Optical clearing of human skin for the enhancement of optical imaging of proximal interphalangeal joints

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ABSTRACT

We are proposing a new method for enhancement of optical imaging of proximal interphalangeal (PIP) joints in humans at skin using optical clearing technique. A set of illuminating laser diodes with the wavelengths 670, 820, and 904 nm were used as a light source. The laser diodes, monochromatic digital CCD camera and specific software allowed for detection of the finger joint image in a transillumination mode. The experiments were carried out *in vivo* with human fingers. Dehydrated glycerol and hand cream with urea (5%) were used as optical clearing agents (OCAs). The contrast of the obtained images was analyzed to determine the effect of the OCA. It was found that glycerol application to the human skin during 60 min caused the decrease of contrast in 1.4 folds for 670 nm and the increase of contrast in 1.5 and 1.7 folds for 820 nm and 904 nm, respectively. At the same time, the hand cream application to the human skin during 60 min caused the decrease of contrast in 1.1 folds for 670 nm and the increase of contrast in 1.3 and 1.1 folds for 820 nm and 904 nm, respectively. The results have shown that glycerol and the hand cream with 5% urea allow for obtaining of more distinct image of finger joint in the NIR. Obtained data can be used for development of optical diagnostic methods of rheumatoid arthritis.

Keywords: rheumatoid arthritis; optical diagnostics; optical clearing; transillumination mode; proximal interphalangeal joints.

1. INTRODUCTION

Rheumatoid arthritis (RA) is an autoimmune disease that results in a chronic, systemic inflammatory disorder that may affect many tissues and organs, but principally attacks flexible (synovial) joints. It can be a disabling and painful condition, which can lead to substantial loss of functioning and mobility if not adequately treated. The pathology of the disease process often leads to the destruction of articular cartilage and ankylosis (fusion) of the joints. RA can also produce diffuse inflammation in the lungs, the membrane around the heart (pericardium), the membranes of the lung (pleura), and sclera, and also nodular lesions, most common in subcutaneous tissue[1]. To be able to start with the treatment of RA on time, the disease must absolutely be diagnosed at an early stage, which is not always easy. In most cases, such techniques as x-rays and sonography are the only imaging tools[2].

The development of digital technologies combined with mathematically and physically based imaging software constantly opens up new possibilities to find and differentiate between pathological and healthy states more reliably than the human eye would be able to do[2]. One of the most perspective imaging methods is optical imaging due to its absolutely safety, high sensitivity, and unique specificity in comparison to other biomedical imaging modalities. Optical imaging in biomedicine is governed by the light absorption and scattering interactions with microscopic and macroscopic

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constituents of the medium. Therefore, light absorption and scattering characteristics of human tissue may correlate with disease development[3-5].

Transillumination is one of the existing methods for optical imaging of the internal structure of biological tissue[6]. However, strong lights scattering in tissues causes image blurring. By using of NIR radiation within the tissue's "transparent window", i.e., 800-950 nm, transillumination technique can be improved[3,7]. In addition, the relatively low contrast and poor spatial resolution of the technique, which do not allow one to find the difference between healthy and pathology joints precisely, could be improved by reduction of skin light scattering at using of OCAs[7,8].

It is known that the basic reason of light scattering in tissues is refractive index mismatch between scatterers (cell compartments, collagen and elastin fibers) and the ground material (interstitial fluid and/or cytoplasm) of a tissue[7,8]. The optical immersion technique allows one to control effectively optical properties of tissues[9,10]. Administration of OCA into tissue causes partial substitution of interstitial fluid by immersion solution and follows up matching of refractive indices of tissue scatterers and their environment and, therefore, decreases scattering. An OCA can also induce local dehydration of tissue due to its osmotic properties that results in matching of refractive indices of tissue components and their ordering too[7,8].

As an OCA glycerol is widely used at skin clearing[8-10] because of its high refractive index and hyperosmotic properties. Glycerol is a highly effective tool for optical clearing of tissues that providing the major mechanism of optical clearing caused primarily by the osmotic dehydration of tissues. The water outflow leads to an increase of the concentration of salts and proteins dissolved in the interstitial fluid, increasing the refractive index of the interstitial fluid, thus the refractive indices of scatterers and the interstitial fluid matching, and to the skin optical clearing[10]. In spite of the high viscosity of glycerol, it was shown that glycerol penetrates into the interstitial fluid of the skin[11], therefore refractive index matching can be enhanced.

