

3D Microscopy, Image Processing, and Analysis of Lymphangiogenesis in Biological Models

Authors : Thomas Louis, Irina Primac, Florent Morfoisse, Tania Durre, Silvia Blacher, Agnes Noel

Abstract : In vitro and in vivo lymphangiogenesis assays are essential for the identification of potential lymphangiogenic agents and the screening of pharmacological inhibitors. In the present study, we analyse three biological models: in vitro lymphatic endothelial cell spheroids, in vivo ear sponge assay, and in vivo lymph node colonisation by tumour cells. These assays provide suitable 3D models to test pro- and anti-lymphangiogenic factors or drugs. 3D images were acquired by confocal laser scanning and light sheet fluorescence microscopy. Virtual scan microscopy followed by 3D reconstruction by image aligning methods was also used to obtain 3D images of whole large sponge and ganglion samples. 3D reconstruction, image segmentation, skeletonisation, and other image processing algorithms are described. Fixed and time-lapse imaging techniques are used to analyse lymphatic endothelial cell spheroids behaviour. The study of cell spatial distribution in spheroid models enables to detect interactions between cells and to identify invasion hierarchy and guidance patterns. Global measurements such as volume, length, and density of lymphatic vessels are measured in both in vivo models. Branching density and tortuosity evaluation are also proposed to determine structure complexity. Those properties combined with vessel spatial distribution are evaluated in order to determine lymphangiogenesis extent. Lymphatic endothelial cell invasion and lymphangiogenesis were evaluated under various experimental conditions. The comparison of these conditions enables to identify lymphangiogenic agents and to better comprehend their roles in the lymphangiogenesis process. The proposed methodology is validated by its application on the three presented models.

Keywords : 3D image segmentation, 3D image skeletonisation, cell invasion, confocal microscopy, ear sponges, light sheet microscopy, lymph nodes, lymphangiogenesis, spheroids

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