



A pilot 2-photon fluorescence microscopy study on the effect of topical interleukin-1 alpha on collagen deposition in ageing skin

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2-Photon fluorescence microscopy

Method

2-Photon fluorescence microscopy is a fluorescence imaging technique which allows the generation of three-dimensional pictures of biological specimens *in vivo*. It uses visible light at high intensity to elicit fluorescence. Skin can be scanned to a depth of about 250 μm with a resolution of less than 1 μm .

Excitation: Laser pulses of 70 – 80 fs at 80 MHz and a wavelength of 800 nm.

Recordings: Stacks (= vertical skin scans) of autofluorescence (green) and second harmonic generation (SHG, red) images were collected in two separate channels.

The study was carried out by Neurotar Ltd., Helsinki.

Study protocol

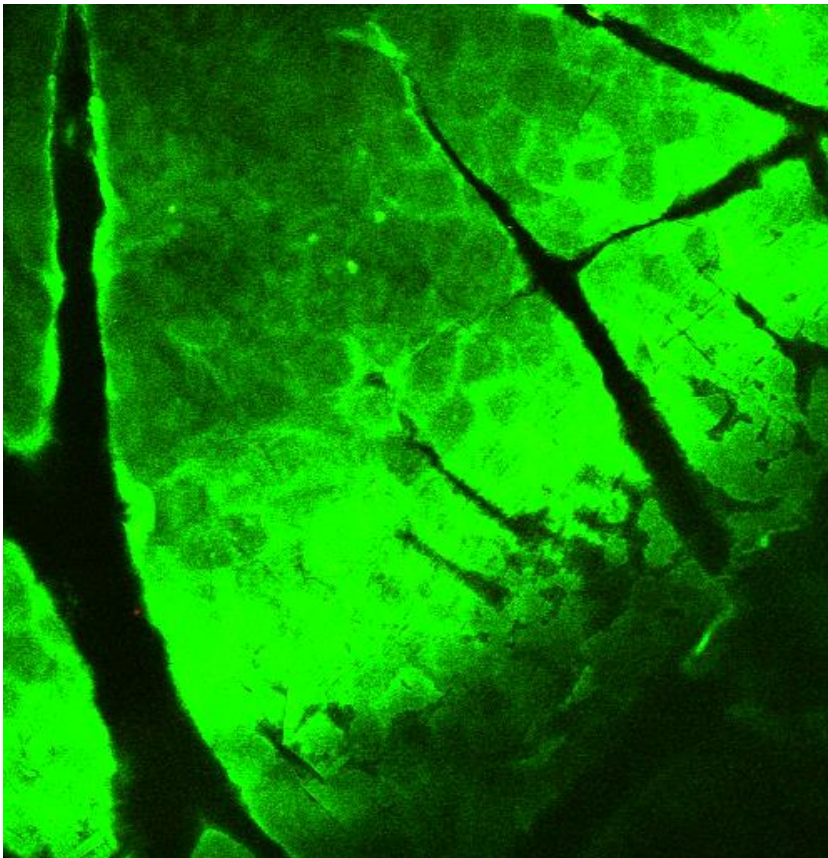
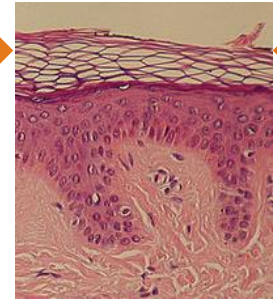
The forearm skin of three human volunteers, aged 63 and 64 years, was analysed longitudinally using 2-photon fluorescence microscopy.

Placebo and verum (30 or 150 $\mu\text{g/L}$ IL-1a) gels were applied twice daily to the left and right forearm, respectively. Fluorescent image stacks were acquired from both forearms at baseline (day 0) and after predefined treatment periods. All imaging parameters were kept identical between imaging sessions. The same skin spot was targeted each time. Statistical analysis was performed using Student's paired t-test.

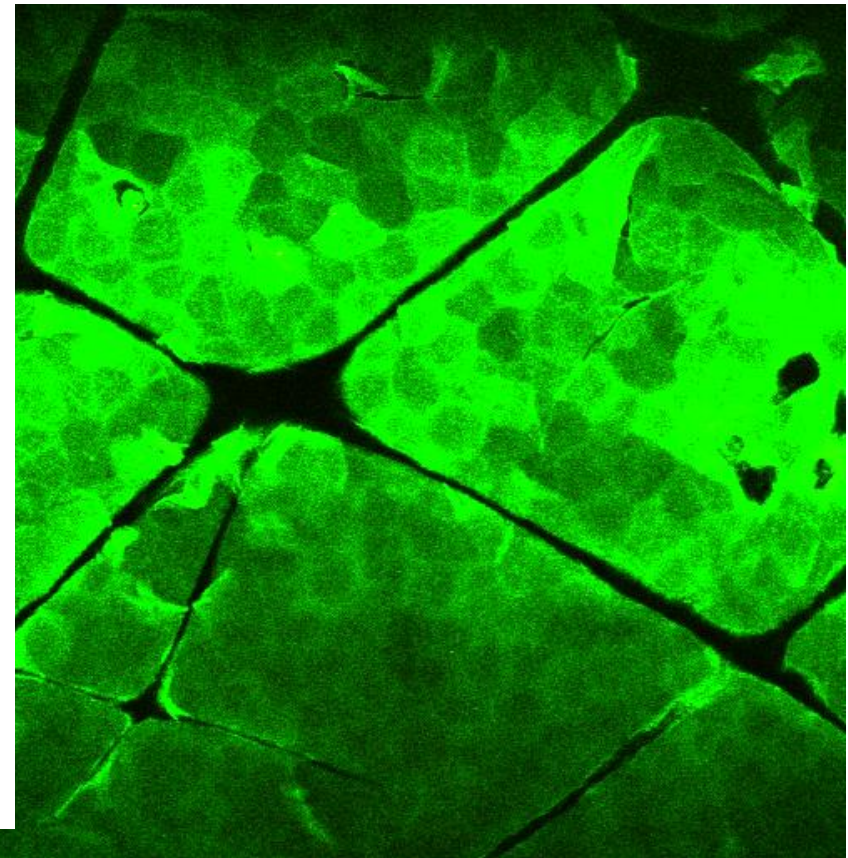
1st Study: Pictures taken after 4-week treatment

