AUTOMATIC ALIGNMENT OF TEM TILT-SERIES WITH AND WITHOUT FIDUCIAL MARKERS

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INTRODUCTION

In order to successfully perform the 3D reconstruction in electron tomography, transmission electron microscope images have to be accurately aligned or registered. So far, the problem is solved by either manually showing the corresponding fiducial markers from the set of images or automatically using simple cross-correlation between the images on several rotations and scales. The manual approach has the disadvantage of being time consuming for the user and the previous correlation-based automatic methods are inaccurate because the transformations are computed between the consecutive images of the tilt series and the errors accumulate along the image series.

METHODS

We have proposed two methods where the registration is automated using ¹conventional colloidal gold particles as reference markers between images, or when fiducial markers are not available, ²tracking high curvature interest points of the intensity surface. We approach the problem from the computer vision viewpoint, and the alignment problem is divided into several subproblems: (1) Finding initial matches from successive images, (2) estimating the epipolar geometry between consecutive images, (3) localizing and matching the gold beads with a graph matching technique, or alternatively, matching the found interest points with a wavelet-based multiresolution technique, and (4) optimizing the transformation parameters for the whole image set.

RESULTS

We tested four tilt series of which two series were aligned by using markers and three by the method without markers. Example images are shown in Fig. 1. The reconstructions by the maximum entropy methods (MEM) are shown in Fig. 2. We evaluated the obtained level of accuracy by computing the standard deviation between the measured marker or feature point locations and the estimated noise-free counterparts. These deviations are summarized in Table 1.

CONCLUSIONS

We recommend to use gold markers whenever possible because gold beads can be localized very accurately. Moreover, the marker tracks are typically very long implying that the number of parameters to be solved is smaller. However, since the other method uses for the first time the true 3D motion model without any fiducial markers, it is the inevitable way for aligning the images in most accurate way when there are no markers available. Since the level of accuracy obtained so far is close to what has been achieved by using markers, the use of markers should become unnecessary from the alignment point of view in future.

REFERENCES

Table 1. Standard Deviation of the Localization Error after the Optimization.

<table>
<thead>
<tr>
<th>Method</th>
<th>Mitochondrion I</th>
<th>Chromosome scaffold</th>
<th>Microvillus</th>
<th>Mitochondrion II</th>
</tr>
</thead>
<tbody>
<tr>
<td>With Gold Markers</td>
<td>0.93</td>
<td>1.3</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>Without Gold Markers</td>
<td>-</td>
<td>2.5</td>
<td>0.80</td>
<td>3.4</td>
</tr>
</tbody>
</table>

Figure 1. Example images from the tilt series. (a) Whole-mounted CPD mitochondrion (120kV), (b) chromosome scaffold (100kV), (c) freely dangling microvillus (120kV), (d) another whole-mounted mitochondrion spanning a hole of the supporting film of the grid (120kV).

Figure 2. MEM reconstructions. The mitochondrion and chromosome scaffold on the top row have been aligned by gold markers. On the middle row the chromosome scaffold has been aligned by corners as well as the top part of the microvillus and the other whole-mounted mitochondrion on the bottom.