Evaluation method of skin model thickness from 3D image by Optical Coherence Tomography

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Abstraction

As a skin model, sheet-like epidermal tissues cultured on insert well are widely used in assays. Thickness of the skin sheets is known as one of these phenotypes, and it is effective feature for capturing morphological changes. As a method for measuring the thickness, it is common to prepare a tissue section by the paraffin embedding method and measure the length from the upper part to the lower part of the tissue with a microscope. However, since this method is invasive evaluation method, subsequent detailed assay cannot be performed. In addition, because the evaluation is based on a tissue section, it is a local evaluation, and it is difficult to capture features such as distribution of tissue thickness. Furthermore, embedding with paraffin is difficult to maintain the original shape of the tissue. Therefore, in order to perform more appropriate thickness evaluation, a new method for non-invasively observing and quantifying the tissue on the insert well is required.

In recent years, optical coherence tomography (OCT) has become widespread as a three-dimensional diagnostic method for the fundus. OCT is a technique for rendering a three-dimensional tissue as a tomographic image, and the near-infrared light, used by OCT as a light source, is suitable for biological permeability and low in cytotoxicity.

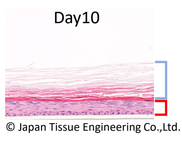
In this study, the OCT imaging device Cell3iMager Estier (SCREEN Holdings Co., Ltd.) was used to acquire 3D images of non-invasively stacked cell tissues, and we aimed to establish a new method for measuring of tissue thickness distribution. The cell sheet on the insert well was observed using Cell3iMager Estier, and a three-dimensional image was obtained. Based on the obtained three-dimensional image, cell regions were extracted by image processing, and the thickness distribution of the entire sheet was calculated. We report that it is possible to measure non-invasively the change of the thickness distribution of the whole cell sheet by the experimental condition.

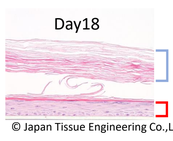
Introduction & Methods

In vitro Skin Models

Skin in vitro model is now available as an alternative to animal experiments in skin irritation tests.

LabCyte EPI-MODEL
(Japan Tissue Engineering Co., Ltd.)
➢ Skin model with epidermal structure





Thickness as one of the Phenotypes

- Only obtainable from invasive and fragmentary information such as tissue sections.
- Three-dimensional form may collapse by creating blocks for tissue sections.
- Cannot be used for further analysis due to invasive evaluation.

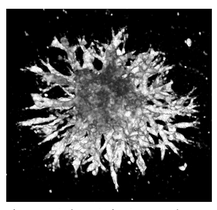
Difficult to imaging without sample damages

OCT Technology

OCT : Optical Coherence Tomography

- Using near-infrared light as light source
  ➢ Less damage to sample
- Acquire cross-sectional images and 3D morphology of tissue
  ➢ Widely used as fundus examination

Cell3iMager Estier
(SCREEN Holdings Co., Ltd)



Spheroid with invadopodia in Matrigel®

Possible to acquire 3D images non-invasively

Measurement of Skin Model Thickness using OCT Technology

Culturing LabCyte EPI-MODEL 24

OCT Imaging by Cell3iMager Estier

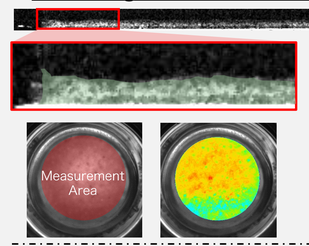
Imaging conditions

Magnification	Resolution	Scan Area (X-Y)
High	3 µm	1000 µm square
Low	10 µm	1000 µm square

Image Processing
Extraction of skin layers/
Calculate Thickness

How to Calculate Thickness

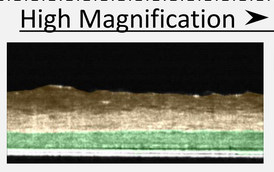
Low Magnification ➢ Imaging whole area of insert



