

# **ANTI-AGING**

#### **Combining Points of View**

Hello,

We are glad to share with you the 3rd **FOCUS**, the biannual discussion panel of Skinobs dedicated to the **Anti-Aging Testing.** The latest Focus was published in June and was considering the <u>Sun care testing for both Clinical and Preclinical evaluations</u>. This new publication is 8 articles, 8 points of view of the today testing evolution for personal care, actives, ingredients and medical devices.

For some years, the beauty industry is gradually leaving the era of **anti-ageing** behind. Today, most consumers are more in the mood for a **well aging**, **slow aging or pro aging approach...** Some brands have used the idea of "improves the appearance of skin quality" and "restore the skin comfort". A new vocabulary of renewal, regeneration, plumpness and "glow" now dominates the language of the antiaging claims.

Beauty then becomes more integrative, it will globalise well-being, the silhouette, the sleep quality, or the lifestyle... resulting in a different look expecting new codes and expressions. In this quest for mindful beauty radiance, what women expect from skin care is emotional pleasure, sensoriality. It is all about supporting the aging process rather than fighting the signs of aging.

#### The Exposome Process beyond the Aging

During consultations with the elderly, the signs of aging observed are mainly: skin atrophy, xerosis, various keratoses. The skin abnormalities that characterise photoaging depend on the light or dark phototype. Light skins become cut instead, dark skin becomes thicker. UVB and UVA ultraviolet rays cause severe damage to the dermis. The exposome can be summed up mainly by the **oxidative stress** caused by different environmental conditions. The pro-age treatment can boost blood circulation, encouraging collagen production and helping lift, plump and **define contours for a firmer appearance**.

### The Studies of Skin Age Perception

To look more closely at the category of anti-aging, it represents the most numerous, precise and specific claims of all skin care products. In addition to the geographical variations of their regulatory status according to each country, their performances generally announce several effects on mechanisms which are:

- Targeted: skin relief, collagen, cellular communication, Dermo-epidermal junction...
- Localised: Crow's paws, Lion's wrinkles, oval face...,
- Focused at different skin depths: epidermis, dermal-epidermal junction, dermis) and
- **Interconnected** in various mechanisms: emotions, cellular and biochemical (microbiota, keratinocytes, fibroblasts, melanocytes, nervous receptors...)

More than classical efficacy on the skin surface and structure, skin care dedicated to mature skin reveal improvements on **emotional**, **well-being**, **sensoriality properties**...but the cutaneous signs of aging are still seriously studied with a great accuracy as a tangible proof of the product performances. The promise is a recovery of the **tissue elasticity**, **the face's shape**, **the plump and volume appearance**. A list of the principal signs of aging influencing the age perception can be established even their importance differs among different ethnicities. Skin tone is generally considered aging among Asian, with skin dullness and hyperpigmentation. Among Caucasian wrinkles – particularly around the eye and forehead area – are the important signs. Sagging skin around the mouth and jawline is also described earlier than other ethnicities.

We suggest categorizing the various and vast anti-aging claims, in 6 categories:

- **Skin colour and pigmentation:** Redness, tone, Radiance, age spots, circles under eyes
- **Skin topography:** Crow's Feet, Furrow Lines, Upper Lip, under-eye, forehead wrinkles, roughness
- **Skin structure:** thickness, epidermal junction, barrier function...
- **Volume of the face and specific zones:** Face, Nasolabial Sagging or Smile lines, puffiness, eyelids...
- Biomechanical properties: firmness, elasticity...
- Emotions and perceptions: well-being, happiness...

#### The Various Ways of Objectivation

In the notion of "well-being" the notion of "anti-aging" comes little, one might as well say "pro-age" or "plain age", the condition of the skin being thought in its entirety. The identification of the properties of these products uses a sensory approach, sometimes neurosensory and holistic. The feeling, the impacts on the quality of life are measured.