Stratum corneum: **corneocytes**

Depth: 5 μm

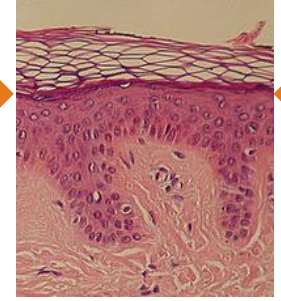


Placebo

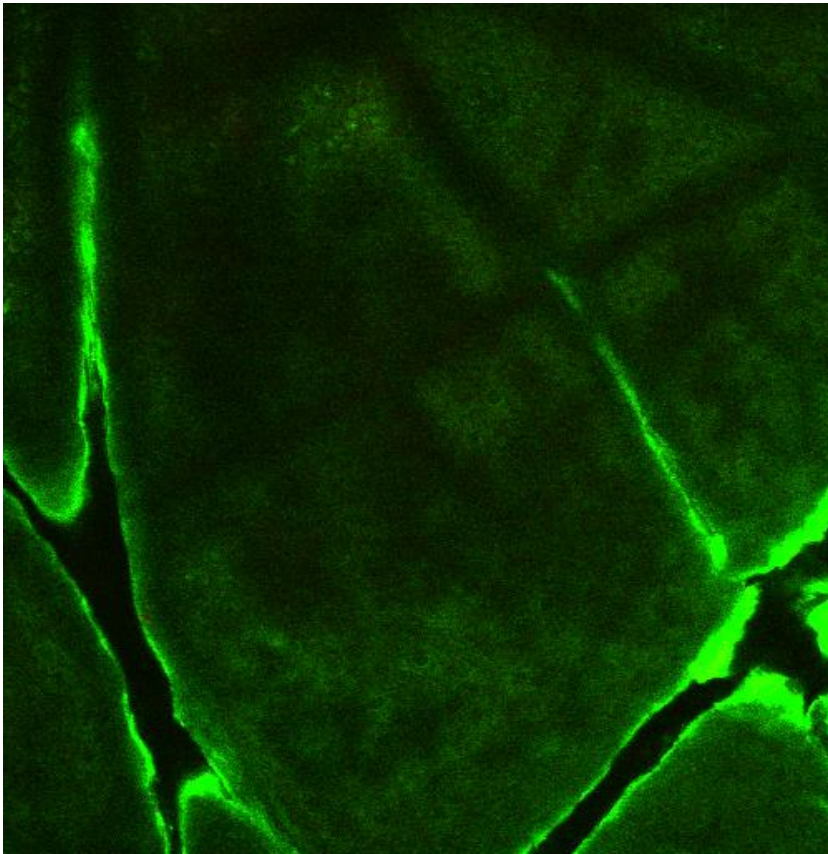


Interleukin-1 alpha

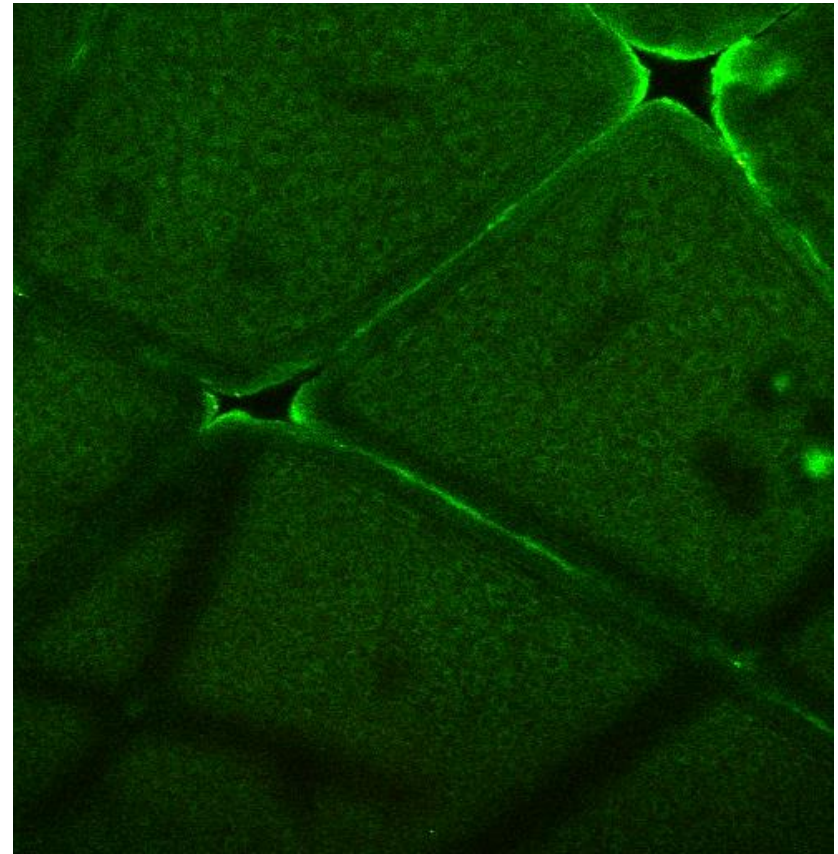
