Heat profiles of laser-irradiated nails

Uwe Paasch
Pietro Nenoff
Anna-Theresa Seitz
Justinus A. Wagner
Michael Kendler
Jan C. Simon
Sonja Grunewald
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Abstract. Onychomycosis is a worldwide problem with no tendency for self-healing, and existing systemic treatments achieve disease-free nails in only 35 to 76% of cases. Recently, treatment of nail fungus with a near-infrared laser has been introduced. It is assumed that fungal eradication is mediated by local heat. To investigate if laser treatment has the potential to eradicate fungal hyphae and arthrospores, laser heat application and propagation needs to be studied in detail. This study aimed to measure nail temperatures using real-time videothermography during laser irradiation. Treatment was performed using 808- and 980-nm linear scanning diode lasers developed for hair removal, enabling contact-free homogeneous irradiation of a human nail plate in one pass. Average and peak temperatures increased pass by pass, while the laser beam moved along the nail plate. The achieved mean peak temperatures (808 nm: 74.1 to 112.4°C, 980 nm: 45.8 to 53.5°C), as well as the elevation of average temperatures (808 nm: 29.5 to 38.2°C, 980 nm: 27.1 to 32.6°C) were associated with pain that was equivalent to that of hair removal procedures and was not significantly different for various wavelengths. The linear scanning laser devices provide the benefits of contact-free homogeneous heating of the human nail while ensuring adequate temperature rises. © 2014 Society of Photo-Optical Instrumentation Engineers (SPIE)

Keywords: laser; fungi; thermography; temperature; nails.

1 Introduction

Dermatophytosis is found in ~20 to 25% of the world’s population. An estimated 2 to 13% of the population suffers from onychomycosis (OM), which is the most common nail disease worldwide and is responsible for approximately half of all nail abnormalities. This condition has a huge impact on the quality of life. To treat the dermatophytes T. rubrum and T. interdigitale (formerly T. mentagrophytes) that are the main causative agents of OM, near-infrared lasers have been introduced because standard systemic terbinafine administration achieves disease-free nails in only ~35 to 76% of cases. In addition, the relapse rates are up to 22.3% within 3 years after completion of the systemic treatment.

Previously, CO2 lasers were found to be effective but unpredictable in terms of efficacy and side effects. Therefore, longer pulsed nonablative near-infrared lasers were thought to have a much better side-effect profile while maintaining their efficacy. Due to their absorption characteristics, potential targets are both water and melanin. This absorption is of interest because T. rubrum, the most common causative agent of OM, expresses a pigment called xanthomegnin that provides a typical color in agar-based culture systems and in nails.

Earlier studies located the pigments into the outer microconidia walls of T. interdigitale. Approximately 0.2% of the wall compounds reflect pigment. It is assumed that the fungal eradication effect is mediated by the heat absorption of water and/or melanin, although heat-resistant (up to 80°C) strains of fungi have been detected recently (Table 1). With lower wavelengths, the absorption of melanin increases, whereas that of water decreases. Overall, a nail does have a water content of 9 to 35% and arthrospores are protected by proteins. However, our in vitro study on the heating effects of common dermatological lasers demonstrated that hair removal lasers operating at 808, 980, or 1064 nm are able to heat liquid pathogens in liquid cultures efficiently if certain parameters are adopted. This finding is of practical importance because lasers using wavelengths of ~800 nm are widely used for hair removal. Moreover, lasers operating at a 1064-nm wavelength are frequently used for vascular treatments and skin rejuvenation in addition to hair removal, and therefore, all of these laser systems have been proven safe for use on human skin if precautions are taken. Finally, the 1064-nm systems are most often Food and drug administration (FDA)-approved for the “temporary increase of clear nails in patients with onychomycosis.” However, the reported clearance rates vary substantially, from 50 to ~100%. In line with this, the pathogen eradication effects observed in vitro were less impressive. To date, many systems that can operate with diverse parameter settings are available, making clinical comparisons difficult.