The investigators can choose the tests between **4 main categories or combine them**: consumer tests, sensory or emotional analysis, biometrological studies, or clinical scores. In the Clinical Testing Platform you can find all the devices enabling a quantitative, semi-quantitative or imaging objectivations. Whether for small or big areas, these biometrological techniques always look for **perfect data acquisition** with the **best repositioning**, the higher resolution, the quicker capture time, and the automatic rotation system.

#### The Bright Future of Skin Science

Now the imaging of the skin from the centimetre to the nanoscale is more and more used. And the analyse of the several elements and structure of the skin: water, lipids, dermal-epidermal junction, extracellular matrix, fibres...is under the light. Whether for aging, radiance, biomechanical properties, the several techniques look for **higher resolution**, **larger measurement area**, **non-invasive**, **contactless**, **and direct methods**. The **algorithms related ti I.A** are the principal future contribution of the success of these new technologies.

The various high-tech biometrological measurements give the opportunity to connect the technology with **the new digital use of personalisation** from the shop to the bathroom. In another way, the industry anticipates the latest and global technological evolution such as genomic, A.I, the Internet of Thing, to develop, product and market these specific skin cares and answer the regulation constraints and the consumer's demand.

#### Easily find the methods and testing labs to substantiate the anti-aging claims

- For the *in-vitro* efficacy tests: "Preclinical Testing Platform"
- For the clinical tests: "Clinical Testing Platform"

Warning Connection: You need to subscribe twice for both platforms

#### Our Partners have the floor

We are glad to introduce the several topics presented by our partners

- Closer to the skin by **Eotech**
- Assessing the Contributing Attributes of Intrinsic and Extrinsic Cutaneous Aging by <u>Eurofins</u>
- Covering anti-aging claims by <a href="PhD Trials@">PhD Trials@</a>
- Conquering photography during the Pandemic by <u>Validated Claim Support</u>
- Combining in-vitro and in-vivo tests to consolidate an anti-aging claim by **Zurko**
- Solution for Wrinkles analysis: How to Measure Ground Truth by Newtone
- The choice of the Relevant Techniques by <u>CERCO</u>
- Objectivation of anti-ageing effects by <u>Complife</u>



#### Eotech closer to the skin

Jean-Jacques **SERVANT** – Commercial Director

**Since more than 20 years,** our company develops, conceives, and offers innovation in the skin measurement especially for anti-aging effects like wrinkles and sagging on the face and body in general. Our solutions are composed of a **positioning device, a very accurate and sensitive 3D scanner and a clinical studies oriented software,** used every day by a big number of company acting in the cosmetic field (manufacturer of active ingredients or cosmetic product or research laboratories) to support claims or measure the anti-aging efficacy of those products.

Each solution can either **measure locally or globally a face and extract any zone of interest** where aging effect are visible (crow's-feet, eye bags, front, peri-oral, glabella or nasal folds, neck). Objective parameters can be calculated from each of these zones to quantify their number, depth or volume of those wrinkles and folds.

Images are also produced to illustrate those effects. The strong advantage of our solutions is the ability to reproduce the same measurement at different time points of the volunteers due to **reliable repositioning**, and the ability of our algorithms to extract the exact zones at these different time points.

Our technology improved significantly the past year to offer all of these capability of the full face by extracting all zone of interest (18) from one set of measurement and give a global evaluation of ageing signs like the wrinkle density witch is calculated by the ration of detected features divided by the total surface of the face.

Contact: jj.servant@eotech.fr

**Discover Eotech profile and instrumentation** on the Clinical Platform: <u>Eotech Link</u>



# Assessing the Contributing Attributes of Intrinsic and Extrinsic Cutaneous Aging

Clotilde Fourault, Anne Sirvent and Michael Anthonavage Eurofins Evic France; Eurofins Dermscan; Eurofins Clinical Research Labs

In the world today, the increase in life expectancy is leading to a real demographic shock: in 2018, the number of people over 65 exceeded that of children under 5. To best serve this growing population, it is important to understand their needs to offer personal care/cosmetic products that specifically meet their expectations.

