6

Markerless Alignment in Electron Tomography

Sami S. Brandt

1. INTRODUCTION

In computing high-accuracy reconstructions from transmission electron microscope (TEM) tilt series, image alignment currently has an important role. Though most are automated devices today, the imaging systems have certain non-idealities which give rise to abrupt shifts, rotations and magnification changes in the images. Thus, the geometric relationships between

Sami S. Brandt • Helsinki University of Technology, Laboratory of Computational Engineering, PO Box 9203, FI-02015 TKK, Finland

the object and the obtained projections are not precisely known initially. In this chapter, *image alignment* refers to the computation of the projection geometry of the tilt series so that most of the above deviations from the assumed ideal projection geometry could be rectified by using simple 2D geometric transformations for the images before computing a tomographic reconstruction.

An accurate way to compute the image alignment is by using the conventional colloidal fiducial gold markers. Marker-based alignment methods are accurate, first, because gold beads can be localized very accurately due to their spherical shape and typically high contrast to the background and, secondly, the localization errors are averaged out if a large number of beads is used (see Brandt *et al.*, 2001b). However, it is not always possible to use markers. The markers, being electron-opaque, can interfere with the reconstruction (Frank *et al.*, 1987). Moreover, even if markers were sprinkled on the preparation, they might not be visible at all in an interesting part of the preparation, or there might be too few of them to compute the alignment parameters (Brandt *et al.*, 2001a). Also, with freely supported objects, the use of markers may be problematic (Liu *et al*., 1995).

In this chapter, we will consider automatic, marker-free image alignment methods designed to avoid the above problems of the marker-based alignment methods. In fact, different kinds of markerless approaches have been proposed. For instance, the cross-correlation-based alignment has been used for a long time to compute the translational alignment for tilt series (see, for example, Frank and McEwen, 1992; Frank *et al*., 1987; Guckenberger, 1982). A modern, completely different approach for the problem is feature-based alignment (Brandt *et al.*, 2001a; Brandt and Ziese, 2006), where automatically extracted points of interest are automatically tracked through the image series to solve the alignment parameters. In our taxonomy, the third category is formed by the 3D model-based methods where the projection geometry is refined with the help of an intermediate reconstruction (Brandt and Kolehmainen, 2004; Brandt *et. al*, in preparation; Dengler, 1989; Owen and Landis, 1996).

The organization of this chapter is as follows. The geometry of the alignment problems is first briefly described in Section 2. In Section 3, we review the cross-correlation-based alignment and the alignment by the common line. Section 4 is devoted to feature-based methods and Section 5 to 3D model-based alignment. In Section 6, we present some alignment examples, and concluding remarks are found in Section 7.

2. THE GEOMETRIC PROBLEM SETTING

For the TEM, a parallel projection model is usually assumed. The general parallel projection model is also known as an *affine camera model* and it includes the orthographic, scaled orthographic and para-perspective