

## Imaging Transient Blood Vessel Fusion Events by Correlative Volume Electron Microscopy

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In a world where the use of high resolution electron microscopy to determine the structure of single molecules has become routine, the challenge of obtaining high resolution information from entire samples of cell cultures, tissues and whole organisms remains largely unexplored.

Recent innovations in Volume Electron Microscopy have led to a shift towards high resolution imaging of large volumes of biological samples. In Focused Ion Beam/Scanning Electron Microscopy (FIB/SEM) [1,2,3] and Serial Block Face/Scanning Electron Microscopy (SBF/SEM) [4,5] a slice of material is removed in situ and the revealed surface is imaged using the scanning electron beam. This cutting and imaging process is repeated sequentially to automatically collect a stack of high resolution images through the sample. In the FIB/SEM a gallium ion beam, which is inherently destructive, is used to ‘mill’ or ‘sputter’ away material whereas the SBF/SEM uses a modified ultramicrotome inside the SEM chamber to remove material using a diamond knife. These techniques exploit different sectioning methods to cut and image slices of material automatically. Much of the work in this area has been driven by neurobiology, where traditional transmission electron microscopy (TEM) inadequately copes with the conflict between volume imaging of complex neural networks and high resolution imaging of neuronal connections[2,5,6]. In general, samples for these methods, are prepared as for traditional TEM. Care is then taken to manually trim the resin block, containing the sample, so that the region of interest of the sample is easily accessible to imaging and further cutting within the microscope.

We set out to develop and apply volume EM techniques to study a highly complex three-dimensional network, that of the developing circulatory system. The formation of new blood vessels, angiogenesis, is crucial in the patterning of the vascular system during vertebrate embryonic development in normal physiology and in pathological settings such as chronic inflammation, tumour progression and metastasis. Here we use live confocal microscopy to identify the point of fusion between growing blood vessels of the transgenic zebrafish *Tg(fli1: EGFP)<sup>γ1</sup>* [6] and subsequently obtain high resolution information by tracing through the structure of the organism using FIB/SEM and SBF/SEM. The resulting data give unprecedented microanatomical detail of the zebrafish, and for the first time allow visualization of the ultrastructure of a time-limited biological event within the context of a whole organism

## References

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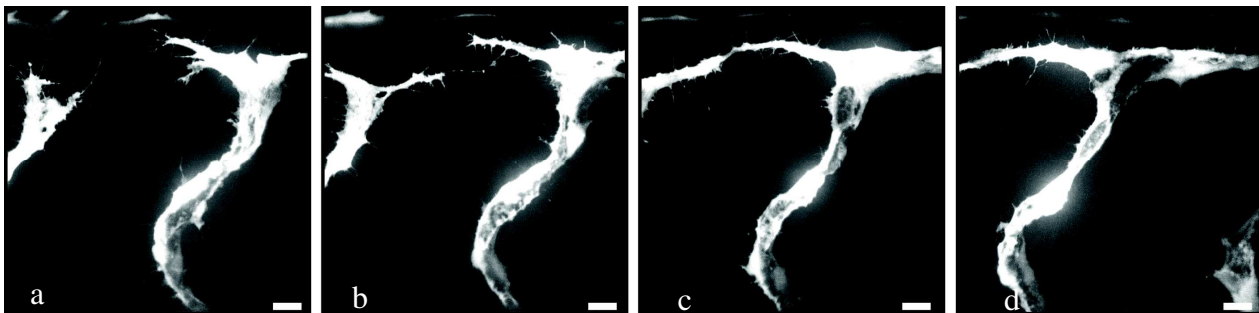


FIG. 1. *In vivo* live confocal imaging of the anastomotic process showing two adjacent ISVs sprouting from the dorsal aorta and extending filopodia which eventually fuse to form the DLAV. Bar = 10 $\mu$ m

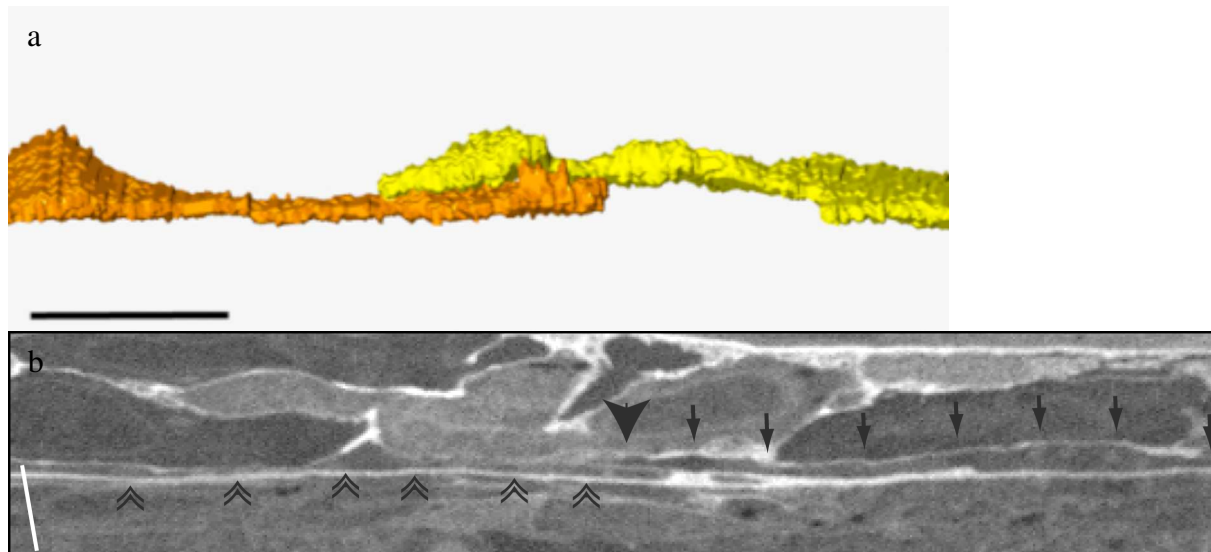


FIG. 2. Features within the FIB/SEM dataset were segmented using Amira (Visage Imaging Inc.). Endothelial cells from adjacent ISVs (2a; small arrows, one cell and double arrowheads the other cell) form a thin layer between the neural tube and the somites. At the point of anastomosis filopodia from each cell overlap and contact (large arrow head). High magnification of the AOI from the CurvedSlice (2b). Bar (a) 3  $\mu$ m, (b) 2.5  $\mu$ m unidirectional.