Wrinkles Treatment with Leorex Approach and Tests

by
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Skin Ageing Bio-Mechanics

In a physical sense, skin is a multilayer medium with an internal networked matrix filled with an intercellular liquid. The sub-layers of the skin can be divided into the following functional categories from outer-to-inner layers:

- Stratum Corneum
- Epidermis
- Dermis, with an addition subdivision into the outer and deep dermis

It is important to note that the interfaces between the skin layers are not flat but rather of a fractal structure with mutually penetrated "protrusions", which provide an extremely efficient adhesion between these layers.

Skin properties demonstrate a high variability depending on origin, age, obesity, lifestyle, exposure to UV radiation, diseases etc. Collagen (~25% of the body proteins) is a major constituent of the skin's dermis layer extra-cellular matrix. Young skin is characterized by the collagen fibers held in orderly bonds forming a kind of net. The glycan network corresponding to the "windows" in the collagen "lattice" is a water-saturated gel in which water-soluble molecules and ions are able to circulate. It is this gel that gives skin its visco-elasticity and its turgidity. It is this layer that allows nutrients to flow from the dermis to the epidermis. As we age, this layer gets flatter (loss of the developed protrusions structure) which reduces the area of exchange interface between the dermis and the epidermis, significantly reducing the overall quality of the skin. The loss of adhesion between these two structures, which is normally provided by collagen IV and collagen VII, results in nutritional exchange deficiencies and a slowing of biological processes in the skin. These are the symptoms of disturbed hormonal equilibrium corresponding to the general organism ageing. This process causes an inner redistribution between the layers thicknesses – epidermis becomes thicker in relation to dermis.

From the skin mechanics point of view the main skin functions are:

- reversible extendibility
- reversible compressibility
- shear resistance

These mechanical characteristics of skin are achieved due to the internal intrinsic skin parameters such as non-linearity, anisotropy, plasticity and incompressibility. The ageing processes considerably affect these properties, and loss of them leads to wrinkles, induced by mimic muscle activity becoming permanent. Mechanical repetitive skin folding on the same site progressively gives rise to permanent wrinkles due to the effect of matrix fatigue. Once initiated, this wrinkle build-up becomes a self-perpetuated and self-containing accelerating (process) phenomenon. Local mechanical stresses under the wrinkle cause further deterioration of dermis-epidermis interface, nutrition supply deficit, pronounced in accelerated thinning of dermis, thickening of epidermis and increased

dead cells production. Therefore, mechanical skin folding initiates wrinkles. Wrinkles provide a permanent mechanical stress, this stress disturbs local liquid flow and exchange (local metabolism deterioration) causing the wrinkle fixation and growth.

The Leorex Concepts and Approach.

The main goal of the Leorex design declared by the product developers was to break this "vicious cycle" of wrinkle initiating-perpetuation mechanism. This positive feedback between the mechanical and biological interdependence of the skin deterioration processes is likely to be eliminated by active outside unfolding of the skin in the wrinkles' area which keeps for several hours daily over a period of several months, during all the restoration of the skin revival. Actual unfolding of the wrinkle area triggers the following skin short and long term restoration mechanisms:

- reduction of pressure on the capillary network

- improvement of blood, lymph and interstitial liquid flow by removing wrinkle induced mechanical obstacles

- restoration of normal supply of nutrients

- enhancement of cellular respiration (gases exchange) due to restoration of gaseous partial pressure in and out of capillary.

Additional crucial functions of the Leorex products and their impact on the skin include the following pre-designed features:

- skin dead cells removal

- deep cleansing

- improving skin gases ("breathing") permeability

Leorex technology exploits low biochemical-active (almost neutral) nanoparticles to meet these goals. The designed material has the following unique features:

- high thixotropy
- developed surface area
- good affinity to skin
- good washing-off ability
- high self-organizing ability
- 2-D into 3-D and 3-D into 2-D reversible transitions of Leorex network matrix

Pre-designed Leorex performance features:

- Applied to skin, Leorex products create 2-D network matrix, with some "roots/protrusions" which slightly penetrate into the stratum corneum adhering the network to the skin.

- Leorex aqueous liquid partially penetrates into the skin and partially evaporates.
- Nanoparticles of Leorex, which do not participate in the matrix, adsorb dead cells of stratum corneum on their highly developed active surface.
- At that stage several processes take place simultaneously:

1. penetrated into epidermis dermis interface, water causes cell swelling with corresponding local mechanical stress relaxation.
2. The networked matrix shrinks and flattens the skin, especially in the wrinkle area.
3. Dead cells are mechanically detached and absorbed.

4. Impurities accumulated in the skin layers (for example accumulated makeup particles) are "sucked out".
5. Products of skin secretion, which usually cover the skin, are detached from the skin surface and absorbed as well.
6. The outer Leorex layer absorbs these "impurities" to be washed out with it at the end of the procedure, leaving only supportive nano-matrix on the clear, vivid and shining skin.

Leorex Aesthetic Effect Tests

Leorex performance tests, were performed on women of various skin types and ages from 35 to 65.

The tests' goal was to quantitative evaluating of:

- Wrinkles Reduction Effect
- Cleansing Effect
- Complexion Enhancement
- Skin Texture Enhancement

The tests were designed to be:

- Non-Destructive
- Non-Invasive
- Remote
- Repeatable
- Reliable
- Sufficiently Sensitive to the Relevant Characteristics
-

Tools used for theses tests were:

- High Definition Digital Photography
- Optimal Lighting
- Reliable and Repeatable Object Fixation
- Advanced Image Processing for Per-Winkle and Overall Skin Enhancement Evaluation
- Spectral Analysis
- Statistical Analysis
- Pattern Recognition and Advanced Decision Making Techniques

The photographs of the "baseline shapes" were taken immediately before Leorex application. Post treatment photographs, were taken after 15 min, when the layer had been washed off, and then several times, within the next 5 hours. Some photographs, were taken on the following day. All the parameters of the scene kept strictly the same: illumination, head position etc. The results were analyzed by special sophisticated software. This testing was performed on different subjects and on various wrinkled areas on the same subjects. It provided a wide variety of wrinkles with a representative range of wrinkle lengths and depths.

