Structure of Smallest Blood Vessel Networks

Registration of Serial Sections for 3D Reconstruction

Even with state-of-the-art imaging techniques in medicine, visualizing the smallest blood vessels in organs like the human spleen or bone marrow has so far not been possible at high resolution. With a new method for the registration of immunohistologically stained serial sections this has become possible. Now it is possible to visualize the highly complex network of these blood vessels in three dimensions.

Introduction

In immunohistology, a common technique in medicine and biology, cell specific glycoproteins are stained in serial sections using antibodies. With this technique, molecules that only exist in cells lining smallest blood vessels (capillaries) can be marked visually. This is however only possible with the aid of thin sections that are made from a specimen using a specialized sectioning device (microtome) and that usually have a thickness of 5 to 7 micrometers. For further digital processing, these tissue sections are first scanned using an optical scanning microscope.

Afterwards, the images of many successive sections have to be registered exactly to reconstruct the three-dimensional structure of the blood vessels. As the sections are very thin, the tissue is inevitably deformed during sectioning. This is a huge problem, since different deformations occur in each section and thus adjacent sections cannot be merged correctly. First, the corresponding regions in adjacent sections have to be detected and then each section has to be rectified digitally [1]. Then a three-dimensional reconstruction of the blood vessel network can be reconstructed using segmentation of the staining colors [2].

Registration and Rectification

The images of the sections have a resolution of more than 1000 megapixel (e.g. 30000 x 40000 pixel) due to the high resolution of the scanning microscopes and the size of the regions examined. First, the sections have to be roughly aligned since they have been placed on the specimen holder by hand and are therefore not oriented consistently. The rotation and translation of the sections with respect to each other is calculated which however does not compensate for the deformations.
For rectification, the specimen is mathematically expressed as deformable surface using a B-spline surface which is commonly used in computer aided design. The rectification is performed at several resolutions. In each step, the total deformation of all sections is minimized in order to reconstruct the original shape as accurately as possible. Due to the chosen representation only a sparse linear equation system has to be solved and therefore the method is applicable for such high resolutions.

For both, alignment and rectification, a feature matching approach is used. First, distinctive image regions are determined using feature detectors like SIFT or SURF. As the features also contain a description of their neighborhood, they can be compared for similarity. In the first step, a rotation and translation is determined that maps as many corresponding features of adjacent slices onto each other as possible. For the rectification, only features in a local neighborhood, that are also similar, need to be considered. In addition to a reduced complexity when searching corresponding features, this particularly solves the ambiguity problem originating from the high self-similarity of the tissue structure. In addition to refining the control grid with every increase of the resolution, the feature size is also reduced. Furthermore, the search radius for corresponding features can also be reduced since larger deformations have already been compensated for at coarser resolutions.

The result of these computations is a series of section images that very accurately fit together. The images can be merged into a three-dimensional volume data set after this registration and rectification. This can then be visualized directly or processed further.

**Three-Dimensional Reconstruction**

The image data are first filtered and cleaned after registration and rectification. Then the surface of the blood vessels can be extracted from the volume data. The pixels are first classified, i.e. it is determined if and with which color they are stained. Then a density distribution in the volume is determined for each color and
a so-called iso-surface is defined at the boundary between stained and unstained regions. The iso-surface is then approximated with a triangle mesh. Finally, the number of triangles in the generated mesh is reduced using a simplification algorithm as the mesh contains too many triangles for further processing. The number of triangles is minimized for a given error bound (usually half of the pixel resolution).

The proportions are realistically depicted when extracting the surface as a triangle mesh. The shape and location of the blood vessels in biological specimens becomes much more apparent in a 3d reconstruction than in a single section. The accuracy and correctness of the reconstruction is finally validated by overlaying single serial sections with the reconstructed surface.

The method is of great interest for medical basic research which has still not precisely understood the complex network of blood vessels in the spleen and bone marrow. In the current state it is however still too slow for use in medical diagnosis due to the enormous amount of data that needs to be processed. Using 3d reconstruction it has e.g. been discovered that the capillaries in the human spleen are open ended and the blood flows freely outside of the blood vessels for a short distance. Using the system, we could also show that the two so far known types of smallest blood vessels in the iliac crest (capillaries and sinuses) do not run in succession but side by side [2]. We clarified by immunohistological methods which antibodies have to be used to completely detect all microvessels in the bone marrow.

The research project was made possible by the donation of iliac crest bone marrow samples by the Department of Oral and Maxillofacial Surgery of the University Hospital of Marburg, with the consent of the patients and the relevant ethics committee. These samples had been left over from operations in which bone disorders were treated using material from the iliac crest. Other tissue samples were from patients whose spleens had to be removed due to life-threatening fissures that resulted from abdominal trauma.

**Application for Research on Lymphatic Organs**

The system will next be used to investigate lymphatic organs like the tonsils and for further examination of blood vessel network sections in the spleen. The lymphocytes are of special interest in this project. These white blood cells, which are crucial to the immune system, form round accumulations in the lymphoid organs called follicles. Lymphoid follicles have a diameter of about one millimeter which is a huge distance in terms of microscopy, and which can only be achieved with several hundred serial sections. For this, the development of methods to
reduce the number of serial sections is necessary to analyze the different cell types in a complete follicle. The goal is to clarify how the lymphocytes in a follicle cooperate in an immune reaction and how they migrate into and out of the tissue and the mucosa.

**References**


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