Extract overall structure of skin model
(Cell layer / Cornified layer)

Measure in 90% Area from center of membrane

High Magnification ➢ Imaging center area of insert



Extract each layer by Deep learning

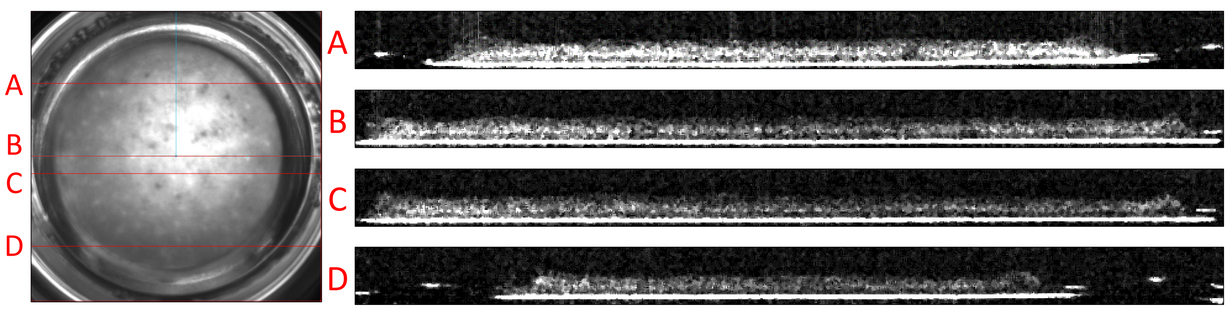
Measure each layer

Evaluated possibility of OCT imaging / thickness measurement

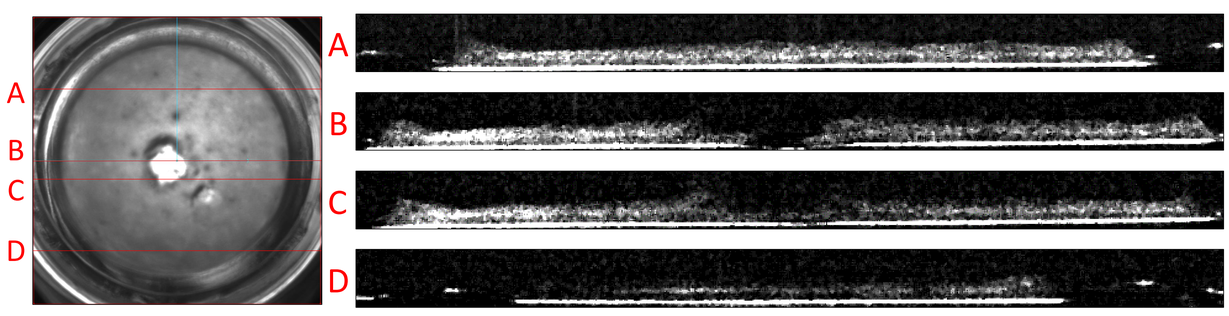
Results

Result 1 : OCT Imaging of Skin Model

【 Control 】



【 Scratched 】

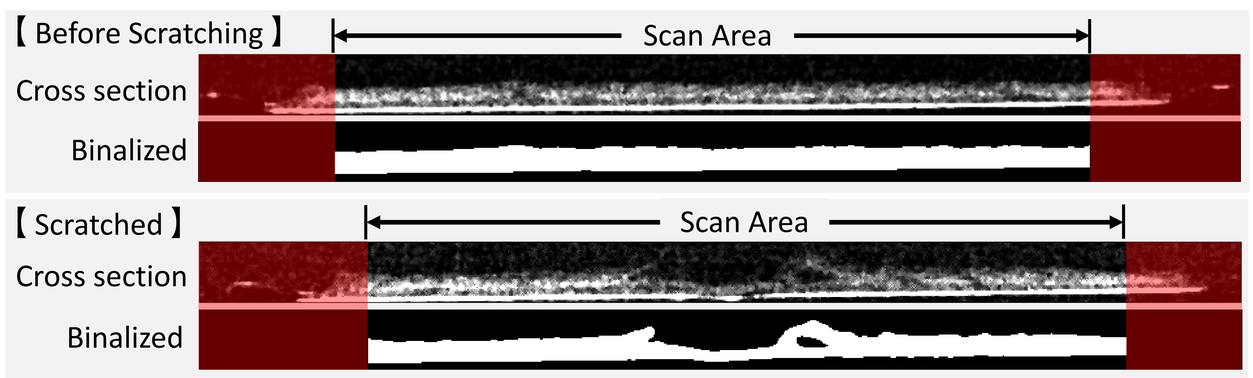