The set of advanced optical tests was based on high-resolution photography of the Leorex treated areas before and after the procedure. Under proper lighting conditions, a wrinkle is shown pronounced on the photography as dark shadows – the deeper the wrinkle the darker and (bigger) wider is the "shadow". Therefore, wrinkled skin is characterized by a wider range of grey tones on the photography with higher content of "dark" tones with respect to smoother and flatter skin areas. Flattening the skin corresponds to the narrower grey scale distribution and higher overall brightness. Therefore, under the same object position and lighting conditions, the higher mean brightness and lower brightness variance correspond to the comprehensively improved skin condition. The variance and the main changes, caused by the treatment, are limited to extent of values characterizing the natural face shape, skin color unevenness, and presence of hair, eye and other facial elements. If the flattening is effective, we can anticipate the variance reduction by the value of wrinkle contribution only.

This testing was performed, for several wrinkles with the various initial depths. During the tests, we did not analyze the small wrinkles/lines below 0.25mm of initial depths, but limited ourselves to wrinkles with depths in a range of 0.25-0.75 mm.

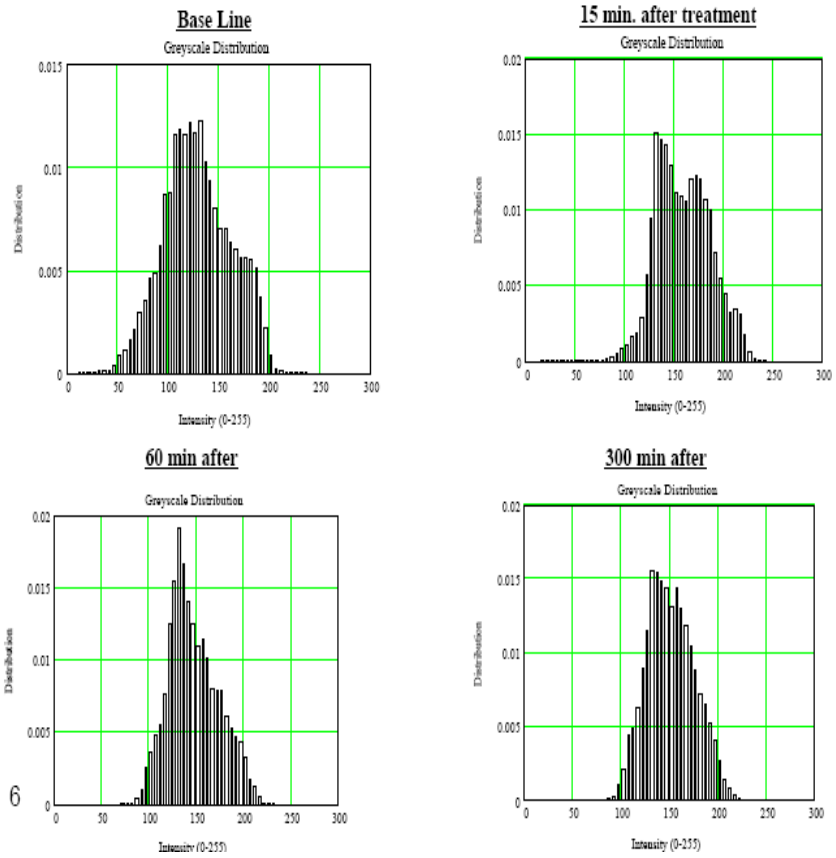
Findings

1. General Skin Condition Improvement

The results are presented in the following graphs and tables:

1.1 Grey scale tones complexion distribution

Fig.1 Skin Grey Scale Distribution Dynamics (histograms)



