Highlight the efficacy of your cosmetic products with Atomic force microscopy

A. DRILLAT, C. PAILLIER, G. RUNEL, J. CHLASTA
BioMeca® SAS, Lyon, France

contact@bio-meca.com

Introduction

Atomic Force Microscopy (AFM) is composed by a sharp tip attached on a flexible cantilever which is used to indent or to scan sample surface. When a known force is applied, the cantilever bends; this process is monitored with the use of a laser beam reflecting from the top of the cantilever into a photodetector. From the process mentioned above, we obtain force–displacement curves which give us accurate results about **mechanical properties** of the sample. It also provides probe displacement on the surface to generate high resolution imaging.

By using **high resolution surface imaging**, **traction force assay** and **Stiffness Tomography** (ST), BioMeca has developing a **non invasive method** to measure the mechanical properties of biological samples (skin, hair etc..): hydration, elasticity, ageing, UV radiation effects, blue light, etc..

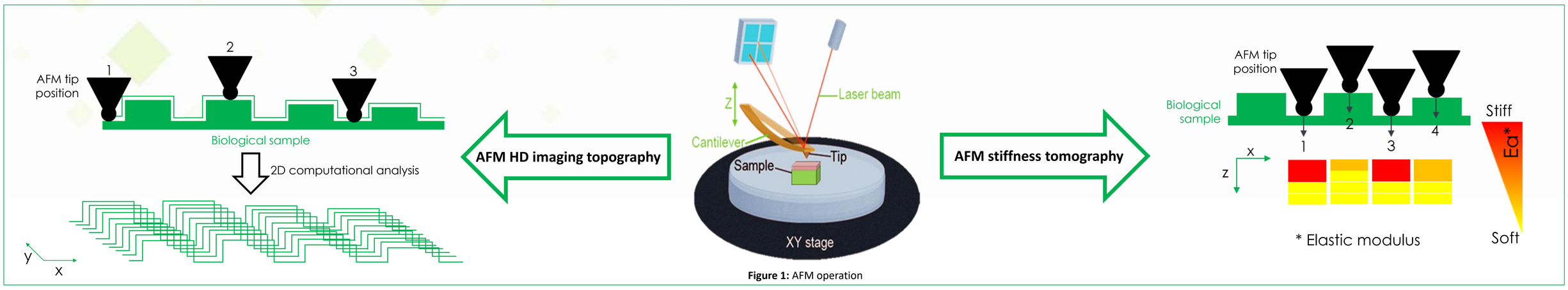

Figure 1: AFM operation

High resolution surface imaging

High resolution imaging enables us to study cells organisation and morphological changes. Regarding studies on hair, High resolution imaging allows us to visualize condition of the outer cuticle. To better understand inner organization, we can also combine high resolution imaging to mechanical properties.

➢ Investigation of corneocytes topography

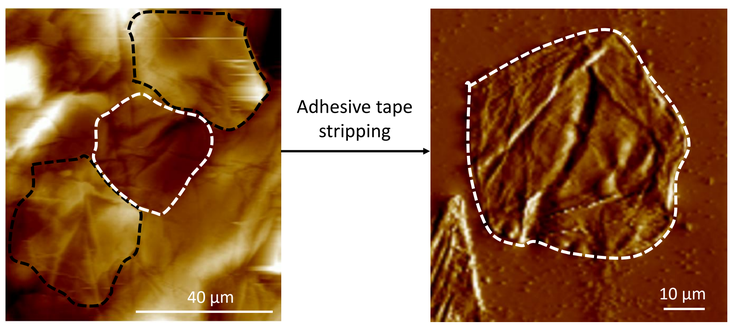

Figure 2: High resolution imaging of skin surface. The dotted line marks out the corneocyte and the colour corresponds to the layer. On the left, we have the surface imaging of an isolated corneocyte harvested by tape stripping.