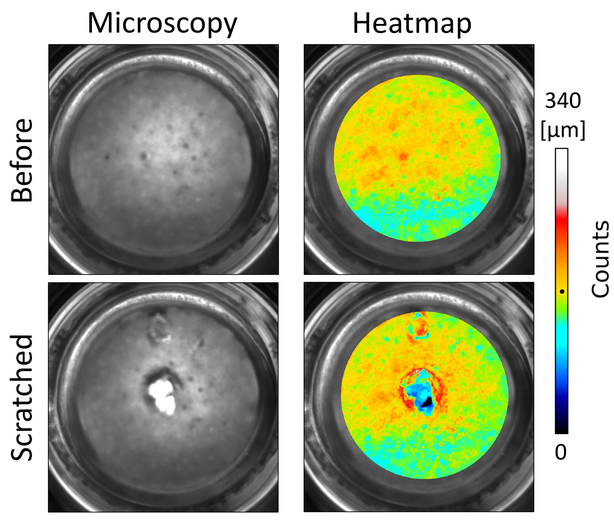
Skin models in 24-well inserts were imaged by OCT. (Low magnification)
For comparison, skin model (under; Scratched) was scratched tip of micropipette tip.
(Left) Microscopy images
(Right) Cross section images (OCT); At positions A~D in microscopic image

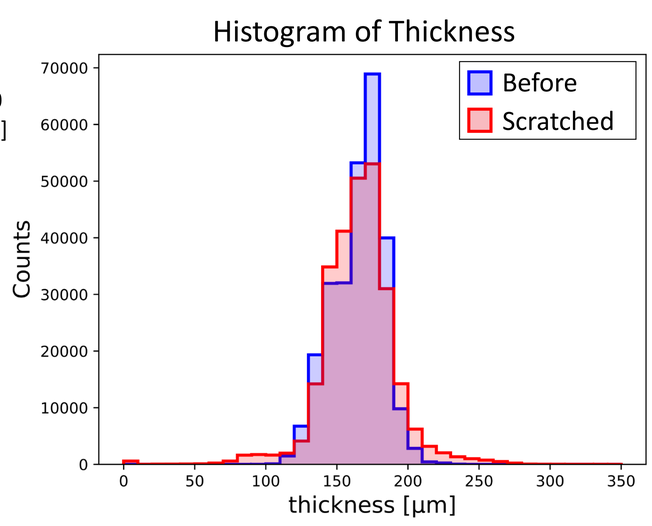
Imaging Condition
Resolution(X-Y); 10 µm / pixel Scan Area; 8000 µm × 8000 µm

Three-dimensional imaging of the entire skin model in a 24-well insert was possible.