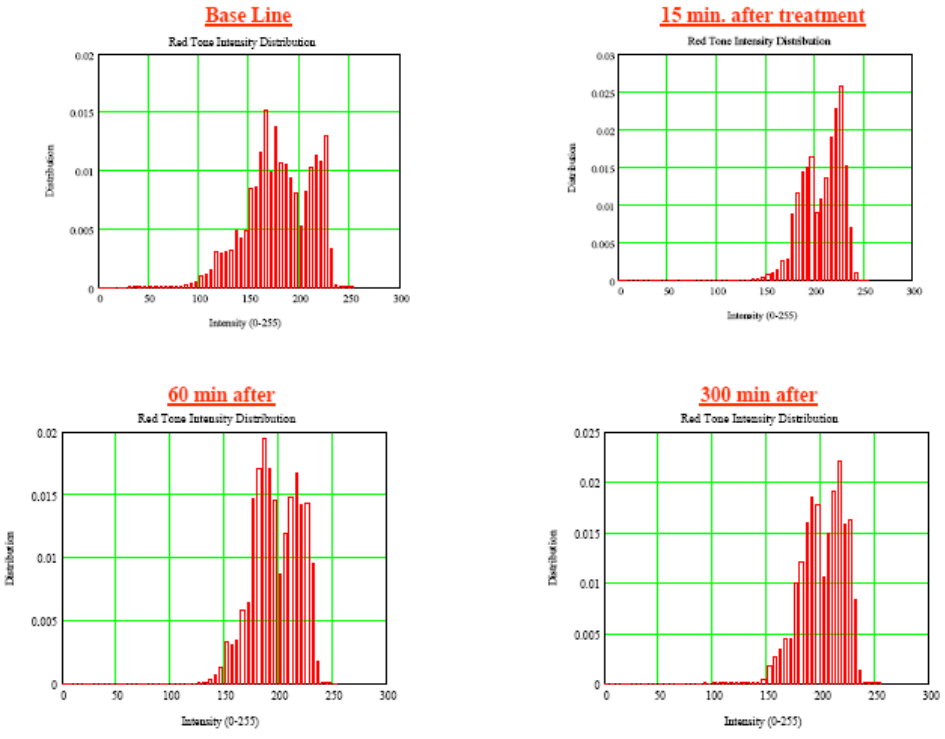
The grey gamma scale range was from zero ("absolute darkness") to 255 ("absolute whiteness"). The grayscale before-treatment (base line) distribution demonstrate that the grey tones spectrum is concentrated in the intensity range of about 50-200, while all the after-treatment distributions are characterized by the range of 100-220. This demonstrates the "dark" part of the spectrum elimination, i.e. reduction of the dark shadows and spots - flattening effect, and enhancement of the treated skin light reflection ability (increase in the bright skin tones) which corresponds to more vivid, even and healthy skin appearance. It is important to stress the stability of the effect – the spectrum remains practically the same for at least 4 hours after reaching a full effect (an hour from Leorex application). The consecutive tests were an expansion of the grey-tone analysis to a "before and after" color analysis of the complexion.

This analysis shows additional information regarding the Leorex treatment effects.

1.2 Skin Complexion Dynamics (histograms)

Fig. 2a.

Red Tone



As it is easy to see the red-tones shift to higher intensities from 100-230 to 150-230 range, demonstrating stable, more even red-tones spectrum.

Fig. 2b.

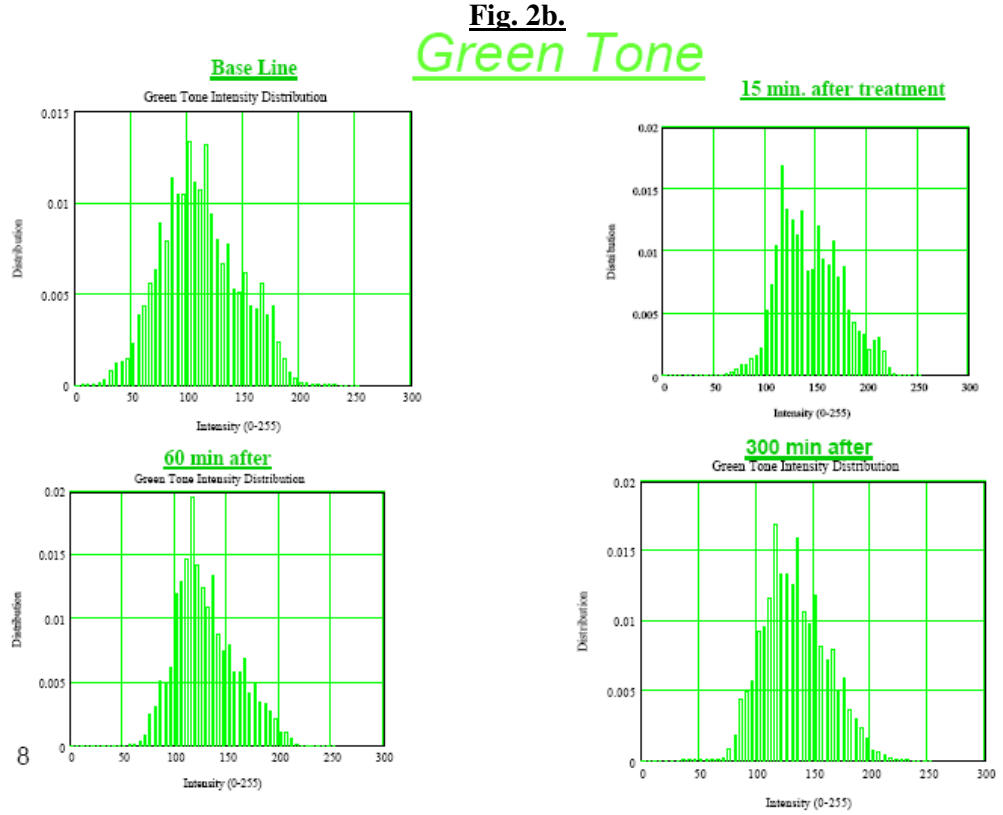
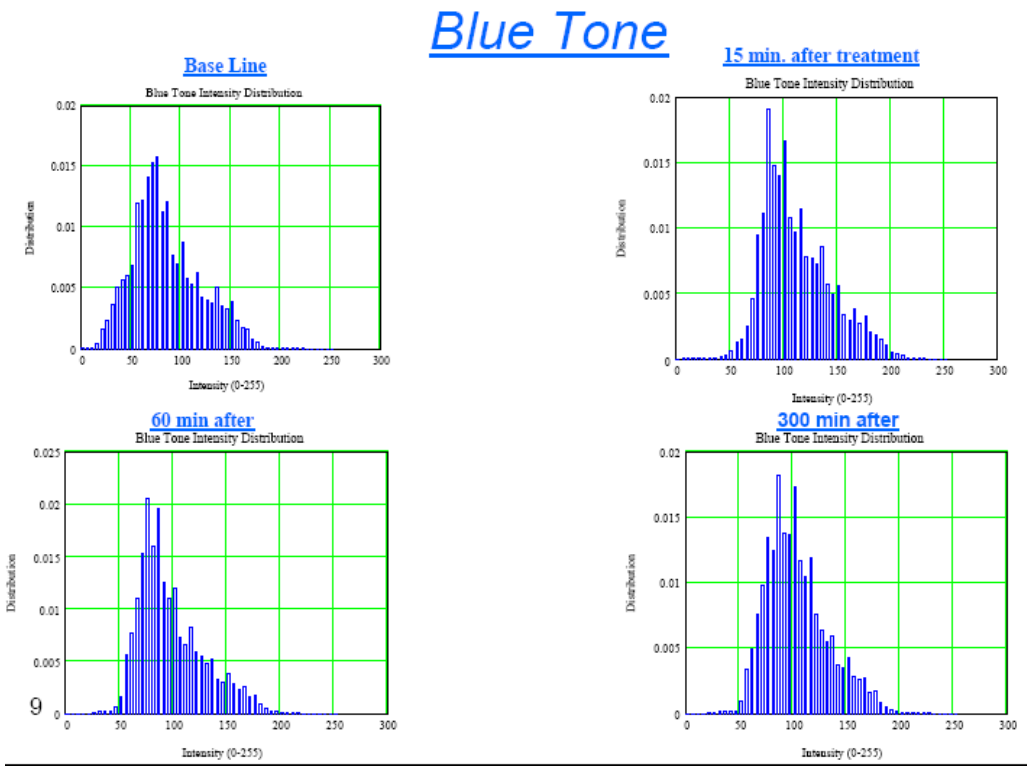