Skin aging is a physiological process influenced by 2 types of factors, each with specific cellular mechanisms and clinical aspects.

- Intrinsic factors are mainly age-related; with variable expression according to genetic factors and hormonal status (i.e. oestrogenic deficiency induces a loss of 30% of collagen in the 4 years following the menopause). At the cellular level, the length of telomeres at the ends of chromosomes gradually shortens resulting in cellular alterations in function. DNA repair capacity and proteins such as P53 decrease resulting in less cell renewal. Clinically, we observe on non-photo exposed areas, a skin that is thinned, drier, withered, less pigmented, and less toned.
- Extrinsic factors are mainly UV exposure-related, which induces lesions at the epidermis (UVB) and dermis (UVA) but also includes pollution (ozone) exposure, alcohol and tobacco use. At the cellular level, these factors are a source of free radical production (i.e. oxidative stress), with deterioration of DNA and repair systems. Clinically, a solar elastosis is observed on photo exposed areas resulting in skin that is rough, yellow, flabby, atrophic with deep wrinkles and irregularities of pigmentation.

Whatever the skin aging factors are, **the clinical scoring** of skin density, dryness, firmness, wrinkles/ fine lines, complexion homogeneity/pigmented spots, and complexion radiance allows for the assessment of products focused on improving these aging parameters.

Biometrology is a fiels of non-invasive evaluation technics focused on skin's innate biophysical
responses using various imaging and applied energy technologies .Prime examples of these are
Laser Doppler, Corneometer, Ballistometry and Ultrasound to name a few. Data are obtained
with precise controls which are based on reproducible, calibrated and controlled processes of
data collection.

The application of skin metrology spans from normal healthy skin function to stressed and even pathological conditions. Because the skin is a highly variable organ designed to acclimate and respond to environmental stresses both acute and chronic, tracking these changes, to understand or correct the aging process, is paramount to the development of any new technology.

Several bioengineering methods have been proposed to objectively, precisely, and non-invasively measure skin aging, and to detect early skin damage that is sub-clinically observable, in order to support new product development: computerized imaging, biophysical instrumentation looking at color, firmness, roughness and density all tie together for a global picture of skin function. Application of skin metrology can be used to even screen subjects prior to the initiation of studies to identify appropriate users, and/or those prone to adverse reactions based on their pre-existing skin conditions.

Cosmetic products play a growing role in the well-being of individuals as they age. As early as
1994, WHO defined an individual's health as a complete state of well-being - not only physical
but also psychological and social collectively. This triad is particularly true for seniors who
care less about their physical appearance than their physical condition and quality of life (general
form, mood and attractiveness).

Today, advertising and new product developments **highlight integrative beauty** that sublimates beauty at any age. Anti-aging claims are abandoned in favor of pro-aging, creating new opportunities for growth. As a result, it becomes essential to assess the impact of cosmetic products on the **emotional aspect**. Emotions can be understood through 3 components: **expressive (behavioral), visceral (physiological) and subjective (psychometry).** This last component is very often employed in cosmetics, due to its ease of use and its relevance. Thanks to standardized and objective questionnaires, subjects are asked to document and quantify the intensity of their emotional engagement (joy, relaxation, excitement, etc.) or the frequency of well-being situations.

**Understanding the mechanisms behind skin aging** has led to creative technologies design to slow down the signs of aging, accompany physical changes and promote sense of well-being. Developing studies that encompass the physiological/biophysical, behavioral and subjective aspects of skin care product performance to understand the relationship between mechanism of action and consumer perception are key to successful product development. **Adopting standardized processes** as part of a comprehensive testing protocol not only contributes to a better reliability of research results, but also allows for the industry to properly peer review published results.

By developing and using **cutting-edge measurement tools and methods**, we at Eurofins are applying our expanding knowledge of skin and skin measurement science to help create new therapies, as well as making current ones more effective.

