

LASMIK laser biorevitalization: mechanisms and therapeutic experience

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Abstract

LASMIK laser biorevitalization is a well-known technology developed in Russia that is based on laser phoresis as a method to enhance percutaneous penetration of substances. The article describes the mechanisms that act here and factors that determine the optimal parameters of the technique. The research conducted for 2 years allowed to optimize the parameters of the laser phoresis technique, testing the LASMIK laser biorevitalization technology. Our findings indicate the stimulation of facial skin blood microcirculation and, consequently, an increase in the oxygen tension in the skin, oxygenation of the facial skin blood, and an increase in the trophic support to tissues. The skin becomes 5-15 years younger, a noticeable reduction of facial wrinkles is demonstrated. The studies enabled to optimize the parameters of gels and laser exposure for the maximum efficiency of procedures that are in great demand both by cosmetologists and customers, due to non-invasiveness, simplicity and good results.

Introduction

The article should start with a brief explanation of the terminology, since the name of the technique (*laser biorevitalization*) in the title has two components. The first component denotes the well-known ability of Low-Level Laser Therapy (LLLT) to significantly enhance the penetration of Biologically Active Substances (BAS) through the skin. The second reflects the pronounced synergism, when the effectiveness of two factors (LLLT and BAS) is significantly higher than of each one taken separately and of their simple sum.¹ As a result, we get the “revitalization of the life” – the apparent tautology of the term (biorevitalization), since real rejuvenation occurs,

the skin becomes 5-15 years younger, if we consider the objective parameters of age-related physiology.² The efficiency of oxygen metabolism of facial skin cells is increased, the structure of collagen and elastin improves. According to these indicators, facial skin begins to correspond to the age of 20-25 years instead of 40-45 years.^{2,3}

Let us consider how this happens, what mechanisms act here and what factors determine the optimal parameters of the technique that was registered 10 years ago under the name of LASMIK and ensure its high efficiency and popularity. For example, in Russia at least 3,000 cosmetology centers successfully use LASMIK laser biorevitalization, and the number of supporters of our technology continues to grow.

Laser phoresis

Percutaneous laser phoresis is a well-known and very effective technique (in Russia, which has an almost 200-year history of the development of physiotherapy and balneology as scientific areas in medicine) for the combined use of various therapeutic physical factors (Electromagnetic Radiation (EMR) of various frequencies are implied; several dozen options are currently used).^{4,5} The mechanisms of percutaneous laser phoresis, the ways and conditions for the penetration of biologically active substances, based on an understanding of the skin structure, function and physiology, have been thoroughly researched.

Substances can penetrate through the skin in three main ways: i) transepidermal; ii) intercellular; iii) and an additional one, through shunts: substances are transported through the sweat glands and hair follicles.

One of the main functions of the skin is to protect the body from external exposure; therefore, transepidermal penetration of aqueous solutions of various substances, (*i.e.*, literally directly through the layer of epidermal cells), is practically impossible.⁶ The third way is undoubtedly most important for the penetration of major substances; therefore, it is crucial to understand the properties which macromolecules must have in order to be able to penetrate into the skin. In addition, there are other factors that influence penetration: i) specific cutaneous factors (place and area of application; human age, skin condition, temperature and degree of hydration; blood supply intensity, *etc.*); ii) characteristics of the substance (molecular weight, chemical structure, conformation, hydrophilicity); iii) available external factors (frequency and type of electromagnetic radiation, energy characteristics and exposure). Whereas the transepidermal route through the intercellular spaces is practically impossible, the situa-

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Key words: low-level laser therapy; laser phoresis; skin; rejuvenation.

Conflict of interest: the authors declare no potential conflict of interests.

Received for publication: 26 October 2020.
Accepted for publication: 15 December 2020.