This situation reflects the lack of knowledge of a highly interesting clinical laser application. Assuming that heat is the underlying mechanism, the application and propagation of heat via lasers needs to be studied. The peak and average temperatures should be investigated to answer the question of whether the proposed laser treatment regimens have the potential to eradicate the fungi and spores within the entire nail plate. Because spores are known to survive at 60 to 80°C, the laser must be able to heat the entire area to this threshold value. However, heat generates pain. Pain is inflicted by...
the current OM laser treatments, and this physiological reaction determines the clinical endpoint of treatment. Therefore, temperature profiles for individual laser systems are of interest to define safe and effective heating regimens for larger and smaller nails that ensure the lowest pain intensity. Finally, homogenous heat distribution is highly desirable to achieve complete pathogen clearance.

To address these issues, this study aimed to measure nail temperatures during laser irradiation (1) to estimate the peak temperatures using two wavelengths, (2) to establish temperature profiles for all of the toes immediately before and after laser irradiation during consecutive treatment passes, (3) to analyze the heat propagation during laser treatment, and (4) to investigate histological changes in nail explants. These investigations will help to rank the value of the investigated wavelengths for their suitability in OM laser treatment, to define concepts for application, and to analyze the potential risk of insufficient treatment due to inhomogeneous irradiation. To address these questions, an advanced real-time videothermography system was used. Additionally, nail explants were subjected to histological investigation.

2 Materials and Methods

The objective of this study was to define the ability of 808- and 980-nm linear scanning lasers, using proven safe and effective parameter settings established for hair removal procedures, to deliver heat to nails on human feet \textit{in vivo} to treat OM. The patients were selected after informed consent was given to also have a thermographic (EasyIR-9™, using software IRBIS 3plus, InfraTec GmbH, Dresden, Germany) video record made during the routine treatment procedure using CE certified devices. To compare temperatures additionally a contact-free temperature measurement (Voltcraft IR-1000L, Germany) was performed in another group of patients treated with either 980-nm linear scanning laser or a long pulsed 1064-nm Nd:YAG laser with a cooled contact hand piece.

### 2.1 Pain Evaluation

Because the method used is based on heat application to nails to eradicate the pathogens that cause OM, pain determined the clinical endpoint of the treatments performed in earlier studies. Pain was quantified using a visual analogue scale (1 to 10). Patients were asked to report the highest pain score during each treatment per foot.

### 2.2 Thermography Measurements

Thermography was performed by using a device for measuring the power of incident electromagnetic radiation due to the heating of a given structure with a temperature-dependent electrical resistance. This method was invented by the American astronomer Samuel Pierpont Langley in 1878.

The thermography system (InfraTec mobileIR E9, InfraTec, Germany) used was a bolometric camera equipped with a 25-mm lens field of view (FOV) (22 x 16)/instantaneous FOV 1.0 mmrad and an uncooled microbolometric focal plane array detector with a spectral range of 8 to 14 \mu\text{m}. The measurement accuracy was given as \pm2 K for 0 to 100°C, and \pm2% for <0 and >100°C at a temperature measuring range of \pm20 to 250°C. The temperature resolution at 30°C was determined to be better than 0.06 K (thermal sensitivity). The thermograms had an image format of 384 x 288 pixels at an IR frame rate of 50 Hz. Real-time video recording was performed in all of the treatment sessions. The Irbis3Plus software (InfraTec) was further used for processing the primary images. The video streams were uploaded and examined for quality control. Then, the video streams were analyzed manually by frame-by-frame analysis to note the temperatures of interest by setting a continuously adjusted region of interest for calculation of the following: (1) peak temperatures of all of the toes during laser irradiation in all of the passes, (2) average nail temperatures of all of the toes immediately before and after laser irradiation during all of the passes, and (3) qualitative analysis of heat propagation during the laser treatments.

### 2.3 Temperature Measurements

Foot nails of 11 patients were evaluated using an infrared thermometer (Voltcraft IR-1000L, –50.0 to 1000.0°C, Germany) in a fixed position at 13 cm distance from digitus I of both feet to ensure measuring at the whole nail plate. Measurements were taken before intervention (t0), immediately after the last laser pass to measure the temperature maximum (Temp. max), and 30 s postintervention (Temp. post).