Green Tone
Base Line
Green Tone Intensity Distribution
Distribution
Intensity (0-255)
15 min. after treatment
60 min after
300 min after
8

Fig. 2c.


Blue Tone
Base Line
Blue Tone Intensity Distribution
Distribution
Intensity (0-255)
15 min. after treatment
60 min after
300 min after
9

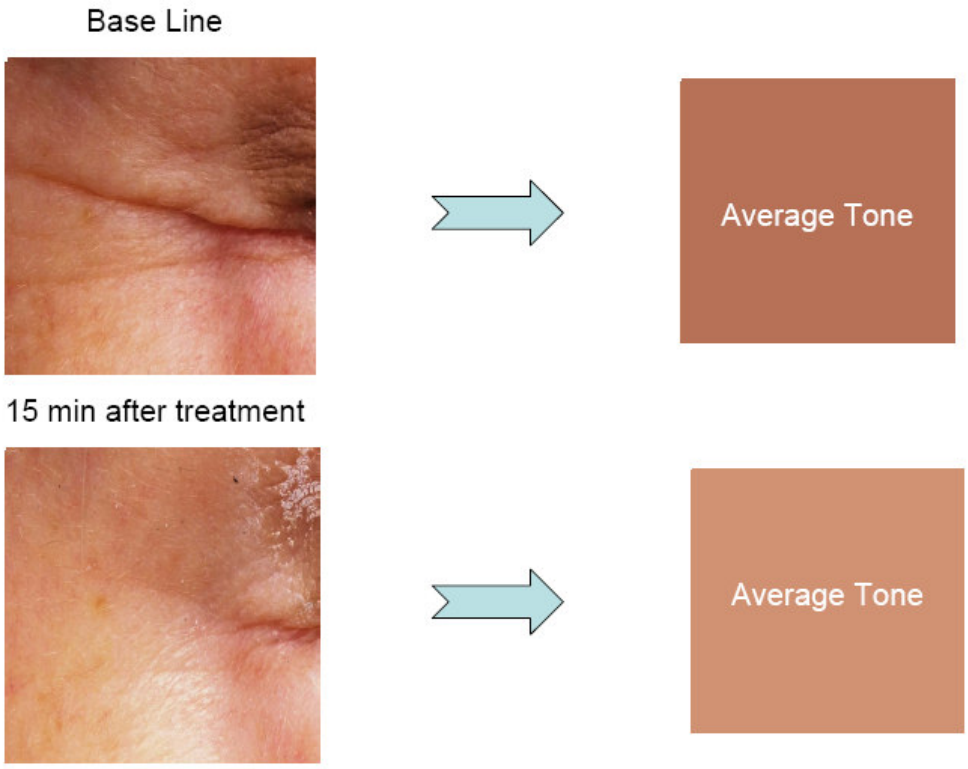
The changes of green and blue tones intensity are even more dramatic. Here, we see the low (dark) tones shift from green intensity of 30 to 75 (2.5 fold), and from 20 to ~60-70 for blue tones (change of more then 3 fold).This shows the glowing effect of the Leorex treatment on the skin's appearance.

Table 1. Statistical Results of Complexion Tests

Tone	Base Line		After 15 min		After 60 min		After 300 min	
Parameter	Int.**	St. Dev.*	Int.	St. Dev.	Int.	St. Dev.	Int.	St. Dev.
Grey	130.4	33.2	160.7	27.1	148.2	26.5	152.1	24.8
Red	181.5	31.9	208.0	20.2	199.0	21.3	202.6	19.5
Green	112.9	34.4	145.5	30.6	131.8	29.2	135.6	27.4
Blue	86.9	33.75	114.5	31.6	99.3	29.8	105.1	28.5

St. Dev.* – Standard Deviation of the Color Intensity
Int.** – Average Color Intensity