**Contact:** ASI@dermscan.com, cosmetics@eurofins.com

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#### **Covering Anti-aging Claims by PhD Trials®**

Pedro PINTO - General & Scientific Manager

Skin aging is a complex process involving intrinsic (personal and genetic) and extrinsic (environment, i.e. sun exposure) factors that result in the well-known signs of aging.

In order to improve the knowledge of the changes that occur in the skin with the aging process and to evaluate the effects that an anti-aging product produces on the skin after its use, covering the multiple possible anti-aging claims, **PhD Trials® carries out a series of instrumental objective evaluations.** 

The main structural changes perceived on skin are regarding **texture and wrinkles**. For its assessment, methods of surface texture measurements as **AEVA-HE** (Eotech) can be used. Tridimensional images of the skin topography are obtained by a stereo camera combined with a fringe projection system, giving us a quantitative skin profile data. Interesting parameters such as Wrinkle count, Wrinkle volume, Wrinkle average depth, Ra and Rz can be found.

Another problematic skin aging effect is regarding **skin firmness and elasticity loss**, related with structural changes on fibers network. Skin biomechanical evaluation can be performed by the **Cutometer**® (C+K) probe. This system uses negative pressure which deforms the skin mechanically and parameters skin firmness and skin elasticity can be found.

Also, to evaluate structural changes inside the skin a **Reflectance Confocal Microscope – RCM** (MAVIG) can be used. With this system the fibers network or the epidermis thickness can be found and a 3D reconstruction of the top of the dermis and the collagen fibers network can be obtained. An **Ultrasonography** (Dermscan C system) that generates ultrasounds images with a 20MHz probe is also possible to be used in order to obtain quantitative parameters such as skin density, skin thickness or SubEpidermal Low Echogenic Band (SLEB).

Changes on skin pigmentation leading to a decrease on luminosity and the appearance of hyperpigmented spots are also an important concern related with the aging process. To assess **skin luminosity**, a colorimetric method such as **Chromameter®** (Minolta) can be used and data concerning Visible, Brown (hyperpigmentation) and UV spots (photodamaged induced spots), is obtained by image analysis of standard digital face photography using the **VISIA® system** (Canfield). And last but not least, aging process also brings skin hydration decrease. To assess objectively and quantitatively skin hydration the method of choice is the well-known probe **Corneometer®** (C+K) based on skin capacitance.

These techniques allow **PhD Trials**® to have a global approach of skin aging effects and an objectivation of the different aspects of antiaging effects, properly covering the multiple possible product antiaging claims.

Contact: geral@phdtrials.com

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# **Conquering Photography during the Pandemic**

Jane TERVOOREN - Vice President

It is no secret that our eyes are often one of the first areas to show the signs of aging. Our skin there is significantly thinner than the rest of our face, making it more delicate. When we smile or squinch our eyes, crow's feet (or aptly named "smile lines") become more visible. There are a multitude of eye serums and eye products claiming to reduce the appearance of crow's feet, so the big question is, have the claims you are seeing been the result of the most accurate third-party testing?

A common method to measure the improvement of reduction of the fine lines and wrinkles involves "inside the box" photographic solutions with a wide range of underlying lighting conditions. While these **imaging systems are outstanding for a myriad of parameters,** often times they are inherently limited when it comes to crow's feet claims. The fact that the volunteers must squinch their eyes shut to avoid a close-range strobe flash when an image is taken prevents a true and accurate measurement of the area.

There is no way to produce a consistent before and after image because it is impossible to control how hard the volunteer closes their eyes at each timepoint. Many of these systems are also not particularly COVID friendly since the face of each volunteer is effectively encased inside the device and it would be nearly impossible to properly sanitize between subjects.

**So, what is the solution?** The answer is VCS clinical imaging! At VCS we offer a proprietary system which was created by our clinical photographer who has over 20 years of experience in the business. The **VCS system** allows the subjects to keep their eyes open, and there are no constraints on or around the face which could cause inconsistencies.