Depth: 25 μm



Stratum granulosum: **keratinocytes**

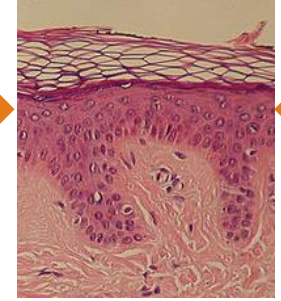


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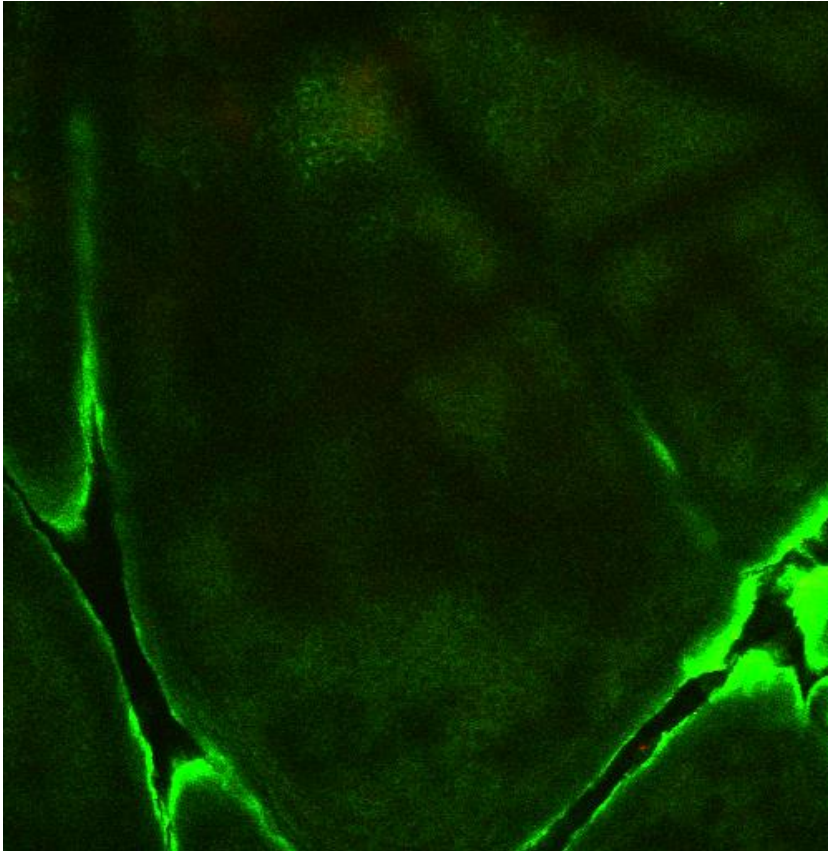


