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Automated keratinocyte identification on *in vivo* reflectance confocal microscopy images of the epidermis

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INTRODUCTION

Reflectance Confocal Microscopy^{1,2} (RCM) is a real-time non-invasive *in vivo* technology that allows the visualization of the epidermis and upper layers of the dermis at cellular level. It provides information on the geometrical and topological properties of the skin, which play important roles in the makeup of the skin barrier.

In most cases, analysis of RCM stacks is done manually, providing qualitative observations. However, manual analysis is time-consuming and subject to human interpretation and inter-expert differences. Thus, we could benefit from automated methods to quantitatively analyze RCM images.

Our approach focuses on RCM images of the *stratum granulosum* (SG) and *stratum spinosum* (SS) where viable cells, called keratinocytes, appear in a honeycomb pattern. They are both characterized by a dark center and a grainy cytoplasm due to micro-structures, surrounded by bright grainy membranes.

Attempts at the automated identification of epidermal cells on RCM images have been made^{4,5}. Unfortunately, the amount of noise and heterogeneity of RCM images hinders the development of accurate segmentation methods.

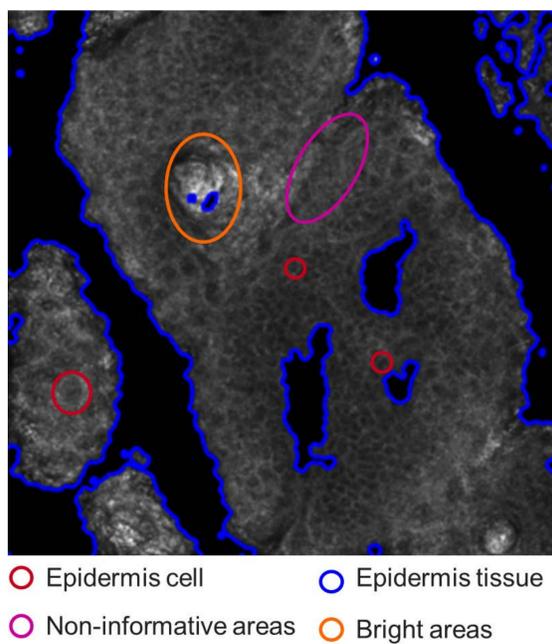


Fig. 1 RCM image of the *stratum spinosum* of light-pigmented skin. In blue, the border between tissue and background. In pink, non-informative areas. In orange, bright spots. And in red, epidermal cells.

We propose a method to automatically detect keratinocytes centers on RCM images of the *stratum granulosum* and *stratum spinosum*.

IMAGE ANALYSIS WORKFLOW

The first step is to identify the region-of-interest (ROI) containing cells. We start by identifying the tissue using the Morphological-Geodesic-Active-Contour method. We further refine the ROI by removing non-informative bright areas sometimes present in RCM images by applying an alternate sequential filter on the binarized blurred image. Other spurious areas are removed using a Support Vector Machine algorithm trained on Grey-level-Co-occurrence-Matrix features.

The second step is to identify individual cells in the segmented tissue area. To perform this task, we use successive application of Sato and Gabor filters. The outputs of these filters are then locally normalized, binarized, and skeletonized. The obtained skeletons (representing cell membranes) are pruned to remove spurious branches.

The third step is a post-processing to improve the detection and remove size outliers (detected areas too small or too large to be accurate detections of cells).

