Three-dimensional visualisation and analysis of the dermal-epidermal junction in young and aged skin

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Introduction:

- The dermal-epidermal junction (**DEJ**) is a three-dimensional (3D) structure which is remodelled in aged skin.
- Current understanding of DEJ structure derives primarily from 2D histological sections.
- Micro-computed X-ray tomography (**microCT**) is a 3D imaging technique which is commonly applied to intact calcified tissues, but is rarely used to visualise soft tissues.
- In contrast to these biopsy-based approaches, non-invasive *in vivo* imaging techniques not only avoid the discomfort and ethical implications of biopsy collection, but also facilitate longitudinal studies. Currently, their ability to image 3D volumes remains under developed.

Aims:

- To compare the ability of *in vivo* (reflectance confocal microscopy) and *ex vivo* (microCT & histology) technologies to visualise, characterise and quantify the 3D structure of the DEJ.
- To determine if measurements taken from the resulting 3D images differentiate between the DEJ in young and aged skin.

Methods:

- Caucasian volunteers were recruited into the study (*n*=10; 18-30 & 65+ yrs old; 6♀ / 4♂; University of Manchester Research Ethics No. 13268).
- An area of skin on the mid dorsal forearm (sun exposed) was imaged using a confocal laser microscope (**RCM**; VivaScope® 1500; MAVIG; 5 stacks of 65 images (500x500µm, 3.05 µm intervals).
- The imaged areas were then biopsied (6 mm) and half of each biopsy fixed and either:

i. Wax embedded, sectioned and haematoxylin & eosin (H&E) stained for **histology** *or*

ii. Stained with Lugol's solution (I_2KI) for 18 hours before wax embedding & **microCT** imaging (Zeiss Versa XRM-510 system).

Result a): 3D reconstructions of the DEJ show in high resolution how rete ridges form long interconnected elevations in contrast to the more discrete protrusions of dermal papillae

