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# Automatic cell identification and analysis on in vivo reflectance confocal images of the human epidermis



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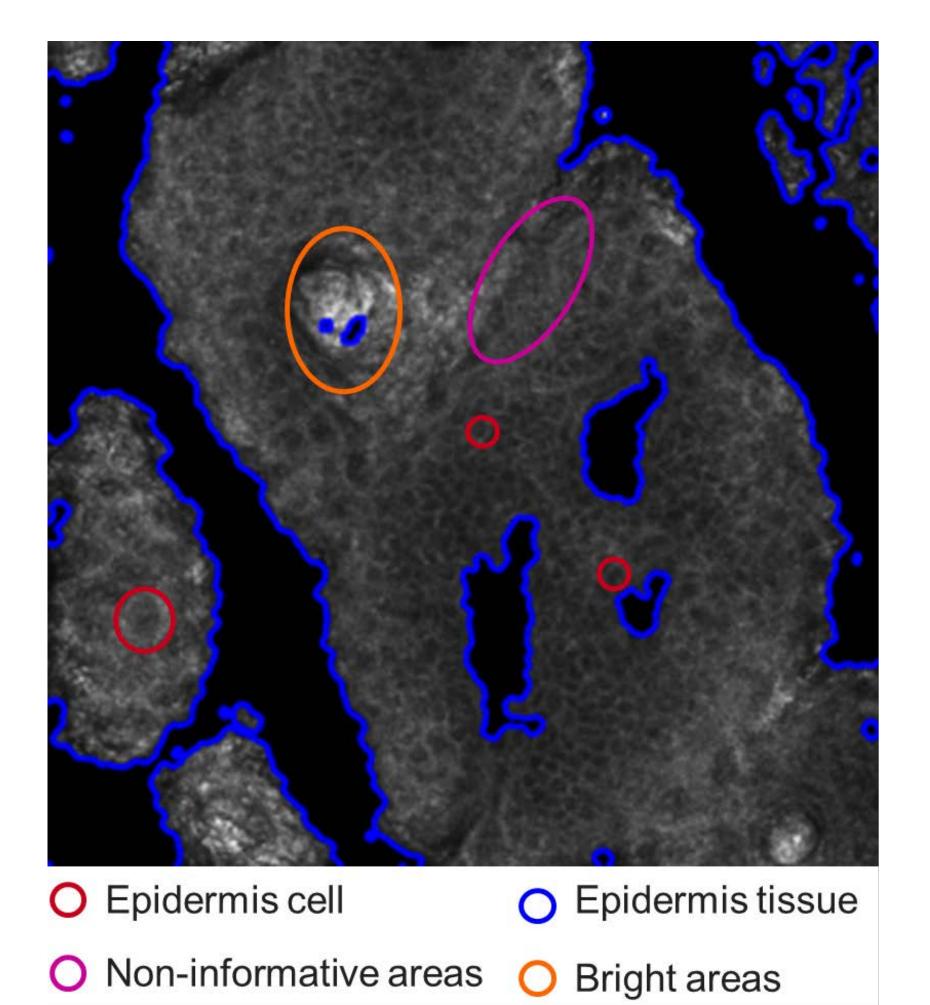
#### INTRODUCTION

Reflectance Confocal Microscopy<sup>1,2</sup> (RCM) is a real-time noninvasive in vivo technology that allows the visualization of the skin 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. Granular cells are typically larger than spinous cells and thus have a higher density<sup>3</sup>. 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<sup>4,5</sup>. Unfortunately, the amount of noise and heterogeneity of RCM images hinders the development of accurate segmentation methods.



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.

automatically propose to keratinocytes centers on RCM images of the stratum granulosum and stratum spinosum.

### IMAGE ANALYSIS WORKFLOW

RCM images tend to be noisy and non-uniform, which hinders the development of automated segmentation methods. To guide our cell detection, we started by identifying the region of interest (ROI), i.e., the regions containing epidermal cells.