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Dermatology Reports 2020; 12:8996
doi:10.4081/dr.2020.8996

tion with cutaneous appendages is completely different. The duct of the Sweat Gland (SG) has dermal and epidermal parts, it opens on the top of the skin combs, the pore diameter is 60–80 μm, and the gaps are 14–16 μm in diameter.⁷ According to different authors, the SG density ranges from 64 to 431 per 1 cm² depending on the localization and national identity of a person; it is greatest on the face – up to 174 per 1 cm², and on the palms – up to 424-431 per 1 cm², and their total amount is from 2 to 5 million. Assuming that the total area of the lumens of the excretory ducts is less than 1% of the skin surface (57-94 cm², *i.e.*, the secretory surface of all SGs has an area of up to 5 m², *i.e.*, 3 times larger the total area of the epidermis. The skin layer in which the sweat-gland glomeruli are located is 1.3-3.12 mm thick, and the entire volume this layer amounts to 3200 cm³.⁸⁻¹²

According to different authors, the density of hair follicles varies widely in different parts of the skin, from 60±40 on the scrotum skin to 830±100 on the cheeks of men depending on age, gender, hair color, nationality, *etc.* The number of visible hairs is much smaller or they are completely absent in some parts of the body (palms, feet, *etc.*).^{8,13,14}

Thus, we see that there are more than 1000 potential “inputs” for macromolecules of significant size on the human body per 1 cm² of the surface, and this is quite enough for the required amount of substance to penetrate. Further, the process gets more active due to an increase in the area of contact with glandular and epithelial cells. Moreover, the fact of the molecular penetration through

the entrance does not automatically mean their further advancement, since it is necessary to pass through the cells of the glands and/or epithelium.

The mechanism that allows this to be implemented is well known; it is called transcytosis (pinocytosis) and involves a process that combines the signs of exo- and endocytosis. An endocytic vesicle (endosome) forms on one surface of a cell; this endosome is transferred to the opposite edge of the cell, becoming an exocytotic vesicle and releasing its contents into the extracellular space. The entire process (complete passage of the substance) takes no more than 1 min. This mechanism is known as the main one, which ensures the absorption of small drops of water, proteins, glycoproteins and macromolecules with a maximum size of up to 1000 nm (1 μ m) by cells and provides the work of the endocrine glands.^{15,16}

Therefore, to implement laser phoresis, the substance must be hydrophilic and have fragments up to 1 μ m in size. It is clear that no problems should arise (nor do any arise) in the case of laser phoresis of aqueous solutions of low-molecular weight compounds, which are mainly used in medicine.² The situation is different with Hyaluronic Acid (HA), which in its natural state is prone to form long filaments, for example, ranging from 450 nm (0.45 μ m) to 4200 nm (4.2 μ m) in a cartilage. However, in an aqueous solution, the same HA molecule (1000 kDa), having an extended length of 2500 nm (2.5 μ m), forms a sphere of only 200 nm in diameter.¹⁷

It is known that thermodynamic triggering of Ca^{2+} -dependent processes is the primary mechanism of Biomodulating Action (BA) of Low-Intensity Laser Light (LILL).¹⁸ During LILL absorption, a local short-term violation of thermodynamic equilibrium occurs, which results in Ca^{2+} ions release from the intracellular depot and their consequent propagation in the form of waves throughout the cell, initiating the activation of Ca^{2+} -dependent processes,¹⁵ which, in particular, are endo- and exocytosis.^{15,19,20} Thus, Ca^{2+} ions release induced by LILL leads to transcytosis activation in general; this is the principal process in the laser phoresis mechanism.

Most notably, laser phoresis is not only the easiest and economically feasible method to implement, but it is also a very effective procedure. Figure 1 shows the comparison between the effectiveness of various physical factors affecting the penetration of carbochromene through the cell membrane, which demonstrates the indisputable advantages of laser phoresis.⁵

Although we are aware of the laser

phoresis mechanisms, an extremely important question refers to the Molecular Mass (MM) of HA molecules, which can be introduced percutaneously, and to the result which can be obtained. It has been shown that in women HA concentration in the skin decreases with aging, which is especially pronounced after 60 years.²¹ Over time skin gets severely dehydrated, the fragility of blood vessels increases, new wrinkles appear and current wrinkles deepen, resulting in decreased skin thickness and turgor.

Presumably this is associated, inter alia, with HA deficiency, which serves as a justification for HA introduction into the skin.