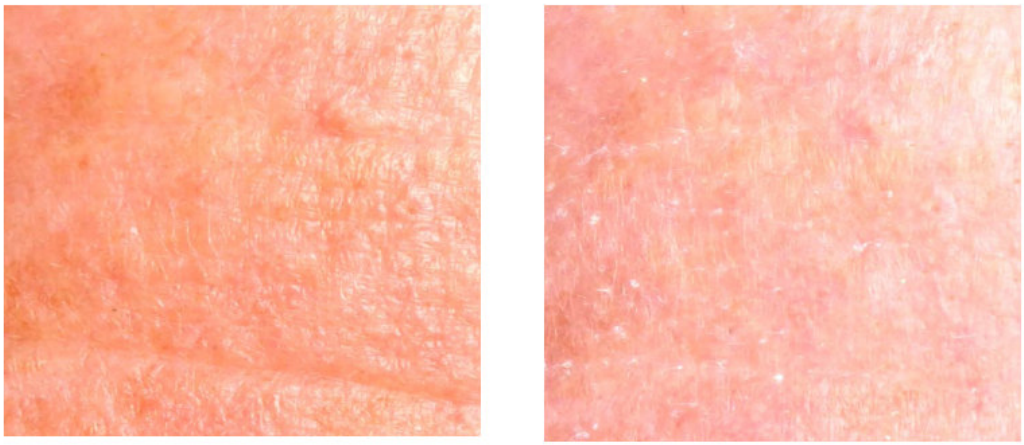
Fig.5. Demonstration of the overall Leorex effect on the skin



This figure shows the changes in the skin appearance in terms of the average skin color: combined effects of skin, flattening, cleansing, and whitening makes the "average" skin tone significantly brighter and whiter, with respect to the base line.

This tests show the color scale shift to higher intensities as well as the spectrum shift into blue zone – whitening effect (see Table 2.)
Enhanced evenness of the complexion is pronounced by a significant reduction in the tones intensity variability – one can see the standard deviation reduction in spite of the overall tones intensity increase.

Fig.6. Actual Complexion Changes by Leorex Treatment



Before Treatment | After Leorex Application

In addition to the overall effect, one can easily see the preferred concentration of the nano-matrix elements at the damaged skin sites – and the tiny fine white lines along the wrinkles and small white spots corresponding to other sunken skin areas, widened pores etc.

Table 2. RGB Analysis of Complexion Tests Results

Case	Color Content in Complexion, %		
	Red	**Green**	**Blue**
Base Line	**47.6**	**29.6**	**22.8**
After 15 min.	**44.4**	**31.1**	**24.5**
After 60 min.	**46.3**	**30.6**	**23.1**
After 300 min	**45.7**	**30.6**	**23.7**

This table shows the changes in color content – reduction of the red intensity in 2-3% in favor of green and blue tones – whitening effect. The same effect can also be seen from the next table – blue-to-green ratio remains the same before treatment and after it, whilst these (blue and green) improve their ratios tones over the red by about 10%.

Case	Proportions Between Colors in Complexion		
	Green-to-Red	**Blue-to-Red**	**Blue-to-Green**
Base Line	0.62	0.48	0.77
After 15 min.	0.7	0.55	0.79
After 60 min.	0.66	0.50	0.76
After 300 min	0.67	0.52	0.78

Table 3. Ratios of Tones Intensity

Average Intensities Statistic

- Leorex treatment increases the overall skin radiance by 20-25% - aesthetic radiant skin effect for 4-6 hours.

- The color scheme (spectrum) "moves" towards green and, especially blue components (blue-to-red ratio gets higher by 60%) leading to a significant whitening effect - even white skin. This effect is more pronounced in the cases of dark or tanned skin.

- Proportions between green and blue colors remain stable, and, practically the same before and after treatment.

Variability Findings:

- It was found that the highest effect in reduction of ton variability by Leorex took place for a red component, which is not surprising taking into account that skin blemishes have a very strong red component
- Leorex application reduces the variability by:
  Red - 30%
  Green - 15%
  Blue – 11%

- Grayscale variability becomes lower by 22% - and this value corresponds to the overall skin flattening effect – wrinkle reduction.

Leorex treatment was found to provide an immediate aesthetic effect:

- Overall wrinkle reduction-
- Increased skin brightness – "radiant" looking skin
- Whitening-Cleansing
- Enhanced skin color evenness

1. Cleansing Effect

This test consisted of 3 procedures:
- Application of skin staining color (ink) to the pre-chosen clean skin area
- Soap washing the area
- Photography of the stained area after washing
- Leorex application for 20 min.
- Water-washing out the Leorex layer
- Photography of the area
- Comparison between the intensities of stain color before and after Leorex application.

It is widely known that dead cells of the outer epidermis and stratum corneum are the most stainable cells of the skin. Therefore, detaching and removal of these cells will be found in the stain color intensity if the outer fat and other secretion products layer is removed before staining. For stratum corneum layer, the stain intensity can be supposed with sufficient accuracy to be proportional to the amount of the stained cells and, therefore, dead cells removal is proportional to the stain color intensity reduction.

