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Helical reconstruction in RELION

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ABSTRACT

We describe a new implementation for the reconstruction of helical assemblies in the empirical Bayesian framework of RELION. Our approach calculates optimal linear filters for the 3D reconstruction by embedding helical symmetry operators in Fourier-space, and deals with deviations from perfect helical symmetry through Gaussian-shaped priors on the orientations of individual segments. By incorporating our approach into the standard pipeline for single-particle analysis in RELION, our implementation aims to be easily accessible for non-experienced users. Although our implementation does not solve the problem that grossly incorrect structures can be obtained when the wrong helical symmetry is imposed, we show for four different test cases that it is capable of reconstructing structures to near-atomic resolution.

1. Introduction

The first biological structure to be determined by three-dimensional electron microscopy (3D-EM), the extended tail of the T4 bacteriophage, had helical symmetry (De Rosier and Klug, 1968; DeRosier and Moore, 1970). An important advantage for structure determination of helical objects over asymmetrical particles lies in the observation that a single projection image of a helical specimen will typically contain all necessary information to perform a 3D reconstruction. Moreover, for helical reconstructions the number of parameters to be determined is, in principle, strongly reduced compared to single-particle analysis. That is because in single-particle analysis one needs to determine the relative orientations for every individual particle projection image, while for helical structures many copies of the repeating, asymmetrical unit have fixed relative orientations. Therefore, once the parameters describing helical symmetry and the orientation of the image of an entire filament with respect to that symmetry have been determined, one can reduce the experimental noise efficiently by averaging over a large number of asymmetrical units.

The determination of helical symmetry and the subsequent calculation of a 3D reconstruction were initially performed in Fourier space. The mathematical description of the Fourier transform of an object with helical symmetry was first proposed by Crick, Cochran and Vand (Cochran et al., 1952), and Klug generalised the theory afterwards (Klug et al., 1958). The initial step is to inspect 2D Fourier diffraction patterns of, sometimes averaged, and preferably long and straight, helical filaments. The helical lattice can be considered as a 2D surface lattice that is curled into a cylinder (also see Fig. 1). The curling effect causes a convolution of the Fourier transform of the 2D surface lattice with a cylindrical harmonic called the Bessel function. In the 3D Fourier transform of a helical object, discrete horizontal lines, called layer lines, arise from the periodicity along the helical axis. Through the central slice theorem, the radially oscillating ring-like amplitudes on each plane perpendicular to the z-axis in 3D Fourier space give rise to symmetrical maxima across the meridian on each layer line in the Fourier transforms of 2D projections. The position of the maxima along these lines, combined with the real-space width of the helical object, can be used to infer the 2D lattice parameters in a process called Fourier-Bessel indexing. After the Fourier transform of a helix has been indexed, a 3D reconstruction can be obtained through Fourier inversion (DeRosier and Moore, 1970). For more detailed reviews on Fourier-Bessel analysis, the reader is referred to (Diaz et al., 2010; Stewart, 1988).

Computer programs for Fourier-Bessel analysis were first developed in the MRC image processing package (Crowther et al., 1996), and have since been adapted in many other packages, such as the Brandeis Helical Package (Owen et al., 1996), Phoelix (Whittaker et al., 1995) and for objects with a break in helical symmetry

Abbreviations: CARD, caspase activation and recruitment domain; EM, electron microscopy; FSC, Fourier shell correlation; IHRSR, iterative helical real-space reconstruction; MAVS, mitochondrial antiviral signaling protein; TMV, tobacco mosaic virus.