Our Validated Clinical Photography Technique utilizes **socially distant, high resolution image capture** (approximately 50 megapixels) as a means of visual claim support in order to demonstrate product efficacy. Panelist facial expressions, focal distances, equipment positioning, lighting, and ambient temperature and humidity are all meticulously controlled, ensuring that the main delta from one image to the next is a function of time and the effect of an individual treatment product.

These images serve as a wonderful "study summary," and when cropped and aligned side by side, they provide an easy to understand and incredibly compelling visual presentation package. Industry professionals, retailers and distributers, and end users and consumers can all clearly and quickly get a feel for what the product is actually capable of doing.

When taken in conjunction with clinical visits and paired with biophysical instrumentation or expert grading, these "direct from a clinical study" images offer a unique bridge between the science behind your products and marketing friendly claim support. With consumers stuck at home and more products going direct to market, there is no time like the present to demonstrate your product's core attributes uniquely and powerfully. As always, remember to back up your labels and marketing materials with a Validated Claim Support laboratory.

Contact: www.validatedcs.com

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**Claims Support** 



# Combining in-vitro and in-vivo Tests to Consolidate the Anti-aging Claim

Sabina Giovannini - Managing Director

The skin aging is characterized by an alteration of structural components of the connective tissue with the consequent appearance of external skin signs, such as wrinkles, loss of elasticity, laxity, roughtextured appearance and heterogeneous pigmentation. Despite the fact that aging signs are visible on the surface of the skin, the core of skin health comes from inside.

Most of these skin aging signs are triggered by **disorganization or damage of the skin structures** partly due to a lack of collagen and elastin, and the changes induced by their oxidation, but also by the decrease of cell proliferation in the epidermis (also known as cell senescence). Anti-aging cosmetic products targeting skin aging must be capable to address at least one of the previously mentioned aspects, as the claim is not well defined. Demonstrating the intended claim can be a complex matter given the number of possibilities.

**The** *in vitro* **approach** provides an in-depth knowledge of the physiological interaction of the cosmetic active with the cells and their processes. A number of these assays are performed on fibroblasts because of their ability to produce most of the key aging-related substances. A typical setup would compare the amount of collagen, elastin and/or hyaluronic acid in cells treated with the product or not. Other important claim is the increase of cell renewal or cell proliferation of fibroblasts, but also of keratinocytes, which form the outer layer of the skin and are more exposed to the environment aggressions.

**The antioxidant activity** does not improve aging itself but decreases the aging speed by playing a protective role. Assessment of this activity in cells is usually performed by quantifying the reactive oxygen species (ROS).

Most of the *in vitro* tests can be made in epidermal reconstructed tissue. This model replicates the different layers of the skin and therefore, introduces additional variables without the need of clinical testing.

*In vivo* assays also offer a great number of possibilities and have the advantage of being performed in humans. The main objective of anti-aging products is the reduction of wrinkles, which we can evaluate with the use of the Primos equipment, since it is usually one of the most visible effects. We must also focus on other variables altered by aging, such as loss of skin density, for which we can use the Ultrascan equipment, loss of firmness and elasticity evaluated with the Cutometer tube, irregular pigmentation, by quantification of melanin and erythema with Mexameter equipment or assessment of skin dryness with Corneometer. All these data can also be supported visually using VISIA equipment.

Combining *in vitro* and *in vivo* tests is essential to consolidate a claim, since we obtain conclusions supported by sufficient scientific evidence.

**Contact:** sabina.giovannini@zurkoresearch.com

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#### Newtone Solution for Wrinkles analysis: How to Measure Ground Truth

Solène TREVISAN – Clinical Study Manager

The Newtone family of solutions specializes in creating and developing increasingly powerful customized interpretation **algorithms** and **acquisition systems**, to help Cosmetic Industry to Innovate, Explore and Develop new products for all countries.