However, the delivery of OCA into the deep skin layers can be a difficult problem at its topical application. It can be explained by barrier properties of living epidermis and its upper layer stratum corneum[12] and relatively slow diffusivity of OCA molecules and water in tissues. For example, in the model of free diffusion of molecules with diffusion coefficient $D = (10^{-5}\text{-}\ 10^{-6})$ cm^2/c (characteristic to small molecules diffusion in dermis and other stromal/connective tissues), the time of diffusion τ_D on the distance l=1 mm should be $\tau_D = l^2/D \cong (10^3\text{-}10^4)$ s, i.e. ~(17-167) min. It was found that topical application of urea and its derivatives allows for reduction of skin barrier function and increasing the penetration rate of topically applied substances into the skin[13]. The mechanism of this phenomenon is caused by keratolytic action of urea. According to this fact, we used hand cream with 5% urea that also contains glycerol to compare the effectiveness of these agents.

In spite of well-established benefits of optical clearing technology, it is not evident that it can help in transillumination imaging, where rather thick organs are typically under investigation, such as human fingers.

The goal of this study is to improve transillumination optical images of capsule synovial structures of a human finger by OCA topical application.

2. MATERIALS AND METHODS

2.1 Samples

The experiments were carried out *in vivo* with finger human skin. The proximal interphalangeal (PIP) joints of index (thickness of 1.6 cm) and middle (thickness of 1.8 cm) fingers of female (25 years) volunteer were investigated.

2.2 Experimental setup

The experimental setup for transillumination imaging of finger joints consisted of illuminating laser diodes (670, 820, and 904 nm) and a monochromatic digital CCD camera (DMK 4002-IR with 1/4" Sony CCD, 640×480 pixel) of an 8 bit dynamic range and variable exposure time. This camera was linked to a CS-Mount lens with 10-mm focal width. The IR-

corrected lens focused the incident light upon the same image point, as a result of which no focus shift occurred. The beam of the laser diodes was focused upon a spot diameter of ≤1 mm on the finger surface. Using laser diodes of a fixed power, the exposure time depended on the diameter of the finger and the manifestation of rheumatoid arthritis. Figure 1 shows the complete setup and the scheme of the transillumination system.

Controlling both, the laser diodes and the camera, specific software displayed a live picture on the monitor. The live picture was provided with crosshairs permitting the joint to be centrically positioned upon the optimum irradiation site so that the finger was irradiated symmetrically around the articular gap. Positioning of the joint can be controlled by the patient in the live picture. For follow-up examinations, an earlier image of the same PIP joint can be transparently superimposed by the live picture. So doing, positioning of the finger can be reproduced at a lateral accuracy of 1 – 2 mm.

a b

Figure 1. The exterior of the experiment (a) and the transillumination system with 3 laser diodes (b).

In addition, further motion artifacts occurred while switching between the different wavelengths. Such artifacts were dependent on the effective exposure time and the current state of the patient. If findings based on different wavelengths are combined, a higher diagnostic hit rate is to be expected compared to optical diagnostics using only one wavelength.

As a result of the experiment a set of images, which represent two-dimensional maps of intensity distribution of the scattered light on the upper surface of the finger, was obtained.

2.3 OCAs

Dehydrated glycerol (G) and hand cream Balea (DM, Germany) with 5%-urea (HC) were used as OCAs for the testing of the possibility of image improvement of the joints. Refractive index of G was measured with Abbe refractometer (Atago DR-M2/1550, Japan) at some wavelengths (450, 589, 680, 1100, and 1550 nm) and interpolated. It was evaluated as 1.469 at 670 nm, 1.466 at 820 nm, and 1.465 at 904 nm. The OCA was applied topically on the investigated skin site during the first 5 min and then was additionally applied before image detection that was made every 5-15 min during one hour.

2.4 Contrast calculation

Processing of the experimental data made it possible to calculate a contrast in the region of interest (ROI) near the middle of the finger joint (fig.2).

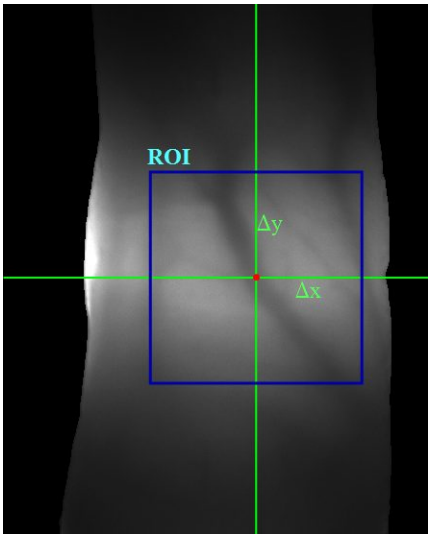


Figure 2. An image of ROI (blue square, fixed to 220x220 pixel) for the contrast calculation.

