REGISTRATION OF ELECTRON TOMOGRAPHY SINGLE-AXIS TILT-SERIES WITHOUT FIDUCIAL MARKERS

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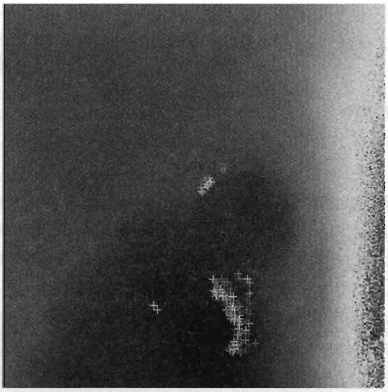
Single-axis tilt series are the standard data collection geometry in three-dimensional tomography of cellular organelles by electron microscopy and X-ray microscopy [1]. The alignment of tilt series is usually performed by the manual tracking of fiducial markers (usually gold beads) along the whole tilt series. This manual tracking is not always easy due to the inhomogeneous distribution of markers leaving areas with nearly no marker and areas with large concentrations of them (further complicating the tracking of the markers from one image to the next). Moreover, the presence of electron-dense gold particles in the micrographs shadows the structural information along the beam line at the particle location. This translates into artifactual stripes in the 3D reconstruction. Automatic image alignment methods that don't need any gold particle have already been published [2,3,4]. However, the challenges posed by the imaging conditions (especially related to the low Signal-to-Noise Ratio of the images) make the automatic image alignment task particularly elusive so that automatic tomography alignment methods have not yet become a standard procedure.

In this work we present an algorithm for automatic single-axis tilt series alignment that addresses the problem in a robust way by first finding an affine transformation between any pair of consecutive images in the series which serves as initialization for a subsequent step of automatic marker search. Specifically, a regular grid of possible markers is superimposed to each image in the series. Using the previously computed affine transformations, each point of the grid is tracked upstream and downstream in the tilt series while a local patch around the studied point correlates above a threshold that is automatically computed from the average correlation level found during the calculation of the affine transformations. During the landmark tracking process, the location of each landmark is locally refined to maximize the correlation of the two corresponding image patches. To avoid slow drifts of the region being correlated, the local patches are not only compared to the immediately adjacent patches but also to patches that are 2, 3 and 4 images away in the tilt series. All these comparisons should result into correlations above the automatically computed threshold. The output of this automatic landmark tracking is a set of thousands of short landmark chains spanning over a range between 5 and 15 images (considering a tilt series with a total number of images between 70 and 100) (see next figure for an example of an image of the tilt series with the automatically detected landmarks).



The chains of 2D landmarks are used to compute the 3D locations of the virtual landmarks and the image alignment parameters using mathematical formulas similar to those of [5], although modified so that to yield more compact algebraic expressions with closed form solutions. During the determination of the image alignment parameters a statistically robust approach has been followed in which those landmark chains with high regression residuals are discarded. This process is iterated three times. The reconstructions obtained with the alignment parameters estimated by our automatic algorithms are comparable to those obtained with a manual selection of the fiducial markers and will be published somewhere else (along with the specifics mathematics describing our model).

It is remarkable that the previously outlined procedure is not able to absolutely determine the location of the true tilt axis inside the microscope column, and it can be "virtually" set at the center of any region of interest in the micrograph tilt series. This fact is indirectly acknowledged in the previous literature but no formal proof has been given. In the following paragraph we outline such a proof.

Let $\mathbf{r}_0$ be a point of the tilt axis, and $\mathbf{u}_0$ its direction. Let $\mathbf{r}_i$ and $\mathbf{r}_j$ two 3D landmarks whose projections onto a given image are $\mathbf{p}_i = HR_\psi(A_{\mathbf{u}_0,\alpha}(\mathbf{r}_i - \mathbf{r}_0) + \mathbf{r}_0) + \mathbf{d}$ and $\mathbf{p}_j = HR_\psi(A_{\mathbf{u}_0,\alpha}(\mathbf{r}_j - \mathbf{r}_0) + \mathbf{r}_0) + \mathbf{d}$, where H is the projection matrix onto the XY plane, R_ψ is an in-plane rotation matrix, $A_{\mathbf{u}_0,\alpha}$ is the matrix accounting for the tilt rotation and $\mathbf{d}$ is an in-plane shift. The distance between the two projection points is $\mathbf{p}_i - \mathbf{p}_j = HR_\psi A_{\mathbf{u}_0,\alpha}(\mathbf{r}_i - \mathbf{r}_j)$, which is independent of $\mathbf{r}_0$, proving that the exact location of the tilt axis cannot be absolutely determined from the landmarks, since the absolute coordinates of the projection landmarks are unknown and can be arbitrarily shifted. Therefore, the only accurate information from the landmarks are their relative location and distances.

References

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