### 2.4 Laser Treatment

Laser treatment was performed using two different systems: an 808-nm linear scanning diode laser (Alma Lasers, formerly Quantel-Derma and WaveLight Aesthetic, Erlangen, Germany) and a 980-nm linear scanning diode laser (Alma Lasers, formerly Quantel-Derma and WaveLight Aesthetic). Both systems are routinely used for hair removal. Both systems are therefore tested to ensure that they would be safe and efficient in clinical routine treatments using a fluence of 30 J/cm², with a pulse duration of 12 ms and a spot size of 12 x 12 mm.²

The laser beam itself is made of a rectangular array of diodes forming a spot of 1 x 12 mm. Using a mirror system, this rectangular spot is moved linearly to cover an area of 12 x 50 mm. Scattering of the light along the 10-mm side of the rectangular...
spot allows a deep penetration in one dimension. At the 1-mm side, the scattering is also present within the second dimension since the spot is moved continuously over the nail. Each area is therefore preheated by scattered photons, and immediately after this, the full beam is heating up the whole area.

The parameter settings used were the following: 808 and 980 nm: fluence of 30 J/cm², pulse duration of 12 ms, spot size of 12 × 12 mm, five (808 nm) or three (980 nm) passes for digits I to V. A fixed number of passes applied was chosen based on the in vitro temperature profiling of earlier studies. The patients were asked to allow an extra pass from the standard treatment in case they had no clear feeling of pain. The treatment was performed by starting pass one at digitus one on a given foot. Then, the laser was moved to the next toe, allowing a cooling period for the recently treated one. After all five toes had been treated, the second pass was begun at toe one. In case of a severe pain sensation, extra time for cooling was given until the patient felt comfortable to continue.

For comparison of temperature measurement results using video thermography and conventional infrared thermometer, a 980-nm linear scanning diode laser (Alma Lasers, formerly Quantel-Derma and Wavelight) and a long pulsed 1064-nm Nd:YAG-laser (Alma Lasers, formerly Quantel-Derma and Wavelight) with a cooled hand piece operated in nail contact were in use. The parameter settings used were the following: 980 nm: fluence of 30 J/cm², pulse duration of 12 ms, spot size of 12 × 12 mm, three passes for digits I to V and 1064 nm: fluence of 70 J/cm², pulse duration of 40 ms, spot size 5 mm, three passes for digits I to V. The number of passes applied was chosen based on the in vitro temperature profiling of earlier studies. The treatment with the 980-nm system was performed as described above, while the 1064-nm treatment was performed by starting pass one at digitus one on a given foot. The whole nail plate was covered with 30% overlap three times having a 5- to 10-s break in between the treatments. Then, the laser was moved to the next toe. In case of a severe pain sensation, extra time for cooling was given until the patient felt comfortable to continue.

### 2.5 Histological Analysis of Laser–Nail Interaction

Basic histological investigation was performed in the human nail explant after six shots with the linear scanning 808-nm diode laser (fluence: 30 J/cm²; pulse duration: 12 ms). An additional nail with mycobacteriologically proven infection was subjected to histology to visualize growth pattern of fungi within the nail plate. The specimens were decalcified and then subjected to buffered 4% formalin for 24 h for fixation. Tissue blocks were embedded in paraffin, cut into 5- to 8-μm slices, and stained with hematoxylin and eosin [H&E and periodic acid Schiff (PAS)] according to standard procedures. Slides were evaluated under a calibrated microscope (BX41, Olympus Germany, Hamburg, Germany) equipped with a digital camera (DP70, Olympus Germany). Dimensions were measured using calibrated CellIF software (Olympus Germany).

### 2.6 Statistics

The statistical analysis of the thermography data was performed using Statistica 8.0 software for Windows (StatSoft Inc., OK). The normality of the distribution was investigated using the Shapiro-Wilkes test. A Mann-Whitney U test was performed to investigate the differences between the groups. Both of the tests were two-tailed, and significance was indicated by $p < 0.05$.

