Revolutionizing Microscopy – One Image at a Time

GPU-based algorithms for quantitative microscopy of liver tissue organization with subcellular resolution

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Automated computer aided workflows have changed how Biologists can understand the inner workings of a cell. Experiments with thousands of parallel samples, all just slightly different, can now be carried out, analysed and influenced in almost real time. The compute power of GPUs has further enhanced this method to open up new dimensions and even increase image resolution beyond the limits of the microscope's optics. The following is an example how the ground-breaking methods, developed by the team of Yannis Kalaidzidis can be used to understand the inner workings of liver cells – one step further to understand how cancer can happen and how it can prevented. The used Motiontracking software is now also GPU enabled, allowing the step from 2D to 3D microscopy.

The liver is a key organ performing tasks vital to life, such as metabolism of nutrients and xenobiotics, as well as detoxification. It is remarkably dynamic, being able to constantly renew its cells and regenerate 2/3 of its mass after partial hepatectomy. Liver function depends not only on the presence of specific cells (hepatocytes, sinusoidal endothelial, stellate and Kupffer cells) in the tissue, but also on their characteristic arrangement into a unique microarchitecture.

Hepatocytes exhibit an apico-basal polarity that is more complex than the one in simple epithelia, e.g. the cells lining the kidney or intestine, which display a "simple" apico-basal polarization state. The apical membranes of hepatocytes, however, form a three-dimensional (3D) narrow belt between adjacent cells, which collectively give rise to the bile canaliculi (BC) network, an essential component for bile secretion and overall liver function. The different cell-cell contacts in the tissue allow the hepatocytes to develop multiple apical and basolateral surfaces per cell giving rise to a complex 3D tissue organization. It is, thus, very important to understand the specific structural organization of hepatocytes and their interactions with the sinusoidal endothelial cells in order to understand liver function and homeostasis. Our aim is to discover the basic rules of how

the liver tissue is built from its cellular constituents and generate quantitative parameters of cell and tissue organization that can best provide mechanistic insights in the analysis of phenotypes.

In order to achieve a comprehensive characterization of liver tissue organization, we used simultaneously up to 4 different markers in high-resolution microscopy of thick (up to 100 µm) slices of liver tissue, using a combination of single- and 2-photon laser excitation (the size of one 3D image is within the range of 1.2÷2 GB).

For the quantitative analysis of cellular and tissue architecture from state-of-the-art 3D fluorescence microscopy images, we have developed a set of GPU-based algorithms. These algorithms were integrated in our image analysis platform, the MotionTracking software (Fig.1). In order to accelerate the algorithm development process we implemented in the development environment the possibility to update the GPU-based code without restarting the application and reloading image data in CPU-running application. To this end, we designed a set of low-level computational procedures that allowed us to align z-stacks acquired in different modalities (2-photon and 1-photon) and correct standard errors of the scanning confocal microscope (e.g, chromatic aberrations, uneven illumination in the large 3D volume, partial acquisition/data transfer failure, high NA objective point spread function (PSF) distortion in the deep slices). Some algorithms, such as the one for the distortion correction and section alignment require **fit free parameters of model**. Fitting leads to hundreds-to-thousands calculations of goal function over the whole image, which would be practically impossible without the GPU.

At a higher level of image analysis, we designed and implemented a toolbox of algorithms to achieve the reliable, high-resolution segmentation of liver structures. We developed a tailored and robust algorithm for the identification of the sinusoidal network (i.e., the small ramified endothelial cell network within the liver lobule), for the identification of hepatic cell walls and for the precise estimation of the BC, the interconnected network of apical surfaces (Fig.2). The cell and canaliculi identification presented different challenges that required the development of specialized solutions to achieve high-quality automated image analysis. For this, we developed active mesh based algorithms for the reconstruction of hepatocytes and their interconnections (Fig.3).

Next step in the GPU development will be the translation of developed algorithms for GPU-clusters for the automated analysis of medium-throughput experiments.