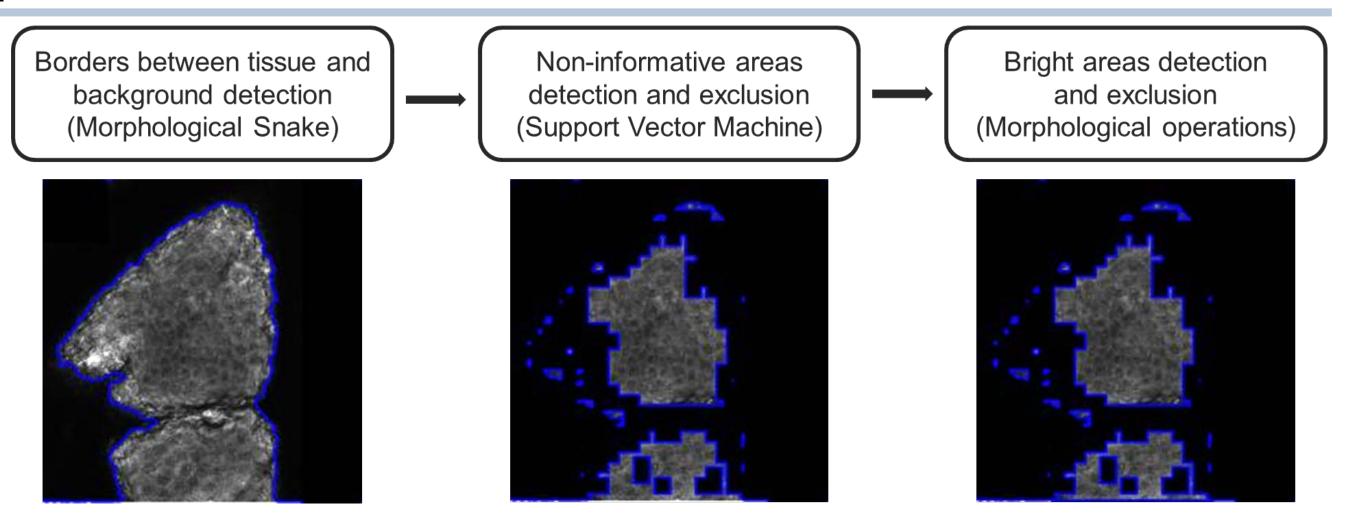


Fig.2 Steps of the identification of the region of interest. In blue the region of interest mask.

After obtaining the region of interest, the individual cells were identified on the ROI by applying a Sato tubeness filter followed by a Gabor filter on the processed image.