Findings

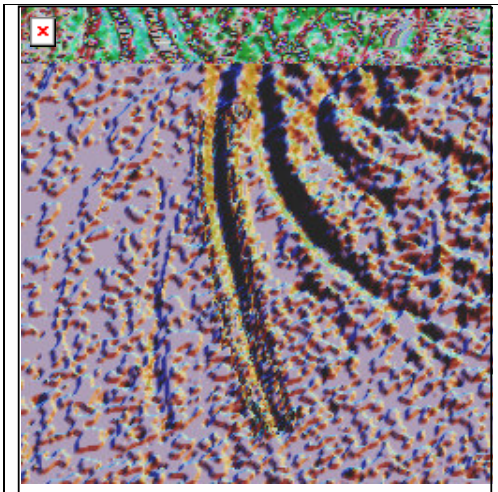
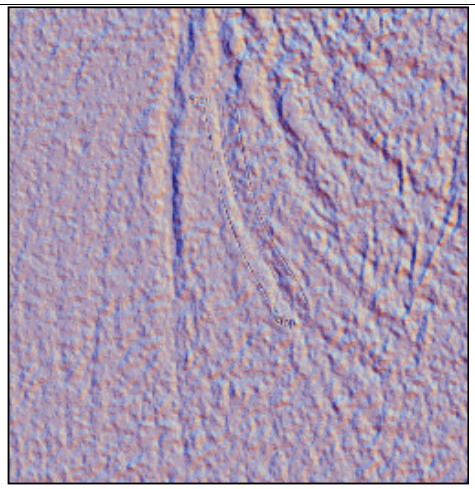
The test demonstrated high levels of stain, and, therefore, dead cell removal efficiency by Leorex. The observed stain intensity reduction values are in the range from 87% to 94%.

3. Skin Surface Texture Test

The following computer processed skin surface pictures demonstrate that Leorex performs surface "scavenging"-soft peeling, in addition to wrinkle flattening.

Quantitative Statistical Analysis of such surfaces shows, that on average, the skin roughness after Leorex application is only about 23% of the before-treatment roughness.

Fig. 7 Skin Surface Roughness

Before	After
	
	m22

4. Per-Wrinkle Tests

One of the major findings from this set of tests, was two different patterns of wrinkles response to the Leorex treatment:

1. small wrinkles/lines below 0.25mm practically disappeared after the treatment
2. wrinkles deeper then of 0.25-0.27 mm, were flattened to the limit of about 0.25 mm depth independently of their initial depth.

The deeper the wrinkle - the higher the flattening effect.

For example, wrinkles with the initial depth of 0.5 mm, were flattened by 50%, those with initial depth of about 0.75 mm demonstrated flattening by 66% and those with 0.35 mm depth were "ironed" only by about 29%.

Reduction in wrinkle depth, was accompanied by corresponding reduction in the wrinkle width (see attached screen output)

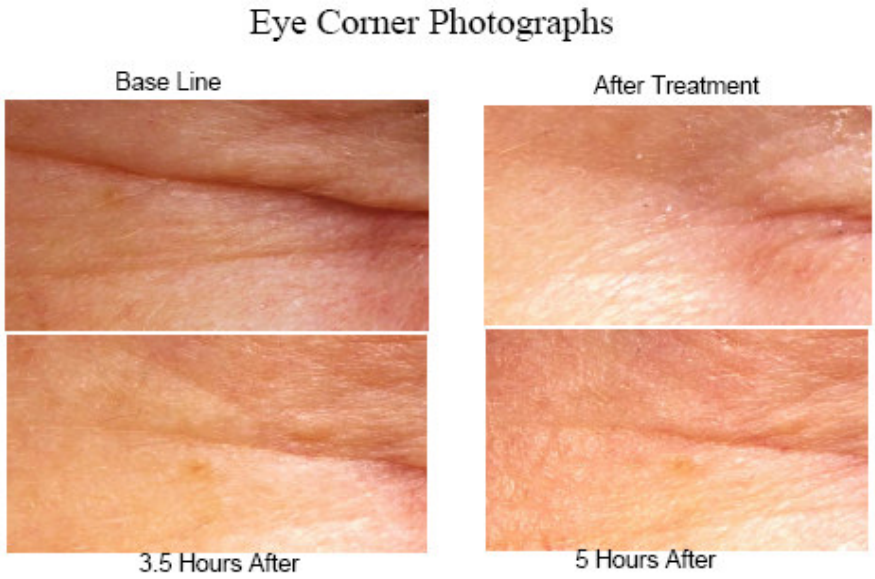


Fig. 9. Before and After

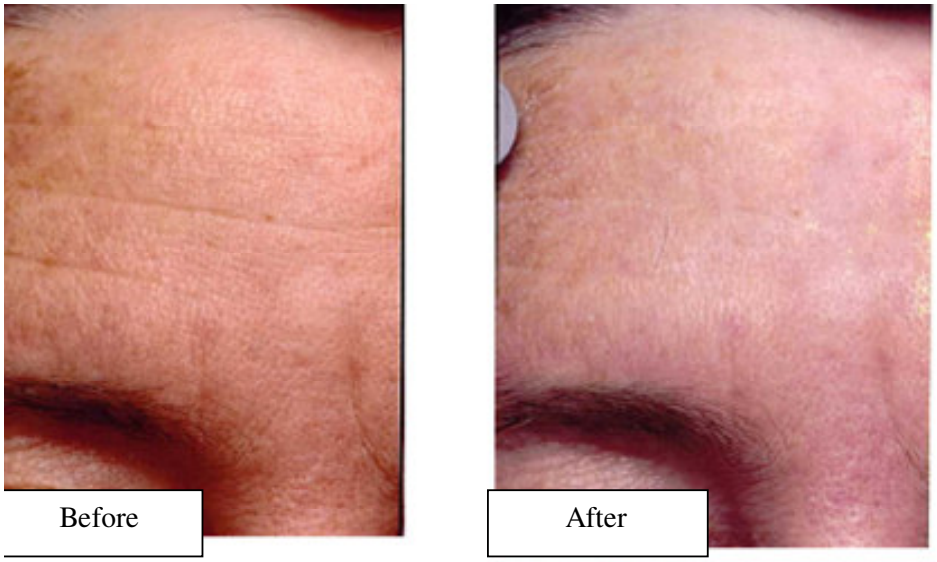
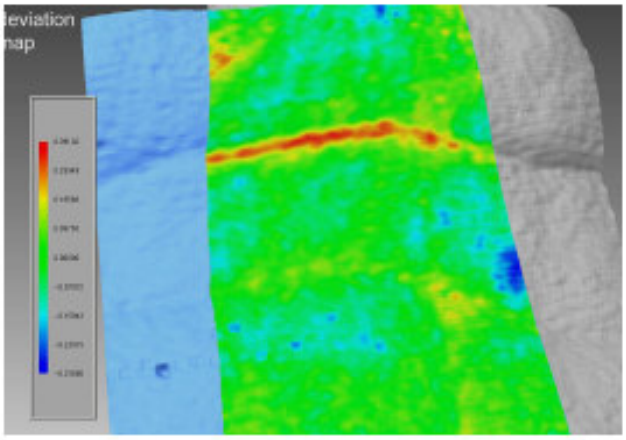