Interleukin-1 alpha

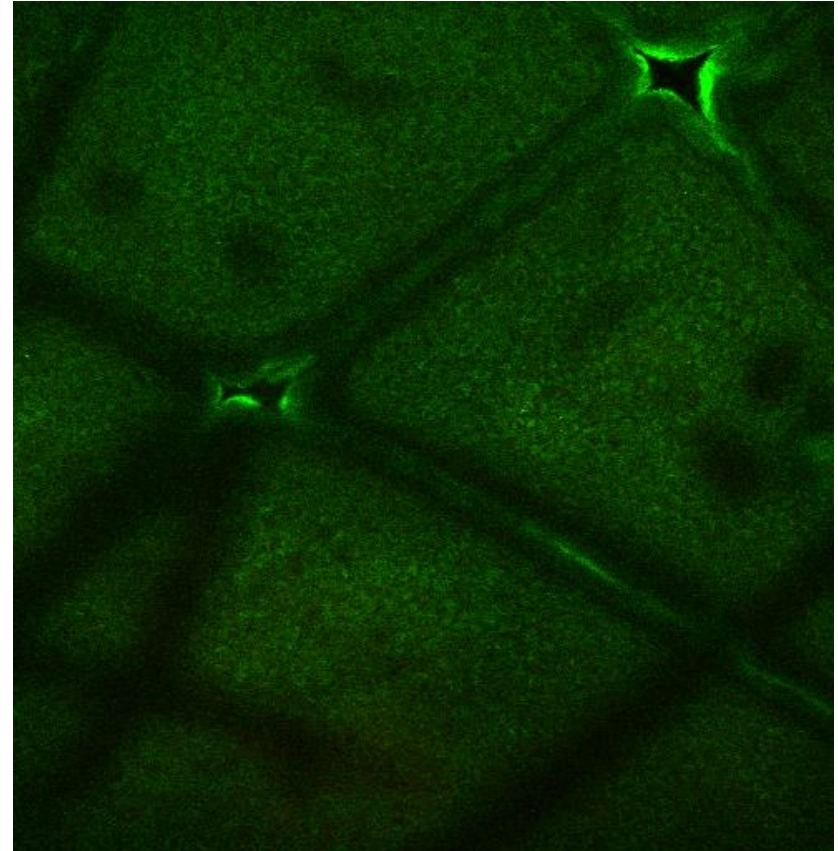
Depth: 30 μm



Stratum spinosum: **keratinocytes**



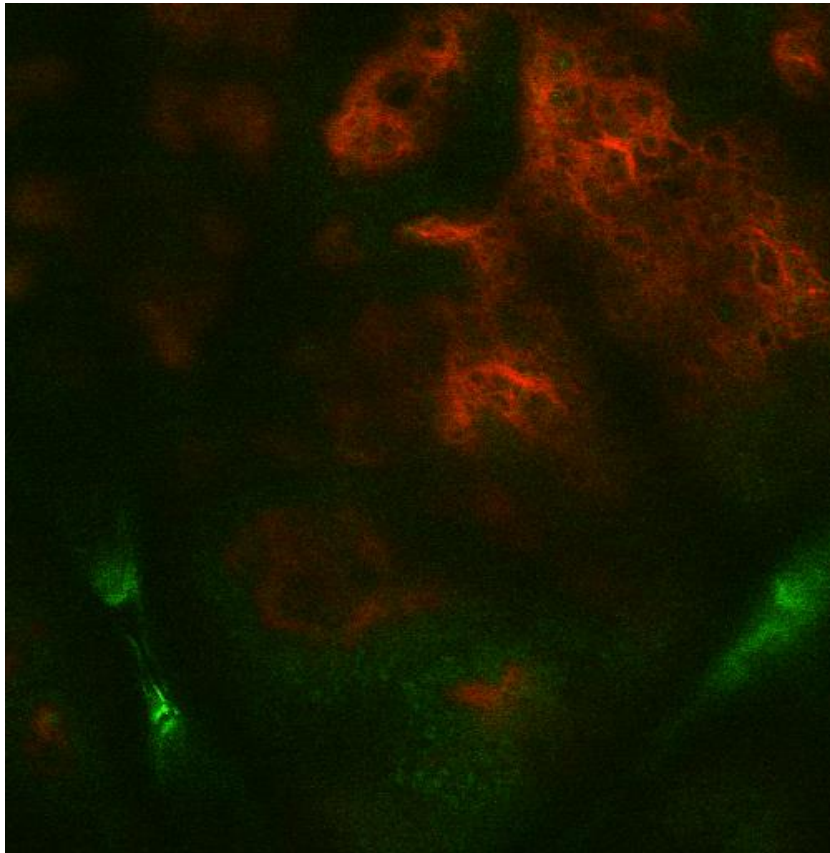
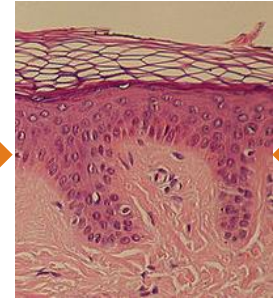
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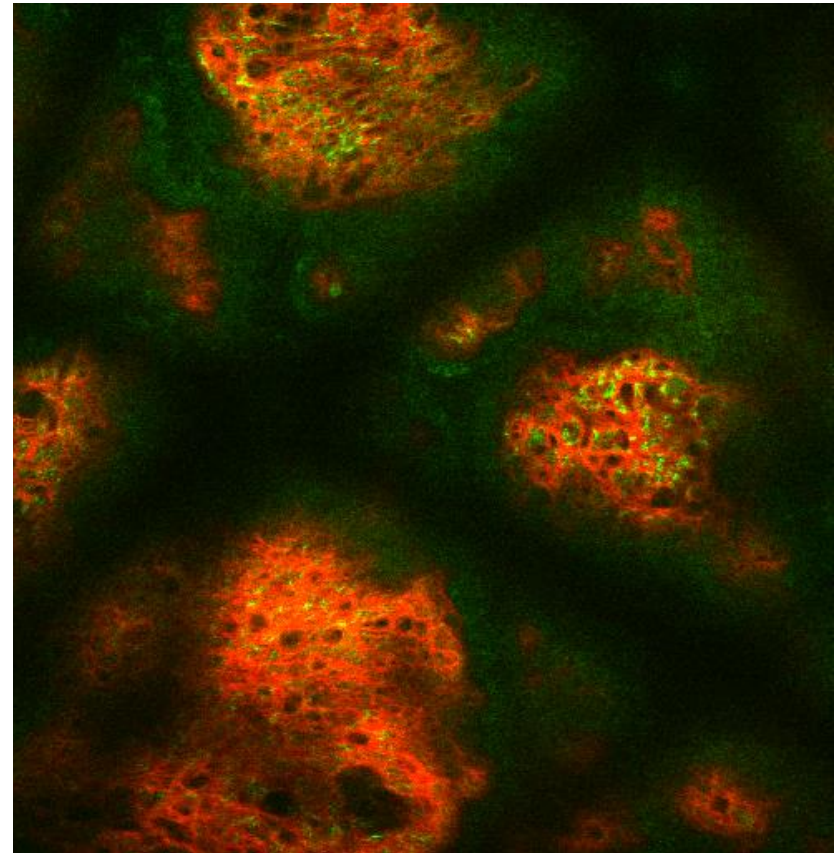
Interleukin-1 alpha