### 3 Results

In total, 187 toes of 11 patients (nine males, two females, all Caucasian, Fitzpatrick skin types I-II, age 61.7 ± 14.2 years) were treated for toe nail fungus confirmed by mycology using a linear scanning diode laser emitting at 808 nm ($n = 125$) or 980 nm ($n = 62$). During the treatment, real-time thermographic monitoring was performed at a frame rate of 50 frames per second (fps). A total of 42.268 (1.083 × 374) video frames were subjected to analysis using Ibris3Plus software.

Overall, the treatment procedures were well tolerated. However, in selected cases, the development of a single subungual hematoma was noted as a side effect separate from the ubiquity of pain.

#### 3.1 Pain Evaluation

Pain, quantified using a visual analog scale, was reported as 6.2 ± 2.2. There was no significant difference ($p > 0.05$) with regard to the application of either the 808-nm (6.1 ± 2.2) or the 980-nm (6.4 ± 2.3) laser.

#### 3.2 Thermographic Measurements

Thermographic video recording was performed in such a way that the linear scan of the laser beam could be followed over time and over the total area of each toe. In case of incomplete visibility (time- or area-wise), the data were not subjected to evaluation. The larger the nail plate was, the easier it was to perform thermographic recording.

#### 3.3 Peak Temperatures

In general, the peak temperatures measured during the movement of the laser beam along the nail plates increased pass-by-pass, starting at a mean of 74.1°C and reaching a mean of 112.4°C after five consecutive passes using the 808-nm linear scanning laser (Fig. 1). Between the passes, while the remaining toes were being treated, the temperatures decreased substantially (Table 2). Despite this decrease, the absolute peak temperatures measured ranged from 260 to 290°C starting with the very first treatment pass. The relatively high SD can most likely be attributed to the fact that at 50 fps, the recording rate of the thermographic system is relatively slow compared to the pulse durations of 12 ms. As a consequence, the increase in the mean peak temperatures did not reach the level of significance. With regard to the different size of the nails, plotting the peak temperature profiles toe-wise showed higher peak nail plate temperatures post first to fifth pass of the laser intervention in digitus I compared to all of the other toes ($p < 0.05$).

In comparison, the 980-nm treatment showed the same trend of stepwise increasing temperatures over four passes, although the trend started at 45.8°C, reached the peak temperature after the third pass (53.5°C), and ended at 42.6°C after the fourth pass. The temperatures reached using the two laser systems were significantly different at each pass. The peak temperatures reached 161.5°C after the third pass. Digitus I showed a significantly higher peak temperature after the second pass compared to all of the other toes. Comparing the two laser systems...
pass-by-pass revealed that the 808-nm system always resulted in significantly higher peak temperatures on the nail surface.

### 3.4 Average Temperature Profiles

The average temperature measured immediately before laser treatment within a continuously adjusted region of interest increased significantly ($p < 0.01$) stepwise from pass to pass using the 808-nm linear scanning diode laser, increasing from 29.5°C (prepass 1) to 38.2°C (prepass 5). The average temperatures measured immediately after a laser pass were higher and increased stepwise from pass to pass (38.4°C postpass 1 and 53.8°C postpass 6). With regard to the different sizes of the nails, plotting the temperature profiles toe-wise showed higher average nail plate temperatures after each pass of laser treatment in digitus I compared to all of the other toes.

The laser energy emitted by the 980-nm system also resulted in a stepwise significant ($p < 0.01$) elevation of the average temperatures measured before laser irradiation increasing from 27.1 to 32.6°C. Immediately after each laser irradiation, the nail temperature was slightly higher than the mean value (31.0 to 35.6°C). However, the maximum average temperatures reached 57.3°C. The temperature profiles plotted toe-wise showed slightly higher average nail plate temperatures after each pass of laser irradiation for digitus I compared to all of the other toes.