The Michelson contrast is defined as

$$C_{Mich} = \frac{I_{max} - I_{min}}{I_{max} + I_{min}}, \tag{1}$$

where $I_{\max}$ is the maximum and $I_{\min}$ is the minimum intensity in any pixel within the ROI according to Fig. 2.

Even the whole image is scanned, only one pixel read out, for example by surface reflection, motion artefacts or CCD-errors can distort the final result completely. Another definition of intensity variations reads

$$C_{mean} = \frac{\sqrt{\sum_{ROI}(I(i,j) - I_{mean})^2}}{220}, \tag{2}$$

where

$$I_{mean} = \frac{\sum_{ROI} I(i,j)}{220x220}$$

according to Fig 2.

Even Eq. 2 reads similar to the common definition of the mean standard deviation, we can interpret the result as the mean contrast within the ROI. Pixel errors of small extent will not change C_{mean} and draws an important distinction in comparison to C_{Mich}. It works well, because OC is like removing the blurring effect of skin based on its overwhelming scattering.

3. RESULTS AND DISCUSSION

Figs. 3 and 4 show transillumination images of volunteer's finger joint before and after OCA topical application. G was used in the experiment with an index finger and HC was used with a middle finger. The result for HC looks similar.

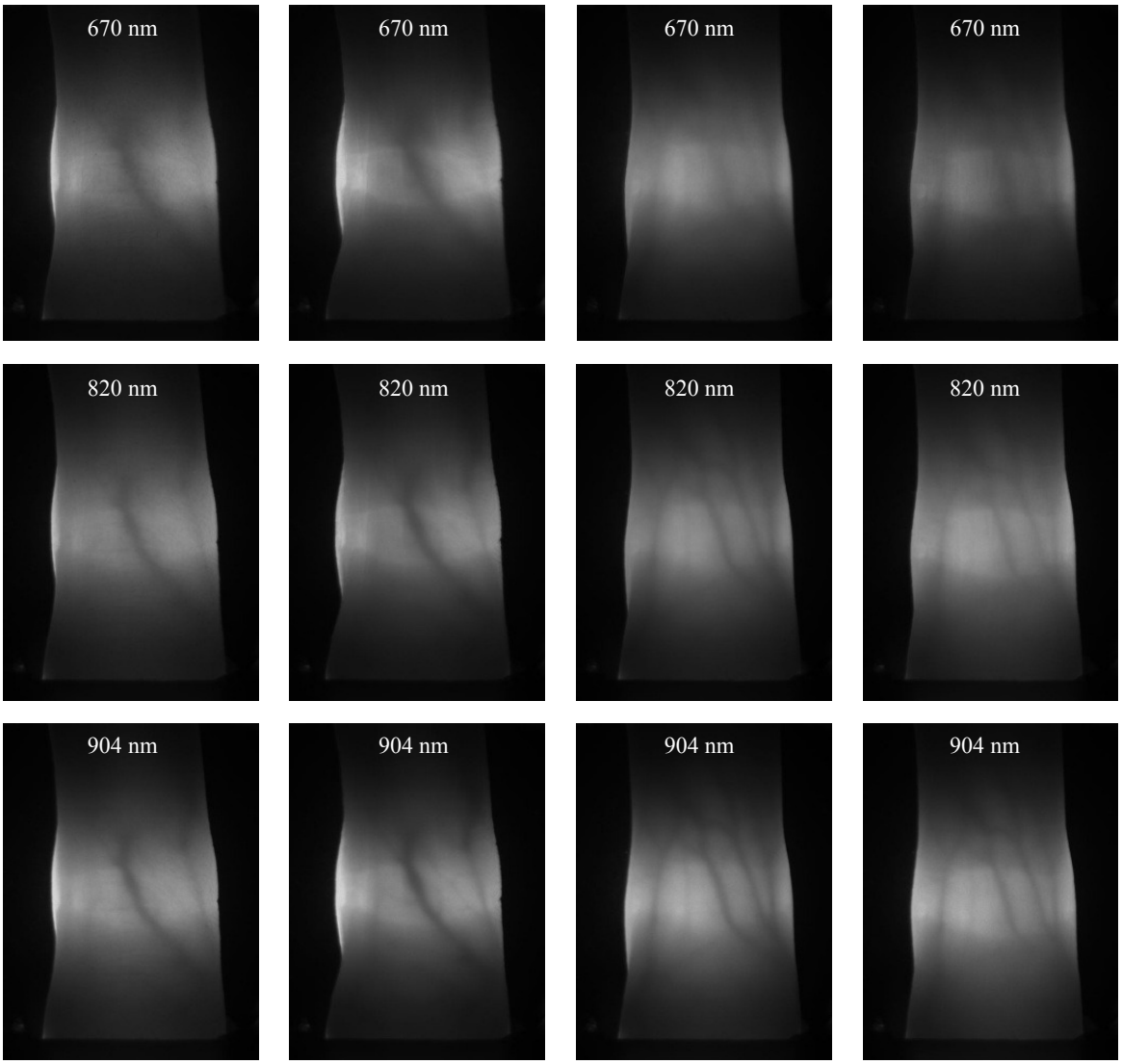
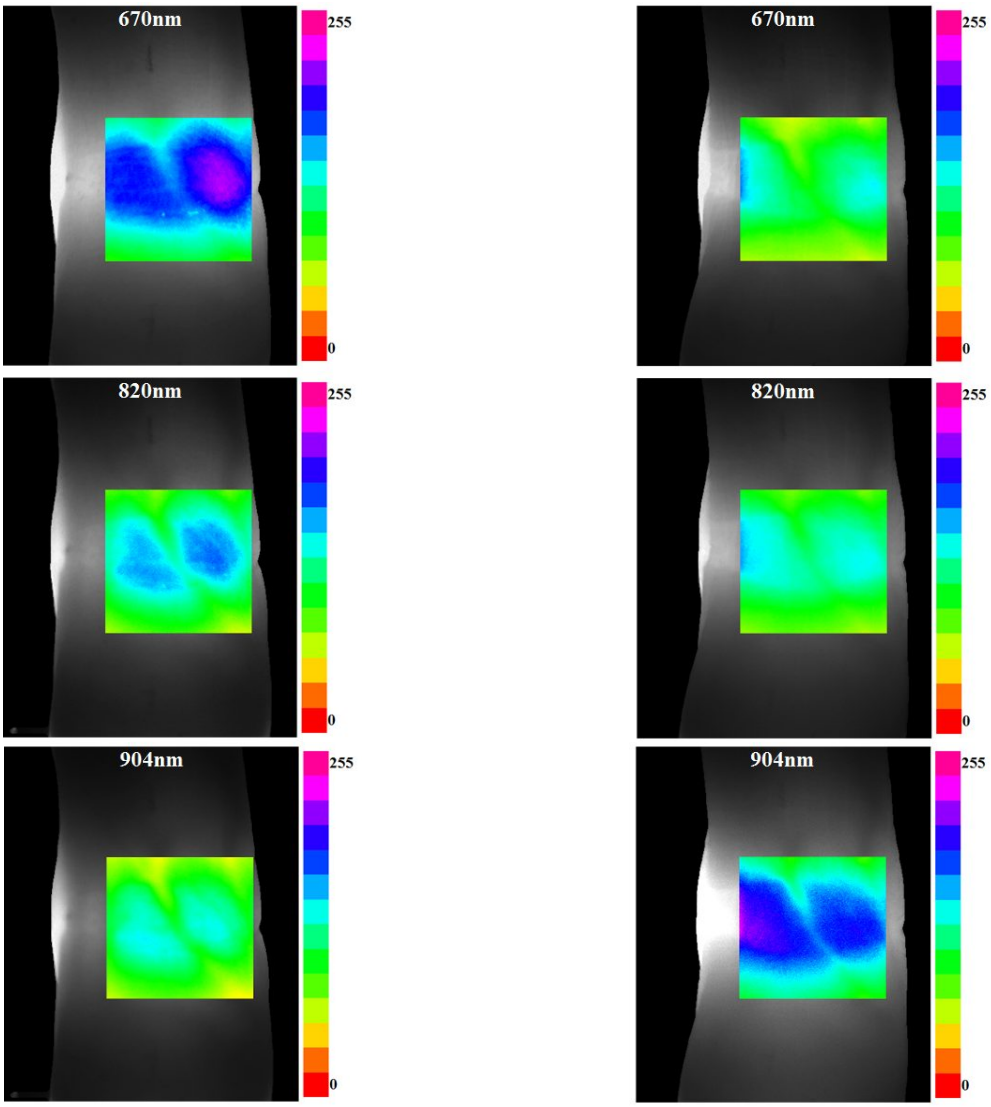


Figure 3. Images of finger joint under the action of glycerol in a transmission mode: before agent application (a), 60 min after agent application (b).

