



FIFLOW®

SKIN STRUCTURE PRESERVATION WITH FIFLOW® PRODUCTS

FIFLOW® products are fully fluorinated Perfluorocarbons, which have a high capacity to carry gases, notably Oxygen, Nitrogen and Carbon Dioxide.

FIFLOWS are completely inert, stable and heavy liquids (density above 1,8). Even the most volatile **FIFLOW®** are safe to use, and they have no flash point.

FIFLOWS have specific surface characteristics. They are not oil soluble or water soluble, but omniphobic repelling other ingredients in the formulation, acting as a co-emulsifier. **Fiflows** create instantly a third phase and stabilizes the emulsion increasing considerably the hexagonal structure of the micelle.

The surface charge of **FIFLOWS** is very negative and hence **Fiflows** are very easy materials to formulate with.

No incompatibility with any cosmetic ingredients is known, as very inert materials, **FIFLOWS** are not sensitive to any chemical reactions.

FIFLOWS provide an instant refreshing and brightening effect to the skin, ideal in Asia, due to its very emollient character, creating a thin film application. They improve texture in formulations offering a very smooth and soft feel.

Fiflow® BTX is based on a blend of medical grade perfluorocarbons (INCI name : Perfluorohexane (and) Perfluoroperhydrophenanthrene (and) Perfluorodecalin (and) Perfluorodimethylcyclohexane)

Tests used

Videoscope®

Vivascope®

Microcirculation

Its performance is based on four mechanisms permitting strong and immediate visible results, but also long term improvement of age lines :

1. Very fast penetration within the skin and short half-life with different molecular weights
2. Instant skin volume increase (15-25%) based on 3D volume structure of PFCs and their omniphobic properties. This phenomena is comparable to ice versus water : ice is structured, volumised water.
3. BOTOX®-type strong muscular relaxation (di-electric effect)
4. Cell cleaning of Carbon Dioxide refreshing the cellular metabolism.

We have based this study on these mechanisms.

Purpose of the study

This study focuses on the long-term efficacy of FIFLOW® products on skin structure preservation as keratinocytes or collagen, which are key components to maintain skin integrity and properties.

SUBJECT INTRODUCTION AND TEST CONDITIONS

The subject is a 63-year old (non-smoker) man who regularly applies FIFLOW® product on his left hand. He does not apply any FIFLOW® product on his right hand.

To have comparable assessments, all measurements have been performed on the same day with the following conditions:

Date	Products Studied	Time of Measurement	Atmospheric Pressure	Outside Temperature	Weather	Outside Relative Humidity	Room Temperature of the evaluation room	Relative Humidity of the evaluation room
10/12/15	Fiflow®	Between 10:30 am and 14:00 pm	1015 hPa	Around 8°C (morning) Around 13°C (afternoon)	Sunny	52% to 71%	21 – 23°C	36 – 45%

VIVASCOPE®

Introduction

The VivaScope® is a Reflectance Confocal Microscope (RCM) equipped with a laser sweep with an imaging software. This non-invasive device makes it possible to have in vivo measurements by optical biopsies and to observe internal skin layers up to the deep dermis. It has a laser probe for observing deep skin layers and blood microcirculation, a macro digital camera VivaCam® allowing to acquire macroscopic skin images and digital high-resolution screen.

Reflectance Confocal Microscopy is the only technique that enables the en-face (horizontal plane) visualization of the skin with a resolution at the cellular level and therefore may provide an alternative to histopathology.

The device uses a diode laser as a source of monochromatic and coherent light, which penetrates into the skin and illuminates a small point inside the tissue. The light is reflected through a small pinhole and forms an image in the detector. This small pinhole does not allow the reflected light (reflectance) to reach the detector from another tissue point. Therefore, only reflected light from the focal region (confocal) is detected. Light from out-of-focus planes is rejected at the pinhole. Here lies the essential difference to conventional microscopy, where the entire light enters the detector where it is transmitted to a computer and displayed by a monitor. With a stepwise movement of the object table, a two-dimensional picture representing parallel sections of the skin is obtained.

The contrast in RCM images relies on the differences in the reflectivity of the tissue, which is depending on chemical and molecular structures. Due to these variations of the refractive index, only a certain portion of the light is reflected. Structures with a higher refractive index appear bright in RCM. Melanin and melanosomes are the strongest natural sources of contrast resulting white in RCM.