One challenge addressed by Newtone is to develop solutions enabling to measure "ground truth": what the consumers actually see in the mirror. To help investigate wrinkles, Newtone developed innovative optical solutions and algorithms, for a combined approach using full face visible light imaging and local 3D imaging.

#### Imaging systems and analysis algorithms

**ColorFace** is a **full-face** imaging system, for high resolution and standardized image acquisition, under 7 lighting modes including one illustration mode "open eyes"). It is commonly used in clinical trials. Connected to a dedicated cloud application (**CiPaaS** - Clinical Image Processing as a Service), it provides direct automatic and highly secured backup of images wherever the study takes place, all over the world. About ColorFace image analysis, Newtone's original approach is to prove product efficacy correlated with the **human perception**. Therefore, analysis parameters are conspicuous (visible) ones.

**Algorithms** are developed to adapt automatically to the type of panel (specific and validated algorithms for different groups of origins), to fit with wrinkles typologies (specific and validated algorithms for nasogenian folds, eye contour, lips contour, forehead etc...) and finally to roughness severity (specific and validated algorithms for line marks, fine lines and wrinkles). All algorithms are developed to be correlated with clinical grading and reflect ground truth (using Newtone PhotoScale online solution for image grading). Newtone likes to advise and offer a **combined analysis of whole face wrinkles** to bring more value to product performance investigation.

New born **SkinCam** is a new nomadic solution for skin evaluation, including analysis of skin micro-relief in 3D. It can be handed over to the subject on the day of inclusion, at the clinical laboratory, for image acquisition and additional local measurements at home, when the laboratory is closed (early morning, every day, late evening, etc.). It provides additional information to ColorFace full face acquisitions by focusing on selected and localized areas, like wrinkles. Its different lighting modalities, as well as 3D for roughness analysis, can be used to acquire images of any area of the face and body skin. Skin smoothness improvement can then be assessed by 3D analysis integrated on the whole surface of acquisition.

#### **Clinical Grading**

With **PhotoScale**, one smart and user-friendly online service for clinical grading, Newtone also brings a supporting solution for clinical experts willing to grade the wrinkles and sagging under standard and reproductible conditions, on standardized images from all over the world. Randomization, image preparation (crops, zooms, morphings etc..), flexible image display and grading options, data export, statistics. This is an all in one packaged solution for smart remote image grading. Images can be acquired with Newtone solutions or independently coming from other imaging systems.

What about AI for wrinkles assessment? Artificial Intelligence is being used in Newtone since several years now, to improve wrinkle segmentation algorithms but also develop customized automatic grading tools for skin characterization and product performance prediction.

Newtone's **Average face** solution is one way to valorize anti-wrinkle product performance, with customized impactful visuals (article illustration: Newtone average face - ColorFace profile views).

Contact: <a href="mailto:studymngt@newtone.fr">studymngt@newtone.fr</a>

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**Technologies Link** 



#### The choice of the Relevant Techniques

#### Pascale BARLIER - General Manager

CERCO has been studying cutaneous relief for more than 40 years; wrinkles are the first visible sign of aging and concern women and men very early on. Studying their evolution over time but especially their decrease under the effect of a cosmetic treatment remains a priority. The methodologies are multiple but must be carefully chosen according to the context. There are many to study the finest furrows to the deepest.

The choice of technique is major in the evaluation of anti-wrinkle effects: **2D photography** combined with image analysis or clinical scoring, **3D profilometry** with or without contact have each of the particularities to consider; more the wrinkles are small, more the methodology should be sensitive.

The measurement kinetics, the targeted facial area are also elements to be taken into account to define the most appropriate methodology.

In young adults between the ages of 20 and 30, the concern is to prevent wrinkles. For this, moisturizing and nourishing the skin and protecting it from the sun are the 3 most important factors. At this age, it is a question of studying the cutaneous micro-relief otherwise known as **micro-depressive network**. The skin is covered by these micro-lines that on areas of permanent fold related to facial expressions, such as the crow's feet, the contour of the lips, the forehead, will be deepened over the years, to become fine lines and then wrinkles.

