Second Harmonic Generation Imaging of Collagen Network in Mouse Pancreatic Tumours

Application Note

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1 SHG OF COLLAGEN

Second Harmonic Generation (SHG) microscopy is a label-free non-linear optical microscopy technique that has been demonstrated as a powerful imaging modality for visualizing collagen architecture in tissues. SHG signal can be used to interrogate changes in collagen’s structural properties (fiber density, arrangement, organization, diameter, order…) which can reveal changes that occur in many diseases such as cancer.

SHG is a process that occurs when two photons are combined in an optically nonlinear medium, lacking in centrosymmetry, creating an SHG photon with a wavelength exactly half of the incident wavelength.

Because of its highly anisotropic structure, fibrillar collagen is very suitable for generating SHG signal.

2 SAMPLE

300 µm vibratome sections of pancreatic tumours obtained from a mouse model depicting Pancreatic Adenocarcinoma (fixed for 24 hours in 4% PFA and kept in PBS until sectioning) were imaged. Sections were mounted on standard microscopy slides and coverslipped using special imaging gaskets and kept hydrated with PBS.

3 IMAGING

SHG imaging was performed using an upright multiphoton microscope (TriM Scope, LaVision BioTec) coupled to a femtosecond Ti:Sapphire Laser (Coherent Chameleon Ultra II) tuned to an excitation wavelength of \( \lambda_{\text{exc}} = 836 \text{ nm} \). A 20 × 0.95 NA water immersion objective (Olympus) was used to focus the excitation beam and to collect the backscattered signal. SHG signal was detected on a PMT through a bandpass emission filter (420/50nm). Using ImSpector Pro software, a 3D mosaic scan (16x, 11y with 10% overlap) was performed to cover the entire tumour section. Each individual tile is a 556 × 556 µm z-stack of 5 µm z-step size covering the entire volume of the section.

4 IMAGE PROCESSING

Individual images were stitched using Arivis Vision 4D software and Maximum Intensity Projection of the entire z-stack was performed. The obtained image (total size 8053.66 × 5554.44 µm) reveals a dense and complex collagen network for the investigated mouse model.

Figure 1. SHG image of the collagen network in a mouse pancreatic tumour - Maximum Intensity Projection

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