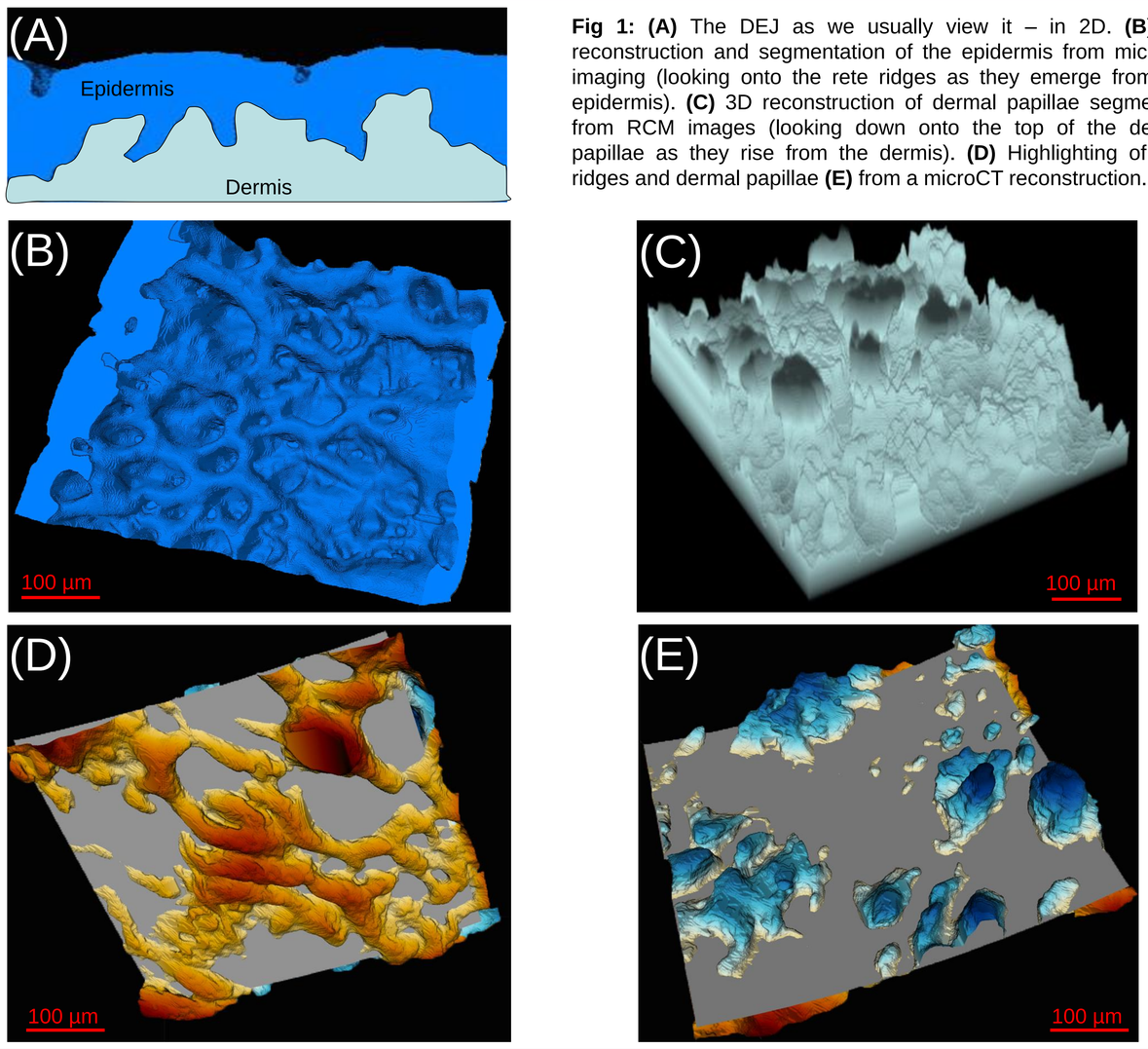

Fig 1: (A) The DEJ as we usually view it – in 2D. **(B)** 3D reconstruction and segmentation of the epidermis from microCT imaging (looking onto the rete ridges as they emerge from the epidermis). **(C)** 3D reconstruction of dermal papillae segmented from RCM images (looking down onto the top of the dermal papillae as they rise from the dermis). **(D)** Highlighting of rete ridges and dermal papillae **(E)** from a microCT reconstruction.

Result b): Calculation of local maxima and minima heights across segmentations provided a rapid method to evaluate rete ridge height over a whole volume. Height profiles were significantly different in young forearm skin compared with aged

