Journal of Structural Biology 192 (2015) 403–417

Contents lists available at ScienceDirect

Journal of Structural Biology

journal homepage: www.elsevier.com/locate/yjsbi

A novel fully automatic scheme for fiducial marker-based alignment in electron tomography

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ARTICLE INFO

Article history:
Received 21 May 2015
Received in revised form 25 September 2015
Accepted 30 September 2015
Available online 1 October 2015

Keywords:
Electron tomography
Alignment
Point set registration
Four point invariant

ABSTRACT

Although the topic of fiducial marker-based alignment in electron tomography (ET) has been widely discussed for decades, alignment without human intervention remains a difficult problem. Specifically, the emergence of subtomogram averaging has increased the demand for batch processing during tomographic reconstruction; fully automatic fiducial marker-based alignment is the main technique in this process. However, the lack of an accurate method for detecting and tracking fiducial markers precludes fully automatic alignment. In this paper, we present a novel, fully automatic alignment scheme for ET. Our scheme has two main contributions: First, we present a series of algorithms to ensure a high recognition rate and precise localization during the detection of fiducial markers. Our proposed solution reduces fiducial marker detection to a sampling and classification problem and further introduces an algorithm to solve the parameter dependence of marker diameter and marker number. Second, we propose a novel algorithm to solve the tracking of fiducial markers by reducing the tracking problem to an incomplete point set registration problem. Because a global optimization of a point set registration occurs, the result of our tracking is independent of the initial image position in the tilt series, allowing for the robust tracking of fiducial markers without pre-alignment. The experimental results indicate that our method can achieve an accurate tracking, almost identical to the current best one in IMOD with half automatic scheme. Furthermore, our scheme is fully automatic, depends on fewer parameters (only requires a gross value of the marker diameter) and does not require any manual interaction, providing the possibility of automatic batch processing of electron tomographic reconstruction.

1. Introduction

Electron tomography (ET) techniques have become an indispensable tool in structural biology. In ET, the three-dimensional density of the ultrastructure is reconstructed from a series of micrographs (tilt series) taken in different orientations. Generally, the geometrical information about the tilt series can be recorded by the electron microscope. However, the projection environment may be affected by mechanical instability, and inevitable transformations and deformations of the sample occur during data collection. Therefore, to obtain high-quality three dimensional density maps, the projection parameters of the tilt series should be calibrated accurately before reconstruction.

Two main types of alignment methods are available for ET: marker-free alignment and marker-based alignment. Each type of method has its own applicability and limitations. Marker-free alignment (e.g., cross-correlation (Guckenberger, 1982) and common lines (Liu et al., 1995) used in coarse alignment, the iterative alignment methods combining cross-correlation with reconstruction and reprojection (Winkler and Taylor, 2006, 2013), and feature-based alignment methods that replace fiducial markers with features (Brandt et al., 2001a; Brandt and Ziese, 2006; Castaño-Díez et al., 2007, 2010; Phan et al., 2009; Sorzano et al., 2009; Han et al., 2014)) does not require fiducial markers to be embedded in the sample. However, the applications of

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http://dx.doi.org/10.1016/j.jsb.2015.09.022
1047-8477/

marker-free alignment are still limited by Signal-to-Noise Ratio (SNR), the intrinsic biological structure of the sample and the contrast of the micrographs. Fiducial marker-based alignment (Lawrence, 1992; Kremer et al., 1996; Frank, 2006) requires that fiducial markers be embedded in the sample. Because fiducial markers have a high contrast with the background, the positions of the markers can be determined precisely, which further improves the alignment accuracy. Therefore, fiducial marker-based alignment is most accurate and has been widely used for high resolution electron tomography. Particularly for cryo-ET datasets with low SNR, fiducial marker-based alignment could be the only choice (Amat et al., 2008). However, to be noted that, the embedding of fiducial markers may interfere with the sample and introduce undesirable artifacts in the reconstruction.

Fiducial marker-based alignment requires the positions and the corresponding relationship of markers in different tilted micrographs. Thus, marker-based alignment should first localize the embedded fiducial markers in micrographs and then track these markers throughout the tilt series. Although fiducial marker-based alignment has existed almost as long as structural biology, there are still no robust automatic schemes to perform the detection and tracking of fiducial markers. With the advent of the subtomogram averaging technique (Briggs, 2013), batch tomographic reconstruction becomes an urgent demand, and fully automatic alignment is the main technique for batch tomographic reconstruction.