This micro-relief, hardly visible to the naked eye can only be studied with very resolving technologies such as the **projection of fringes** associated with the skin replica. We can thus study the smoothing effects on less marked relief.

When fine lines become visible with the naked eye, other measurement methods can be used such as **high-resolution photography** combined with **image analysis** or **clinical scoring**, based on wrinkles severity scales and trained experimenters. We thus move from studying a small area by an replica to the measurement of a whole face and the study of all the lines included. These measurements are currently based on a motionless and inexpressive face.

The next step is the assessment of the variations of the folds **in «dynamic» mode**, that is to say during the facial movements. The study of the anti-wrinkle effects on a face in motion will be more representative as close as possible to the image showing when we express ourselves. The difficulty lies in achieving the same movement before and after application of a cosmetic treatment. But this will be a major breakthrough in the impact of a cosmetic or surgical anti-wrinkle effect. Limit the digging of folds during our expressions without making disappear the facial expressions and emotions.

Contact: <a href="mailto:pascale.barlier@cercotests.com">pascale.barlier@cercotests.com</a>

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# **Objectivation of Anti-ageing Effects**

Christine Braud - General Manager

Nowadays, the increase of life expectancy is correlated to a higher demand of anti-ageing skin care. The appearance of ageing skin is highlighted by skin roughness, wrinkling, pigmentation change, loss of elasticity and decreased firmness. **Wrinkles are the results of a combined impact of internal and external factors** on skin ageing, such as diet, smoking, sleeping disorders, photo-ageing, chronic and oxidative stress. A highly polluted environment can also be viewed as an extrinsic factor accelerating the skin ageing process.

These multifactorial causes could **accelerate the natural decline of skin structure and functions** and are growing evidence that environmental factors associated with lifestyle and comorbidities deserve greater attention from dermatologists and may require new approaches in the management of skin ageing. This environment encourages the improvement and development of more personalized formulations with additional benefits for general skin health and age associated skin signs.

Considering consumers needs the **new generation of cosmetics** has been developed **offering a multipurpose formulation** comprised of unique active complexes toward pollution, pollution induced inflammation, anti-oxidant and anti-ageing concerns, with beneficial results demonstrated by *in-vitro* (culture model with primary human skin cells), - *ex-vivo* studies with full-thickness human skin and *in-vivo* clinical trials.

In particular, the clinical objectivation of similar multifunctional cosmetic formulas entails a combination of measures. Indeed, to the dedicated **wrinkle-depth measure by profilometry** (Primos® 3D small and large field capture), it is common to associate **skin hydration** (Corneometer®), **skin color and gloss** (CM-700D, Konica-Minolta), **skin firmness and skin elasticity** (Cutometer®), **transepidermal water loss** (Tewameter®), **skin density** (ultrasound Aloka alfa6 pro-sound, Hitachi), skin microrelief (Visioscan®)...

**Image analysis** performed with dedicated softwares on highest resolution facial photographs taken by Visioface®RD or Visia® Skin Analysis System, as well as clinical evaluations performed by dermatologists and expert technicians are a complementary analysis methods.

**The beneficial anti-ageing effects of probiotics** are now widely reported. A recent study has proved their significant anti-ageing effect by increasing the skin water content, the skin elasticity, the skin gloss and decreasing transepidermal water loss as well as the wrinkle depth. The analysis on skin microbiome performed by PCR technology could be a valid support for objectifying the action of probiotics.

All the described methods could also be used for nutraceuticals presenting new nutritional strategies for anti-ageing care.

**Contact**: <u>info@complifegroup.com</u>, <u>christine.braud@complifegroup.com</u> **Discover Complife Group profile and tests on the Clinical Platform:** <u>Complife Group</u>