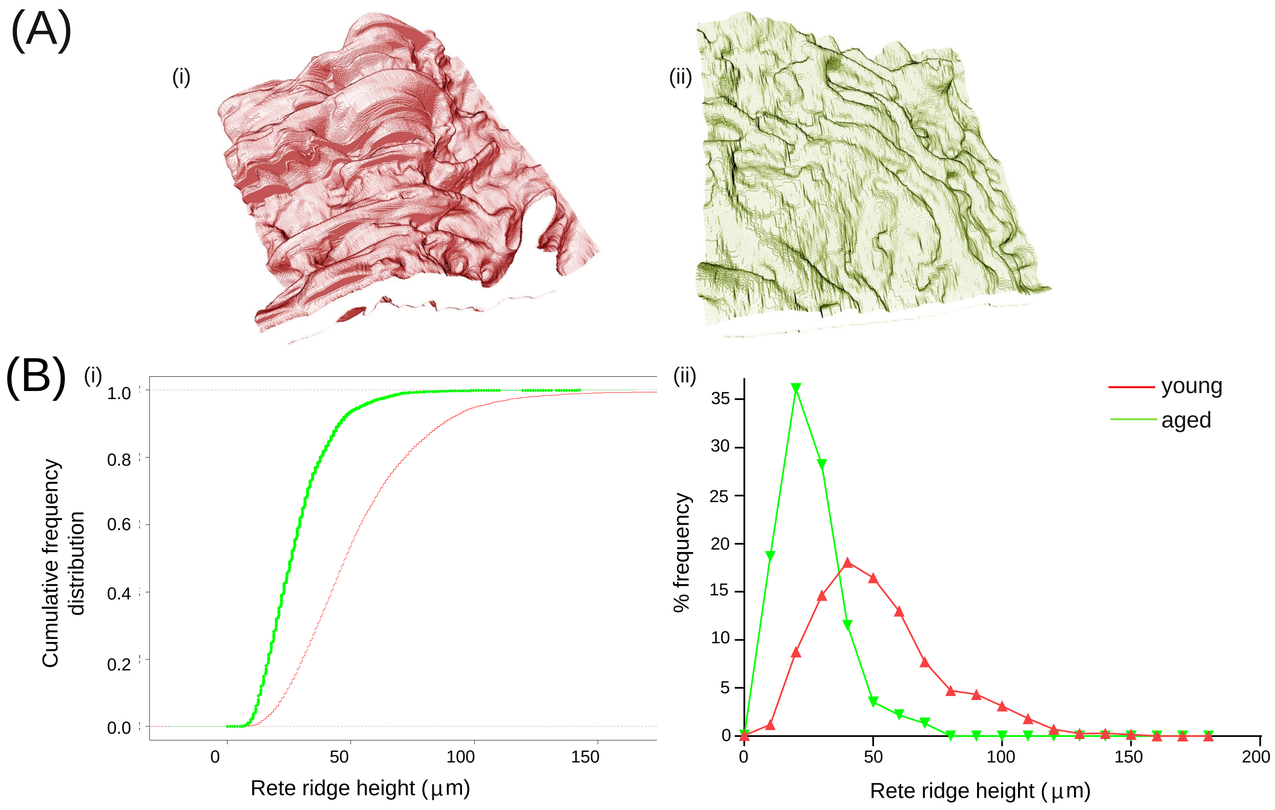

Fig 2: **(A)** Example images of rete ridges in the forearm in young (i) and aged (ii) skin reconstructed and segmented from microCT images. Images represent a 470 µm³ volume of skin. **(B)** Cumulative frequency distribution (i; D=0.48, p<0.0001 Kolmogorov-Smirnov test) and histogram (ii) of rete ridge height distributions in young and aged forearm skin. Comparison of rete ridge heights measured using histology images and those obtained using local minima and maxima of microCT segmented epidermises correlate well (p>0.0001, r = 0.86, n=17, Pearson's r correlation, data not shown).

Result c): Using the median plane to define rete ridges and dermal papillae allows easier analysis of these structures and results in differences in volume being detected between young and aged forearm skin when using microCT generated 3D images, but not those from RCM