It is known that high-molecular weight HA (more than 2000-6000 kDa) is applied to intradermal injections; in contrast, HA with a molecular weight of up to 600 kDa can penetrate directly through the skin.^{16,22} The research by M. Farwick *et al.* (2008)²³ showed that HA possesses skin-friendly properties that can be controlled by the use of HA with various MM. Thus, low-molec-

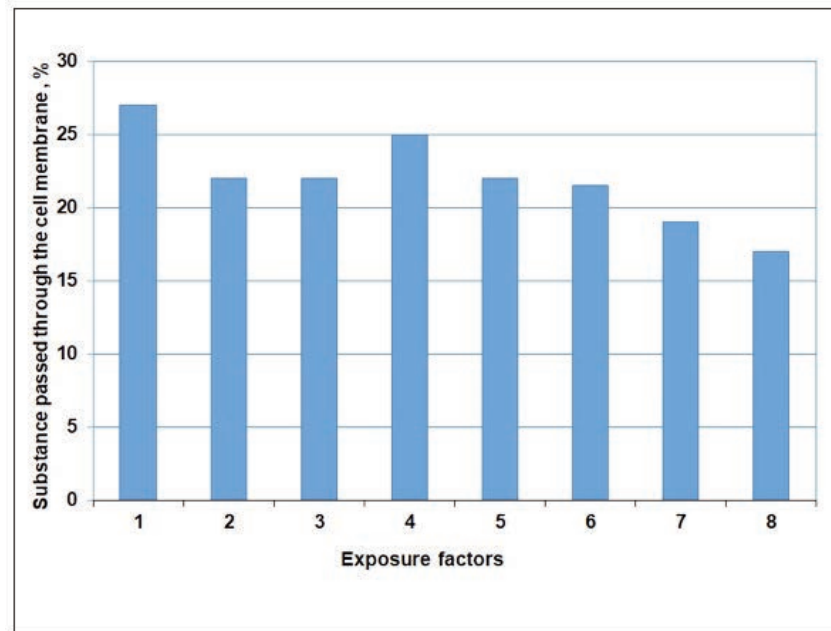


Figure 1. The effect of various physical factors on the phoretic mobility of carbochromene in various physical fields (exposure time 5 min). 1: helium–neon laser illumination (633 nm, 20 mW); 2: short-wave ultraviolet illumination (254 nm, lamp power 220 W); 3: ultrasound (0.88 MHz, 0.6 W/cm²); 4: EMR 460 MHz, 2 W; 5: EMR 40.7 MHz, 15 W; 6: alternating magnetic field (50 Hz, 35 mT); 7: constant magnetic field (30 mT); 8: direct electric current (electrophoresis).⁵

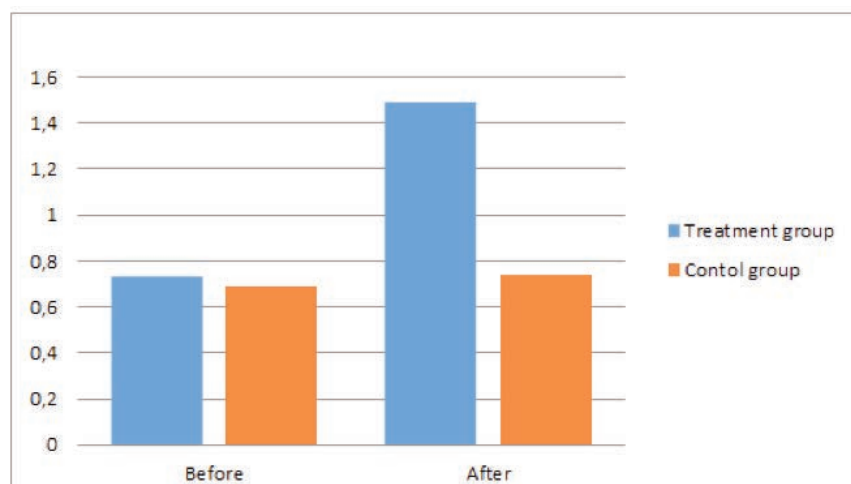


Figure 2. Indicators of the activation coefficient of syntoxic adaptation programs in the treatment and control groups (87 patients).

ular weight HA (50 kDa) is better transported through the skin cover than HA with high MM (800 kDa); it also activates a larger number of keratinocyte genes, including genes responsible for the differentiation of keratinocytes and the formation of complexes of intercellular contacts, which decreases in photo-damaged and aging skin. The moisturizing effect and increased skin elasticity are more inherent in high-molecular weight HA, while low-molecular weight HA showed the wrinkle-tightening effect. The authors explain the increase in activity with the decreasing molecular weight of HA through better transepidermal penetrating ability for smaller HA molecules.