The deeper a skin section is the more laser power is needed to penetrate the skin. The VivaScope® 1500 uses a laser diode with a near-infrared wavelength of 830 nm. Laser power of 830 nm causes no damage to tissue, but limits the imaging depth to 200-300 µm, which corresponds to the papillary dermis, depending from the skin site. Higher laser power would provide an increased signal and contrast but be hazardous for the skin or the eyes.



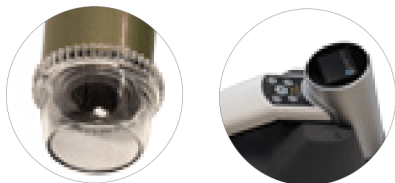
MACROSCOPIC SKIN ASPECT

Assessment protocols

The subject regularly applies **FIFLOW®** for around 20 years on his left hand, and does not apply it on his right hand.

A macroscopic picture of both hands is taken with the Videoscope® (macro-camera in non-polarized light with x50 of magnification) before product application on the four different locations

The digital camera VivaCam® is a macro hand-operated camera, giving a macroscopic skin image, which corresponds to the confocal microscope image. It makes it possible to show detailed and comparative examinations of selected skin areas. VivaCam® gives a 5 megapixel image with a field of 10 mm. With this magnified view, it is possible to make a differentiated exam of observed skin areas. The VivaCam® can be placed on the skin with the standard ring to match macroscopic image with confocal images produced by the VivaScope®.



Results with Videoscope®



Right hand without FIFLOW® application

Large and deep skin furrows with amorphous corneocytes. Skin furrows appear more parallel.

Left hand after 20 years of FIFLOW® application

Smaller skin furrows. Polygonal-shaped corneocytes.

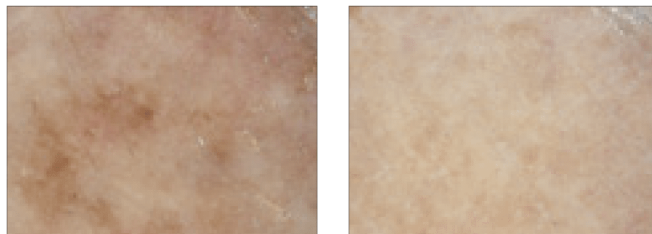
Conclusion

On the right hand without **FIFLOW®** application, skin furrows appear more parallel with amorphous corneocytes. This is a typical characteristic of skin aging, where skin furrows appear wider and more parallel.

On the left hand after 20 years of regular use of **FIFLOW®** products polygonal-shaped corneocytes with well-defined and thin skin furrows can be seen.

The use of **FIFLOW®** products maintains a polygonal structure of corneocytes.

Results with VivaCam®



Right hand without FIFLOW® application

Many age spots. Dilated vessels and reddish skin.

Left hand after 20 years of FIFLOW® application

Very few age spots. Lighter skin and non-visible vessels.

Conclusion

The right hand presents a lot of dark spots, which are senescence spots. Moreover, skin appears quite reddish because of the dilated vessels. The number of active melanocytes decreases, increasing skin sensibility to UV rays. Melanocytes are also less active but melanosomes are bigger. They pile up consequently forming pigmentation spots, which become permanent. These characteristics are not observed on the left skin (with regular **FIFLOW®** application). The skin is visibly lighter without age spots.

This means that **FIFLOW®**, by maintaining melanocytic functions, visibly reduces this kind of skin aging signs.

MICROSCOPIC VIEW OF SKIN STRUCTURE

Assessment protocols

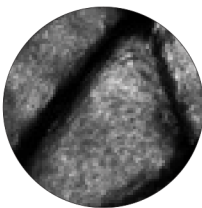
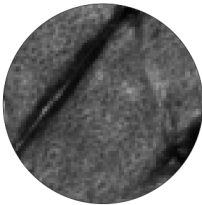
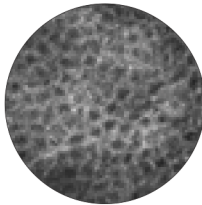
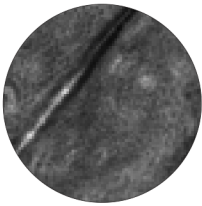
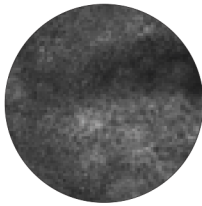
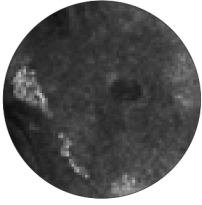
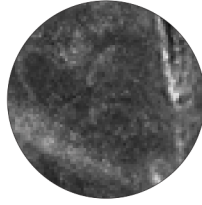
Reflectance Confocal Microscopy (RCM) images are obtained horizontally from the skin. Each single image displays a 500 µm x 500 µm large field-of-view on the screen. RCM allows an automated vertical sequence of images, each 3 µm can be captured in depth, providing a three-dimensional view of a certain area. This is called a VivaStack.

