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SPRING – An image processing package for single-particle based helical reconstruction from electron cryomicrographs

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A B S T R A C T

Helical reconstruction from electron cryomicrographs has become a routine technique for macromolecular structure determination of helical assemblies since the first days of Fourier-based three-dimensional image reconstruction. In the past decade, the single-particle technique has had an important impact on the advancement of helical reconstruction. Here, we present the software package SPRING that combines Fourier based symmetry analysis and real-space helical processing into a single workflow. One of the most time-consuming steps in helical reconstruction is the determination of the initial symmetry parameters. First, we propose a class-based helical reconstruction approach that enables the simultaneous exploration and evaluation of many symmetry combinations at low resolution. Second, multiple symmetry solutions can be further assessed and refined by single-particle based helical reconstruction using the correlation of simulated and experimental power spectra. Finally, the 3D structure can be determined to high resolution. In order to validate the procedure, we use the reference specimen Tobacco Mosaic Virus (TMV). After refinement of the helical symmetry, a total of 50,000 asymmetric units from two micrographs are sufficient to reconstruct a subnanometer 3D structure of TMV at 6.4 Å resolution. Furthermore, we introduce the individual programs of the software and discuss enhancements of the helical reconstruction workflow. Thanks to its user-friendly interface and documentation, SPRING can be utilized by the novice as well as the expert user. In addition to the study of well-ordered helical structures, the development of a streamlined workflow for single-particle based helical reconstruction opens new possibilities to analyze specimens that are heterogeneous in symmetries.

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1. Introduction

Structure determination of large macromolecular assemblies embedded in vitreous ice using electron microscopy (EM) is becoming increasingly popular as evidenced by the steady increase in the number of structure depositions into the EM databank (EMDB) (Lawson et al., 2011). Depending on the molecular weight and order of the assembly, a series of three-dimensional (3D) structures at near-atomic resolution have become available in the past decade. Pioneering work on highly symmetric structures derived from two-dimensional arrays, helical or icosahedral assemblies (Henderson et al., 1990; Unwin, 2005; Zhang et al., 2008) have demonstrated the potential of electron cryomicroscopy (cryo-EM) based structure determination. Historically, the first 3D reconstructions were computed from electron micrographs of helical assemblies (De Rosier and Klug, 1968). These assemblies have the advantage that a single helix already represents many views of the asymmetric unit whose structure needs to be determined.

Currently, helical assemblies make up ~10% of the determined structures in the entire EMDB. This is due to the fact that only a limited number of proteins form arrays of helical symmetry. Nevertheless, many of these are functional in the helical state and as such, are of fundamental importance to the cell (Moore et al., 1970; Nogales et al., 1999). Several structures that mediate the modulation of membrane shapes have been determined with the protein coat assembled at the membrane in a helical geometry (Frost et al., 2008; Low et al., 2009). In addition, there are examples of helical assemblies that form protein crystals in the context of a tubular membrane (Korkhov et al., 2010; Unwin, 1993). Such assemblies have also been successfully formed by affinity-tagged membrane-associated proteins (Wilson-Kubalek et al., 1998).

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Methods for structure determination of helical assemblies have significantly evolved since the birth of 3D electron microscopy. In the past, the procedure relied on entire and straight filaments, filamentous viruses or tubules that were processed in Fourier space by indexing the helical lattice and extracting the amplitudes and phase from the corresponding layer lines. Multiple helices were averaged and brought to a common phase origin and a 3D reconstruction was computed by Fourier inversion of the structure factors. For a more comprehensive description consult (Stewart, 1988). More recently, adapted Fourier-based techniques and real-space approaches that treat helices as small segments have significantly improved the attainable resolution (Beroukhim and Unwin, 1997; Ge and Zhou, 2011; Sachse et al., 2007; Yonekura et al., 2003). In addition, some helical assemblies deviate from their ideal straight path and can also vary in their helical symmetries because of inherent flexibilities (Fujii et al., 2010; Sachse et al., 2008). In certain cases, Fourier-based helical 3D reconstruction can be complicated by particular symmetries. First, in cases of long helical pitches many layer lines are required to represent the entire helical structure as in the case of amyloid fibrils. Second, several layer lines can interfere on a single reciprocal pixel line and the resulting Bessel overlap makes the assignment of Bessel order impossible. Nevertheless, real-space helical reconstruction can cope with these complications and determine the 3D structures of these helical assemblies (Jiménez et al., 1999; Sachse et al., 2008).