We developed custom hyaluronic acid (2% sodium hyaluronate) with a molecular weight of 250-750 kDa that is applied by LASMIK technology. A mix of HA with different MM enables getting a quickly visible result in the form of tightened fine wrinkles, and provides a long-term effect that lasts up to 1-3 months.^{24,25} This will be discussed further.

Laser phoresis, including HA-based preparations, has long been successfully used in medicine: dentistry (TMJD, stomatitis, periodontitis),²⁶⁻³³ otorhinolaryngology (sinusitis, tonsillitis)^{34,35} cardiology (arterial hypertension),³⁶ andrology,³⁷ gynecology³⁸ and other fields. However, it is cosmetology and dermatology that are the main areas of application of this technique.^{39,40}

Laser biorevitalization

Having understood the essence and mechanisms of the processes occurring during laser phoresis, as well as the optimal parameters of HA, we continued research on the optimization of the laser illumination technique parameters with an objective assessment of the results of the skin response to the combined exposure.

E.A. Ryazanova (2007)²⁵ showed that after applying electromyostimulation prior to laser phoresis by Matrix-Cosmetologist Laser Therapeutic Complex produced by Research Center "Matrix" (Moscow, Russia), the neuroreceptor and muscle apparatuses were activated and blood microcirculation improved. These changes contribute to an active response to the exposure to Low-Intensity Laser Illumination (LILI) and the penetration of HA and Succinic Acid (SA) deep into the tissues. It turned out that low concentrations of HA and SA provide a very significant effect of enhanced microcirculation and the general influence of LLLT on the human body, increasing its adaptive capabilities (Figure 2).⁴¹ There is a significant (almost 2-fold) increase in the number of open pores, which we demonstrated with a thermal imaging camera (Figure 3).⁴¹

We conducted the research for 2 years (2010-2011) to optimize the parameters of the laser phoresis technique, testing the LASMIK laser biorevitalization technology, which was later patented but according to existing legislation, the method can be used

by anyone without any restrictions⁴² Various parameters of blood microcirculation and face skin fluorescence were measured using special equipment (Laser Doppler flowmetry with the function of evaluating cellular metabolism and protein structure). The trial involved 85 women, and was carried out in 3 stages: i) measurements were taken in 25 women aged 20-25 years (control group) and 60 women aged 45-55 years (treatment groups) without external influence, ii) measurements were taken after applying a gel with HA (the 1st treatment group), iii) measurements were taken after the LILI and laser phoresis (in two other treatment groups).^{2,3}

Laser illumination parameters were as follows: 780 nm wavelength, continuous mode, power of 50 mW, power density of 50 mW/cm², exposure time of 30 s per zone, total procedure time of 10 min (15 zones on the face), treatment course consisting of 10 daily procedures. For laser phoresis, 1.5% HA gel (MM 25-750 kDa) was applied to the zone shortly before the illumination.

The mean value of the parameters and the error of mean were determined. A correlation analysis was performed, one-way analysis of variance, Student t-test was used. To analyze the contingency tables of nonparametric features, the χ^2 criterion was used. $p < 0.05$. Our findings indicate the stimulation of facial skin blood microcirculation and, consequently, an increase in the oxygen tension in the skin, oxygenation of the facial skin blood, and an increase in the

