

# *In vivo* measurements of epidermal thickness by reflectance mode confocal microscopy to assess cellular proliferation induced by topical agents

Kevin G. Phillips

<sup>a</sup> Dermatology, Oregon Health and Science University, 3181 SW Sam Jackson Park Rd. L468R, Portland, OR, USA;

## 1. INTRODUCTION

The thickness of the epidermis is important in understanding normal and pathological processes in the skin including cell proliferation in the epidermis; a process indicative of inflammation. The determination of epidermal thickness from imaging methods such as rCSLM and optical coherence tomography (OCT) has been motivated by the desire to emulate histological examination of skin sections non-invasively. rCSLM and OCT both elucidate skin structure in axial reflectance profiles:<sup>4</sup> an entrance peak in the depth resolved axial reflectance profile (A-scan) as the signal crosses into the stratum corneum, with a second peak occurring at greater depth and presumed to correspond to the papillary-reticular dermis junction (PRJ)<sup>4</sup> or to fibrous structures in the upper dermis.<sup>5</sup> In Figure 1 we present a sagittal view (B-Scan) and corresponding A-scan obtained by averaging with identification of the prominent structural features. Comparative studies between OCT and cryostat histology suggest the local minima (valley) between the entrance peak and the secondary peak corresponds to the dermal-epidermal junction (DEJ).<sup>6</sup>

We report on our preliminary efforts to monitor changes in epidermal thickness in mice skin (ear) using rCSLM imaging. Topical application of 12-O-tetradecanoylphorbol-13-acetate (TPA) in wild-type mice models is used to induce inflammation and thickening of the epidermis.<sup>7</sup> TPA is a phorbol ester tumor promoter which stimulates cell proliferation through rapid activation of protein kinase C (PKC).<sup>8</sup> The use of rCSLM imaging provides a means to monitor epidermal changes in murine models without recourse to biopsy or sacrifice.

## 2. DATA COLLECTION AND PROCESSING

The rCSLM system consists of an argon ion laser,  $\lambda = 0.488$  [ $\mu\text{m}$ ], with an average output power of 10 [mW], providing illumination through a water-coupled objective lens with numerical aperture (NA) 0.90 and  $\times 60$  magnification. ( $x, y$ ) translation of the focus was achieved using a scanning assembly consisting of two galvanometer

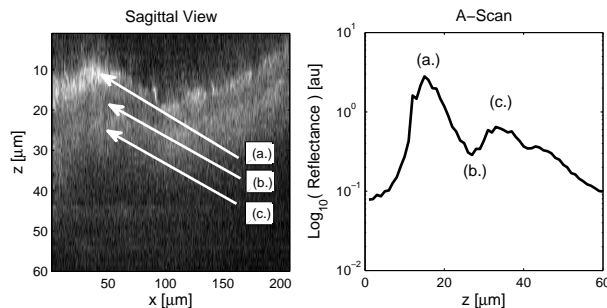


Figure 1. *Left*: Sagittal view of mouse ear obtained by rCSLM imaging. *Right*: Axial scan obtained by averaging over the region from 100 [ $\mu\text{m}$ ] to 200 [ $\mu\text{m}$ ] in the transverse,  $x$ -direction. In both figures (a.) is the entrance peak, (b.) is the valley corresponding to the DEJ, (c.) is the secondary peak in the upper dermis. The epidermis is the region between (a.) and (b.), the upper dermis is the region beyond (b.).

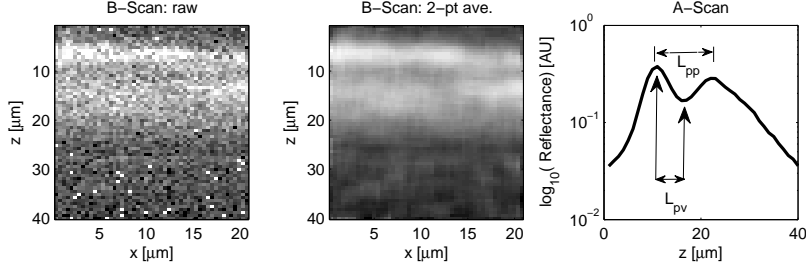


Figure 2. *Left*: Sagittal view of the ROI: raw data. *Middle*: Sagittal view of ROI: 2-point moving average smoothing. *Right*: A-Scan obtained by averaging over ROI.  $L_{pv}$  is the peak-to-valley distance along  $z$ .  $L_{pp}$  is the peak-to-peak distance along  $z$ .

