

Quantification of Liver Fibrosis by SHG Microscopy

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[Introduction] Assessment of liver fibrosis mainly relies on tissue staining. However, it is difficult to obtain detailed information on liver fibrosis, such as fiber orientation or the volume of fibrous tissue, only by the staining methods. Therefore, a new quantitative imaging technique is now required in medical field. When collagen, the major component of fibrous tissue, is irradiated with an ultrashort pulse laser, the light with twice frequency of irradiated light is generated, which is called second harmonic generation (SHG). In the present study, we propose a new method to quantify liver fibrosis by SHG microscopy.

[Methods & Results] Liver tissue were obtained from mice with steatohepatitis that was induced by high-fat feeding (SH group), and also from mice with liver cirrhosis that was induced by intraperitoneal injection of carbon tetrachloride (LC group). Then the liver tissue were sliced to 5- μ m thickness. We customized SHG microscope equipped with a 810-nm femtosecond pulse laser. The laser power was set to 20 mW at the sample on the stage, and Galvano mirror was set to scan a 162 μ m square range. The samples were exposed to the laser for 10 seconds and 256 pixels square images were obtained. The intensity of SHG signal and fiber orientation based on SHG images were analyzed by Image J software. There was no significant difference in the integrated intensity of SHG between SH group and LC group. However, the fibrosis pattern was different between the two models; linear fibrosis was found in SH group, while dotted fibrosis was observed in LC group. The dispersion of fiber orientation was significantly large in LC group (27.6 ± 4.5 , $n=6$) when compared to SH group (12.8 ± 3.1 , $n=7$, $p=0.018$ vs. LC group), suggesting that fibers are randomly constructed in LC group.

[Conclusion] We have successfully detected liver fibrosis using SHG in two different pathological liver disease models. It has been shown that SHG microscopy could be a powerful tool for qualitative and quantitative assessment of liver fibrosis in medical field.

Keywords: second harmonic generation, liver fibrosis