Result 2 : Examination of Thickness Measurement Method of Skin model







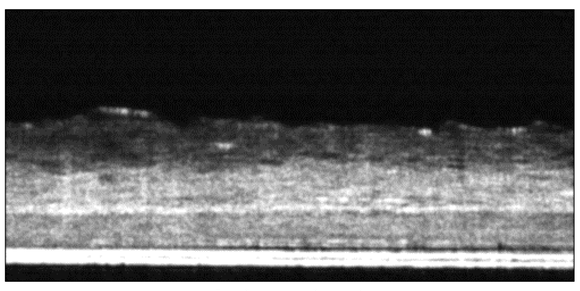
The skin model thickness was measured from the OCT imaging results before and after scratching the skin model inside the 24-well insert with the tip

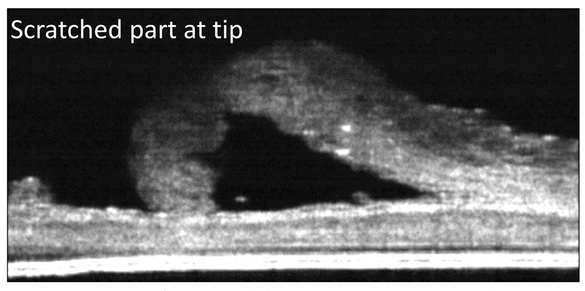
From the histogram of thickness, change in thickness was observed before and after scratching at the tip in the section from 0 to 100 µm and 200 µm~.
It was suggested that this method could catch the difference in thickness distribution of whole inserts.

Result 4 : Segmentation of Cell Layer/Cornified Layer in OCT Image.

Skin models in 24-well inserts were imaged by OCT. (High magnification)

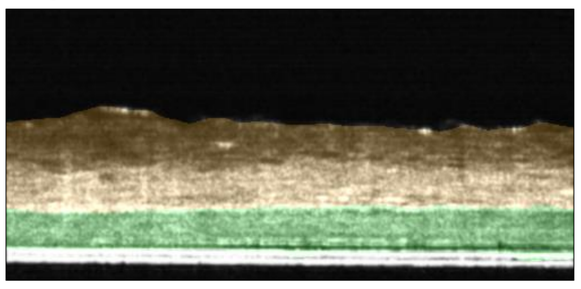
Imaging Condition
Resolution(X-Y); 2 µm / pixel Scan Area; 1000 µm × 000 µm

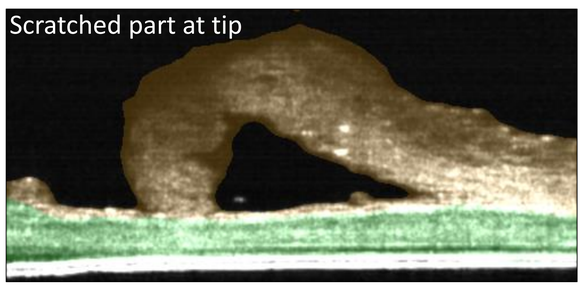




It was suggested that the layer structure boundary of the skin model could be observed.

＜Segmentation Result＞





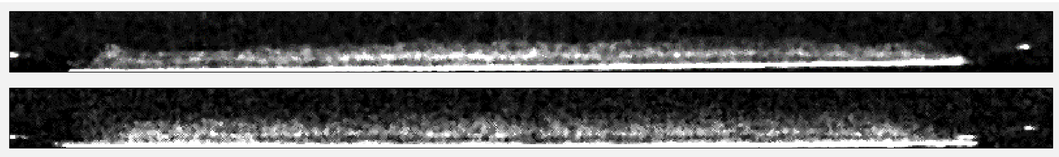
Examined whether layer structure can be extracted using Deep Learning Segmentation. ➢ Suggested that it is possible.

