# Coherency Image Analysis to Quantify Collagen Architecture: Implications in Scar Assessment

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## Supporting Information

**Table S1 – Current quantitative methods to determine collagen architecture in the skin**

| Method | Reference paper | Summary of Key Findings |
|---|---|---|
| **OrientationJ Analysis (ImageJ)** | | • Successfully differentiates between normal and scar modelled environments *in vitro*<br>• Greater sensitivity when compared to Fourier analysis methods<br>• Suitable for analysing coherency differences in the collagen deposited by human keloid scars<br>• Quick and easy quantitative analysis method of assessing collagen structure in scarring |
| **Fourier Analysis and Collagen Orientation Index (COI)** | Van Zuijlen et al.[1] Verhaegen et al.[2] | • Study compared the accuracy of Fourier analysis of confocal images with individual observers of polarised light and the confocal images of scar tissue and normal skin.[1]<br>• In follow up work they used the COI to try and differentiate between normal skin, normotrophic, hypertrophic, and keloid scars.[2]<br>• Fourier analysis was able to achieve a superior measurement of collagen orientation compared with subjective histological evaluation by several experts in the field.[1]<br>• The COI (based on Fourier analysis) was significantly less for normal skin when compared to scar but was unable to define differences between the scar types.[2] |
| **Second Harmomic Generation (SHG) imaging** | Tanaka et al.[3] | • *In vivo SHG* imaging of dermal collagen fibres following burns in a rat model.[3]<br>• Similar to *ex vivo* analysis of skin sections, SHG imaging is able to discriminate between the effects of thermal denaturation of collagen molecules following a burn injury.[3]<br>• Expensive specialised equipment required. |
| **Confocal Microscopy** | Khorasani et al.[4] | • Scar collagen morphology comparing differences in full thickness burns and normal tissue using fractal dimension and lacunarity analysis was achieved.[4]<br>• Confirmed with transmission electron microscopy for comparison.[4]<br>• More sensitive than Fourier analysis for quantification of scar morphology. |
| **Histological Staining (e.g. Masson's trichrome or Herovici)** | Rawlins et al.[5] Sanders et al.[6] | • Able to determine the differences in mature burn scars with normal skin.<br>• Herovici staining can differentiate type I collagen (red) from type III collagen (blue).[5]<br>• Masson's suitable for measuring differences' in collagen density in mechanically stressed vs normal skin with computer aided image processing.[6]<br>• Quantification of collagen possible with post image analysis software of pixel colour thresholding.<br>• Unable to be used *in vitro.* |

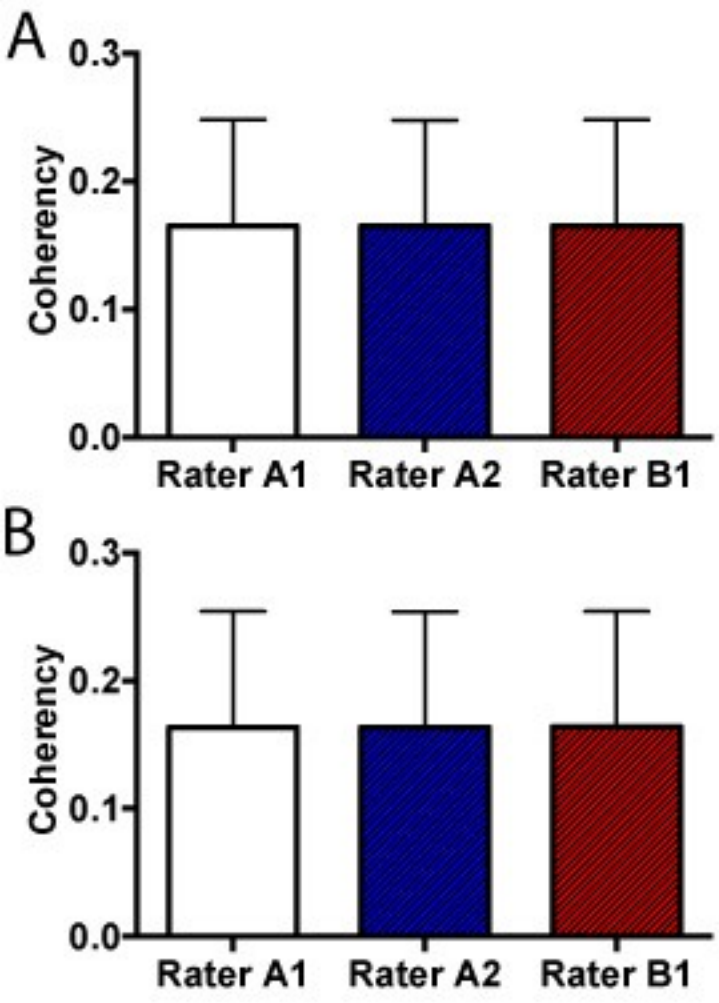


**Figure S1 –** Inter and intra-rater reliability of the coherency measurement for *in vitro* collagen deposition in a scar like environment (A) and skin tissue sections (B). No significant difference was observed between rater 1's repeated measures or between rater 1 and rater 2 for the *in vitro* (n=18 images*)* or the *in vivo* samples (n=50 images). Data displayed as mean ± SD and statistically assessed with a one-way ANOVA followed by a Bonferroni comparison test ($p<0.05$).

**Supporting Information References**


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