Figure 4. Images of finger joint under the action of hand cream in a transmission mode: before agent application (a), 60 min after agent application (b).

In the images blood vessels, intra-joint gap and – in a small measure - skin surface structure is visible. We can see differences between images of the joints before agent application and in an hour. Effect of G and the HC on quality of the images is also different. During the optical clearing, immersion agent firstly fills up the natural sulci of skin and stratum corneum. At that, G provides a superficial refractive index matching with the immediate elimination of skin superficial scattering. The quality of the images rises as the clearing agents penetrates through the stratum corneum. Thus, improvement of the images is achieved at longer G exposition for all studied wavelengths. At the application of the HC as an immersion agent we cannot see the improvement of the image quality.

To enhance the differences between Fig. 3(a) and Fig.3 (b), and Fig.4 (a) and Fig.4 (b) visually, we used a pseudo color presentation (Figs. 5 and 6). Metrics in the HSV color spaces makes the differences more clearly in comparison to the well known rainbow scaling (not shown).



a b

Figure 5. Images of finger joint with pseudo color imaging in HSV color space within ROI region under the action of glycerol in a transmission mode: before agent application (a), 60 min after agent application (b).

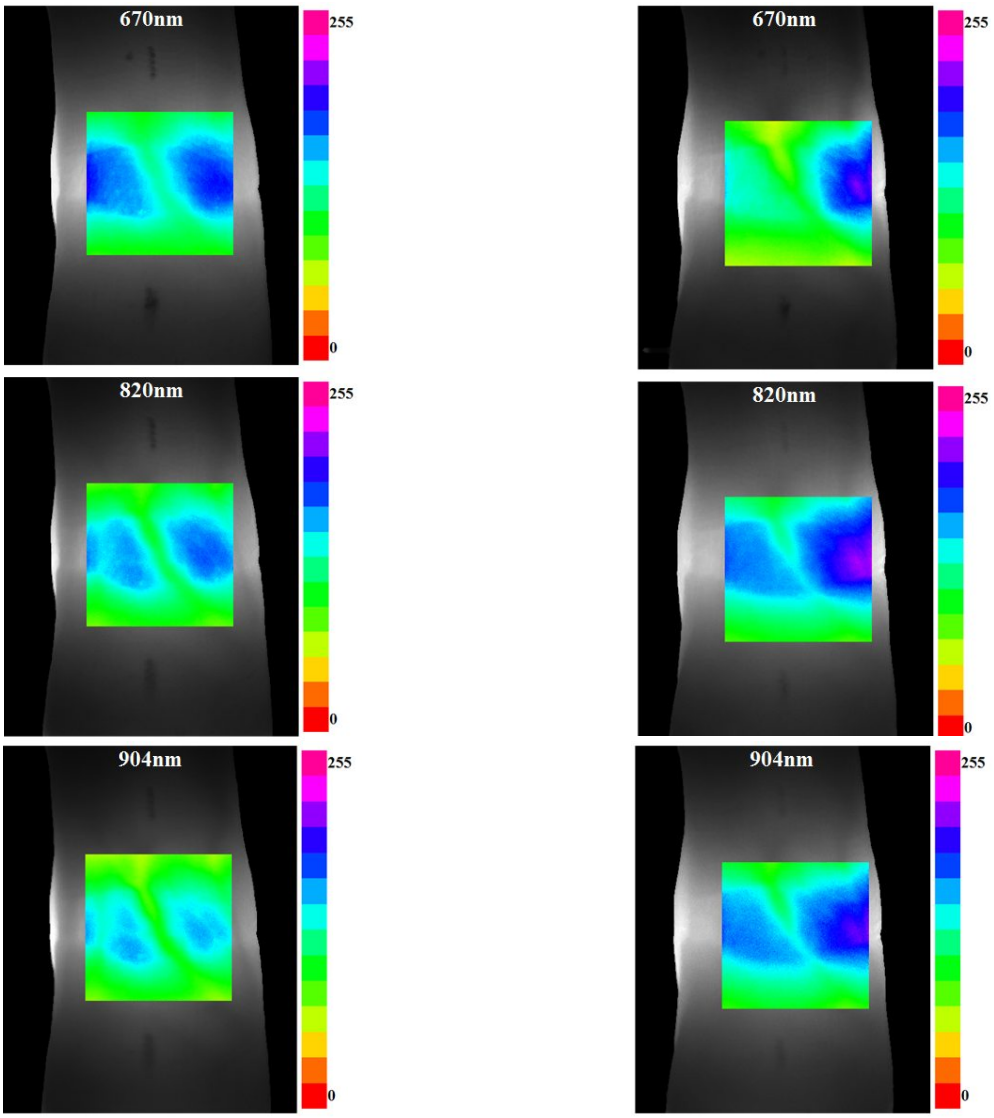


Figure 6. Images of finger joint with pseudo color imaging in HSV color space within ROI region under the action of hand cream in a transmission mode: before agent application (a), 60 min after agent application (b).