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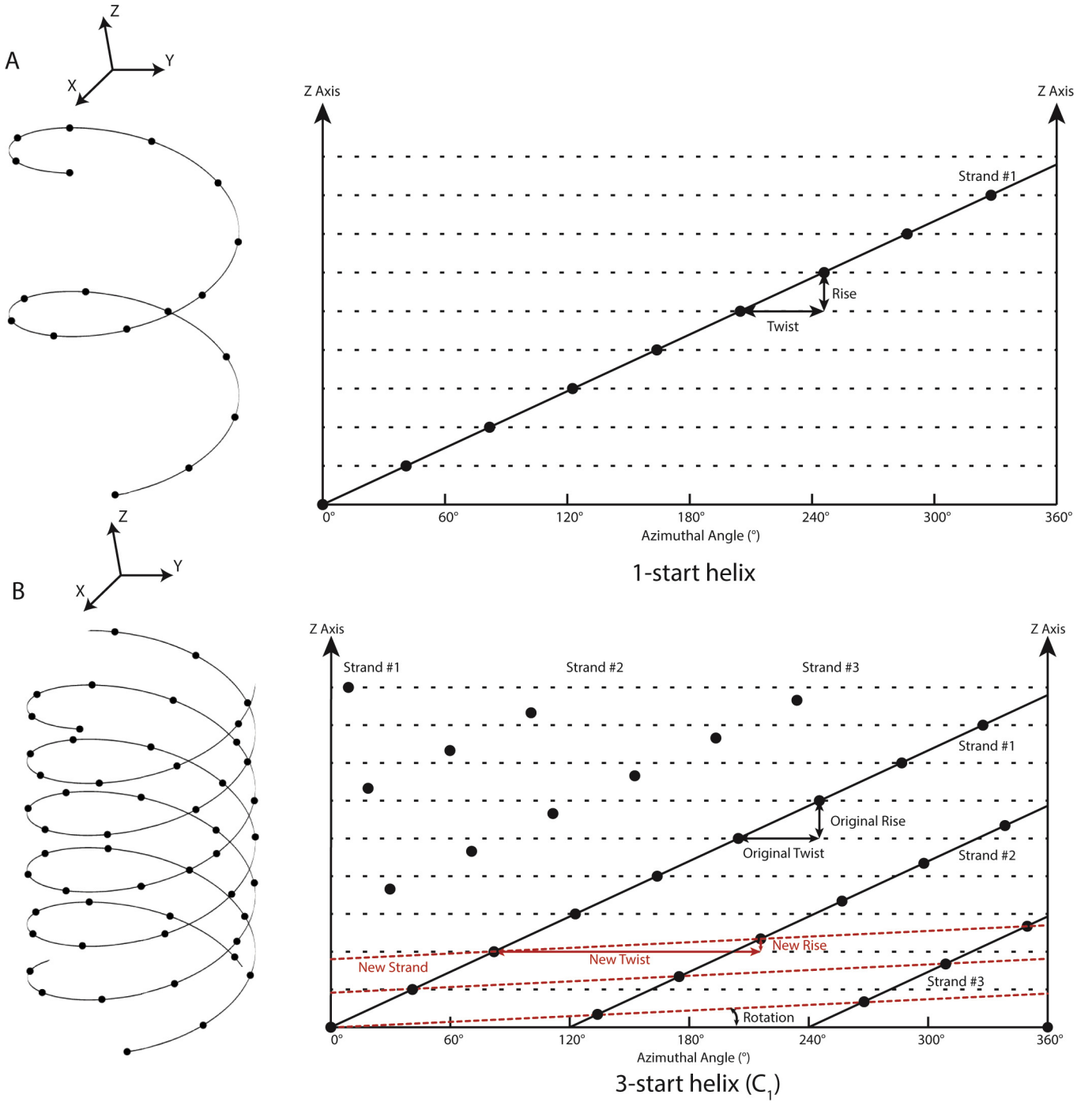

Fig. 1. Definition of helical symmetry for *n*-start helices. A. Example of a 1-start helix with no point group symmetry (C1). The 3D helical structure on the left can be 'unfolded' into a 2D plot of the *z*-height against the azimuthal angle φ on the right. The dots represent the centres of the subunits in the 2D surface lattice. B) Example of a 3-start helix (with C1 point group symmetry), where instead of using the original twist and rise of the individual three strands (in black), RELION expresses the helical symmetry using a third of the original rise (in red).

known as a seam in Ruby-helix (Metlagel et al., 2007). Fourier-Bessel analysis of helices is often considered laborious and difficult, as small inaccuracies in the indexing may lead to incorrect reconstructions. Indexing is particularly prone to errors when the filaments are not lying flat, the lattice is somehow distorted by bending or flattening of the filaments, or by short-range disorder in the helical symmetry. Therefore, the Fourier-Bessel approach only performs well when the specimen adopts a close to perfect helical structure. In practice, many helical assemblies are far from perfect due to molecular flexibility and distortions induced by the sample preparation process. To reduce the effects of long-range distortions on the Fourier-Bessel reconstruction of AChR filaments, Beroukhim and Unwin introduced the idea of dividing helical filaments into short segments, which are independently aligned against a reference structure (Beroukhim and Unwin, 1997).

Analogous to single-particle analysis, one can also align separate helical segments in real-space against projections of a 3D reference model. Early applications of real-space helical reconstruction were performed on haemoglobin fibrils (Bluemke et al., 1988) and on microtubules (Sosa et al., 1997). The introduction of the iterative helical real-space reconstruction (IHRSR) algorithm by Egelman opened up new horizons for application of this