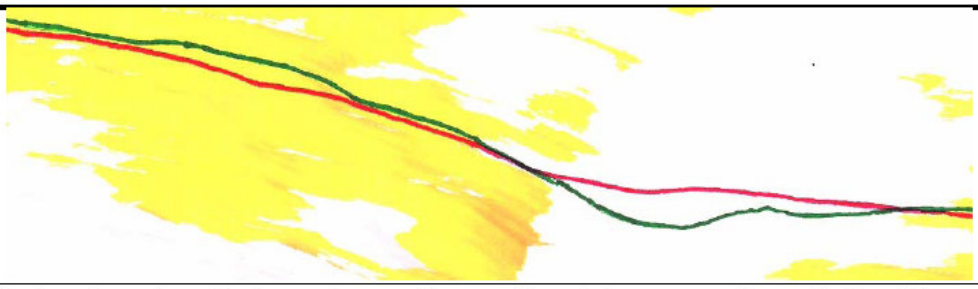
Fig. 9. Before and After

Fig. 10. Before and After Wrinkles Map Changes



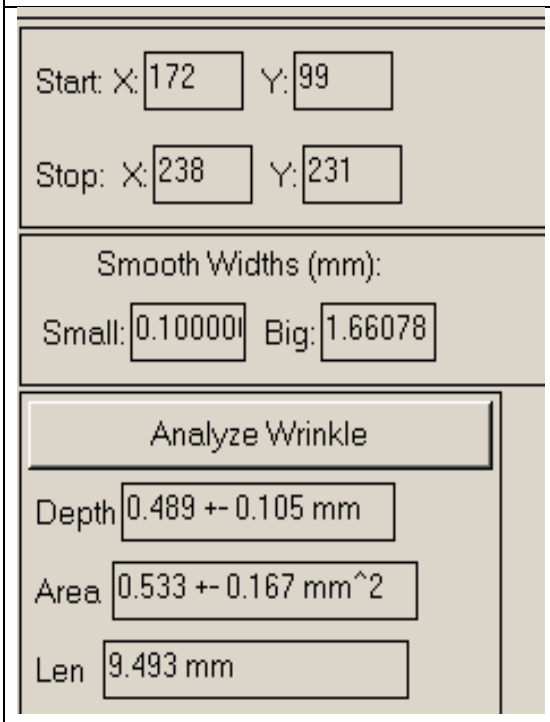
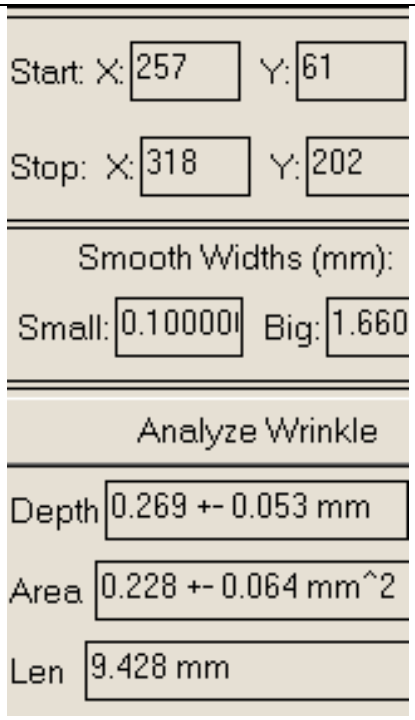
Color map in the middle area represent actual skin surface position change – colors from green to red – skin elevation and green-to-blue range corresponds to the skin depressing.
Elevation in the region of the upper wrinkle gets up to 0.4 mm and the same order of the protrusions reduction can be easily observed on the map

Fig. 11. Before and After Wrinkle Profiles



Let us imagine that we draw a line on the skin across the wrinkle. Then:
Green line is drawn before the treatment,-
and
- Red line is drawn after Leorex application.
Deviation extremes of the green line from the red one are:
-0.52mm on the left side of picture – protrusion was pushed down
+0.63mm on the right half – groove (wrinkle) was pushed up