The image contrast values $\boldsymbol{C_{mean}}$ before and after G and HC topical application are presented in tables 1 and 2, respectively. Figs. 6 and 7 allow for comparison of kinetics of the image contrast at G and HC application at different wavelengths. In different, $\boldsymbol{C_{Mich}}$ contains to considerable fluctuations over time that does not allow a reliable approximation to a mathematical function (results are not shown).

Normalized values of $\boldsymbol{C_{mean}}$ in time dependence for G and HC topical application are presented in Figs. 8 and 9, respectively. Symbols correspond to the experimental data and solid curve is a fit approximation by double exponential formula.

Table 1. C_{mean} in a ROI near the middle of the joint for the case of glycerol topical application

GLYCEROL: C_{mean} in ROI			
	670nm	**820nm**	**904nm**
No agent	0.114	0.054	0.072
0 min	0.122	0.065	0.096
5 min	0.119	0.060	0.090
10 min	0.115	0.066	0.104
15 min	0.111	0.069	0.105
20 min	0.110	0.075	0.120
30 min	0.101	0.076	0.117
40 min	0.095	0.080	0.118
50 min	0.089	0.080	0.120
60 min	0.084	0.081	0.123

Table 2. C_{mean} in a ROI near the middle of the joint for the case of hand cream topical application

HAND CREAM: C_{mean} in ROI			
	670nm	**820nm**	**904nm**
No agent	0.090	0.069	0.097
0 min	0.093	0.065	0.087
5 min	0.094	0.070	0.094
10 min	0.092	0.073	0.103
15 min	0.090	0.074	0.103
20 min	0.091	0.075	0.104
30 min	0.079	0.075	0.108
40 min	0.081	0.079	0.106
50 min	0.077	0.083	0.111
60 min	0.084	0.089	0. 111

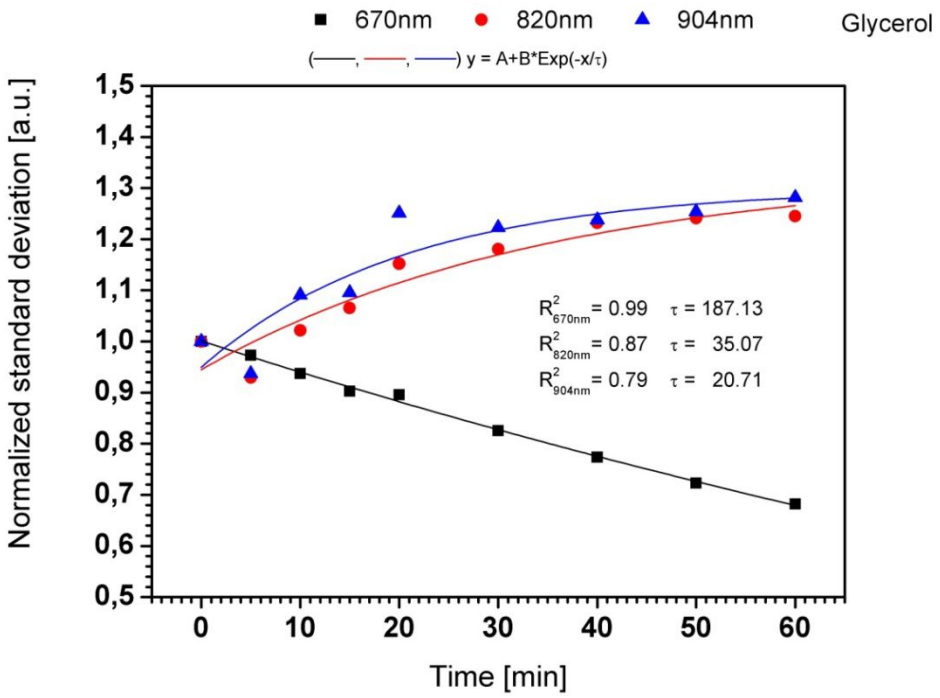


Figure 7. Normalized C_{mean} in time dependences for glycerol with a fit approximation by double exponential formula. $\boldsymbol{R^2}$means the coefficient of determination in statistics.