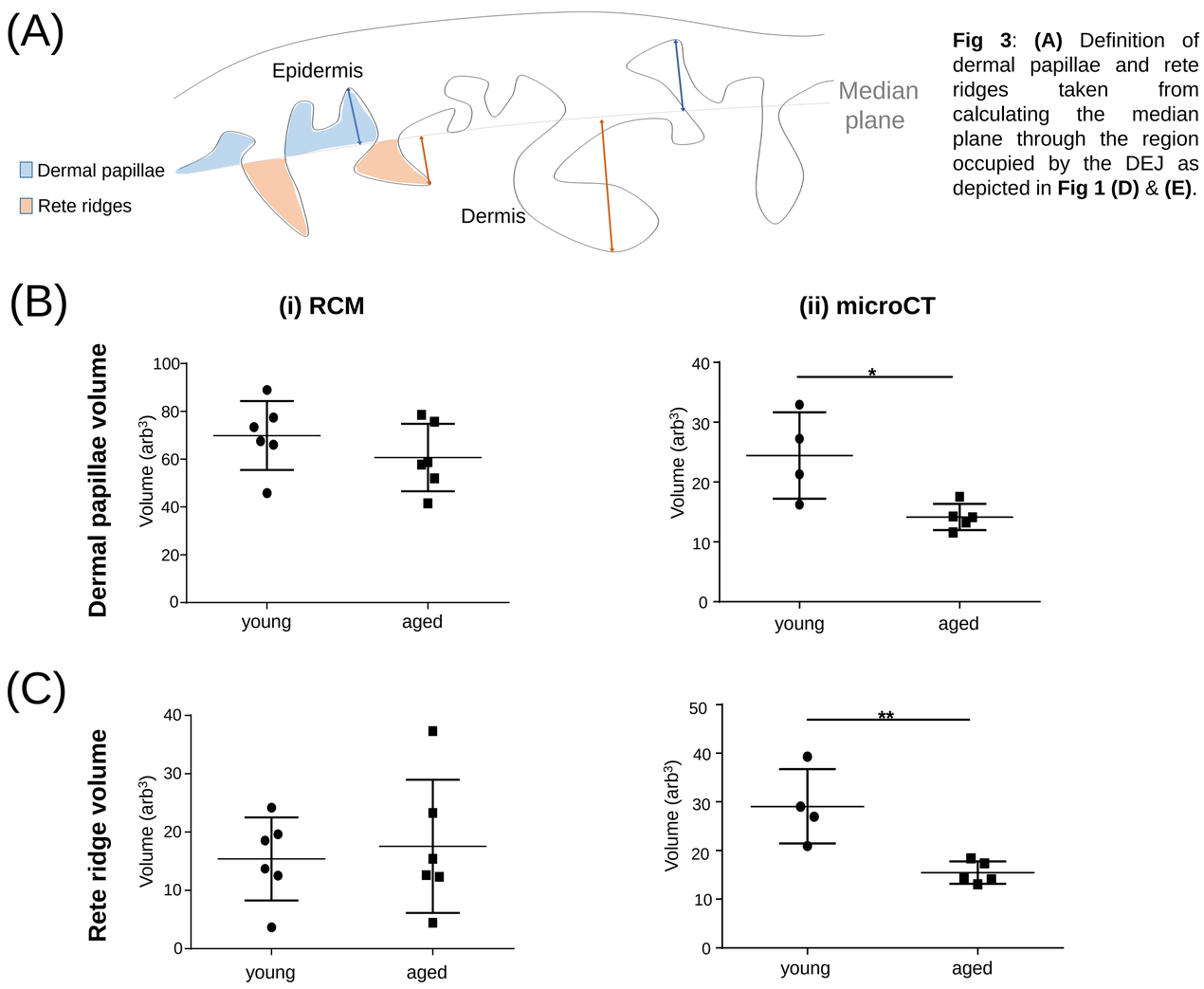

Fig 3: **(A)** Definition of dermal papillae and rete ridges taken from calculating the median plane through the region occupied by the DEJ as depicted in **Fig 1 (D)** & **(E)**.

Fig 3: **(B)** Comparison of dermal papillae volumes segmented from RCM (i) and microCT (ii) images. **(C)** Rete ridge volume comparison in young and aged skin taken from segmentations of RCM (i) and microCT (ii) images. **p<0.01, Student's Unpaired *t*-test, *n*=4-5.

Discussion:

- Both RCM and microCT approaches allowed reconstruction of DEJ 3D structure.
- 3D reconstructions give insight into the complexity & the distinct structural natures of rete ridges compared with dermal papillae, which cannot be appreciated if the DEJ is viewed only in 2D.
- Use of the median plane to define dermal papillae & rete ridges provides an easier mode of analysis than determining a top & bottom/full length for each individual dermal papilla & rete ridge.
- *In vivo* RCM avoids sample preparation artefacts & enables longitudinal studies, but, potentially due to limitations in dermal papillae resolution, age-related volumetric differences were only quantifiable using microCT 3D reconstructions in this study.
- Use of 3D imaging of the skin is likely to become more commonplace as we further address the technical difficulties associated with these techniques. This will give us a more realistic window through which to view the skin & so improve our understanding of skin ageing and corresponding topical treatments.

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