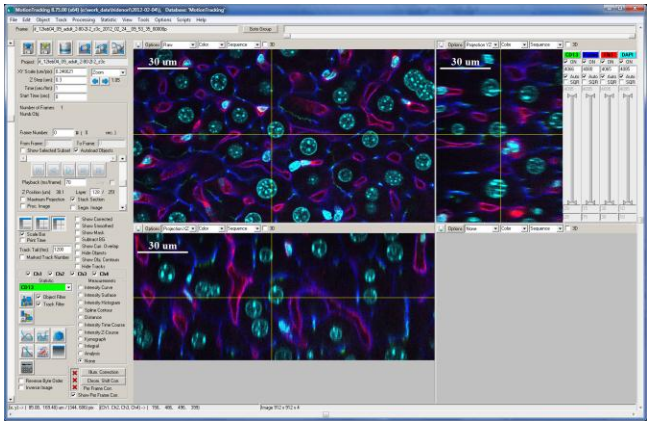

Fig.1 3D 4-color image of liver section in MotionTracking

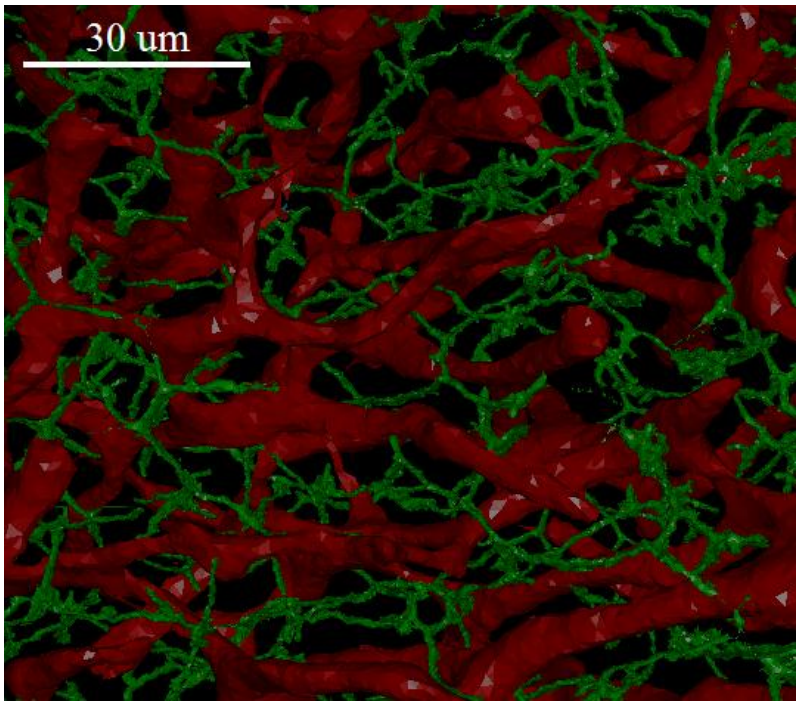

Fig.2 – triangulated sinusoidal (red) and bile canaliculi networks

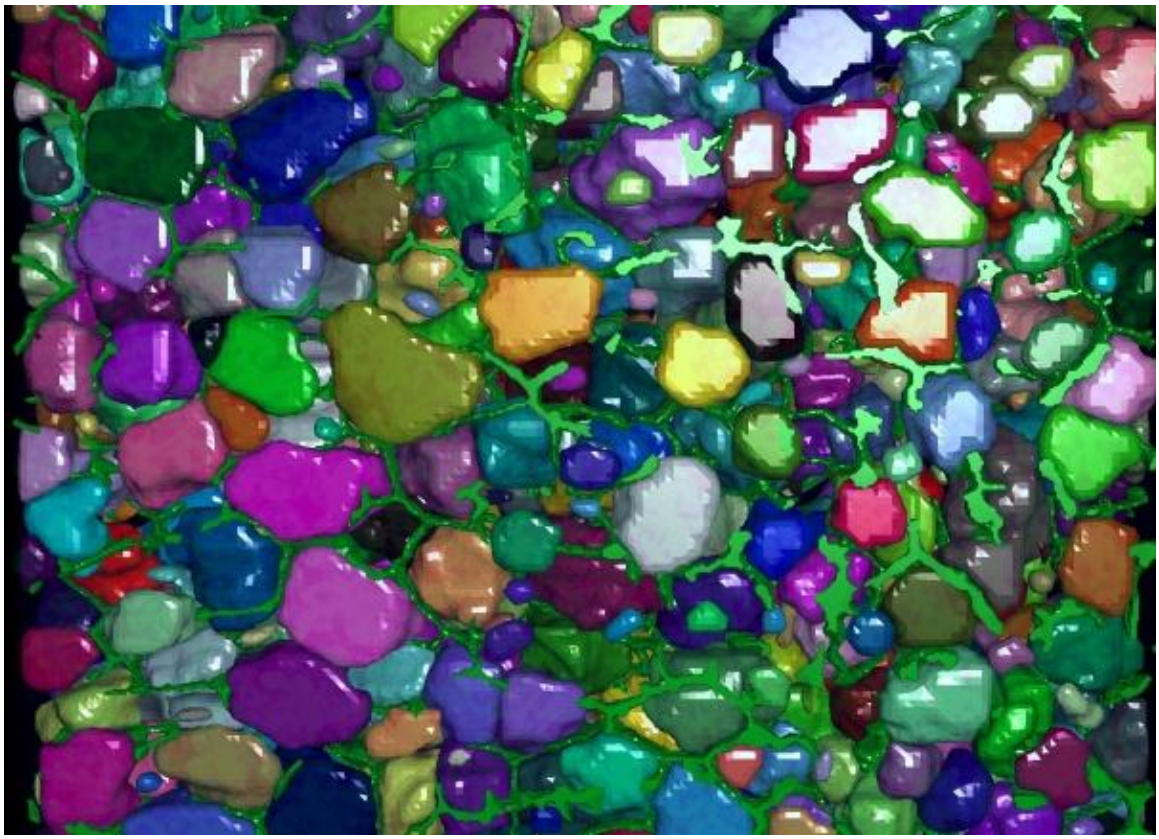

Fig.3 Hepatocytes shape reconstruction by active mesh.