Assessment of collagen in human idiopathic pulmonary fibrosis using second harmonics on Genesis 200

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ABSTRACT

Background It is important to quantify collagen in lung disease as it is associated with severity, progression & loss of organ function. Histochemistry relies on recognition of collagen by dyes, & exhibits variability & other limitations for quantification & characterization of collagen. The HistoIndex Genesis 200™(G200) is a 2nd harmonic imaging instrument designed for tissue sections but hasn't been used in fibrotic lung disease (idiopathic pulmonary fibrosis;IPF).

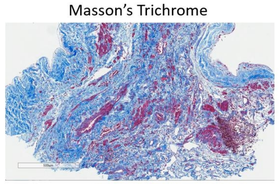
Aim Assess collagen in IPF & donor controls using G200/FI,& compare with conventional histological techniques.

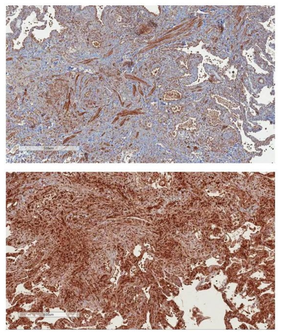
Methods Formalin-fixed paraffin-embedded(FFPE) specimens of IPF & normal donors (n=10) were obtained from the Alfred Lung Fibrosis Biobank. Sections were scanned on the G200 (laser power=0.6,TPE&SHG sensitivities 0.8&0.7)& analysis performed using FibroIndex™(FI) image quantification. Comparison was made with Masson trichrome(MT) analysed using ImageJ.

Results FI analysis of G200 images&MT detected similar collagen area ratio(CAR) in controls, but, FI analysis was able to detect greater collagen area in IPF using the same setting. FI analysis was able to detect a greater CAR, than MT staining ($p<0.001$) in IPF samples with comparable detection in normal donor controls. FI analysis was also able to detect greater collagen overlap with tissue and collagen fibre density in IPF v controls.

Discussion Genesis200, in conjunction with FibroIndex, can be used to quantify collagen in IPF. It may have advantages in sensitivity, reproducibility & efficiency compared to morphometric quantification of collagen from MT-stains. This technology may have application in the characterization of collagen in IPF and other diseases.

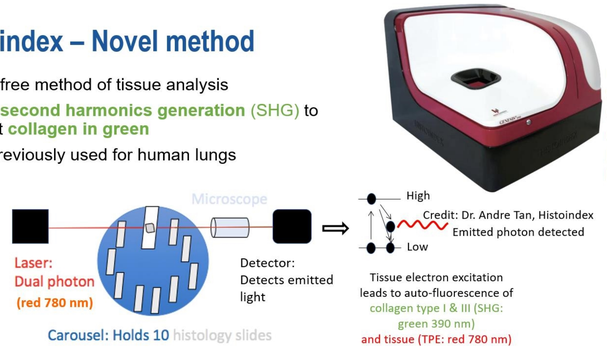
CONVENTIONAL METHODS FOR IPF TISSUE ANALYSIS