The manual intervention in marker detection and tracking is the bottleneck of marker-based alignment for high-throughput tomographic reconstructions. A number of studies have focused on the marker detection and tracking issue. The fiducial marker detection is usually performed by template matching (Kremer et al., 1996; Brandt et al., 2001b; Zheng et al., 2007; Amat et al., 2008; Cao et al., 2011). However, the result of template matching is usually affected by the value of the threshold used to determine the similarity between the image patches and the fiducial marker template. Furthermore, the value of the threshold used in the template matching usually varies for different datasets, and an unsuitable threshold results in an unacceptable output. Current studies on fiducial marker-based alignment also have invested minimal effort in the refinement of the marker diameter parameter. Usually, the diameter parameter is set by users, and an incorrect value also can corrupt the result. The fiducial marker tracking is usually performed by local search between consecutive images, using the underlying local geometry information or local similarity of patches (Ress et al., 1999; Brandt et al., 2001b; Amat et al., 2008; Cao et al., 2011). The reason why previous studies employ local search to track fiducial markers is that if the problem is modeled as a globally consistent tracking, the required computational resources will be extremely large. The use of local geometry information can improve tracking speed, but it cannot guarantee global consistency. Here, we present a novel fully automatic scheme for fiducial marker-based alignment.

First, we developed a complete algorithm to solve the detection problem automatically. Two types of parameters are used in the detection of fiducial markers. The first type includes the information of fiducial markers, such as the marker diameter and the shape of the fiducial marker. The second type of parameters includes the thresholds used to detect of fiducial markers. A robust automatic detection of fiducial markers depends on reasonable adjustment and estimation of these parameters. First, we developed an algorithm to refine the marker diameter. Then, we convert the maker detection problem into a sampling and classification problem. By solving this problem, we can automatically detect the fiducial markers, estimate the number of markers in each micrograph and eliminate the dependence of the threshold used in the detection. Thereafter, we developed an algorithm to refine the localization precision of the detected markers.

After fiducial marker detection, we propose a global optimization algorithm to solve the tracking problem. Under the weak perspective projection model, corresponding image features in two respective 2D views from the identical planar surface are related by an affine transformation (Huttenlocher and Ullman, 1990; Koenderink and van Doorn, 1991). Based on the affine transformation relationship of the two projections, we reduce the tracking problem to an incomplete point set registration problem and propose a novel method to solve the matching of corresponding fiducial markers. Unlike traditional studies (Ress et al., 1999; Brandt et al., 2001b; Amat et al., 2008; Cao et al., 2011) that treat the matching of corresponding fiducial markers as a tracking problem and use the local geometry or similarity of patches to accelerate the tracking, our solution does not use the local geometry information. Because the optimization of the point set registration is globally consistent, our tracking method is independent of the initial state of the tilt series and allows inputs with unrefined fiducial marker positions (possibly contaminated by outliers and missing data). Furthermore, the optimization of the point set registration can be solved by common computational resources in an acceptable time.

Finally, we combine the detection and tracking methods into a fully automatic alignment scheme and evaluate the scheme with experimental data. The theoretical analysis and experimental results show the efficiency of the proposed scheme.

2. Method

The steps of our alignment scheme are illustrated in Fig. 1.[1] The first step is to refine the parameters of the fiducial markers and detect the fiducial markers in the tilt series. We develop an algorithm to estimate the diameter parameter of the fiducial markers. A novel sampling and classification algorithm is used to ensure the exhaustive detection of the fiducial markers, and a new algorithm used to refine the final positions of the extracted fiducial markers is also illustrated as a post-detection subprocess. The second step is to match corresponding markers. We design a novel algorithm based on the idea of incomplete point set registration to guarantee a globally consistent matching of two marker sets. The third step is to track matching pairs consistently across the tilt series and to stretch the tracks as long as possible. A strategy that accelerates the tracking and compensates for the missing of fiducial markers is used in this step. The final step is to optimize the projection parameters with the determined tracks and, when necessary, to transform the images geometrically.

2.1. Marker parameters refinement and marker detection

To detect fiducial markers, we need a clear definition of the appearance of fiducial markers in ET: the shape of a fiducial marker in a micrograph is a circular or nearly circular area with an outstanding contrast to the nearby area and the shape of an identical fiducial marker does not change substantially with

[1] Our system uses the inverted color image. For example, if the pixel value ranges from 0–1, 0 presents white and 1 presents black; if the pixel value ranges from 0–255, 0 presents white and 255 presents black.