The average temperature elevation per pass of laser irradiation did not differ significantly between the laser systems for the first three passes. As early as pass 4, no significant increase in temperature was detected for the 980-nm system ($p < 0.01$). The same trend was demonstrated for the 808-nm system beginning at pass 6, whereas the temperature elevation was significantly lower at pass 6 than at pass 5 ($p < 0.01$). The cooling rates were always lower during laser pass 1 to 5, whereas the opposite was true for the last pass when the 808-nm system was used. The highest cooling rate was visible between passes 2 and 3 in the 980-nm group (Table 2).

### 3.5 Heat Distribution

In general, the linear scanning laser devices with a spot size of $12 \times 12$ mm were easy to handle in terms of the nail treatments performed in this study and clearly had the advantage of allowing a contact-free and very rapid procedure (Fig. 2). Real-time evaluation of the thermal effects in >40 video streams revealed that exact positioning of the laser is crucial to achieve stepwise homogeneous heating of the nail plates. If placed correctly, uniform heating was observed as long as the nail plate was free of rough areas. With regard to the wavelength, there was some delay in lateral heat diffusion within the toe correlated with the higher wavelength. Although the result was not statistically significant, the 980-nm system was rated as more painful, resulting in a lower number of passes applied.

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Temperature reduction (mean values) between passes of laser irradiation.</th>
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<tbody>
<tr>
<td></td>
<td>808 nm</td>
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<td></td>
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<td>125</td>
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Fig. 2 Frames of interest from a videothermographic recording of six passes of an 808-nm [right foot, (a) to (f)] and four passes [left foot, (g) to (j)] of a 980-nm linear scanning laser using a spot size of 12 x 12 mm.
3.6 Alternative Temperature Measurements

Nail temperature was measured after laser irradiation (Digitus I foot left, 1064 nm, 70 J/cm², 40 ms, 5 mm spot, three passes having a 5- to 10-s break in between the treatments, ultrasound gel coupling, contact cooling, 30% overlap; Digitus I foot right, 980 nm, 30 J/cm², 12 ms, 12 × 10 mm spot, three passes having a 5- to 10-s break in between the treatments, no cooling) using an infrared thermometer (Voltcraft IR-1000L, −50.0 to 1000.0 °C) at a fixed distance of 13 cm. Measurements were taken before intervention (t₀), immediately after the last laser irradiation pass (Temp. max), and 30 s post last treatment (Temp. post).

Table 3 Nail temperatures measured after laser irradiation (Digitus I foot left, 1064 nm, 70 J/cm², 40 ms, 5 mm spot, three passes having a 5- to 10-s break in between the treatments, ultrasound gel coupling, contact cooling, 30% overlap; Digitus I foot right, 980 nm, 30 J/cm², 12 ms, 12 × 10 mm spot, three passes having a 5- to 10-s break in between the treatments, no cooling) using an infrared thermometer (Voltcraft IR-1000L, −50.0 to 1000.0 °C) at a fixed distance of 13 cm. Measurements were taken before intervention (t₀), immediately after the last laser irradiation pass (Temp. max), and 30 s post last treatment (Temp. post).

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<th>Temp. T₀</th>
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<th>ΔT max</th>
<th>p T₀ versus T max</th>
<th>Temp. post</th>
<th>ΔT post</th>
<th>p T max versus T post</th>
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<tr>
<td>1064 nm</td>
<td>25.0 ± 2.9</td>
<td>42.5 ± 4.9</td>
<td>17.5 ± 4.7</td>
<td>&lt;0.01</td>
<td>29.8 ± 2.1</td>
<td>4.8 ± 2.6</td>
<td>&lt;0.01</td>
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<tr>
<td>980 nm</td>
<td>25.0 ± 2.9</td>
<td>44.3 ± 7.0</td>
<td>19.4 ± 6.1</td>
<td>&lt;0.01</td>
<td>30.7 ± 2.7</td>
<td>5.7 ± 2.7</td>
<td>&lt;0.01</td>
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3.7 Histological Analysis of Laser–Nail Interaction

Basic histological investigation of a human nail explant clinically diagnosed with OM revealed rather long septed hyphae with a small diameter of ~1 μm (Fig. 3) located everywhere from the surface down to the nail bed within the nail plate.