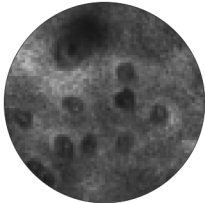
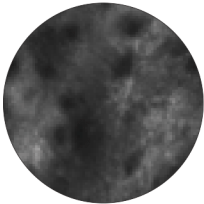
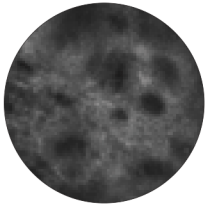
A VivaStack has been performed on each hand to compare the skin structure variations at each skin layer depth:

Stratum Corneum, Stratum Granulosum, Stratum Spinosum, Stratum Basale, Dermal-Epidermal Junction, Papillary Dermis.

Results

The following table compares, at the same skin depth, the skin structure of the hand untreated vs. treated with **FIFLOW®** products.

Skin Layer	Layer	Right hand without Fiflow®	Left hand, Fiflow® applied for 20 years
Epidermis	Stratum Corneum (Top Surface = 0 µm)	 Large skin furrows with big corneocytes. Brightness differences with irregular observation (only Stratum Corneum on the right of the picture and in the middle, we begin to see the Stratum Granulosum). This is due to a lack of skin hydration caused by skin aging.	 Thin to wider dark linear skin furrows. Very clear polygonal shape of corneocytes and regular brightness due to a more hydrated skin with Fiflow®.
	Stratum Granulosum (Depth 15-20 µm) Pictures depth: 20 µm	 Irregular honeycomb pattern of keratinocytes with irregular shape of keratinocytes. Cytoplasm is less bright and less defined outlines of keratinocytes.	 Well-demarcated outlines keratinocytes that form a grid that resembles a honeycombed pattern. More regular honeycomb pattern of keratinocytes. Bright grainy structure of the cytoplasm like a ring surrounding the round to oval dark central nuclei.
	Stratum Spinosum (Depth 20-100 µm) Pictures depth: 35 µm	 Same structure than Stratum Granulosum but is typically smaller. Irregular honeycomb pattern of keratinocytes with irregular shape of keratinocytes.	 Same structure than Stratum Granulosum but is typically smaller. Regular honeycomb pattern of keratinocytes with smaller keratinocytes.
	Stratum Basale (Depth 50-100 µm) Pictures depth: 55 µm	 Mottled pigmented keratinocytes due to a lot of melanin inside related to age spots.	 Uniform basal cells in size and shape, smaller in size with a higher refractive than spinous keratinocytes because of the melanin caps forming bright disks on the top of the nuclei. Some bright melanin inside keratinocytes. No or few age spot.

Skin Layer	Layer	Right hand without Fiflow®		Left hand, Fiflow® applied for 20 years	
Dermal-Epidermal Junction (DEJ)	Dermal-Epidermal Junction (Depth 60-100 µm)		Not clearly defined and elongated dermal papillae. Capillary loops are less visible.		Well-defined dermal papillae with a round shape, centered by dermal capillary loops with individual blood cells. Observation of the blood flow in the dermal papillae during real-time examination may further facilitate recognition of the DEJ.
	Pictures depth: 70 µm (left hand) 80 µm (right hand)				
Dermis	Papillary Dermis Superficial Dermis (Depth 100-200 µm)		Less dense collagen network with thicker collagen fibers. Less bright.		Strong and dense reticular network of collagen with bright long and thin fibers. Bright bundles
	Pictures depth: 120 µm				

Conclusion

According to the age, solar exposure or lesions, skin has typical structure in RCM.

We can notice that Dermal-Epidermal Junction pictures have not been taken at the same depth. Dermal-Epidermal Junction (DEJ) becomes flatter with age. As we observe the skin from the top with RCM, the DEJ appears deeper on the hand without FIFLOW®, which presents more signs of skin ageing. On the left hand with regular FIFLOW® application, the wavy structure of the DEJ is preserved and so dermal papillae are visible less deeply.

Polygonal corneocytes, regular honeycomb pattern of keratinocytes, well-defined dermal papillae and dense collagen network with thin and long fibers are typical for healthy “young” skin.