Despite the previous successes of helical structure determination, a simple standardized workflow for 3D helical reconstruction is still lacking. The most widely used approach is the implementation of the iterative helical real-space reconstruction (IHRSR) based on the SPIDER package (Frank et al., 1996) and additional tools for helical symmetry determination and imposition (Egelman, 2000). In the meantime, other packages such as SPARX have adapted the IHRSR algorithm (Behrmann et al., 2012). Moreover, several structures have been determined by extending and modifying the original IHRSR approach significantly with additional SPIDER operations (Sachse et al., 2007). Using a full correction of the contrast-transfer function, alignment restraints and an adapted 3D symmetrization procedure, a series of structures were determined (Bharat et al., 2012; Korkhov et al., 2010; Low et al., 2009; Sachse et al., 2007; Sachse et al., 2008). In order to condense the adapted procedures into a generally usable workflow, we describe here a package for single-particle based helical reconstruction termed SPRING (Single particle reconstruction from images of known geometries). We demonstrate the full functionality of the package by processing a subset of previously published micrographs of Tobacco Mosaic Virus (TMV) (Sachse et al., 2007) (http://grigorieff-flab.janelia.org/datadownload). SPRING contains programs that determine the microscope parameters, analyze and classify the segmented helices, explore helical symmetry at low resolution, refine high-resolution symmetry and determine the 3D structure.

2. Overview

SPRING aims to provide a comprehensive workflow for processing electron micrographs of helical specimens from micrographs to 3D structure analysis and interpretation. The workflow has been subdivided into three separate suites of programs: "Springmicrograph", "Spring2d" and "Spring3d" (Table 1). In SPRINGMICROGRAPH, digital micrographs can be analyzed and processed. The extraction and analysis of helical segments is implemented in the second suite, SPRING2D. The third suite of programs, SPRING3D, generates, refines and analyzes 3D structures. The individual programs can be operated from a graphical user interface (GUI) (Fig. 1), from the command line prompt, from command line options or using a simple text file as input parameter file. In all programs, the user can specify three levels of expertise: beginner, intermediate and expert. The beginner level reduces the complexity of the input parameters by using sensible default values. As their familiarity with the processing operations increases the user can choose to add more parameters. In the current implementation of SPRING, a significant effort was invested to streamline analysis and diagnosis of the obtained results in a user-friendly manner. Where possible, either condensed graphical plots are generated or more complex data representations can be browsed interactively (Fig. 2).

The SPRING package is entirely written in object-oriented python and uses EM-related libraries and functions from SPARX and EMAN2 (Hohn et al., 2007). Microscope parameters are determined by CTFFIND and CTFTILT (Mindell and Grigorieff, 2003). In addition, scientific computing tasks are performed by Numpy and Scipy functions (numpy.scipy.org). For parameter storage sqlite3 databases are used and interfaced by SQLAlchemy (www.sqlalchemy.org). Interactive and diagnostic plots were made with the plotting libraries of matplotlib (http://matplotlib.sourceforge.net). SPRING's GUI has been built using PyQT libraries. SPRING is optimized to run in a multi-CPU environment on high-performance computer cluster implemented by Mpi4Py (http://mpi4py.scipy.org).

Python can be used as a scripting language as well as a structured programming language. Both of these features make the usage of isolated existing functions in a new processing context and the easy modification of SPRING possible. In addition, the widespread use of python as a programming language and the excellent interfaces to scientific computing libraries such as Numpy and Scipy are a great advantage for prototyping any numerical computations and thus promoting further development of the package. The python programming language facilitates code structuring and readability and the code is directly documented and available on SPRING's website as a detailed reference (Fig. 1B). Python has become a popular tool to master the scripting and programming tasks in a variety of other EM software packages such as PyTOM and Xmipp (Hrabe et al., 2012; Scheres et al., 2008).

3. Initial analysis of micrographs and segments

The EM operator records electron micrographs in several different ways. Currently, film, CCD cameras and direct detectors are the common sources of EM data. After film has been digitized all types of data are available as images in various formats. SPRING accepts all the formats of micrograph data that EMAN2 currently supports such as standard MRC, IMAGIC, SPIDER, TIF formats. MICEXAM examines the micrographs by analyzing the power spectra tiles to exclude images that suffer from poor information transfer at higher resolutions due to charging or drift (Hohn et al., 2007). MICCTFDETERMINE determines the CTF of the micrographs by interfacing with CTFFIND initially and optionally refines parameters using CTFTILT (Mindell and Grigorieff, 2003). The program captures a reduced output of CTFFIND and CTFTILT and the results are stored in the SPRING database to be retrieved for further processing.

After the selection of high-quality micrographs, helices need to be extracted from the images. For this purpose, the helices are interactively picked using external programs. In the past, EMAN's HELIXBOXER or BOXER with the helix option was used (Ludtke et al., 1999) (EMAN2 has an updated version named E2HELIXBOXER). BSOFT is also capable of picking filaments with significant curvature and recording their helix paths (Heymann and Belnap, 2007). The program SEGMENT from SPRING extracts a complete data set of overlapping segments using the provided coordinates from either EMAN, EMAN2 or BSOFT, applies CTF correction by either phase-flipping or convolving the segments with the determined CTF and stores coordinates and the derived in-plane

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