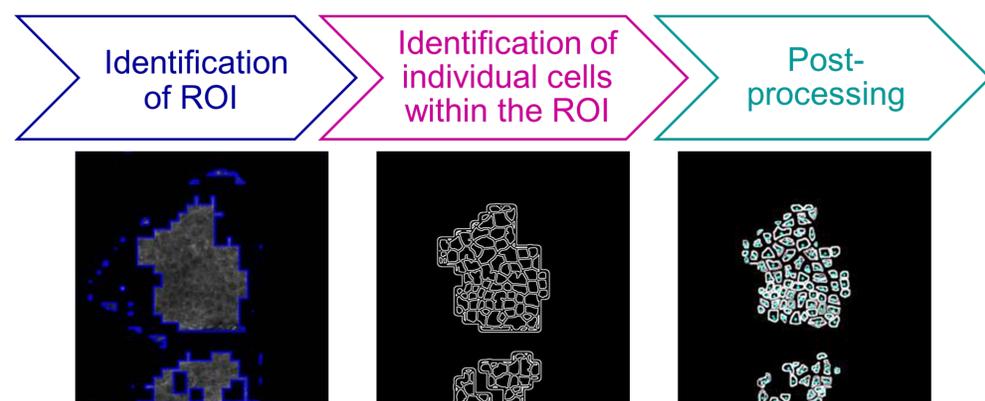


Fig. 2 Steps of automated cell identification

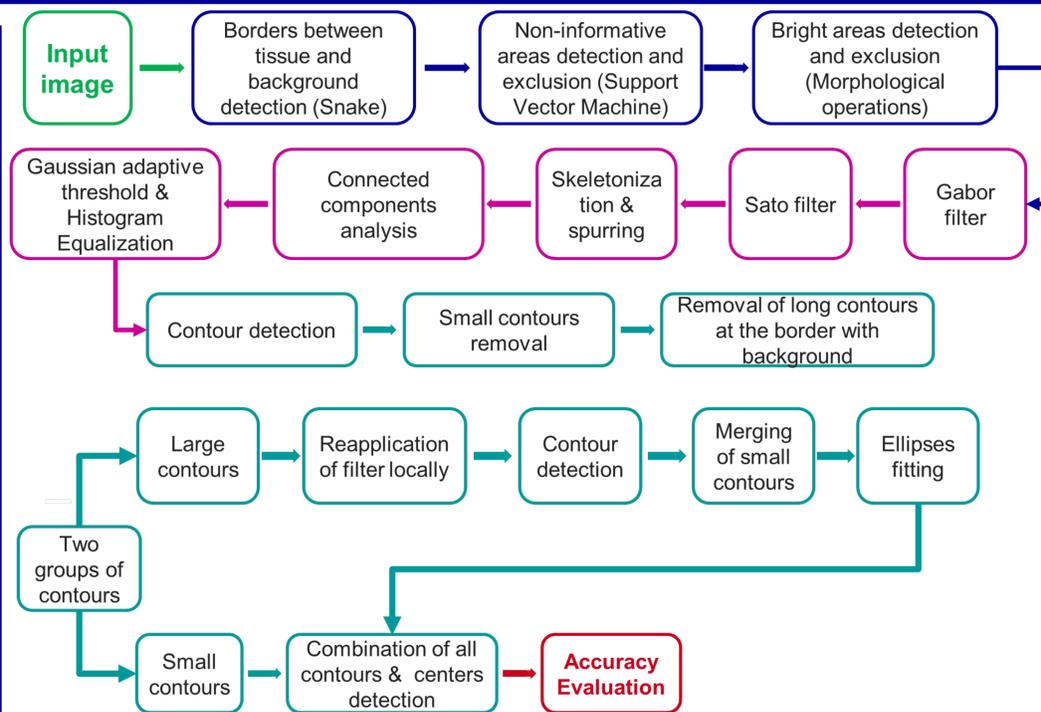


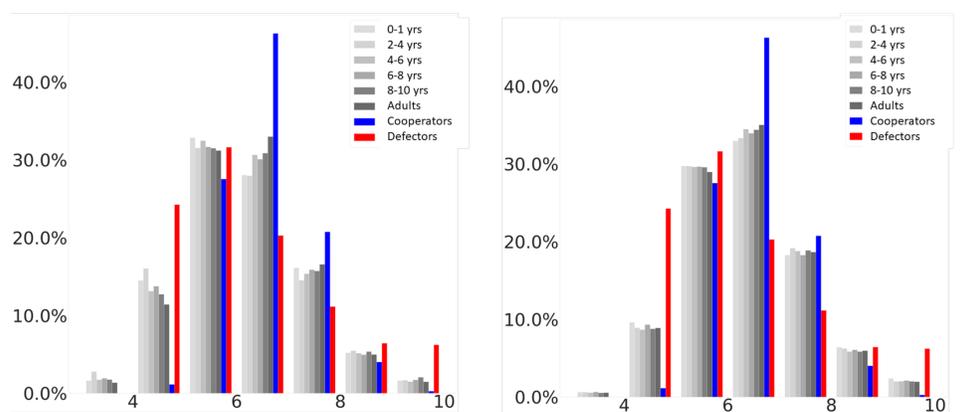
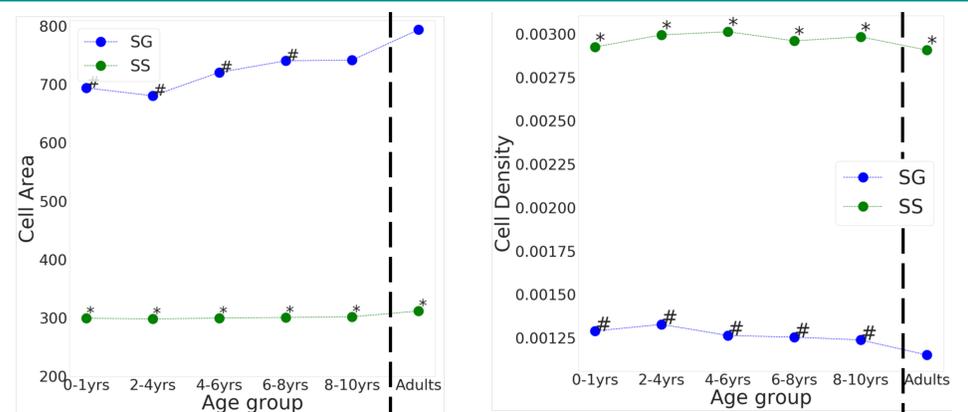
Fig. 2 Steps of automated cell identification

RESULTS

Image	Precision	Recall
Image 1	86%	72%
Image 2	71%	82%
Image 3	81%	86%
Image 4	72%	91%

We have shown that the automated detection of keratinocytes on RCM images of the SG and SS is achievable and comparable to expert's manual identification.

HEALTHY SKIN MATURATION



* indicates that the SS value is significantly different from the SG value; # indicates that group value is significantly different from the adult group value.

The epidermis continuously changes between 0 and 10 years-old with important inter-person variability. Cell geometry changes with age and per epidermal layer but topology remains the same.

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