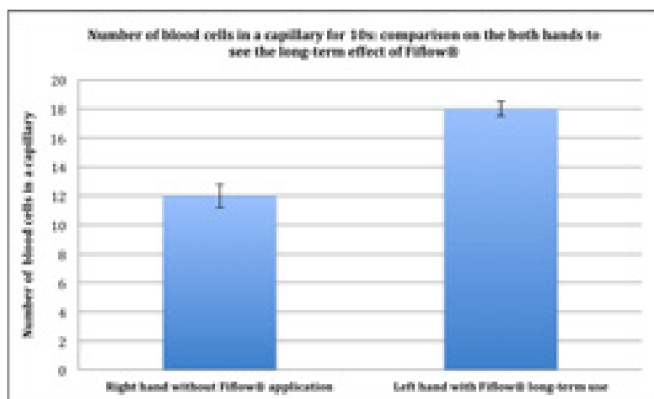
MICROCIRCULATION OBSERVATION IN REAL-TIME

Assessment protocols

Both hands have been observed with the VivaScope® (Reflectance Confocal Microscope). 10 second videos are recorded for each area at the Dermal-Epidermal Junction, where blood capillaries are visible. The number of blood cells seen in the same capillary in 10 seconds is evaluated for each place.

Results

The following graph shows the long-term effect of **FIFLOW®** on microcirculation:



Conclusion

The graph above shows that **FIFLOW®** use strongly increases the microcirculation. In this case, the number of blood cells in a capillary for 10 seconds has increased by 50% after 20 years of regular use of **FIFLOW®** products. The results are significantly different from each other (t-test, $P < 0,05$). This is due to the oxygenating power of **FIFLOW®** products, enabling the cells to improve their breathing.

CONCLUSION

The table below summarizes the results of the entire study:

Date	Right hand skin with no application	Left hand skin with regular Fiflow® application
Macroscopic aspect	- Large, deep and parallel skin furrows with amorphous corneocytes - Many age spots - Dilated vessels - Reddish skin	- Polygonal-shaped corneocytes well-defined with thinner skin furrows - Very few age spots - Non-visible vessels - Lighter skin
Microscopic aspect	- Large skin furrows with big corneocytes - Lack of skin hydration caused by skin aging - Irregular honeycomb pattern of keratinocytes with irregular shape of keratinocytes - Mottled pigmented keratinocytes due to a lot of melanin inside related to age spots - Not clearly defined and elongated dermal papillae. Capillary loops are less visible - Less dense collagen network with thicker collagen fibers. Less bright.	- Thin to wider dark linear skin furrows. - More hydrated skin with well-demarcated corneocytes outlines - Keratinocytes that form a grid that resembles a honey-combed pattern. Regular honeycomb pattern - Uniform basal cells in size and shape, smaller in size with a higher refractive. Some bright melanin inside - Well-defined dermal papillae with a round shape, centered by dermal capillary loops - Strong and dense reticular network of collagen with bright long and thin fibers.
Real-time microcirculation	Good microcirculation	Microcirculation is increased by 50% with faster blood cells

Regarding to the previous table, it appears that the right hand without any **FIFLOW®** application presents a lot of skin aging signs as dark spots, wrinkles, flatter dermal-epidermal junction, unstructured epidermis or less dense collagen network.

All of these phenomena are caused by the natural skin ageing induced with a slowdown activity and efficacy of structural proteins (keratin, melanin, elastin), which are the essential components of the skin. Moreover, the fibroblasts producing collagen are less active too.

FIFLOW® products are fully fluorinated perfluorocarbons stable and inert due to the strong C-F bond. They have the key property of carrying gases, which is comparable to the function of respiratory gases in human body.

FIFLOW® products can solubilise Oxygen and Carbon Dioxide, mimicking the function of hemoglobin, related to the partial pressures of these gases. The solubility of the gases in **FIFLOW®** is a fully reversible phenomenon based on physical gas diffusion (Fick's law).

Due to this function, **FIFLOW®** has the power to oxygenate the blood cells and consequently improve microcirculation. Cells improve their breathing and the skin is visibly lighter. Moreover, it stimulates fibroblasts and collagen network density.

All of these properties explain the visible reduction age spots and wrinkles. Therefore, we can observe on the VivaScope® images that skin structure on the left hand presents polygonal corneocytes, regular honeycomb pattern of keratinocytes, well-defined dermal papillae and dense collagen network with thin and long fibers, which are typical for healthy "young" skin.

To conclude, **FIFLOW®** is a key ingredient for cosmetics products to preserve essential skin properties. If the benefits on wrinkles and microcirculation are visible fastly after application, the regular and extended use of **FIFLOW®** products is vital to observe the long-term effects on internal skin structure.