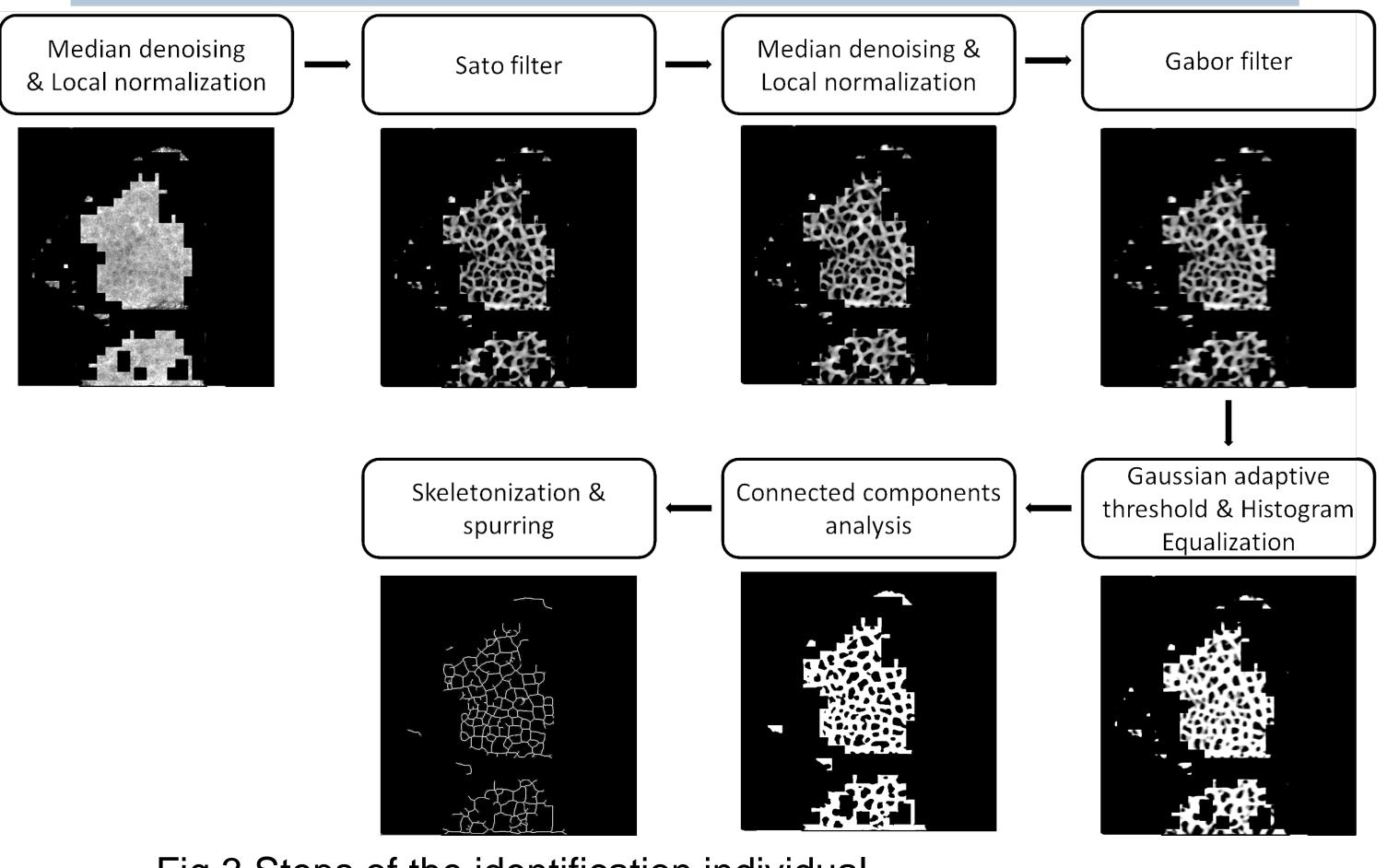


Fig.3 Steps of the identification individual

The obtained skeleton was then cleaned, and its contours, i.e., cells were identified and corrected, and their centers detected.

