ced Leginon use / ning appointments

Facility use and Safety Training Sample preparation [neg-stain & cryo] Leginon intro/use of screening microscopes

> Appointments: Tue @3pm, Thurs @3pm & @3:30pm

Training for independent use of the microscopes and other SEMC resources





appion.org

http://emg.nysbc.org/redmine/projects/nysbc-semc-user-group/issues

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appion.org

Register an account

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appion.org

http://emg.nysbc.org/redmine/projects/nysbc-semc-user-group/issues

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Appion workshops Single particle

Part I

Tools to generate a particle stack and an initial model from micrographs.









Data generation at SEMC **EM** instrumentation

FEI Titan Krios#1 / #2 / #3 Falcon3 x3 | K2 x3

FEI Tecnai F20 DE20 | TVIPS 4K CMOS

> FEI Tecnai Biotwin **TVIPS 4K CMOS**

JEOL 1230 Gatan US4000 CCD

FEI Helios 650 Quorum cryostage

























particle picking

Remote data transfer



Remote data transfer



Create an account at globusid.org

Remote data transfer



Login at globus.org

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emgweb.nysbc.org

Login: Password:

Start your web browsers

test appion2019



Appion & Leginon webtools

EMGWEB Appion and Leginon Tools



emgweb.nysbc.org







Hole Template viewer





Example: Single-particle workflow

During EM session



After EM session







Processing workflow During EM session



Appion Data Processing

Project: SEMC - Glutamate Dehydrogenase (C Session: 17jan26c - Krios2 gdh phase plat Image Path: /gpfs/leginon/rice/17jan26c/rawdata

Hide | Expand | Contract

- Object Selection : 1
- CTF Estimation
- Stacks : 3
- Particle Alignment
- **Ab Initio Reconstruction**
- Refine Reconstruction
- Helical Processing
- **Tomography**
- **Direct Detector Tools**
- V Import tools
- **Upload particles**
- **Upload template**
- 23 available
- **Upload CTF**
- **Upload stack**
- **Upload reconstruction**
- PDB to Model
- **EMDB to Model**
- Upload model
- 1 available



3/4 users who use Leginon also have Appion sessions





Appion web interface

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e Expand Contract Object Selection : 3	
elect Particle Picker	Cluster Stack List
4 complete	
epeat an image loop run	Show Composite Page
CTF Estimation	
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5 complete	Type: Xmipp KerDen SOM
1 running	
ansfer results to another preset	
epeat an image loop run	
Stacks : 9	
ack creation	
11 complete	
ore stack tools	
Particle Alignment	View montage of self-organizing map
elect Particle Alignment	 View montage as a stack for further processing (ID 39)
2 complete	
In Feature Analysis	
3 complete	Clustering Info: coran1 (ID: 12) with 2 clusters
In Particle Clustering	Type: SPIDER Coren
4 complete	Method:
in MaskitOn	Factor list:
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Ab Initio Reconstruction	 80 Class Averages [variance] (ID 40)
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In Single-Model Refinement	Type: Xmipp KerDen SOM
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n Multi-Model Refinement	



Single particle analysis

Collect Data







Single particle analysis





Single particle analysis

3/4 users who use Leginon also have Appion sessions









Going from Appion to RELION/cryoSPARC



CTF Estimation

/gpfs/appion/[username]/[sessionname]/CTF/

/gpfs/frames/[username]/[sessionname]/rawdata/

/gpfs/leginon/[username]/[sessionname]/rawdata/

/gpfs/appion/[username]/[sessionname]/stacks/



Selecting particles – box-files

Hide | Expand | Contract V Object Selection : 1 Run name Dog Picking stackFINAL 1 complete Output directory **Template Picking** /gpfs/appion/eeng/16jul26b/stacks Manual Picking Description **Object Tracing** "just box-files" Signature Repeat an image loop run TF Estimation esn-a \$ Preset Estimate the CTF Wait for more images after finishing 2 complete Repeat an image loop run Max number of images to process Images to process: V Stacks : 5 Do not process hidden or rejected ima Stack creation All images independent of status 6 complete Exemplar and keep images only more stack tools Image order: Forward Particle Alignment Reverse **Run Alignment** Shuffle 2 complete Continuation: Continue unfinished run (default) **Run Feature Analysis** Reprocess all images 2 complete **Run Particle Clustering** Commit results to database 6 complete Run MaskitOn Density modifications: Invert image density **Template Stacks** Normalization Method Ab Initio Reconstruction edgenorm: normalize by mean 0 stdev 1 OptiMod Common Lines 2 complete EMAN Common Lines SIMPLE Common Lines Refine Reconstruction Run Single-Model Refinement Run Multi-Model Refinement Helical Processing Helical Image Processing V Direct Detector Tools Create frame stack 1 running Launch Alignment Catchup Launch DE Frame Alignment Launch Particle Polishing V Import tools

