SPA data collection with the Osaka framework

INTRODUCTION

Cryo-EM has enjoyed an enormous ground swell in popularity ever since the advent of more stable and automated electron microscopes, suitable movie-type cameras, and improved acquisition software. Results obtained so far have been nothing short of spectacular as illustrated by several structures in EMDB and EMPIAR solved by cryo-EM to resolutions better than 1.5Å, such as EMD-31314, EMD-33707 and EMD-35984, the latter of which reaching true atomic resolution. This note describes the workflow used in Single Particle Analysis (SPA) cryo-EM workflows with the Osaka framework, i.e. a set of scripts that work with SerialEM.

Created to meet tomography requirements on the 1 MeV JEOL scope in Boulder, CO initially, SerialEM has since become one of the key factors in driving the success of SPA[1] in part thanks to its enormously rich scripting environment and support for nearly all cryoEM-related hardware. Currently, SerialEM is being used on a daily basis and around-the-clock for data collection on hundreds of electron microscopes. Although SerialEM supports a scriptless workflow for SPA, this Application Note deals with the scripted version, colloquially known as the Osaka framework.

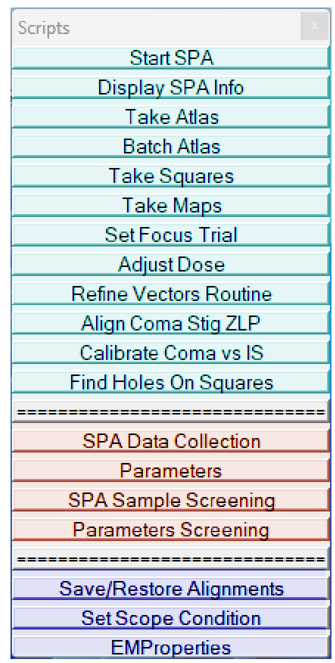

Fig. 1: The SPA toolbar of the Osaka framework

The original Osaka framework was developed by Fukumura, Makino and Marzec. It consists of a series of logically connected scripts arranged in a top to bottom fashion in a toolbar (Fig. 1). The framework essentially guides the user through all the necessary steps for setting up the SPA workflow starting with executing the Start-SPA script and finishing with the script to automatically find holes on all square or polygon maps (the cyan section). For the SPA workflow, the user executes one of the two scripts in the red section, either the main SPA Data Collection script to collected high-resolution data, or the SPA Sample Screening script to screen many samples automatically. All instrument and setup-specific items reside in one location (EMProperties in the blue section). Additional scripts (not all shown) allow the operator to probe the accuracy of each step in the workflow individually.

The development of the Osaka framework was guided by the desire to eliminate as much as possible any human interaction and/or decision making, thus effectively streamlining the entire workflow. To that end, predefined imaging and illumination conditions are used throughout the workflow. Also, since all of the SPA work involves the use of holey grids, algorithms were developed to automatically determine and refine the lattice vectors that describe the hole pattern of these particular grids. The Osaka framework was gently modified to include help and guidance, to improve robustness, to support non-CRYO ARM types of electron microscopes (i.e. without a Cold FEG, autoloader or Omega energy filter), and finally, to support multi-camera setups and scopes without automated apertures, in other words, as generic a version of SPA solution as one could possibly imagine.

[1] Mastronarde, D.N. 2005. Automated electron microscope tomography using robust prediction of specimen movements. *J. Struct. Biol.* 152:36-51.

The SPA workflow starts with the creation of an atlas, an overview of the entire grid acquired in LOWMAG mode used to evaluate and select areas with suitable ice thicknesses (Fig.2). Since CRYO ARMs are equipped with an autoloader, atlases can be acquired from multiple grids with a single script (Batch Atlas). The next step is to acquire images of selected grid squares, sometimes referred to as square images. The square images are often acquired in LOWMAG mode depending on the microscope. Depending on the hole targeting strategy, montages of a grid square can be used instead. These montages, often referred to as MMM or Medium Mag Montages, are always done in regular MAG mode using a Low Dose View condition and at a magnification low enough to keep the number of tiles at a minimum. The operator can automatically process both types of maps, i.e. the square images or the MMMs, to pick candidate holes and apply the imaging pattern defined in the multi-record setup. This greatly speeds up the setup as the time-consuming manual picking of holes can be avoided. The imaging pattern is pre-defined in the scripts but adjustable by the operator. To probe the accuracy of the vector refinement and further speed up the setup, a montaging option is available that reveals immediately the chosen imaging pattern (Fig. 3).

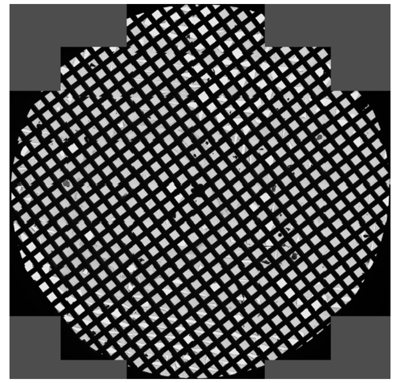

Fig. 2: Atlas of a 300 mesh holey carbon grid with apo-ferritin acquired by Take Atlas script on a JEM-3200FS at IU.

The low-dose setup is greatly simplified as beam positions in Low Dose are computed and set using the refined lattice vectors of the holey grid. The beam diameter is automatically set to a diameter suitable for quick beam centering. Focus can be preset to be within a specific range of values. The dose for the multi-record is automatically set in the Set Dose script that generates warnings if dose rates exceed the accepted levels of coincidence loss. Hole targeting during the SPA run is accomplished using either an Align To Template, when using square images, or using the program's built-in Realign To Item. The latter procedure requires a map of the grid square for hole targeting, the MMM, and the Low Dose View condition to be set appropriately. Various scope-related items are constantly monitored during the workflow, such as the alignment of the scope, coma and zero-loss position of the energy filter (post as well as in-column), as well as flashing the gun and refilling LN2. Eventually these items can cause the workflow to be paused and resumed wherever appropriate. High-resolution data is acquired using the built-in multiple record but a customized multirecord is also available. Data is corrected for coma for shots between holes but not for those within a single hole. Imaging within a hole is possible because the scope employs Köhler mode, an illumination mode characterized by parallel electron beam conditions without visible Fresnel fringes from the condenser aperture. Energy-filtered, high-resolution data is acquired from the main camera (in the event of a multi-camera setup) and stored on either a local device or a framestore. Options are present for a watch folder to be assigned for preliminary data processing.

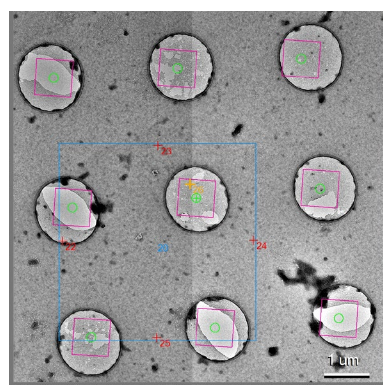

Fig. 3: Montage across a 3x3 hole pattern showing the multi-record positions acquired on a JEM-3200FS at IU.

The Osaka framework is currently employed on nearly all JEOL CRYO ARMs[2].

[2] All inquiries should be directed at serialem@jeol.com.