3D registration of Light Sheet Fluorescence Microscopy datasets using decomposition to 2D projections

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ABSTRACT

A very popular microscopy technique is Light Sheet Fluorescence Microscopy (LSFM, SPIM) [1] where a light-sheet is used to illuminate a sample in sequential slices vertically to a camera system. The resulting stacks of slices render a tomographic representation of the sample. To remove regions affected by occlusion or scattering, scans from different viewing angles (0°, 90°, 180°, 270°), are usually acquired. To utilise the complementary information image registration is necessary to resolve any misalignment issues and transform different sets of data into one common coordinate system. The registration of 3D volumes especially when common information between the volumes is sparse and obstructed is a difficult task and added landmarks, such as fluorescence beads [2] have been used in the past to aid the registration process, by transforming the problem of the registration of the sample to the one of registering landmarks that reside in the empty space around the sample. Yet, suitable landmarks are not always available and their introduction increases the complexity of the imaging methodology.

We propose a method that reduces the dimensionality of the 3D problem of aligning the various viewpoints to the sequential registration of 2D projections (for example, maximum intensity projections) created from the initial volumes. We should note that the individual registration of the projections can be performed using any fast 2D image registration algorithm and the current implementation has the option to choose either Random Sample Consensus (RANSAC) based on automatic SIFT points algorithm or image based methods such as square differences or mutual information. Finally, for difficult datasets, where not much common information exists among views, an easy semi-automatic selection of few artificial 2D landmarks (usually 4-5) can be used. Using the projections registration we managed to create volumetric images of high information for multiangle LSFM, and overcome the problems arising from the high complexity of the 3D algorithms. We achieved fast and accurate results without the need for the introduction of artificial fluorescence beads. Our implementation can be easily used by an experimentalist, and it has shown to work even on samples with minimal common information between angles.

REFERENCES

1. M. Rieckher PLoS ONE 10, (2015) .
2. S. Preibisch, *Nature Methods*, **7, (**2010)