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The Precise Alignment and Auto-fusion of Correlative Cryo-SXT and Cryo-FM.

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Abstract:
Cell's sub-cellular architecture and function can be revealed by Cryo soft X-ray tomography (Cryo-SXT) and Cryo-fluorescence microscopy (Cryo-FM) respectively. To understand the connection between cells' structure and function, correlative Cryo-SXT and Cryo-FM has been applied. Here we introduce a semi-automatic precise alignment and fusion technology of Cryo-SXT and Cryo-fluorescence microscopy.

Introduction:
Cryo-SXT is a rapidly developing high resolution three dimensional image technology to reveal sub-cellular architecture and organization in a near-native state. Within 'water window' (2.34nm-4.4nm), water is relatively transport, and soft X-ray can readily penetrate biologic specimens up to 10 μm with high contrast. During the data collection of tomography, specimens need to be immobilized to avoid blurry details due to the movement of specimen's contents. Cryofixation is a suitable method with the capability of preserving the specimen in situ avoiding the artefacts caused by chemical treatment [1] and of reducing the dose-dependent radiation damage [2,3]. It's not enough to understand interconnected molecular interactions and chemical reactions in cells only having the knowledge of the sub-cellular architecture and organization [2]. As a vital complementary, the Cryo-FM can locate molecules which are essential for cell functions. The correlative method, integrating both the sub-cellular architecture and the information of specific molecules within one framework, connecting the cells' structure and function, is a useful technology for cell organelle discrimination, cell pathopoiesia and drug therapy mechanism researches.

We have built a Cryo-FM by installing a cryo-stage on a wide field fluorescence microscopy. With its air lenses and long work distance, it's difficult to get fluorescent images which are easy to be aligned with X-ray projections precisely [4]. To improve the alignment accuracy of X-ray projections and fluorescent images, we develop a precise alignment and auto-fusion method.

Method:
FluoSpheres (carboxylate-modified polystyrene microspheres, red fluorescent proprietary dye, excitation max.: 580 nm, emission max.:605 nm, 2% solids, catalog No. F-8810, Invitrogen, China), which have high contrast in X-ray projects and bright red fluorescence in fluorescent images, are used to provide positions in both images. After adding some FluoSpheres to the standard electron microscopy grid and plunging the grid into liquid ethane, the grid was transferred to be imaged by Cryo-FM and Cryo-SXT.

The resolution of Cryo-FM is not matched with the one of Cryo-SXT, however, if an object is small enough to be treated as a single 'point-like' particle, then the center of it can be located precisely by some algorithms [5,6] and the accuracy can be much better than the spatial solution of the fluorescence microscopy. Here we used a MATLAB script to help dealing with these images. First, the rough regions containing fiducial markers were captured manually, then centers of FluoSpheres were located with the accuracies (σ_X: the accuracy of locating FluoSpheres in X-ray projections; σ_F: the accuracy of locating

FluoSpheres in fluorescence images) by quadratic fitting. Next an algorithm based on similar triangles was used to align images with different modalities according to the positions calculated before, and the alignment accuracy σ_A was calculated [7]. σ_X, σ_F and σ_A, can be treated as independent. Thus, the overall uncertainty is given by $\sigma_T = \sqrt{\sigma_X^2 + \sigma_F^2 + \sigma_A^2}$. After the alignment of images, we fused aligned images by an algorithm based on HSI (Hue, Saturation, Intensity) color space.

Result:
Figure 1 shows a 2D projection of FluoSpheres. Processed by methods mentioned above, we got the final fused image and the accuracies. The result shows that the overall accuracy is about 100 nm, better than wide field fluorescence microscopy.

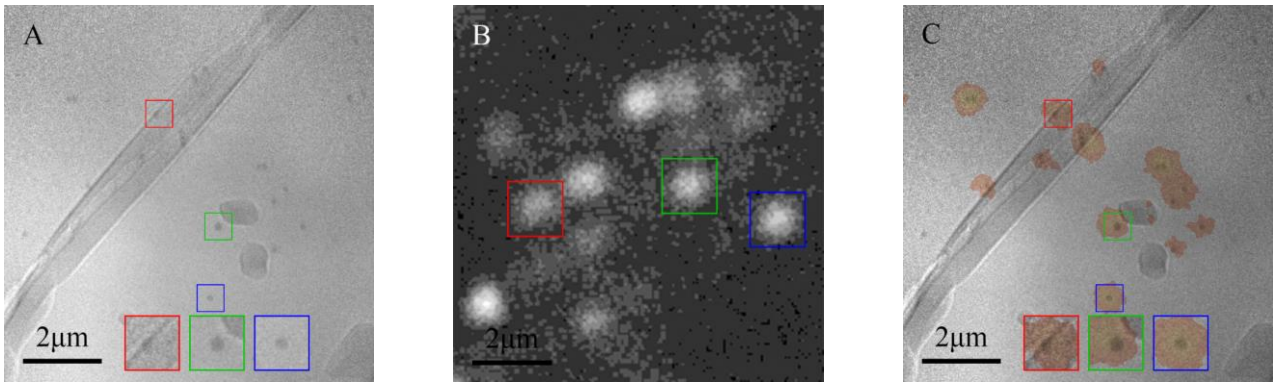

Figure. 1. Images of FluoSpheres (200 nm) captured by Cryo-SXT (A) and Cryo-FM (B), and fused image (C). Colored squares indicate three different FluoSpheres. The accuracies σ_X, σ_F and σ_A are 31 nm, 42 nm and 85 nm, the overall uncertainty σ_T is calculated as 100 nm.

by density, distribution and the manual choice of FluoSpheres. The alignment accuracies are also limited by the 2D fluorescent imaging method, because FluoSpheres are probable not in the focal plane at the same time, and this is not matched with the 3D imaging capability of SXT. The accuracy would be improved if both image were obtained by 3D methods.

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