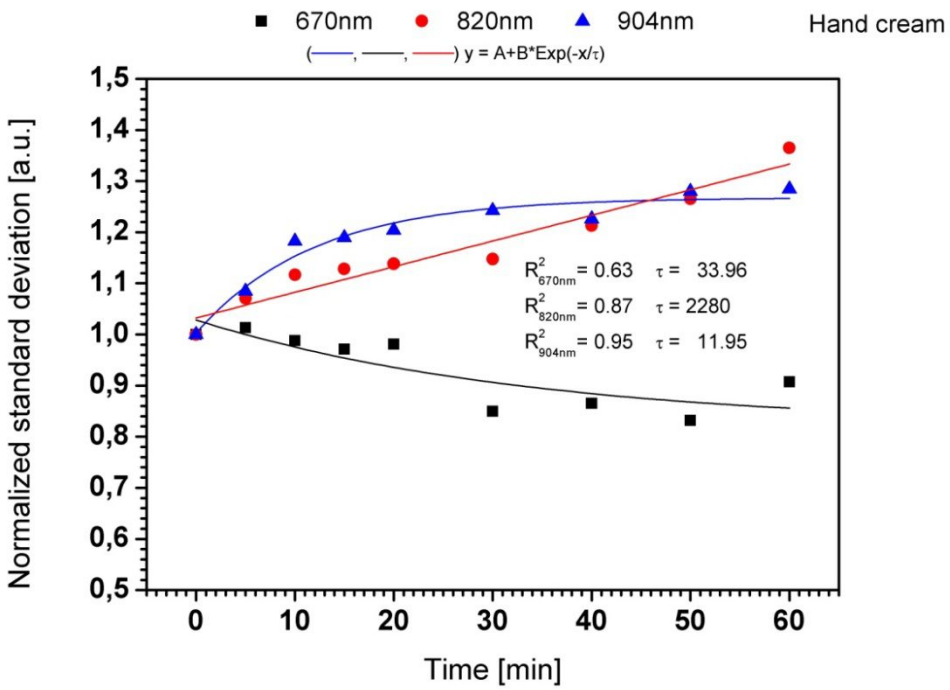


Figure 8. Normalized $\boldsymbol{C_{mean}}$ in time dependences for hand cream with a fit approximation by double exponential formula. $\boldsymbol{R^2}$ means the coefficient of determination in statistics.

The underlying physical process of OC is diffusion, which is an exponential process with saturation. At the beginning of the clearing it can be approximated as the concentration of the OCA that enters the tissue or water that exits the tissue, resulting again in an excess of immersion substance, salts, proteins, etc.

$$C_a(t) \cong C_{a0}[1\text{-}\exp(\text{-}t/\tau_1)\ ], \tag{3}$$

but with time elapsed it should be saturated with further decrease, what can be seen for the hand cream and what can be associated with water coming from in tissue depth.

In Eq. (3) C_{a0} is the concentration of OCA (or its equivalent in the dehydration model) which is applied and, ultimately, the concentration that will be in the tissue at infinite time, or the final concentration after complete dehydration in the dehydration model. τ_1 is essentially the diffusion constant.

To describe urea cream action the process of rehydration should be taken into account, which compensates optical clearing. Therefore, a one more exponential term with a bigger $\tau_2 = l^2/D$ has to be added, because water needs some time to come from in deeper tissue layers.

In principle, the same two-stage physics is valid for glycerol too, but it just comes later and it can be already seen in the hourly interval. Both processes are balanced with some coefficients A, B, C. So, both processes, optical clearing (skin dehydration and OCA penetration) and tissue rehydration (decay of clearing with time), are balanced with two exponents $A - B \cdot \exp\{-t/\tau_1\} + C \cdot \exp\{-t/\tau_2\}$. The extent of experimental data has to be increased, to proof the hypothesis $\tau_2 > \tau_1$.