Depth: 50 μm

Stratum basale: **keratinocytes**, **elastin**, **collagen**



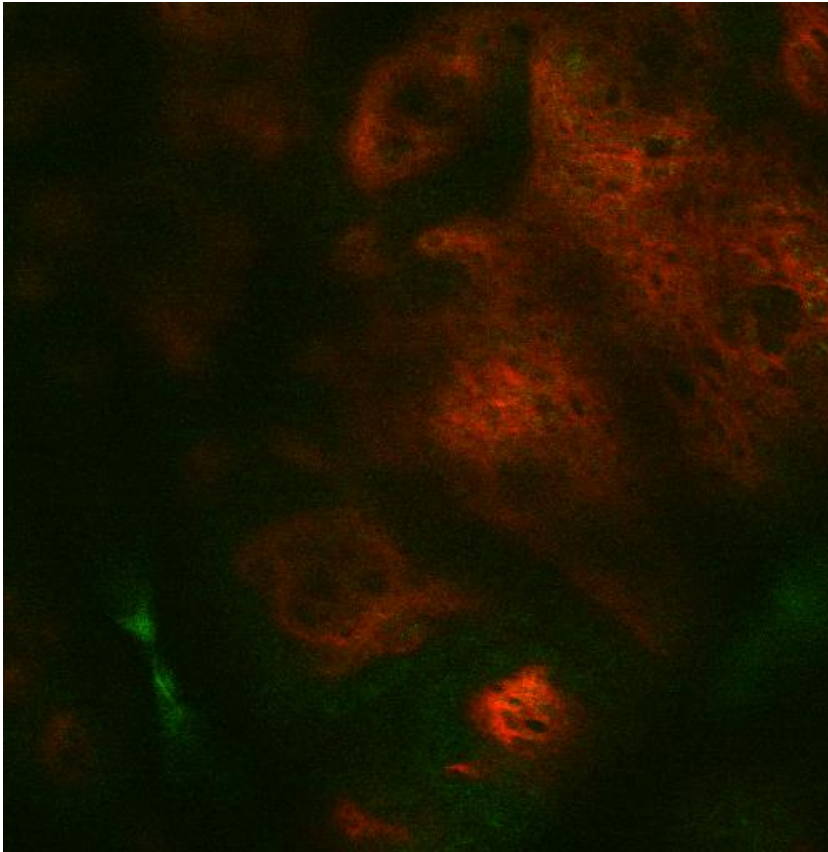
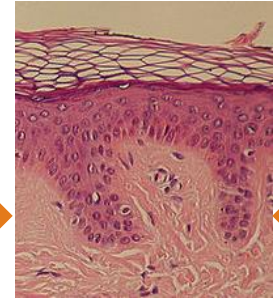
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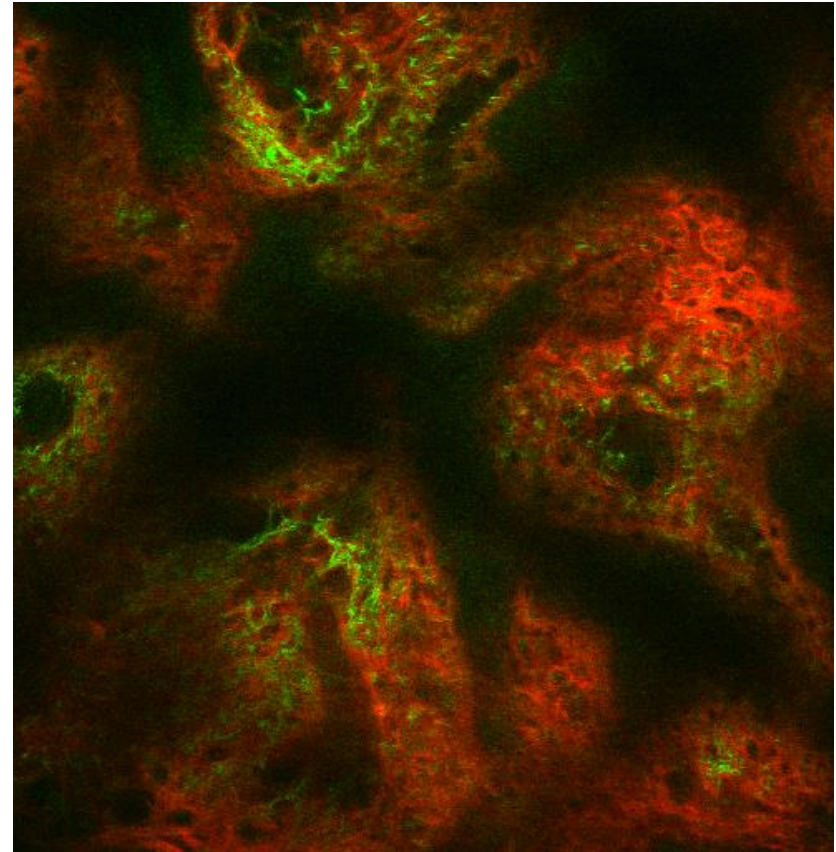
Interleukin-1 alpha

