

# IMPROVEMENT OF SECOND HARMONIC GENERATION MICROSCOPE USING PULSE COMPRESSOR

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## 1. Introduction

Second Harmonic Generation (SHG) and Two photon excited fluorescence (TPEF) imaging has been used over the past few years to **visualize extra cellular matrix (ECM) distribution** and tissue and cellular architecture [1, 2]. The near infra-red excitation **reduces scattering** and **increases depth of imaging**. SHG/TPEF imaging does not require any **extraneous staining**. Thus we can avoid the time consuming and tissue altering staining procedures. Tissue damage or photo bleaching is greatly reduced as energy is delivered to the samples in femtosecond pulses.

## 2. Aim

Our aim is to **reduce the problems of dispersion and hence improve the sensitivity of the imaging system to collagen in the ECM**. By reducing the Group Velocity Dispersion (GVD) the pulses are made shorter and all the wavelengths present in the beam reaches the sample at the same time. This ensures that higher average power is delivered to the sample, resulting in a more pronounced non linear optical phenomenon such as second harmonic generation and two-photon excited fluorescence.

## 3. Methods

Our SHG microscope is a confocal microscope with additional mode locked Titanium laser, which provides excitation wavelength in the range 710-990 nm. We used 900nm as our excitation wavelength. A pulse compressor was added to the beam path and the system was aligned to work in the presence and absence of the pulse compressor. Samples consisting of phantom collagen gels, muscle and liver samples and breast tumour tissues were imaged both in the presence and absence of the pulse compressor.

## 4. Results

In the presence of the pulse compressor in the beam path the signal to noise ratio (SNR) was 1.5 times better than the original system for both SHG and TPEF. The improved system can detect smaller strands of collagen present in tissue samples. The excitation power can be further reduced ensuring no tissue damage.

## 5. Conclusion

The improved SNR of 1.5 enables detection of small changes in collagen distribution. This is crucial in quantitative ECM distribution studies in in-vitro and in-vivo systems.

## 6. References

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- [2] Lee HS, et al, *Imaging human bone marrow stem cell morphogenesis in polyglycolic acid scaffold by multiphoton microscopy*, Tissue Engineering, 12 (10), 2006