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Localization and Classification of Repetitive Structures in Electron Tomograms of Paracrystalline Assemblies

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1. INTRODUCTION

Electron tomography offers opportunities to study structures that are not amenable to 3D imaging by any of the classical methods, such as single-particle reconstruction (Frank, 1996), helical reconstruction (Egelman, 2000; DeRosier and Moore, 1970) or electron crystallography (Glaeser,

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1999) that require either a repetitive structure, or multiple copies of identical structures. Since electron tomography can produce a 3D image of a single copy of a structure, it is finding wide application in cell biology and material science. Paracrystalline specimens constitute another class of structure for which electron tomography can be particularly useful for obtaining detailed 3D images (Taylor *et al*., 1997). Paracrystals (para—Greek prefix meaning faulty) are arrays with various kinds of intrinsic disorder. Spatial averaging of such specimens usually blurs or even erases the disordered component, which may eliminate the functionally interesting feature. For this chapter, we define a paracrystalline specimen as one with partial ordering such that one component of the specimen may be highly regular while another may be irregular due to either low occupancy, lattice irregularity or both.

Striated muscle is one example of a paracrystalline structure found in nature. Striated muscles consist of hexagonal arrays of two types of filaments: thick, myosin-containing filaments, and thin, actin-containing filaments. Interactions between myosin heads and the actin filament are responsible for filament sliding and muscle shortening (Geeves and Holmes, 1999). Muscle is paracrystalline because the filaments themselves have a well-ordered structure, but have a disordered arrangement within the lattice (Squire, 1981), or the interactions between filaments, which usually involves the myosin cross-bridges, are highly variable. Structures that contain actin filaments are often poorly ordered because the actin helix can have variable twist (Egelman and DeRosier, 1992; Egelman *et al*., 1982). However, even in the instances where the actin filament has a well-ordered 28/13 helical structure, such as in insect flight muscle (IFM), the myofibrils display considerable disordering among the myosin cross-bridges. Other types of natural structures with paracrystalline ordering include the cross-linked actin arrays found in microvilli (Tilney *et al*., 1980) or various *in vitro* 2D actin assemblies (Taylor and Taylor, 1994; Taylor *et al*., 2000).

The asynchronous flight muscles of various species of the large water bug *Lethocerus* are perhaps the best ordered muscles in the animal kingdom and are therefore ideal both to study muscle contraction and to develop methods for image classification in 3D. IFM contains an hexagonal array of thick filaments with actin filaments interdigitated between thick filament pairs at pseudodiad positions in the unit cell (Fig. 1b). This filament arrangement differs from vertebrate striated muscle where the actin filaments lie at trigonal positions within the unit cell. The IFM lattice arrangement facilitates the cutting of several types of thin section that have been extremely useful for obtaining information on the arrangement of myosin heads in different muscle states (Fig. 1b). Two kinds of 25 nm thick longitudinal section can be cut parallel to the filament axis (Reedy and Reedy, 1985). One of these, the myac layer (Fig. 1a), contains alternating thick and thin filaments. Another, the actin layer, contains only actin filaments, but these divide evenly into two groups that differ by azimuthal rotation of 60° and axial translation of 12.8 nm. The third type is a 15 nm thick section cut