mirrors and a pair of relay lenses that directed the laser beam into the rear of the objective lens at varying angles. Confocal detection of light originating from the focus in the mouse tissue was achieved by recollimating the reflected signal and then returning the signal through the optical train until the beam was re-directed by a beamsplitter toward a lens/pinhole/photodetector assembly. The  $z$  position of the sequential  $(x, y)$  scans was incremented in  $1 \text{ } [\mu\text{m}]$  steps by computer control of the translation stage. The spatial resolution along directions  $x$  and  $y$  is  $0.4146 \text{ } [\mu\text{m}]$ . Data was acquired using an A/D converter controlled by Labview software, and image reconstruction was conducted using MATLAB software.

A mouse was placed onto the translation stage of the rCSLM system whereupon the reflectance profile from the ear was resolved using confocal detection. Images were taken prior to and 24 hours after  $2.5 \text{ } [\mu\text{g}]$  application of TPA to the ear. To eliminate motion artifacts, the mouse was anesthetized using vaporized isoflurane. The breathing rate of the mouse was regulated by providing  $0.2 \text{ } [\text{l}/\text{min}]$  oxygen and  $0.8 \text{ } [\text{l}/\text{min}]$  air mixed with the isoflurane vapor ( $1.5 \text{ } \%$  V/V at  $1 \text{ ATM}$ ). The head of the mouse was secured using rubber tubing when imaging the ear. A metal coverslip was placed on the far side of the ear to flatten and further restrict movement of the tissue volume under investigation.

The reflectance from the rCSLM system is stored in the three dimensional array  $\mathbf{B}(i, j, k)$ . Here  $i, j, k$  denote pixel values and are indices of the matrix  $\mathbf{B}$ . The pixel values  $(i, j)$  correspond to transverse  $(x, y)$  locations while  $k$  denotes axial depth along  $z$ . The true spatial location of a pixel value is the product of the pixel value, the resolution along each spatial direction and a correction factor accounting for refractive index mismatch between the water coupling lens ( $n_0 = 1.33$ ) and the mouse tissue ( $n = 1.4$ ):<sup>4</sup>  $x = \frac{n}{n_0} \times i \times dx$ . The same treatment is used for  $y, z$ . Hence  $\mathbf{B}(x, y, z) = \mathbf{B}(\frac{n}{n_0} \times i \times dx, \frac{n}{n_0} \times j \times dy, \frac{n}{n_0} \times k \times dz)$ . The numerical determination of the epidermal thickness from confocal images is carried out in several steps: 1.)  $\mathbf{B}(x, y, z)$  is first divided into local regions of interest (ROI) typically  $20 \text{ } [\mu\text{m}] \times 20 \text{ } [\mu\text{m}] \times 100 \text{ } [\mu\text{m}]$  in size. 2.) Each ROI is smoothed through a 2-point moving average procedure. In Figure 3 we present the raw and the smoothed B-Scans. 3.) Next, an average A-scan is obtained for each ROI by averaging the local axial reflectance profiles for each  $(x, y)$  location in the ROI, see Figure 3. 4.) The thickness of the epidermis is monitored by two means: the peak-to-valley distance,  $L_{pv}$ , in the average A-scan for each ROI, and the peak-to-peak distance,  $L_{pp}$ . See Figure 2.

### 3. RESULTS AND DISCUSSION

To quantify the changes in epidermal thickness, the confocal images collected in the three dimensional array  $\mathbf{B}(x, y, z)$  were divided into 100 ROIs of size  $20 \text{ } [\mu\text{m}] \times 20 \text{ } [\mu\text{m}] \times 100 \text{ } [\mu\text{m}]$ . The thickness of the epidermis was determined by taking the peak-to-valley,  $L_{pv}$ , as well as peak-to-peak,  $L_{pp}$ , distance observed in the average A-scan for each ROI. Figures 3 and 4 presents a summary of the rCSLM results: a 1.68 fold increase in the peak-to-valley thickness and a 1.62 fold increase in the peak-to-peak thickness over a 24 [hr] period. The data is consistent with expected thickening of the epidermis following TPA application.<sup>7</sup>