Dermis: elastin, collagen

Depth: 70 μm



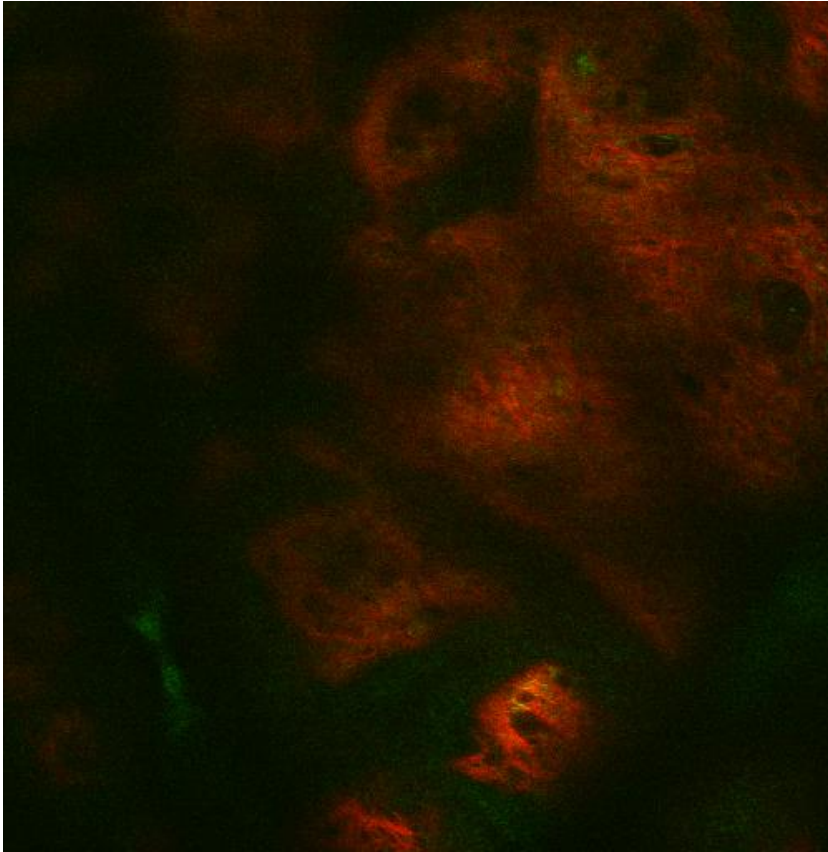
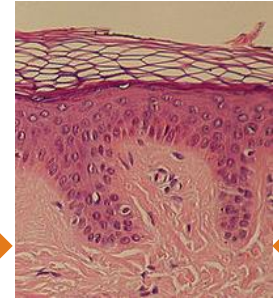
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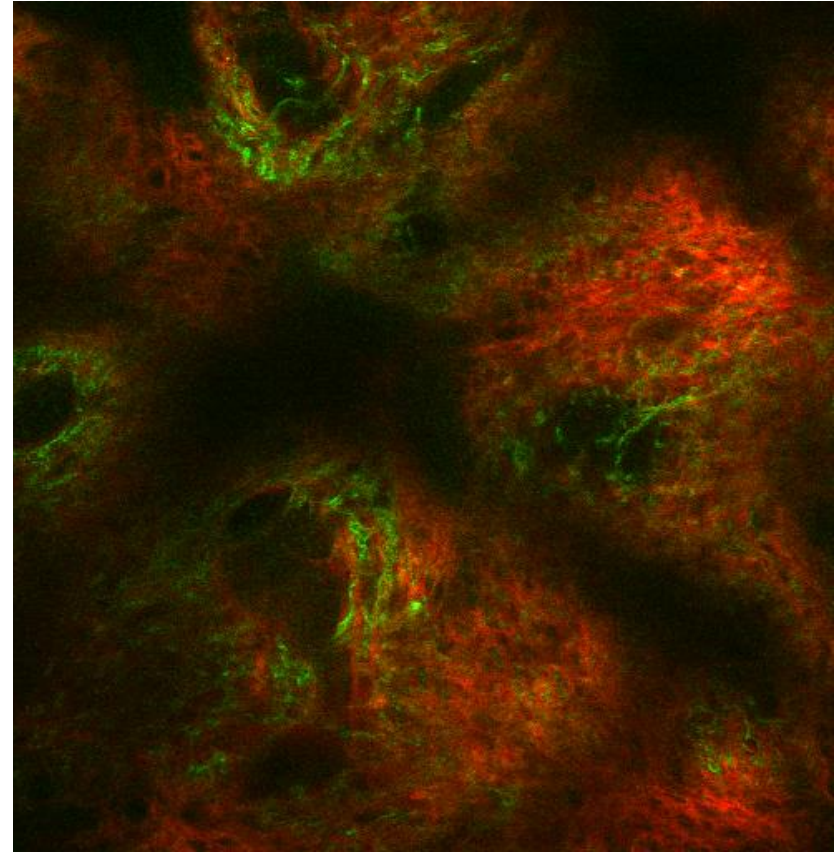
Interleukin-1 alpha

