ALTERATIONS IN EXTRACELLULAR MATRIX COLLAGEN OF HUMAN LUNG EXAMINED BY SECOND HARMONIC IMAGING.

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We aim to study the structure of the extracellular matrix (ECM) collagen in asthmatic, chronic obstructive pulmonary disease (COPD) and non-asthmatic airway tissue to determine if the three dimensional structure is altered, using a combination of second harmonic (SHG) and confocal imaging.

SHG imaging has been in use in biological systems for over a decade. One of the most common proteins imaged is collagen. It is the most important extracellular protein of the animal body. While there are several forms of collagen only Types I, II, III and V form fibrils. Type I collagen is highly crystalline which makes it an ideal candidate for SHG imaging [1]. In human lung types I, III, IV and V are present. In the asthmatic airway it has been identified that there is enhanced deposition of collagens I, III and V but collagen type IV is decreased [2]. We are therefore able to study the 3 dimensional structure of the fibrillar collagens with SHG imaging and simultaneously collect the fluorescent signal from immuno-labelled collagen IV. Human lung tissue was formalin fixed, paraffin embedded and cut to 30µM. There appears to be an increased amount of collagen in asthmatic compared to normal airway. Trypsin treatment is necessary for unmasking the antigen site to label collagen type IV. We investigated if this treatment caused a change in morphology of fibrillar collagen as revealed by the SHG signal. No morphological change was seen at this resolution. Also the autofluorescence signal recorded between 500 and 550 nm was not affected.

The COPD airway appears to have less collagen (as detected by SHG) compared to the normal airway yet the literature states that there is an increased amount of collagen in COPD (3). Thus it is possible that there is a different composition of collagen in COPD, with Type I (which gives the strongest SHG signal) reduced and other types increased.

Further studies are in progress, quantifying these differences and determining the 3D structural relationship with collagen IV and I.