Automated histological segmentation of hair follicles via hierarchical PLS-DA and FTIR hyperspectral imaging

J. Poulain[1], J. Avila[2], C. Sandt[3], S. Roussel[1], T. Bornschlögl[2]

[1] Ondalys, 8, av de l'Europe, 34830 CLAPIERS, jpoulain@ondalys.fr

[2] L'Oréal campus Aulnay : 1 Avenue Eugène Schueller, 93600 Aulnay -sous-Bois, jocasta.avila@loreal.com

[3] Synchrotron Soleil, Ligne SMIS, synchrotron SOLEIL, RD128, Saint Aubin, France, sandt@synchrotron-soleil.fr

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1 Introduction

The hair follicle is a complex, multi-compartment mini-organ responsible for the production of hair fibers. It can be divided into anatomically and biologically distinct regions: the dermal papilla (DP), the hair cortex that arises due to cell differentiation of keratinocytes situated in the matrix, along the transition zone (TZ) and the pre-cortex, the outer root sheath (ORS), inner root sheath (IRS), and the conjunctive tissue sheath (CTS). Recent advancements in infrared (FTIR) microscopy have established this label-free technique as an important tool for characterizing *ex-vivo* follicles, evolving from structural identification to the detection of biochemical markers and energy-storing carbohydrates [1]. This study aims to automate the identification of these tissues using hyperspectral imaging to better assess the impact of cosmetic active ingredients on the follicle health.

2 Material and methods

Transmission-mode FTIR microspectroscopy was performed on 49 hair follicles using an Agilent Cary 620 microscope coupled to a Cary 670 spectrometer (3900–800 cm^{-1}), located at the SOLEIL Synchrotron Facility. To capture the full morphological extent, spectral images were acquired as concatenated mosaics (up to 384 × 128 pixels), enabling chemical mapping across the longitudinal structure. Data were baseline-corrected using Air-PLS [4], and follicles were segmented from the resin background via Particle Size Analysis (MIA Toolbox, Eigenvector Research Inc., USA).

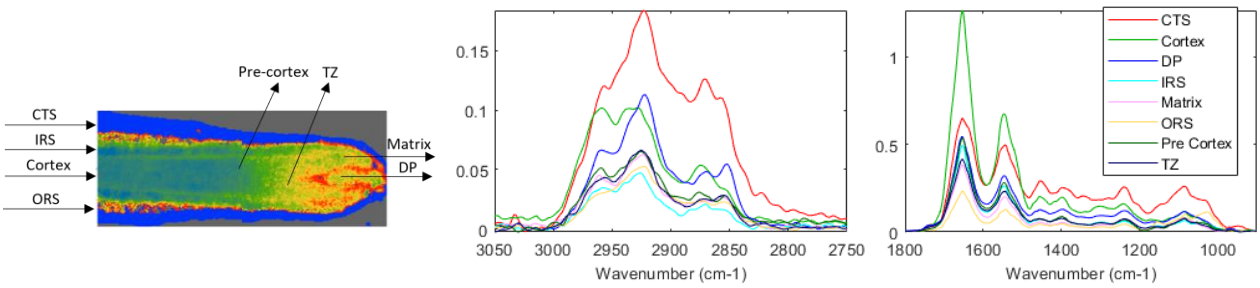

Figure 1 – Theoretical segmentation of hair follicles into 8 tissues and corresponding spectral profiles.

For model construction, reference pixels were manually selected based on the theoretical anatomical location of the 8 target tissues (Figure 1). Nine control samples were randomly selected for the calibration set, and validation was performed using block cross-validation per follicle. A **PLS-DA** [3] **double hierarchical approach** was implemented: the first level discriminates k classes versus all others, followed by a second model for the remaining classes. The double hierarchy corresponds to the fact that for each PLSDA model, the class is attributed hierarchically by first examining the probability of belonging to a class A, then to class B, and so on, until all classes have been checked in a specific order. This process is particularly useful for pixels with a high probability of belonging to multiple classes. Pixels belonging to none of the classes are excluded.

3 Results and discussion

Although eight tissues were initially targeted, the biological continuum of cell differentiation [4] and significant spectral overlap led to the merging of two adjacent tissues, resulting in a 7-class model with high coherence across different samples (Figure 2).

Occasional pixel misassignments and class spreading were observed, which can be attributed to several factors: the inherent difficulty of obtaining perfectly aligned longitudinal sections, the lack of a true reference method for pixel selection, and the high chemical similarity between transitional tissues. These factors contribute to a localized model error, particularly at tissue boundaries. To further elucidate the biochemical drivers of this segmentation, the interpretation of discriminant coefficients is currently underway.

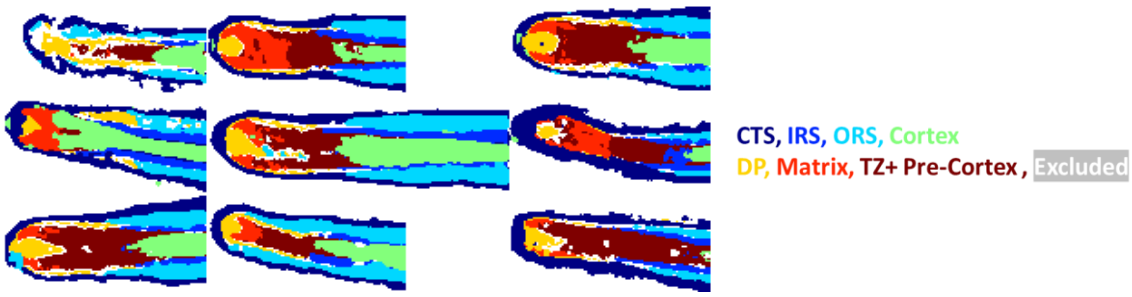

Figure 2 – Identification of 7 tissues in some follicles.

4 Conclusion

Despite the variability between hair follicles (biological samples, donors, sectioning heterogeneity, measurement campaigns) and the lack of true label-free references, multivariate data analysis based on hierarchical discrimination models has demonstrated its great suitability for the automatic segmentation of the different hair follicle tissues.

This methodology provides a robust framework for future studies evaluating the molecular impact of cosmetic active ingredients on hair growth.

5 References

[1] Sandt, C. and Bildstein, L. and Bornschlögl, T. and Baghdadli, N. and Thibaut, S. and Fazzino, P. and Borondics, F. Spectral histology of hair and hair follicle using infrared microspectroscopy. Internation Journal of Cosmetic Science 2024, *46*, 949-961.

[2] Z Zhang, S Chen & Liang Y. Baseline correction using adaptive iteratively reweighted penalized least squares. *Analyst*, issue 5, 2010.

[3] M Barker & Rayens W. Partial least squares for discrimination. *Journal of the Chemometrics Society*, volume 17, issue 3: 166–173, 2003.

[4] Tissot N., Genty G., Santoprete R., Baltenneck F., Thibaut S., Michelet J., Sequeira I., Bornschlögl T. Mapping cell dynamics in human ex vivo hair follicles suggests pulling mechanism of hair growth. *Nature Communications* 2025, *16*, 10267.