Automatic image analysis of third harmonic generation neuropathology images

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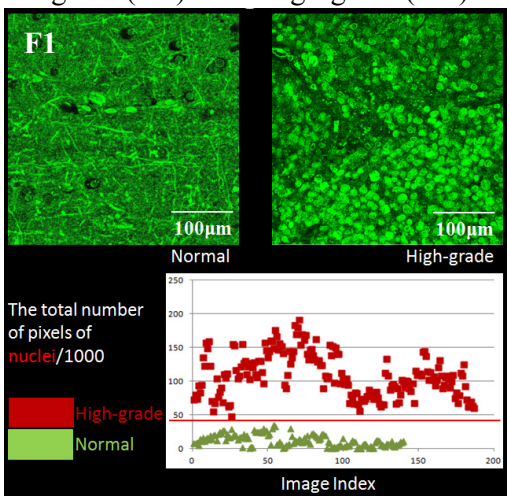
Gliomas are the most devastating type of human brain tumors as they show very extensive growth into the surrounding brain areas. New imaging techniques for visualizing tumor margins during surgery are needed to improve surgical outcomes. THG was shown recently to yield label-free images of *ex-vivo* human brain tumor tissue of histopathological quality. The observed morphologies, e.g. increased cellularity, nuclear pleomorphism and rarefaction of neuropil, are similar to the standard histopathological criterion currently used by pathologists, making the transition from the current practice to THG images relatively easy [1]. To strengthen the potential of THG for *in-situ* pathology of human brain tumors, we have developed several novel automatic image processing tools to reconstruct the noise-free THG images and to extract the pathological relevant features presented in normal brain [2,3].

In this work, we propose an integrated workflow to quantify the pathological relevant features presented in THG images of normal brain and tumor tissues. In brief, histogram truncation and local histogram equalization are applied to enhance the image contrast. The enhanced images are denoised and segmented by novel anisotropic diffusion and active contour algorithms as detailed in [2,3]. Pattern describers such as object size, sphericity and eccentricity, are used to discriminate cells, nuclei and neuropil, and to enable the quantification of each feature.

We applied the proposed workflow on around 500 2D THG images of 2 normal brain, 6 low-grade (LG) and 2 high-grade (HG) tumor tissues. The pathological relevant features were quantified with high accuracy. We found that the THG images of normal brain, LG and HG tumor can be distinguished by pairwise comparison, using one single feature. One relatively easy example of differentiating HG tumor from normal brain is shown in **F1**. The HG tumor is characterized by high nuclei density while the nuclei density in normal brain is relatively low.



We have developed in this work an integrated workflow to differentiate THG images of normal brain, LG and HG tumor from each other. Our work facilitates the application of THG images to *in-situ* visualize and interpret tumor margins because no human expert is needed.

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