➢ Hair surface imaging and mechanical properties

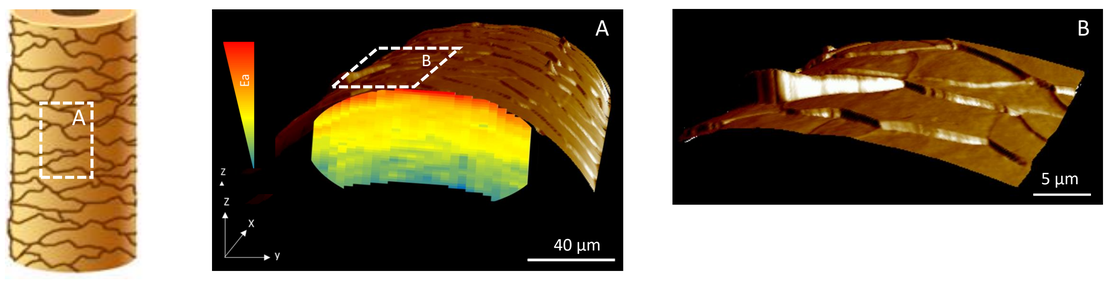

Figure 3: A, 3D high resolution imaging of hair surface associated with hair mechanical properties. B, zoom on the outer cuticle of hair.

Fibroblast traction assay

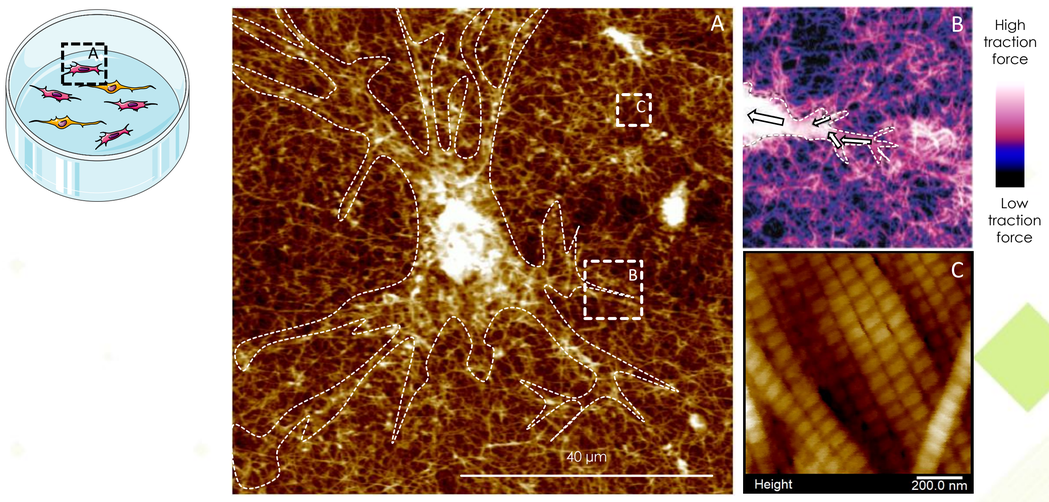

Figure 4: Measurement of the extra cellular matrix fibre stress induced by fibroblasts. A, fibroblast and collagen coated on a petri dish topography by high resolution imaging. B, heat map of traction forces exerted by fibroblast on the matrix. The white colour corresponding to a high traction force exerted by the cell is localised next to cytoplasmic processes. The dotted line marks out the fibroblast, arrows show direction of the traction force. C, zoom on collagen banded periods in the petri dish.

Skin mechanical properties

➢ **Finite element method** is used to simulate the mechanical behaviour (distortion) of biological samples when a force is applied with an AFM tip. The objective is to mimic the skin distortion and displacement during indentation.

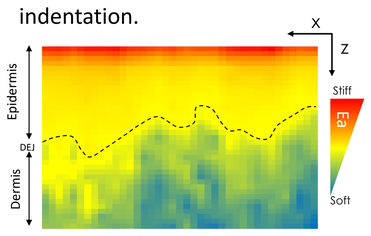

Figure 5: Skin stiffness tomography. Red corresponds to high stiffness, blue to a low stiffness.