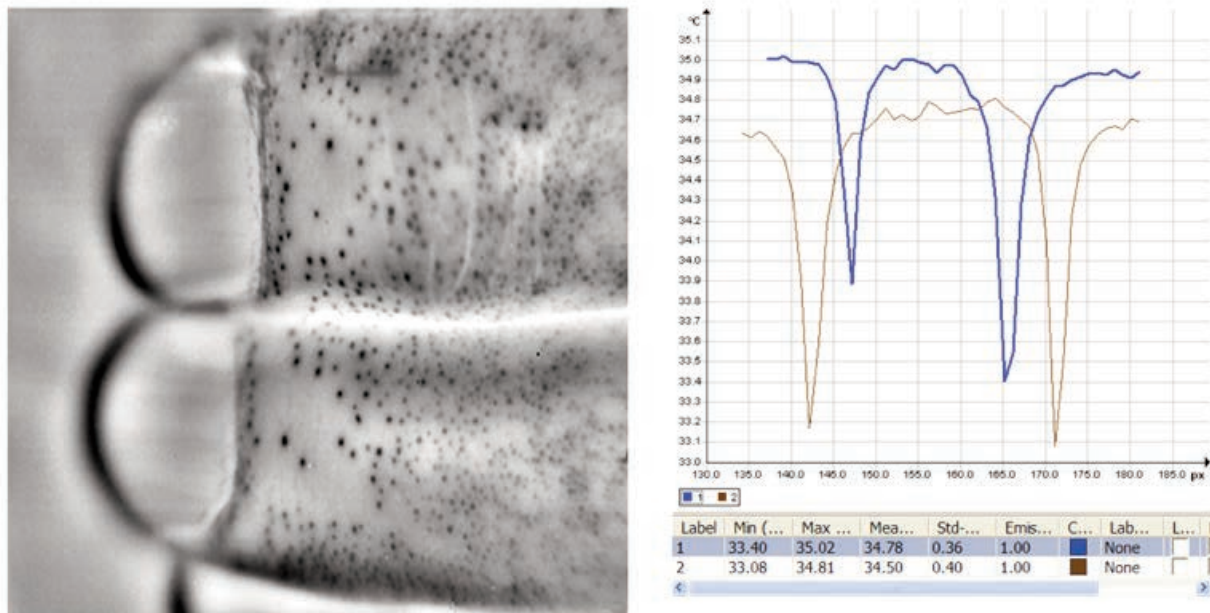


Figure 3. Open sweat channels (left) and temperature profiles through the pores (right) after electromyostimulation and laser phoresis.

trophic support to tissues. All these secondary effects were caused by LILI and the initiation of Ca^{2+} -dependent processes, in particular, stimulation of NO synthesis by endothelium, resulting in endothelium-dependent vasodilation and increased perfusion.³

The comparative analysis of the effects of LILI and laser phoresis exposure indicates a significantly more pronounced effect of the combined technique. Thus, it was confirmed that LILI enhanced the effectiveness of the positive biological effect of HA on the facial skin blood microcirculation in women aged 45-55 years, *i.e.* there was synergy.

Initially, in treatment groups, the integral indicator – the Efficiency Of Oxygen Metabolism (EOM), was reduced by more than half compared to the control group (aged 20–25 years), (Figure 4),⁴¹ which is the physiological age norm. The research results revealed an increase in the EOM by 36% after applying gel with HA in women aged 45-55 years, and on average by 23% after exposure to laser illumination; the EOM almost doubled after laser phoresis with HA (Figure 5),⁴¹ virtually reaching the norm, corresponding to 20 years younger age, which is clearly demonstrated by a noticeable reduction of facial wrinkles (Figure 6). Our findings indicate an improvement in local blood circulation, stable oxygenation of the skin, an improvement in the efficiency of oxygen consumption by skin cells, stabilization of the energy metabolism of skin cells, a slow decrease in the concentration of oxidized flavoproteins and an increase in the concentration of reduced pyridine nucleotides, which causes an increase in the EOM of the facial skin.

The observations showed that, in terms of various indicators, the effect lasts from 1 to 3 months, which explains the need to carry out the procedures at least once per month, and the need to conduct a course consisting of at a minimum five daily, or alternate-day, procedures once every 6 months.

A comparative evaluation of the LILI of various spectra was carried out, and the influence of modulation and other parameters of the technique was studied with the purpose of its optimization; these data were published in specialized journals, and four monographs were printed.^{2,24,40,41}

Conclusions

Thus, an understanding of the mechanisms of laser phoresis and Biological Effect (BE) of LILI at the cellular and tissue