Recently, using transepidermal water loss and erythema index measurements it was clinically proved that topically applied immersion optical clearing agents provide effective tissue dehydration during the time period of 8-10 min for a forearm skin site, but after that an intensive rehydration process was followed up[14]. Therefore, to get a noticeable skin dehydration, i.e. optical clearing *in vivo*, the agent should not be applied on the skin site for a long time and should not interfere with the evaporation of water from the skin surface. This conclusion well correlates with data received in this study that in dependence of applied agent and imaging wavelength, optical clearing of finger skin could be effective in

the time scale from 5 to 60 min. The wavelength dependence is also important in understanding of optical clearing efficiency, because both processes photon diffusion and molecule diffusion are spatially-dependent.

4. CONCLUSIONS

In this paper we demonstrate for the first time that topical application of pure glycerol or hand cream on the basis of glycerol and urea to human skin in projection of a finger joint allows one to improve contrast of finger optical transillumination images during 5-30 min of OCA application. In particular, it was found that glycerol application to the human skin during 60 min caused a decrease of contrast in 1.4 folds for 670 nm and increase of contrast in 1.5 and 1.7 folds for 820 nm and 904 nm, respectively. At the same time, hand cream application to the human skin during 60 min caused a decrease of contrast in 1.1 folds for 670 nm and increase of contrast in 1.3 and 1.1 folds for 820 nm and 904 nm, respectively. The results have shown that glycerol and hand cream with 5% urea allow for obtaining of more distinct NIR images of finger joints in optical transillumination mode. Probably, obtained data can be used for development of optical diagnostic methods of rheumatoid arthritis.

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REFERENCE

[1] Majithia, V., Geraci, S. A., "Rheumatoid arthritis: diagnosis and management," Am. J. Med., 120(11), 936–9. doi:10.1016/j.amjmed.2007.04.005. PMID 17976416 (2007).

[2] Zabarylo, U., Minet, O., "Pseudo colour visualization of fused multispectral laser scattering images for optical diagnosis of rheumatoid arthritis," Laser Phys. Lett., 7(1), 73-77 (2010).

[3] Tuchin, V. V., [Tissue Optics: Light Scattering Methods and Instruments for Medical Diagnosis], second edition, PM 166, SPIE Press, Bellingham, WA, USA (2007).

[4] Tuchin, V. V., [Handbook of Optical Biomedical Diagnostics], PM107 SPIE Press, Bellingham, WA, USA (2002).

[5] Vo-Dinh, T., [Biomedical Photonics Handbook], CRC Press, Boca Raton, FL, USA (2003).

[6] Barozzino, T., Sgro, M., "Transillumination of the neonatal skull: seeing the light," Canadian Medical Association Journal, 167(11), 1271-2, (2002).

[7] Tuchin, V. V., [Optical clearing of tissues and blood], Bellingham, WA, SPIE Press, PM 154, 254 (2006).

[8] Genina, E. A., Bashkatov, A. N., Tuchin, V. V., "Tissue optical immersion clearing," Expert Review of Medical Devices, 7(6), 825-842 (2010).

[9] Zhong, H. Q., Guo, Z. Y., Wei, H. J., Zeng, C. C., Xiong, H. L., He, Y. H., Liu, S. H., "Quantification of glycerol diffusion in human normal and cancer breast tissues in vitro with optical coherence tomography," Laser Phys. Lett., 7, 315–320 (2010).

[10] Wen, X., Mao, X., Han, Z., Tuchin, V. V., Zhu, D., "In vivo skin optical clearing by glycerol solutions: mechanism," J. Biophotonics, 3(1-2), 44-52, (2010).

[11] Genina, E. A., Yaroslavsky, I., Altshuler, G. B., Bashkatov, A. N., Korobko, A. A., Zubkova, E. A., Tuchin, V. V., "Optical clearing of human skin: comparative study of permeability and dehydration of intact and photothermally perforated skin," J. Biomed. Opt., 13(2), 021102 (1-8), (2008).

[12] Schaefer, H., Redelmeier, T. E., [Skin Barrier], Karger, Basel (1996).

[13] Williams, A. C., Barry, B. W., "Urea analogues in propylene glycol as penetration enhancers in human skin," *Int. Pharm*. **56**(1), 43-50 (1989).

[14] Kolesnikova K.N., Galkina E.M., Karakaeva A.V., Utz S.R., Kolesnikova E.A., Tuchin V.V. In vivo clinical study of human skin dehydration at topical application of biocompatible hyperosmotic agents// Saratov Fall Meeting (SFM'13) XVII International School for Young Scientists and Students on Optics, Laser Physics & Biophotonics, Saratov, Russia, September 24-27, 2013.