Dermis: **elastin**, **collagen**

Depth: 80 μm



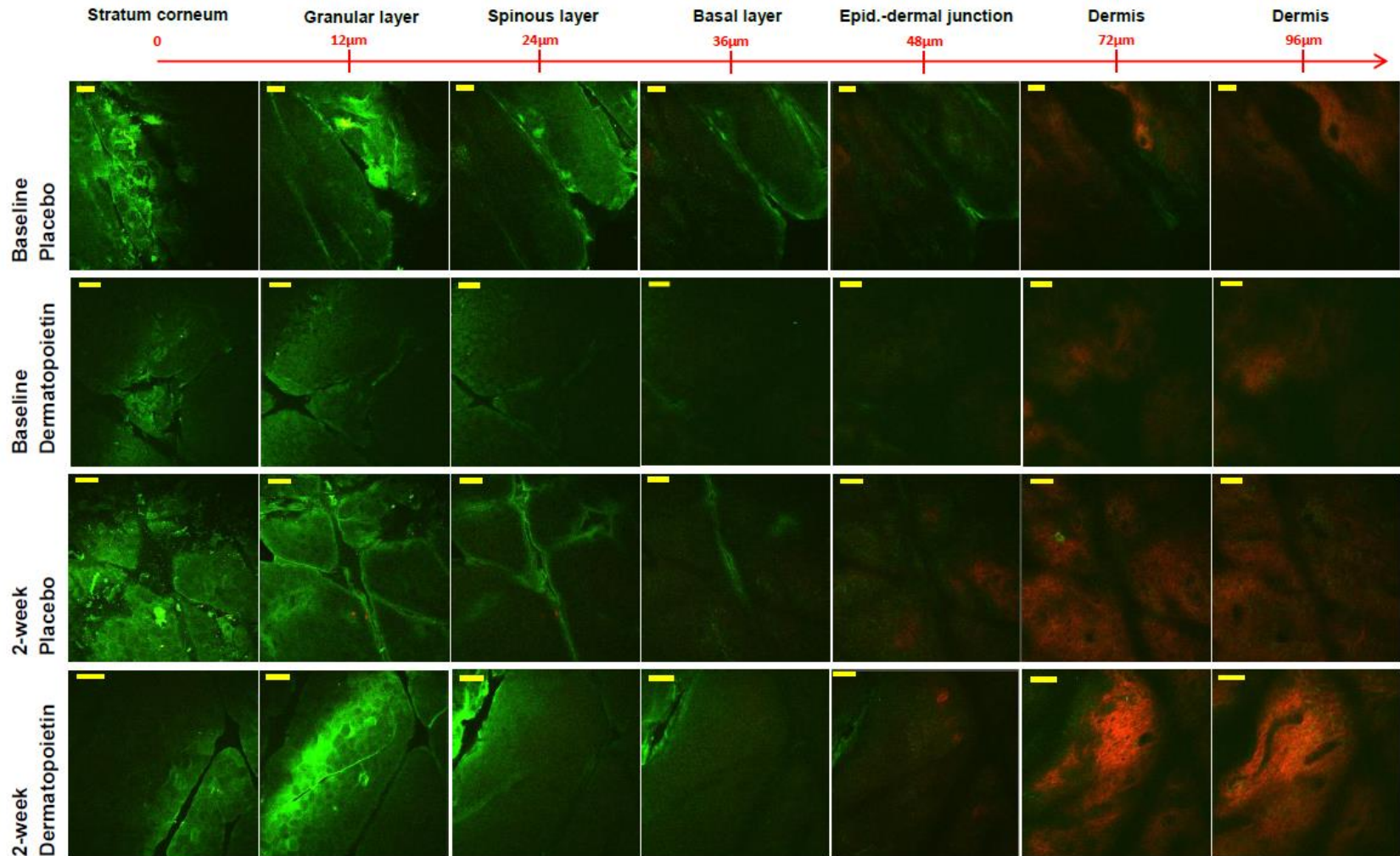
Placebo



Interleukin-1 alpha

2nd Study

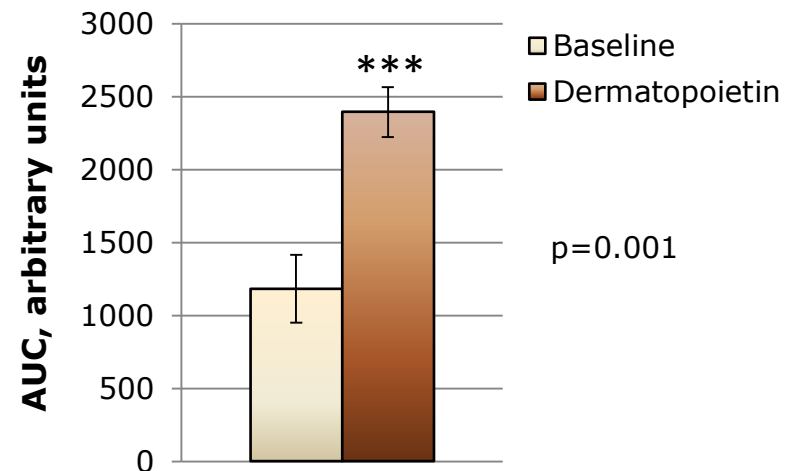
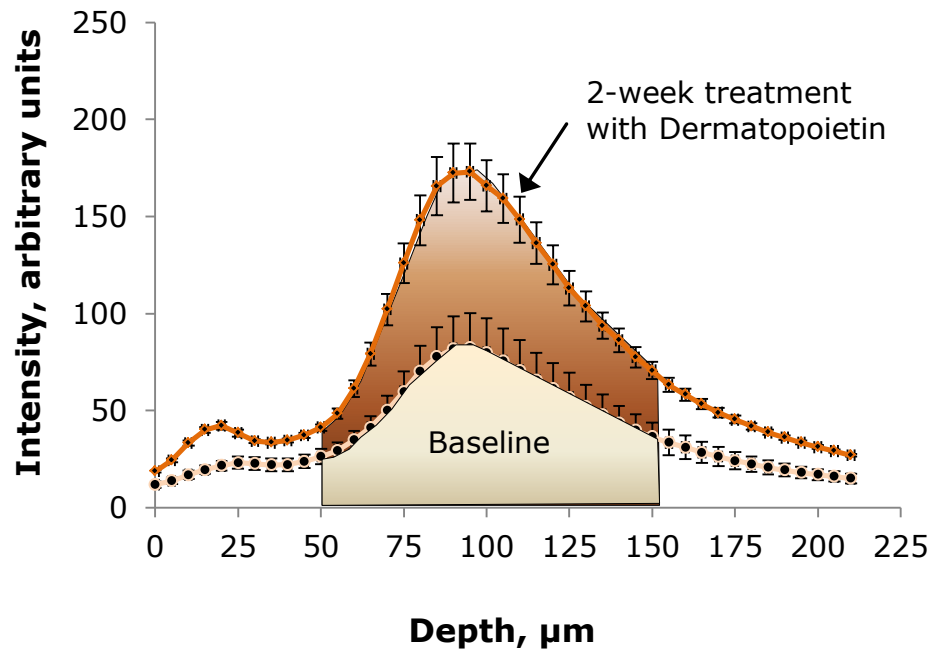
Comparison of placebo *versus* verum and baseline *versus* 2-week



