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Label-free Imaging of Mouse Cancer Tissues by High-speed Stimulated Raman Spectral Microscope

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1. Introduction

Stimulated Raman scattering (SRS) microscopy enables us to observe biological specimens without chemical labeling because of the higher sensitivity and lower background signal compared to spontaneous Raman microscope [1-3].

We have been developing the key technologies for higher sensitivity and high-speed SRS imaging. The existent SRS microscope generates Raman images at the speed of 30 frames/sec with different Raman shift (ca. 300 cm^{-1} of bandwidth). We also found the multivariate analysis such as PCA (principal component analysis) and ICA (independent component analysis) resulted in picking up tissue structures such as cell nucleus, cytoplasm, lipid droplets and protein fibers from large amounts of SRS data [4].

We applied these approaches to the observation of mouse tumor lesion for the first time.

2. Materials and Methods

Mouse Tissue and Sample Preparation

Cancer grafted nude mouse (*balb/cAcJ nu/nu*) was prepared by injection of human pancreatic carcinoma cell lines (SUIT-2, 1E6 cells/mouse) into the tail of pancreas. After 32 days of the growth periods, tumor-bearing tissues (pancreas and liver) were resected and fixed in 10 % formalin. Formalin-fixed tissues were cryo-sectioned with a thickness of 100 μm. The tissue sections were sandwiched between two cover slips with phosphate buffered saline.

Data Acquisition and Multivariate Analysis

Spectral imaging was performed to obtain 91 Raman imaging data for a single area at 30 seconds. In order to obtain large scale image, the data acquisition was continued for the 24 adjacent areas. The datasets of different area were combined and analyzed. The number of data set was 24 for the data shown in Figure 1. The original software was developed with LabVIEW (National Instruments). Both of PCA and ICA generated the PC images and IC images, respectively. The IC image was colored as noted in [4].

3. Results

The IC image of the pancreas tumor lesion is shown in Figure 1. This composite image was prepared by merging three independent IC images which were specified arbitrarily. The cell nucleus, cytoplasm, blood cells and fibers with different colors were clearly visualized. The shape of tissue differed substantially between the cancer and normal region. The acinar cells inside normal region indicated rounded shape and small particles which seem to be zymogen granules which were distributed inside cytoplasm. On the other hand, the cancer cells inside cancer region indicated indefinite shape. The fibril formation between normal and cancer region was also observed. The detail of these images and the results of the metastatic focus in the liver will be also presented.

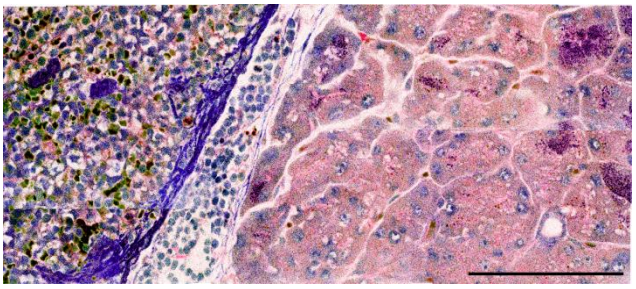

Figure 1
Spectral imaging of mouse pancreas cancer tissue. Image was produced by ICA. The right and left half area are corresponding to normal and cancer region, respectively. The scale bar is 100μm.

4. Conclusions

We have demonstrated the label-free imaging of mouse cancer tissues by both of high-speed SRS microscope and multivariate analysis. The characteristic images indicated the possibility of the differentiation between normal and cancer area of tissue without chemical staining technique.

References
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