	Particles: None +
	Stacks: stack4-centered4 (4,696 parts) \$
	128 Box Size (Unbinned, in pixels)
	1 Binning
	Fast Insert
	Ctf Correct Particle Images
	CTF Confidence Cutoff
	Use Values Above: 0.8 (between 0.0 - 1.0)
les	CTF Correction Method
	The a trianer time trible insign
	CTF Sorting Method
	OP choose a cff rup:
	CTF Resolution Cutoff
	Resolution range at 0.8 criteria:
	between and (in Angstroms)
	Resolution range at 0.5 criteria: between and (in Angstroms)
	Defocus Limits Min 2 9 2 & Max 6 21 2 6
	-3.98-7 G MAX -0.718-0
based on edge pixels	
	No Mask Assessed for this Session
	Raw frame processing if available: start frame: start frame total frame: total frames
	Filter Values:
	Low Pass (in Angstroms)
	High Pass (in Angstroms)
	4 Pixel Limit (in Standard Deviations; 0 = off)
	Particle Correlation Cutoff
	Remove particles with CCC below: 0.5 Remove particles with CCC above: 1.0
	Defocal pairs: Use defocal pairs
	Limit # of particles to:
	Only create EMAN boxfiles

Helical Alignment:



Selecting CTF

V Object Selection : 7 Select Particle Picker... 7 complete Repeat an image loop run **V** CTF Estimation Estimate the CTF... 4 complete Transfer results to another preset Repeat an image loop run V Stacks : 15 Stack Creation... 16 complete **V** Particle Alignment Select Particle Alignment... 1 complete Run Feature Analysis... **Run Particle Clustering...** 7 complete **Run MaskitOn Template Stacks** 1 complete

[show all statistics]

Summary of confidence values from all runs



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- download best ctf data
- download best ctf EMX file

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efocus2		443	0.11 µm	7.56 µm	1.80 µm	0.66 µm					
ale astigmatism		443	-89.991	83.662	-42.236	30.574					



Test set

Sample: Glutamate Dehydrogenase (GDH) **Reconstruction:** Relion 2 3D refine



Imaging statistics: 20 eV slit, stage shift 17feb14h experiment : 528 esn images, 523 esn-a-DW images used 443 enn-a-DW images used from Appion 16,191 particles = 3.16Å (using a CTF conf 0.8 >8Å CTF cut off)

Imaging parameters: Grid loaded: 17feb13 Session: 17feb14h Pixel size: 0.43Å superres bin2 - 0.854905Å/px

Camera: K2 counting, 400 ms - 32 frames Camera settings: 12,800 ms exp Dose: 35.73 e⁻/Å²





Schedule September 19, 2019 10am-4pm

A practical workshop that continues the singleparticle analysis workflow from the Part 1 workshop. The topics include Appion advanced features, moving into commonly used packages and validation methods.

- Overview of SPA processing and web tools
- Advanced Appion features
- Photo
- - RELION
- cryoSPARC
- [if time allows] • 3. Validation & additional tools - FSC & 3D-FSC / local res & ResMap / other methods
- 4. Closing [Q&A with SEMC staff]

• 1. Workshop intro

2. Going from Appion to other packages

2D classification

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2D classification 3D initial model 3D classification				Regularisation	parameter T	: 2	-0	?
3D auto-refine 3D multi-body CTF refinement Bayesian polishing				Number Use fast subsets (for large	of iterations e data sets)1	: 50 – ? Yes		
Mask creation Join star files Particle subtraction Post-processing				Mask (diameter (A)	: 200 -		
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001: Extract/run0_288-144px/		Scheduled jobs 010: Refine3D/run0_288-144px_2D-	it42_3E	Output from this job				

2D classification

Class2D/job002/run_it050_model.star				

Class2D/job003/run_it050_model.star				

Initial model

	X RELION-3.0-beta-2: /gpfs/appion/sworkshop/relion3/run0
File Jobs Autorun	I/O CTF Optimisation SGD Compute Running
Import Motion correction CTF estimation Manual picking Auto-picking Particle extraction Particle sorting Subset selection 2D classification 3D classification 3D auto-refine 3D multi-body CTF refinement Bayesian polishing Mask creation Join star files Particle subtraction Post-processing Local resolution	Number of classes: ? Mask diameter (A): 200 Flatten and enforce non-negative solvent? Yes Symmetry: C1 Initial angular sampling: 15 degrees Offset search range (pix): 6 Offset search step (pix): 2 Offset search step (pix): 2

Initial model

Stochastic Gradient Descent algorithm for initial 3D model generation

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3D classification

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of iterations: 50 e data sets)? Yes		
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-step to (A): -1	?	
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RELION 3 workflow

Workshop workflow : Real time demo test set – 17feb14h (~1/2 day run)