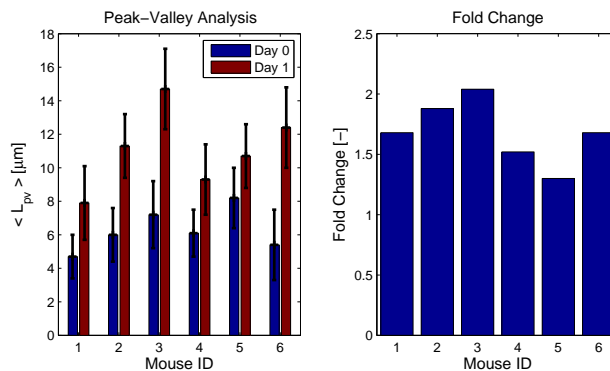


Figure 3. *Left*: Mean peak-to-valley distance obtained from confocal A-scans at day 0 and 1. Error bars denote one standard deviation. *Right*: Fold increase in the peak-to-valley thickness. The average fold increase for the 6 mice is 1.68.

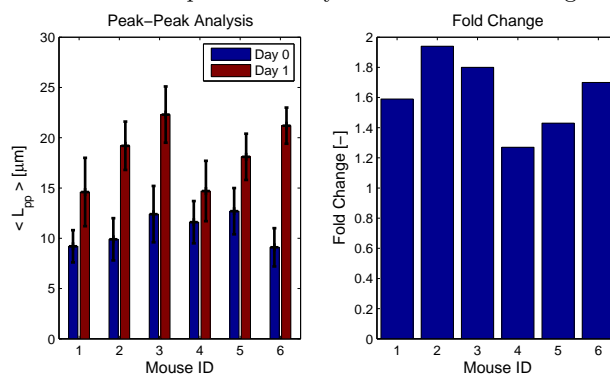


Figure 4. *Left*: Mean peak-to-peak distance obtained from confocal A-scans at day 0 and 1. Error bars denote one standard deviation. *Right*: Fold increase in the peak-to-peak thickness. The average fold increase for the 6 mice is 1.62.

## REFERENCES

- [1] González, S., Gill, M., and Halpern, A. C., [*Reflectance confocal microscopy of cutaneous tumors*], Informa Health Care, United Kingdom (2008).
- [2] Meyer, L. and Lademann, J., “Application of laser spectroscopic methods for *in vivo* diagnostics in dermatology,” *Laser Phys. Lett.* **4**(10), 754–760 (2007).
- [3] Daukantas, P., “Using optics to detect skin cancer,” *Optics and Photonics News*, November, 28–33 (2007).
- [4] Neerken, S., Lucassen, G. W., Bisschop, M. A., Lenderink, E., and Nuijs, T., “Characterization of age-related effects in human skin: A comparative study that applies confocal laser scanning microscopy and optical coherence tomography,” *J. Biomed. Opt.* **9**(2), 274–281 (2004).
- [5] Gambichler, T., S.Boms, Stucker, M., Kreuter, A., Moussa, G., Sand, M., Altmeyer, P., and Hoffmann, K., “Epidermal thickness assessed by optical coherence tomography and routine histology: preliminary results of method comparison,” *JEADV* **20**, 791–795 (2006).
- [6] Gambichler, T., Moussa, G., Regeniter, P., Kasseck, C., Hoffmann, M., Bechara, F., Sand, M., Altmeyer, P., and Hoffmann, K., “Validation of optical coherence tomography *in vivo* using cryostat histology,” *Phys. Med. Biol.* **52**, N75–N85 (2007).
- [7] Thuillier, P., Anchiraico, G. J., Nickel, K. P., Maldve, R. E., Gimenez-Conti, I., Muga, S. J., Liu, K.-L., Fischer, S. M., and Belury, M. A., “Activators of peroxisome proliferator-activated receptor- $\alpha$  partially inhibit mouse skin tumor promotion,” *Carcinogenesis* **29**, 134–142 (2000).
- [8] Kolb, T. M. and Davis, M. A., “The tumor promoter 12-o-tetradecanoylphorbol 13-acetate (tpa) provokes a prolonged morphologic response and erk activation in *tsc2*-null renal tumor cells,” *Toxicol. Sci.* **81**(1), 233–242 (2004).