**Evaluate and implement a processing pipeline to fuse multi-focus non-gridded volumetric dataset**

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Ever since the invention of expansion microscopy (ExM)[1], the physical magnification of the specimen itself has become the new norm. Yet, its gigantic size compared to receptive fields of common objectives causing one must endure the endless gridded volumetric acquisition and 3D image stitching. For lightsheet microscopes, which are well-known for their optical performance, one way to solve this problem is to extend their lightsheet coverage, but sacrificing its optical sectioning ability will become inevitable. Luckily, the invention of tiling lightsheet selective plane illumination microscopy (TLS-SPIM)[2] utilizes discontinuous lightsheets to decrease the number of tiling requirements per planes, alleviate one from choosing between excessive gridded scan or inferior axial sectioning (Figure 1a). This leaves one last problem to solve – an efficient way to stitch multiple in-focus regions spanning across volumes.

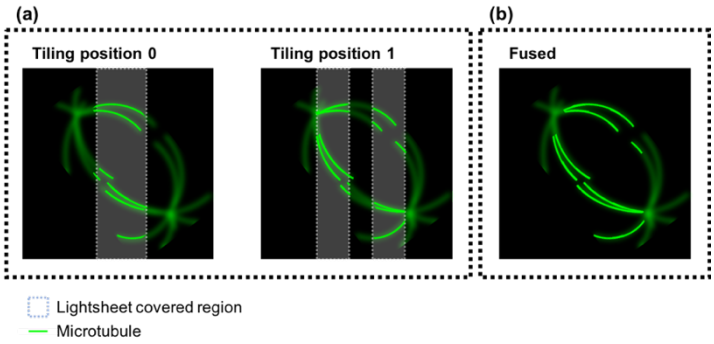


Figure 1: Schematic figure of the fusion process. (a) Different tiling positions yield different in-focus regions. (b) Fuse different in-focus regions from (a).

Despite the fact that image stitching is widely applied in smartphones, those sophisticated algorithms have yet to be adapted for monochrome low signal-to-noise ratio (SNR) volumetric datasets. Here, we provided a complete processing pipeline that can register non-gridded 3D datasets, determine in-focus region from overlapped regions and fused these regions using region mosaicking on Laplacian pyramids (RMLP)[3] as an all-in-focus image (Figure 1b). In this talk, we will walk through how normalized cross-correlation of multiple maximum intensity projections (MIP-NCC) was decided to be adopted in this pipeline, and the differences between various algorithms and available programs. Next, we will compare different fusion schemes for volumetric data in terms of authenticity and visual quality. In all, we would like to demonstrate how one can evaluate and build up a processing pipeline that adapts algorithms for specific bioimage needs in the Python ecosystem, and eventually, pack as a library to share with the bioimaging community.

**REFERENCES:**

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