Scale bar: 50 μ m

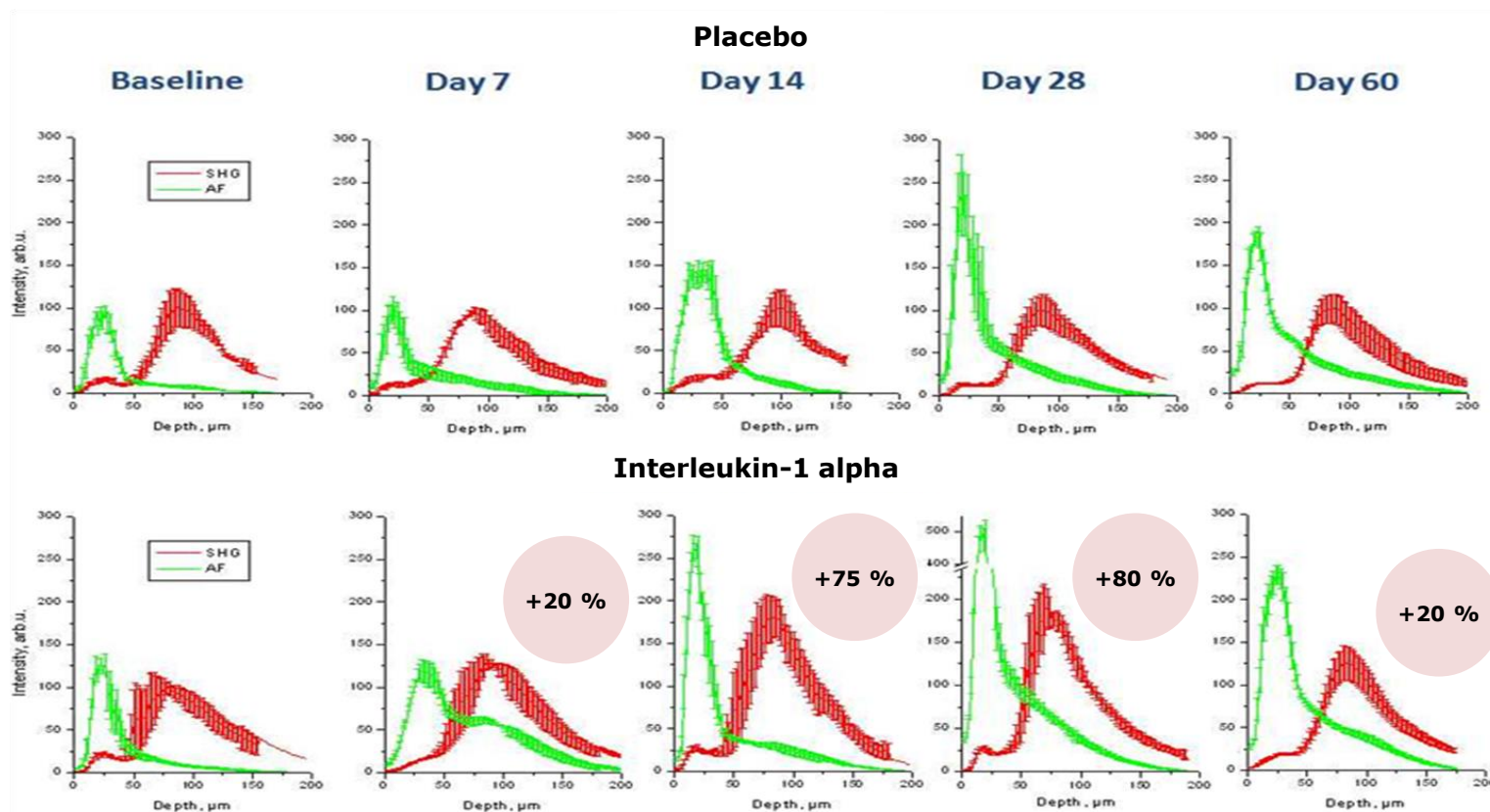
Quantitative measurement of collagen content:

Area under the curve (AUC) of the measured fluorescence intensity as function of skin depth across the dermis



Time course of collagen build-up

Treatment from day 0 – day 28 / No treatment from day 29 – day 60



Collagen almost doubles within 3–4 weeks of topical administration of IL-1a

Legend: Epidermis: 0 – 60 µm; dermis: 60 – 200 µm

Green curves: NADH, NADPH, FADH₂; in addition: keratin (epidermis), elastin (dermis)

Red curves: collagen (Dermis)

Conclusion and Discussion



1. Topical administration of interleukin-1 alpha increases the collagen (and elastin) deposition in the dermis by almost 100% within 3-4 weeks.
2. This finding is consistent with the hypothesis of a physiological role of IL-1a in skin homeostasis. IL-1a acts in the stratum corneum as sensor for the wear and tear of skin. Upon release it propagates itself through the epidermis by paracrine interactions and provides eventually the signal to the fibroblasts in the dermis for skin renewal.
3. The increase of collagen is associated (data not shown) with an improved elasticity and smoother texture of skin.
4. Given the excellent tolerability the combination of topical interleukin-1 alpha and hexadeltine offers itself as 'cosmeceutical' for ageing skin.