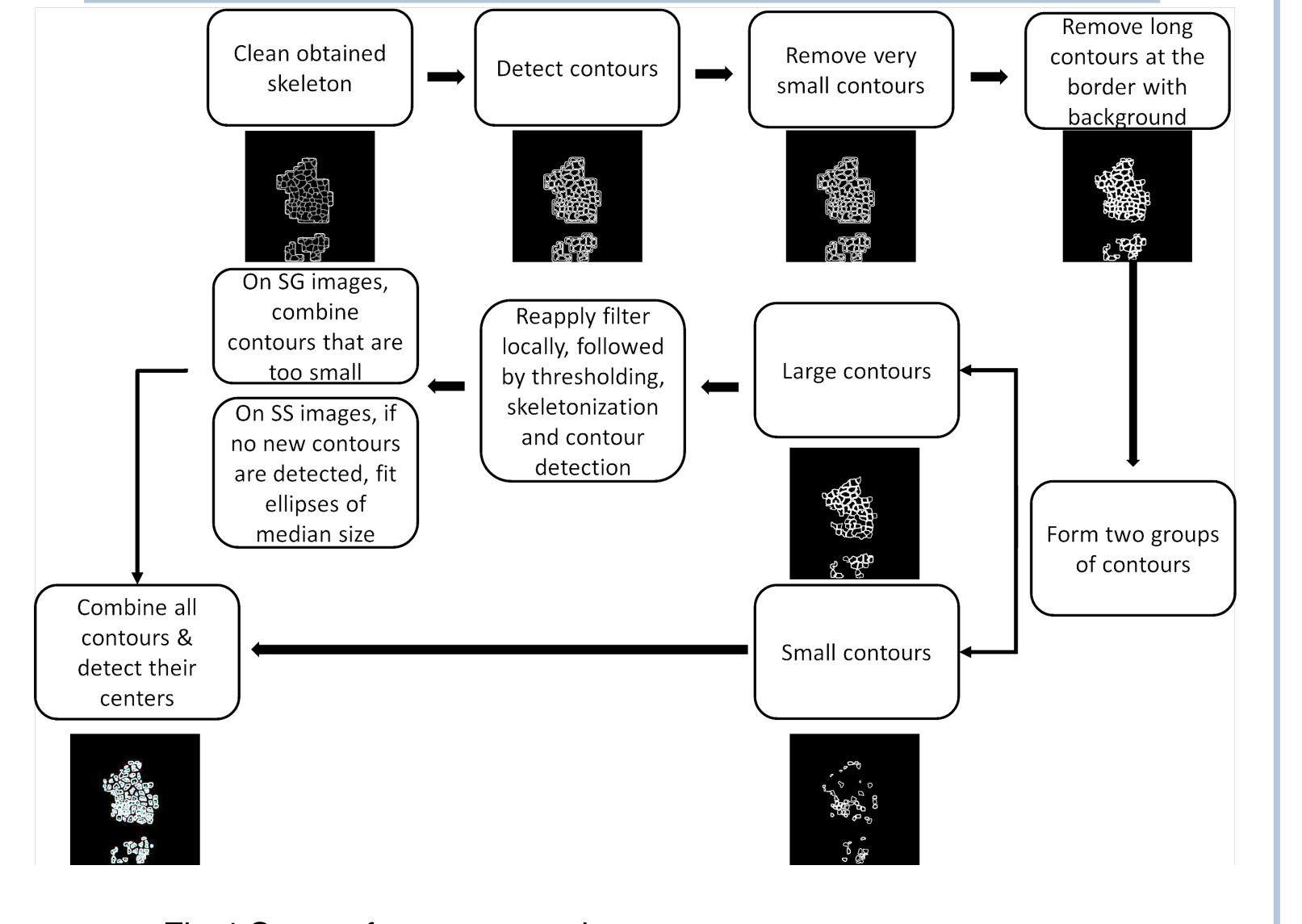


Fig.4 Steps of post-processing

The obtained cell centers were used to initiate a markercontrolled watershed<sup>6</sup> on the ROI which generated a labeled image which was compared against a manual ground truth. Two accuracy metrics were evaluated: precision and recall.

## RESULTS

We evaluate the performance of our approach with respect to two experts. When looking into the differences between the experts, we notice that expert 2 is less sensitive in his detection especially in SS images. The obtained results are more consistent with expert 1 and prove to be accurate compared to inter-expert variability.

Table 1. Table of the cell detection accuracy on stratum granulosum and stratum spinosum RCM images for two different experts

		Precision	Recall
Stratum granulosum RCM image	Detection vs. Expert 1	77%	85%
	Detection vs. Expert 2	64%	82%
	Expert 1 vs. Expert 2	52%	48%
Stratum spinosum RCM	Detection vs. Expert 1	85%	67%
image	Detection vs. Expert 2	75%	30%
	Expert 1 vs. Expert 2	20%	28%

#### DISCUSSION

Our approach gives accurate (precision>75%, recall>65%) results on RCM images of the granular and spinous layers. Performance can be hindered by:

- The presence of multiple layers per image, which makes parametrization of the different steps complicated, although, parameters for a given layer are the same.
- Noise and non-uniformity of the images have a great impact on the method performance.

Computational time is about 10mn depending on the size of the ROI, which is a major advantage when compared to the time required to identify keratinocytes manually on RCM images.

We have shown that the automated detection of keratinocytes on RCM images of the SG and SS is achievable, which is an important step towards the quantitative study of these images and of skin.

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