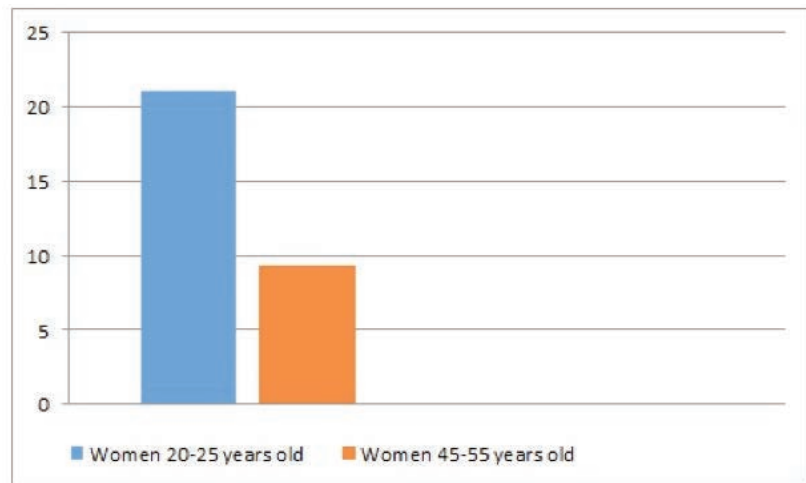


Figure 4. The efficiency of oxygen metabolism of facial skin cells in women of different age groups ($p < 0.05$ compared to another age group).

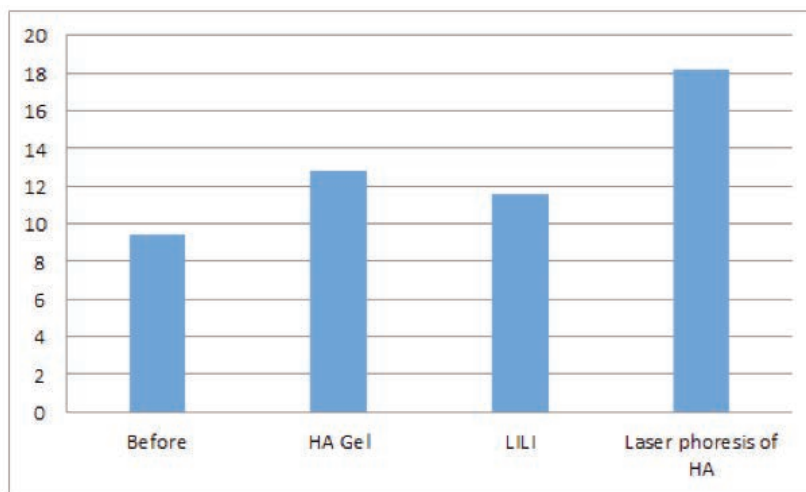


Figure 5. The effectiveness of the oxygen metabolism of facial skin cells in women after application of the HA gel, exposure to LILI and laser phoresis with HA ($p < 0.05$ compared to the control group).



Figure 6. Reduction of facial wrinkles (a 45-year-old male).

levels, as well as the long-term experience of thousands of specialists, allow us to confidently formulate the basic requirements for substances and parameters of the laser illumination technique that provide the most effective implementation of LASMIK laser biorevitalization.^{43,44} i) Substances penetrate into the skin through sweat glands and hair follicles by means of transcytosis. Since transcytosis is a Ca²⁺-dependent process and the LILI BE mechanism is also based on their activation,^{1,18} laser phoresis justifiably is the most effective way to enhance transdermal transport, which is possible only for hydrophilic molecules with molecular masses up to 750 kDa. ii) It has been shown that it is most optimal to use a mix of HA with a molecular weight of 250-750 kDa. iii) The concentration of HA in an aqueous solution should not exceed 2-3%, since a large amount of water is necessary for its penetration. iv) Optimum wavelengths (tested by us) make 405, 525, 780 nm. Each wavelength has its own positive qualities; it is rather difficult to identify the most optimal one.¹ v) The optimal power density is 20-50 mW/cm² and depends on the wavelength. More often, a continuous laser mode is used, however, modulation with a frequency of 10 Hz significantly increases the efficiency of the technique. vi) The area of the substance application should be illuminated for no more than 1 min; furthermore, it is necessary to bear in mind that the total time of the laser illumination should not exceed 20 minutes. vii) Laser phoresis procedures should be carried out at least once per month, and every 6 months it is required to take a course consisting of at least 5 daily or alternate-day procedures.

In conclusion, we draw special attention to the fact that for LASMIK *laser biorevitalization* it is necessary to use only *lasers* in accordance with the well-known (in Russia) rules of low-level laser therapy procedures.^{45,46} Unfortunately, we had to face with discrediting of the procedure for many times when incoherent light sources and/or unacceptable illumination parameters were used.

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