

## **Supplementary Information**

### **From morphology to biochemical state – intravital multiphoton fluorescence lifetime imaging of inflamed human skin**

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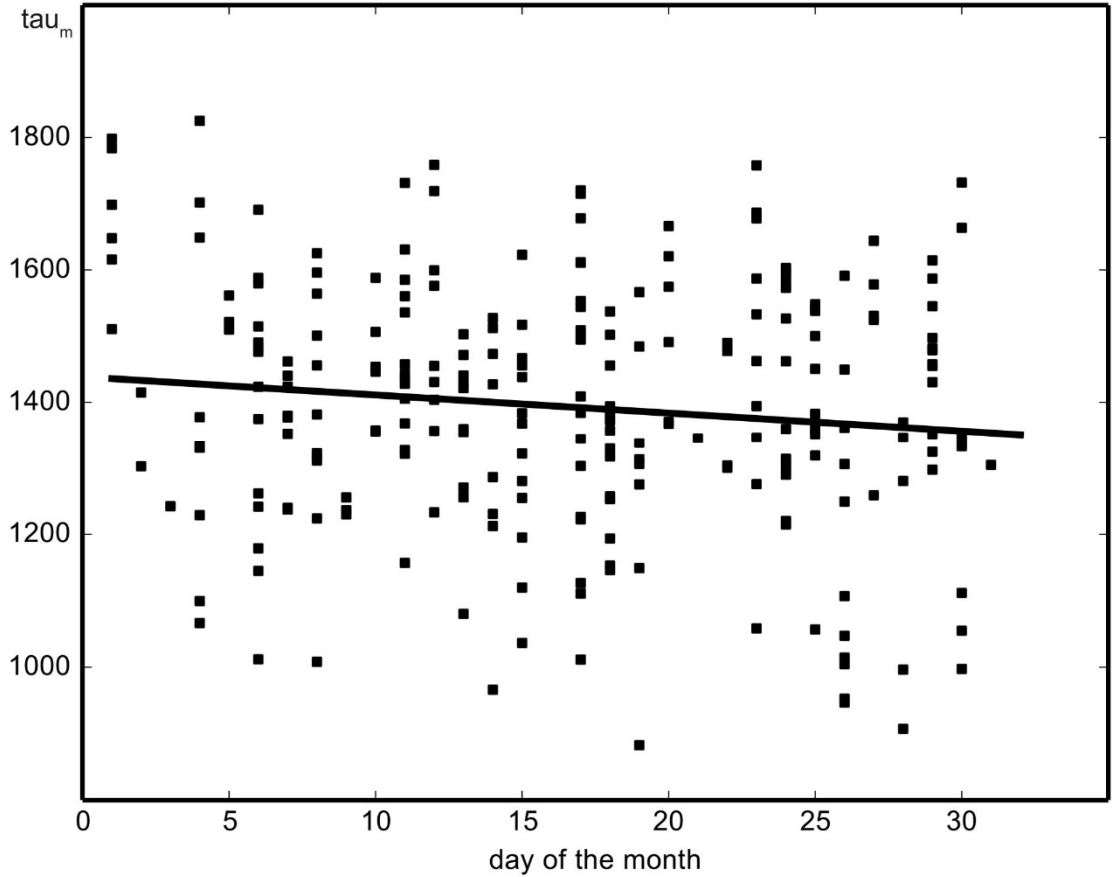
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# Supplementary Figures

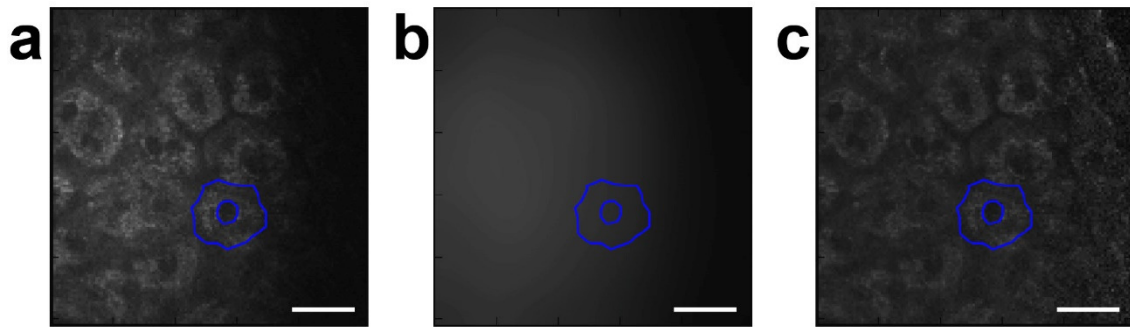
Supplementary Figure S1.



**Control correlation to evaluate the reliability of the study design.**

Correlation between the day of the month of the examination and the tau<sub>m</sub> value.

**Supplementary Figure S2.**



**Shading correction.**

(a) A *Stratum granulosum* greyscale image without corrections and a segmented cell marked in blue. The vignetting effect is visible in the right image area. (b) The image after Gaussian filtering ( $\sigma = 40$ ). (c) The corrected image after the pixel-wise division of (a) by (b). The vignetting effect is removed. Scale bars correspond to 20  $\mu\text{m}$ .

## Supplementary Methods

### Shading correction

Some segmented cells suffer from shading deficiencies due to systemic irregular illumination at the periphery (vignetting) or to a non-optimal sectional plane (Supplementary Fig. S2 a). To address this issue, we utilise a shading correction method based on Gaussian filtering. The continuous Gaussian function for two dimensions is defined as

$$f(x, y) = \frac{1}{2\pi\sigma^2} \cdot e^{-\frac{x^2+y^2}{2\sigma^2}}$$

with  $\sigma$  denoting the standard deviation that controls the strength of the smoothing effect. The photon count image  $I$  (Supplementary Fig. S2 a), is convoluted by a Gaussian kernel with  $\sigma = 40$  resulting in the shading image  $I_{Gauss}$  (Supplementary Fig. S2 b), which represents the general brightness distribution of  $I$ . The corrected image  $I_{corr} = I \div I_{Gauss}$  (Supplementary Fig. S2 c) is then calculated via pixel-wise division and used for further processing.

## Supplementary Movie Legend

**Supplementary Movie S3. Three-dimensional reconstruction of a multiphoton tomographic optical biopsy of healthy human skin.**

Multiphoton-based autofluorescence signals of NADH enable characterisation of the epidermal architecture with a subcellular resolution. Dermal collagen in the *Stratum papillare* is pseudocolour-coded in green. Red scale bar corresponds to 20  $\mu\text{m}$ .