Fig. 12. Sample of Wrinkle Analysis Software Output

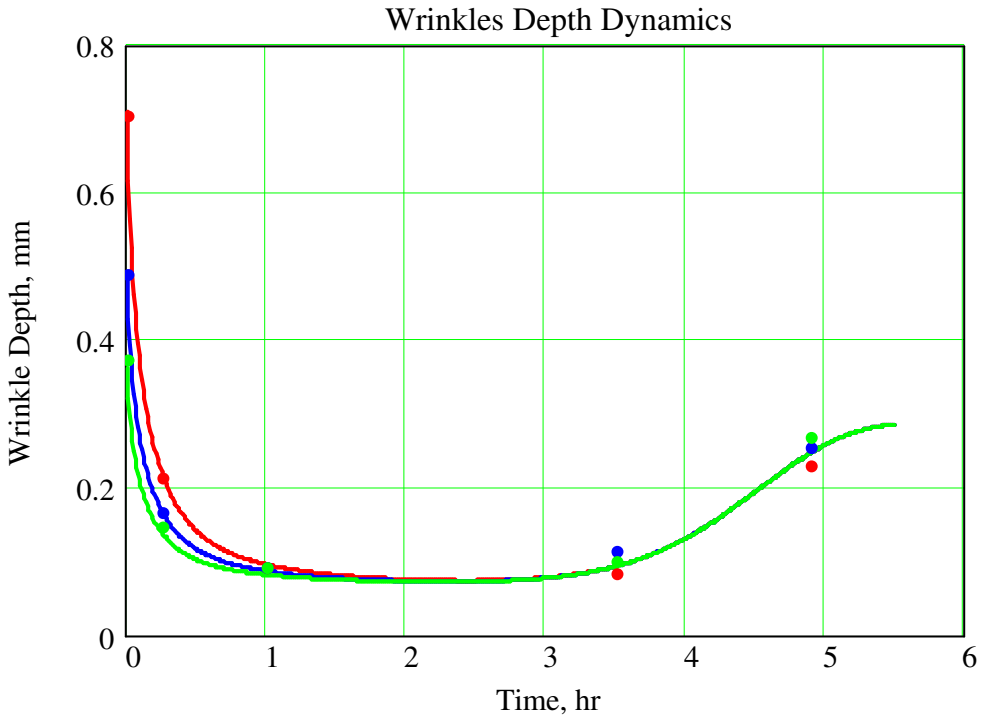
Before	After 4.5 hrs
	

Here we see the reduction of the wrinkle depth by 45%, the area (volume) by ~57% with a corresponding effective wrinkle width reduction of ~22%.

The following graphs demonstrate wrinkle dynamics during the course of 5 hours after the application. The zero time corresponds to the before-treatment conditions

The graph covers the dynamics of three wrinkles with initial depths of 0.71mm (red line and points), 0.49mm (blue line and points) and 0.365mm (green line and points). The points are the outputs of the wrinkle analyzing software and the solid lines are the fitted to these points' curves.

Fig. 13. Wrinkles Depths Graphs



Findings

- There is a rapid reduction of the wrinkle depth right after the Leorex HWNB in a matter of 10-15min.
- The maximal effect is achieved in about 1.5 – 2 hours after the treatment.
- The high level of the wrinkles depression is effective for about 4 hours.
- The wrinkle depth dynamics become independent of the initial wrinkles' characteristics after about 2 hours after the procedure.
- There is no complete wrinkle restoration after more then 5 hours, and our estimation, supported by some measurements, even after 24 hours, which means that some wrinkle reduction effect is permanent.
- The residual wrinkle depth is the same for all the wrinkles in the area and is about 0.25-0.27mm. It probably corresponds to the equilibrium state between the nano-particles network elasticity properties and the local mimic muscles activity (mechanical stress).

Discussion

This discussion presents some scientific interpretation of the achieved results and is of a speculative nature to some extent, however, we believe in its relative proximity to the real underlying mechanisms in the base of the Leorex effects.

Although the Leorex primary effect is of a mechanical-physical nature, the resulting effect has major biological outcomes. One of the main conclusions of the performed aesthetics tests, is discovering the wrinkle depth depending threshold.

It was observed that for any subject, the wrinkles below this threshold ("shallow" wrinkles and mimic lines), practically disappeared right after the treatment. At the same time, wrinkles deeper then this value had a demonstrated significant flattening after the procedure, but the after-treatment wrinkles depth was limited by this threshold independently of their initial depth. Obviously, this final post-treatment depth corresponds to the mechanical equilibrium between the wrinkle reestablishing tendency and the shrinking of the nanoparticles network matrix. Forces leading to the wrinkles restoration are actually manifestations of two biophysical factors – cellular surface tension (elastic force) and interstate gel-colloid system viscosity (plastic forces).

Due to the universal character of skin cells and intercellular homeostasis, we anticipate the universal value of this threshold as well, which has been actually observed, during this set of tests. Therefore, this limit seems to be a universal residue wrinkle depth for any non-destructive treatment.

These advanced optical measurements demonstrated both comprehensive and local metabolism improvement.

Found effects of skin aesthetic enhancement are:

- Gradual removal of the excess metabolites that have been accumulated at the wrinkle zones

- Decrease in the activity of the peripheral nerve cells pronounced in the reduction of mimic muscles activities - Botox-like effect.

- Improvement in under-skin blood micro-circulation. The wrinkle network "presses" on the capillary network disturbing normal blood supply and causing the blood color to look darker and less "vivid". It is due to the fact that stagnant venous blood is crimson dark-red, while, fresh oxygen saturated arterial blood is of a scarlet light red tone. Restoration of a normal subcutaneous blood microcirculation leads to a visible skin whitening effect and provides a more vivid and brighter complexion, which was detected as a rapid, (about 15 min) skin color spectrum changes. Skin smoothing by reduction of skin surface roughness, caused by accumulated dead cells, spikes etc.

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Test Approach - A test approach is the test strategy implementation of a project, defines how testing would be carried out. Test approach has two techniquesÂ Reactive - An approach in which the testing is not started until after design and coding are completed. Different Test approaches: There are many strategies that a project can adopt depending on the context and some of them are: Dynamic and heuristic approaches. Consultative approaches. Model-based approach that uses statistical information about failure rates. Approaches based on risk-based testing where the entire development takes place based on the risk. Methodical approach, which is based on failures. Standard-compliant approach specified by industry-specific standards. Factors to be cons