Result 4 : Application to Irritation Test using Skin Model

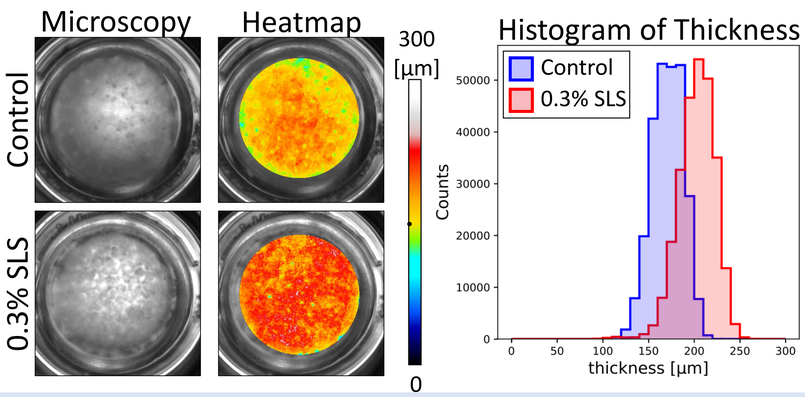
After exposure 0.3% SLS, skin models in 24-well inserts were imaged by OCT.
(High/Low magnification)

Scan Area Low/High Magnification : Whole Inserts/Center Area of Inserts (1000 µm square)

<Results of Low Magnification>



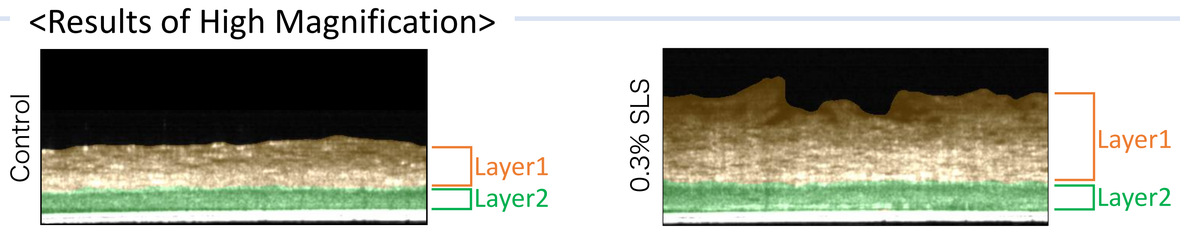
The entire skin models of two conditions were extracted and each thickness was measured.



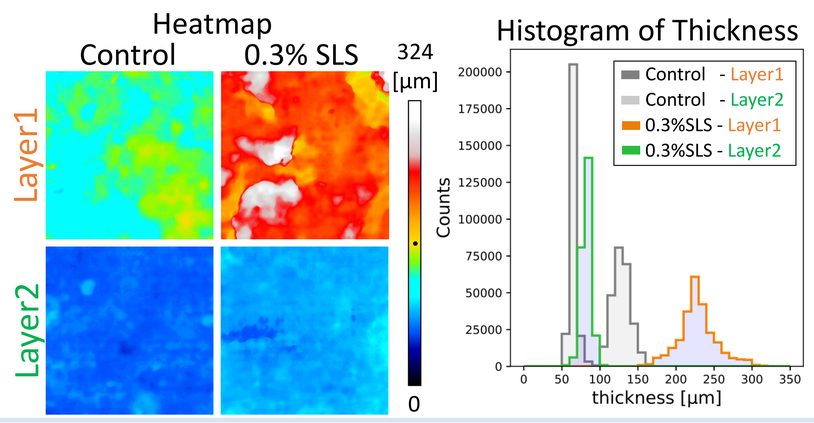
It turned out that the overall thickness tends to increase by SLS exposure.

It was suggested that changes in the overall thickness distribution of the skin model in the irritation test could be captured.

<Results of High Magnification>



For two conditions, each layer structure was extracted and its thickness was measured.



Using high-magnification imaging, it was found that changes in the thickness of each layer due to SLS exposure can be captured.
(Change : Layer1 > Layer2)

It was suggested that the change in thickness distribution of two layers in the skin irritation test could be quantified.

Conclusion

- It was suggested that OCT imaging could be used to non-invasively measure the overall thickness of in vitro skin models in 24-well insert.
  ➢ Low Magnification : Distribution of Whole Insert
  ➢ High Magnification : Distribution of Cell Layer/Cornified Layer