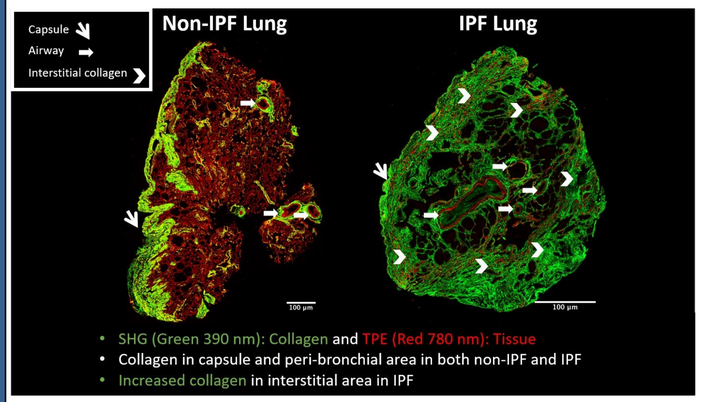


Histoindex – Novel method

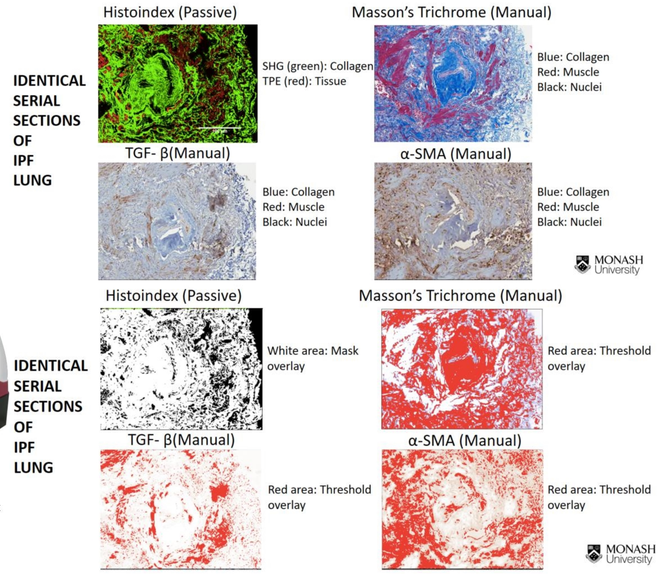
- Stain free method of tissue analysis
- Uses **second harmonics generation** (SHG) to detect **collagen in green**
- Not previously used for human lungs

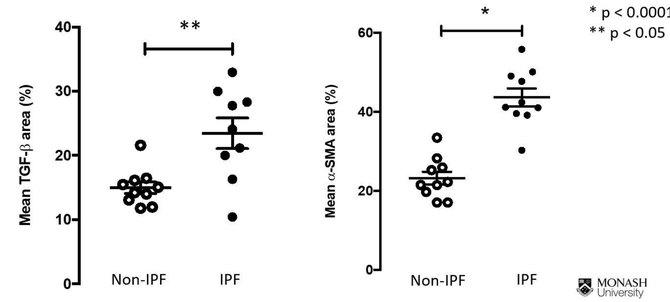


Chu SW, Tai SP, Liu TM, Sun CK, Lin CH. (2009). *J Biomed Opt* 14:010504

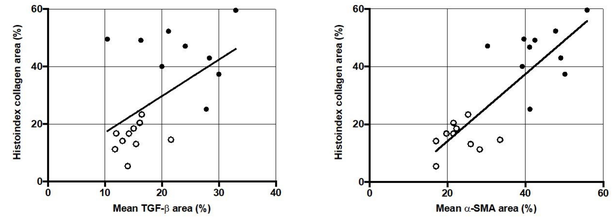


Analysis of regions of interest using Histoindex & conventional methods

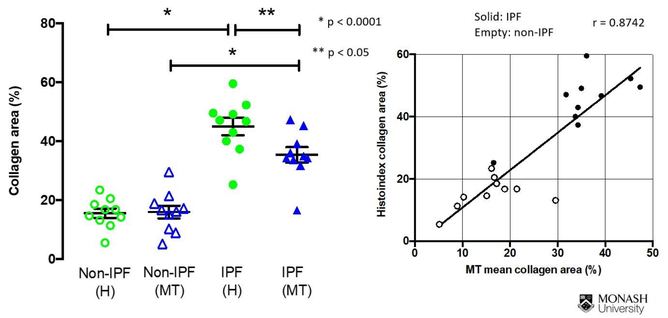




Histoindex correlation with Immunohistochemistry



Histoindex has very strong correlation with MT



Future directions

- Histoindex can be used to compare IPF with bleomycin-induced pulmonary fibrosis models
- Histoindex can be used to compare IPF with chronic lung allograft dysfunction to see if fibrosis levels are similar
- Histoindex could be used during pre-clinical trials as a more efficient way of detecting fold changes in the amount of collagen present in diseased lung or tissue

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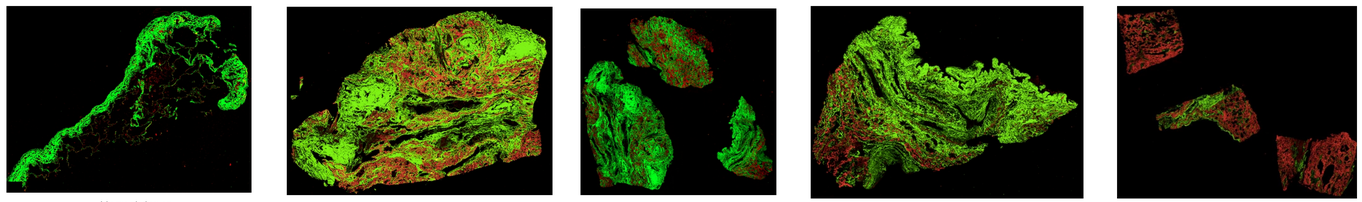
Summary



	HISTOINDEX	MASSON'S TRICHROME
Specificity	Collagen type I and III	Extracellular matrix proteins
Time to stain	n.a	Hours
Time to scan	Hours – days	Hours
Time to analyze	Hours	Hours – days
Analyzing process	Passive	Manual
Efficiency	High	Low
Reproducibility	High	Low

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