Summary

Patients with diffuse glioma are still associated with very poor survival. The widespread nature of diffuse gliomas makes this type of tumors extensively invade into the surrounding normal brain. The prognosis and therapy of patients with diffuse gliomas usually correlate with the extent of resection. The technologies currently used clinically cannot visualize the tumor boundaries of this tumor type. Therefore, a new imaging technique capable of directly revealing tumor boundaries with histopathological quality is highly desirable. Third harmonic generation (THG) is a label-free technique that shows great potential for this purpose. THG microscopy has been shown to provide real-time feedback of tumor boundary in fresh, unprocessed human brain tissues. The morphology observed by THG has an excellent agreement with standard H&E morphology. However, the hardware development is only half the job, and the other half, equally important, is to develop suitable image processing tools to quantify the morphology observed by THG.

In this thesis we focus on processing THG images of brain tissues, especially the automatic diagnosis of brain tumor. The automatic analysis of the acquired THG brain images has not been studied before and is challenging because of the 3-phase segmentation problem, low signal-to-noise ratio, intensity inhomogeneity, low local contrast, post-processing and validation. In **chapter 2**, all these challenges were initially addressed. The salient edge-enhancing model of anisotropic diffusion was developed to reconstruct all the salient dark and bright objects. A novel active contour model was proposed to detect both the dark and bright objects, by introducing the global intensity extremes into the CV model. The resulting model overcome the problem of intensity inhomogeneity by sacrificing some foreground pixels/voxels. THG images of structurally normal human brain tissue were used to test the developed algorithms and pipeline. **Chapter 4** and **chapter 5** generalized and deepened the main idea of active contour and anisotropic diffusion presented in chapter 2. Global intensity extremes were incorporated into more recent active contour models to deal with the intensity inhomogeneity present in THG images. A novel framework was proposed to accelerate the existing anisotropic diffusion models (including the model developed in chapter 2). Also, anisotropic diffusion was reformulated as a convex model, resulting in an efficient and easy-to-code algorithm. In **chapter 6**, the developed image processing tools were applied to detect key pathologically relevant features observed with THG microscopy in healthy and tumor human brain tissues. Statistical analysis of the density of the quantified features revealed the quantitative difference between tumor and healthy tissue. The generated density thresholds of these features enabled detection of tumor infiltration and tumor boundaries with high sensitivity and specificity.

The interpretation of the features is another important issue relevant to brain tumor diagnosis, which is usually linked to more standard imaging techniques. The link between THG and fluorescence/H&E has been established qualitatively only. In **chapter 2** & **chapter 3** of this thesis, we quantitatively compared THG brain images with fluorescence/SHG/auto-fluorescence images acquired simultaneously from the same tissue area. Such a comparison provided quantitative evidence that confirmed the interpretation of dark and bright objects as brain cells.

In summary, this thesis has significantly strengthened the clinical potential of THG microscopy as a tool for brain tumor diagnosis and surgery, in a way that proper image analysis tools have been developed.