Validation by computational analysis

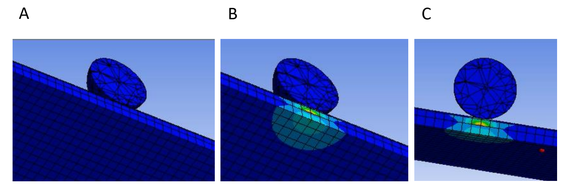

Figure 6: Simulation by using finite element method. The sphere represents the spherical tip probe and the dark blue line corresponds to the skin. A, The tip is not in contact with the skin. B, The tip gets in contact with the skin. With a 3µm indentation, the tip is able to distort the skin until reticular dermis. C, double deformation: epidermis and the dermis.

➢ Mechanical modification in the dermis

A 2mm glass sphere was included under the dermis, at 5mm depth. Indentation was performed above and besides the sphere in order to check if we can sense it with a tip indentation.

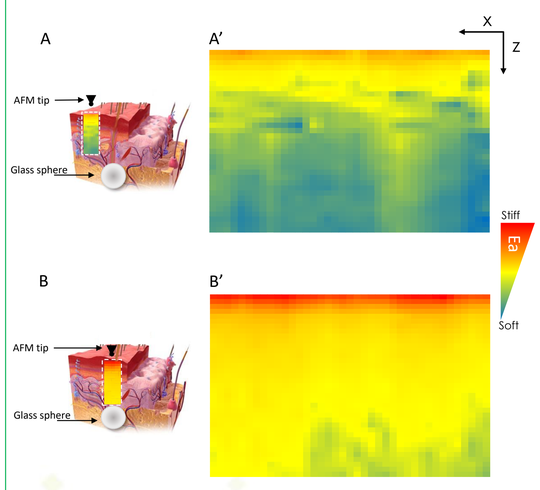

Figure 7: Stiffness tomography of skin with a glass sphere in the dermis. The scales are normalized in order to compare data. A, location of measurement besides the glass sphere. A', soft skin stiffness corresponding. B, location of measurement above the glass sphere. B', stiff skin stiffness corresponding.

➢ Cosmetic products effect on epidermis and dermis

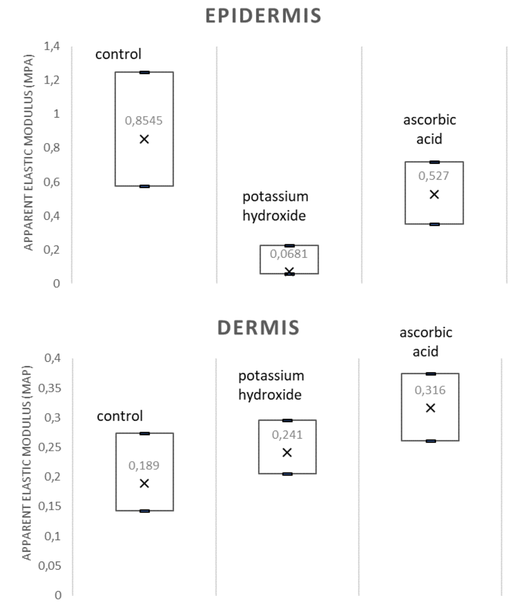

Figure 8: Stiffness of epidermis and dermis treated by aqueous solution of potassium hydroxide 0,2% and aqueous solution of ascorbic acid 20%.

Stiffness tomography and analysis of apparent elastic modulus allow us to highlight the action of active ingredients on the different compartments of the skin regarding rigidity, firmness and tension.

Conclusion

- Reliable method based on high resolution imaging and measurement of mechanical properties along tissue depth.
- Measurement of mechanical changes before/after application of cosmetics product, in terms of traction, tension, rigidity, firmness, hydration…
- Coupling imaging and mechanical mapping to show a unique visual effect of active ingredients.