SPECIFIC IMAGE PROCESSING FOR AUTOMATED ANALYSIS OF HISTOLOGICAL CUTS OF RECONSTRUCTED *IN VITRO* SKIN AND *EX VIVO* HUMAN SKIN

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The standard Hematoxylin-Eosin-Saffron (HES) staining is one of the major stains currently used in histology and is very useful to characterize biological samples, especially the skin. It is often considered as the gold standard since providing information about the **nucleus** (colored purple due to Hematoxylin stain), the **cytoplasm** (colored pink due to eosin stain) and **collagen fibers** (colored orange due to saffron stain). As for **melanin** quantification, a specific reaction is often used, namely Fontana Masson staining, which reveals the melanin as black dots on skin cross sections deposited on a glass slide.

To evaluate the de-pigmenting or pro-pigmenting potential of cosmetic ingredients, these types of staining are used on cross-sections of either *ex vivo* human skin samples or *in vitro* reconstructed skin models such as the Reconstructed Pigmented Epidermis (RPE). On these images, the two upper layers of the skin, namely the *stratum corneum* (SC) and the living epidermis (LED) are of major interest. In order to identify precisely these two layers, an image processing algorithm has been developed to detect three limits: The external surface of the skin (upper limit surface), the junction between the *stratum corneum* and the living epidermis (SC/LED interface), and the basal layer or dermo-epidermal junction (DEJ).

Since the last few years, Whole Slide Imaging (WSI) solutions have been widely used in the field of digital histology. It allows whole slides to being digitized in a fast manner (several hundred slides a day are usually processed). However, it is necessary to develop automated tools that offer a precise analysis of the recorded data.

The present work aims at developing automated image processing tools for HES and Fontana Masson histological images. These developed tools can automatically process a slide image with one or more tissue samples (input: large field image) and generate a formatted measurements file (output: text file) containing all the computed measurements for each tissue sample (see Figure 1).

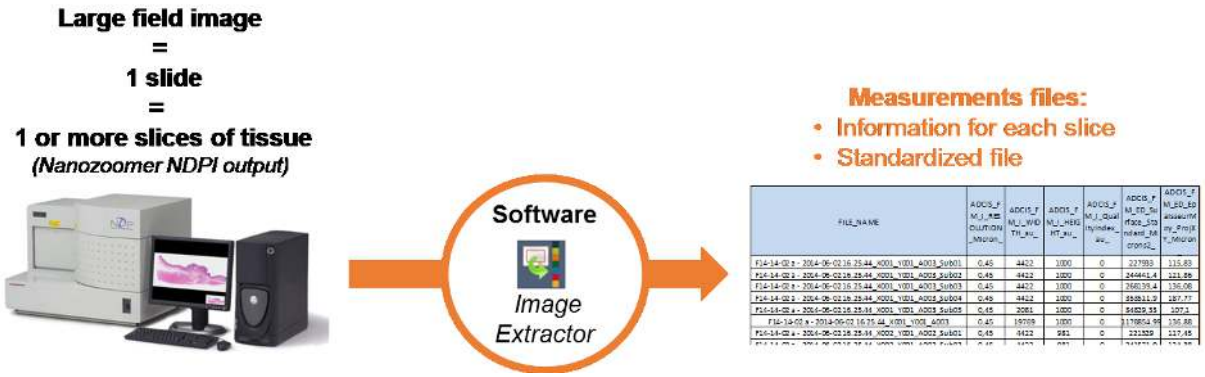


Figure 1: Global overview of the software: from an ndpi image file (Hamamatsu NanoZoomer Slide image format) to measurements.

The final aim of the work is to reduce the global analyzing time and to improve the reproducibility and traceability of the quantification. Specific algorithms are therefore developed for both types of stained images:

- Step 1: To detect and extract all the samples on a slide image ("sample detection", see Figure 2) by creating one image per sample: The slide image (ndpi format) is converted into one sample image (tif file) with some pre-processing to normalize the image including the color rendering correction and the rotation of the sample to always ensure the skin surface is located at the top of the image;
- Step 2: To exclude invalid areas due to a defect on the slide and digitally cut the sample image into several sub-images, that are then processed during the segmentation step as the entire slide is too large to be processed at once;
- Step 3: To segment each sub-image by detecting the **3 limits** (surface, SC/LED and LED/DEJ) and, in the case of Fontana Masson staining, the **melanin** pixels. If needed, the result of automated detections (interfaces and melanin) can be manually edited by the operator;
- Step 4: To compute different measures from these segmented areas, including morphological parameters of the skin layers (area, thickness, ...), melanin density and distribution in each skin layer as well as the melanin profile distribution in the depth of the skin.

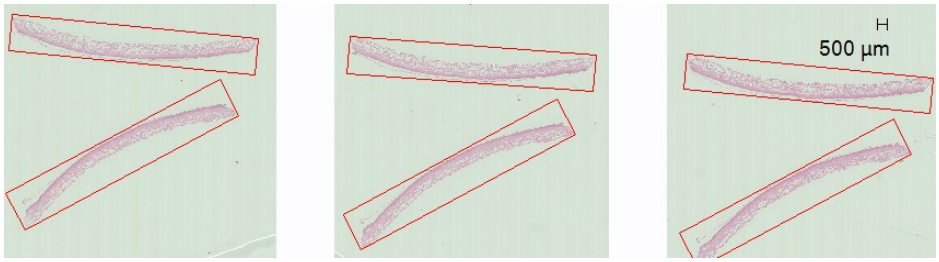
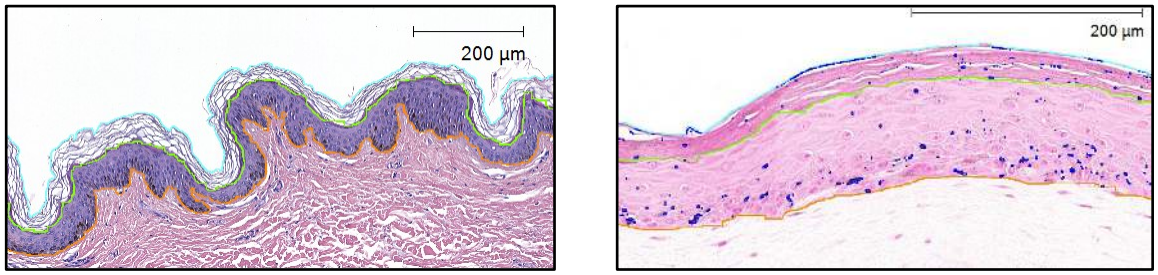


Figure 2: Example of slice detection obtained on Fontana Masson staining image of Reconstructed Pigmented Epidermis (RPE): the red boxes delimit the 6 samples on the slide.

The algorithms that have been developed were adjusted to the specificity of each type of tissue, especially the basal layer, the detection of which depends on the aspect of the basal layer (dermis, support, or membrane). Figure 3 shows some results obtained on different skin tissue samples.



(a) (b)

Figure 3: Example of results obtained on (a) HES staining of ex vivo human skin and (b) Fontana Masson staining of Reconstructed Pigmented Skin (RPS): three limits detected (cyan for skin surface, green for SC/LED and orange for basal layer) and melanin detection (blue).

The results show an accuracy in more than 85% of the processed images database. In the future, *Deep Learning* approaches will be evaluated to improve the accuracy of the detection for the remaining 15%.

These tools allow to automate the analysis of the whole sample, to improve the reproducibility of the quantification, and to reduce the time spent on the analysis. Embedded in a single software, they are very promising and useful to automatically evaluate, as example, the efficiency of cosmetic ingredients in the field of pigmentation and/or skin quality.