5

Fiducial Marker and Hybrid Alignment Methods for Single- and Double-axis Tomography

David N. Mastronarde

1. INTRODUCTION

Accurate alignment of projection images is an important step in obtaining a high-quality tomographic reconstruction. Ideally, all images should be aligned so that each represents a projection of the same 3D object at a known projection angle. Inadequate image alignment will result in blurring or smearing of features in the reconstruction. The problem is made more difficult because exposure to the electron beam during the acquisition of a tilt series induces geometric changes in many samples (primarily plastic-embedded material). For any sample, but particularly for ones that are not

David N. Mastronarde • The Boulder Laboratory for 3D Electron Microscopy of Cells, Department of Molecular, Cellular, and Developmental Biology, Campus Box 347, University of Colorado, Boulder CO 80309, USA

rigid during imaging, a powerful method of image alignment uses the measured coordinates of fiducial markers through the series of images; these can be fit to equations that describe the image projection. Alternative methods are based on cross-correlation of images. The fiducial marker method has the advantage that it guarantees a consistent alignment among the images from the full range of tilt angles. It is also more easily adapted to correct for changes in the sample that occur during imaging.

Various formulations of fiducial alignment have been described; they differ in their levels of complexity and methods of solution and in whether they require each marker to be measured on all projections (Berriman *et al.*, 1984; Jing and Sachs, 1991; Lawrence, 1992; Penczek *et al.*, 1995). The formulation presented below reflects the implementation of fiducial alignment in the IMOD package (Kremer *et al.*, 1996) (http://bio3d.colorado.edu/imod), which provides a superset of features described elsewhere. This chapter will describe the basic method as well as some practical aspects of fiducial alignment, drawing on extensive experience with the method in the Boulder Laboratory for 3D Electron Microscopy of Cells. It shows how the method can be extended to preserve resolution in reconstructions of large, heterogeneous volumes (e.g. Marsh *et al.*, 2001). It also describes the role and limitations of fiducial markers in making reconstructions from tilt series taken around two orthogonal axes (Mastronarde, 1997; Penczek *et al.*, 1995) and the complementary role that cross-correlation can play in this process.

2. DEFINITION OF VARIABLES AND PROJECTION EQUATIONS

Figure 1A depicts the geometry of the projection during a tilt series. There are three coordinates systems to consider: that of the microscope, the specimen and the projection images. The coordinate system of the microscope coincides with that of the specimen before it is tilted; the axes in both will be referred to as x, y and z. The axes of the projection images are designated u and v. The fiducial points are described by the following:

n_T is the total number of fiducial points
$\mathbf{r}_j = (x_j, y_j, z_j)$ are the coordinates in the specimen of the jth fiducial point, $j = 1, \ldots, n_T$
$\mathbf{p}_{ij} = (u_{ij}, v_{ij})$ are the measured projection coordinates of the jth point in the ith view
n_i is the number of points measured in the ith view (not all need to be measured)
V_i is the set of points measured in the ith view
$j \in V_i$ means that the jth point was measured in the ith view

The equations presented next represent a relatively complete model of the projection process, but in practice only a subset of the variables