The nail explant subjected to six passes of 808-nm laser displayed changes in the nail plate structure. The relatively high temperatures caused disruptions and condensed hypereosinophilic areas (Fig. 4).

4 Discussion

Recently, the option of near-infrared laser treatment of nail fungus has become available. Generally, the 1064-nm systems are FDA-approved for the “temporary increase of clear nails in patients with onychomycosis.” The reported clearance rates vary substantially from 50 to ~100%, although the eradication effects observed in vitro are less convincing. However, recent in vitro studies suggested that systems operating at 808 to 980 nm may be effective if temperatures >50°C are achieved. The assumed unifying mechanism is that the heat is delivered to the nail plate and nail bed due to absorption by water and/or melanin. The wide range of reported clinical efficacy might result from the lack of knowledge of how much heat is generated and propagated throughout the nail and nail bed area. However, it is crucial that certain temperature levels be kept constant over a certain time to ensure secure pathogen eradication and to avoid growth induction.

In general, the fast, contact-free treatment at 808 and 980 nm using the linear scanning laser devices with a 12 × 12 mm spot not only ensured the prevention of pathogen transmission, but also allowed the study of temperature development over time and over the area of the entire nail plate.

Fig. 3 Histological specimen stained with PAS, 100× magnification. Septed hyphae are found as rather long structures up to 100 μm with a diameter of ~1 μm within the whole nail plate.

Fig. 4 Impact of 808-nm diode laser treatment (six passes at a fluence of 30 J/cm² and 12 ms pulse duration) on nail morphology. Disruptions and coagulations of the nail plate (hematoxylin and eosin, 40×) have been observed. The changes of the nail structure do reflect the enormous heat action and may explain that living conditions for pathogens stop do be ideal for further growth.
On examining the peak temperatures achieved using both systems, huge differences between the two wavelengths were noted. In general, we conclude that the temperatures, at least those at the nail surface, were high enough to kill spores when the laser energy was safely administered to a human toe. However, it is still not known how long those temperatures need to be maintained to achieve complete pathogen eradication. While in vitro arthrospores as well as microconidia of T. rubrum and T. interdigitale did not survive heat applications >60 to 80°C for as short as 2 to 10 min, the protection by nail keratin might decrease eradication rates and therefore direct us to apply higher peak temperatures or longer heat applications. Specifically, it seems to be important to avoid sublethal temperatures in order to prevent growth induction and to apply temperatures that do kill heat-resistant strains. Interestingly, the shorter wavelength resulted in consistently higher temperatures, although the patients reported a slightly lower pain level and could tolerate more passes. This phenomenon might be attributed to the fact that the higher wavelength may penetrate deeper. Because this leads to a higher pain level, a lower number of passes can be administered. To what extent this is important to reach subungal fungi needs to be evaluated in clinical studies or by invasive temperature measurement. Also, our approach to measure temperatures by thermography helps to determine nail surface heat, but it fails to tell us how much heat is generated within the nail. On a histological level, changes of the nail structure with typical heat-induced coagulation zones were visible using the 808-nm system. This implies that at least the whole nail plate will be heated up, although the water content of a nail plate is lower than that of skin.21,22 Microscopical effects made by the 1064-nm long pulsed laser are characterized by a dissection of the nail plate from the nail bed, confirming a deeper heat propagation.23 Because to date the 1064-nm systems are most commonly used to clear nails suffering from OM, clinical studies comparing the efficacy of various wavelengths would be of interest.

This study adds knowledge to the field by demonstrating the usefulness of real-time thermographic recording during laser interventions. However, there are important limitations of the specific system used. Due to the very short pulse duration and a rather slow recording rate, data acquisition might have been biased. If possible, high-speed cameras should be utilized in future. The comparison to a conventional standalone infrared thermometer measurement showed most probably an underestimation of temperatures reached. The value of an in-built measurement system should be determined. On top of this not only planar temperature profiles are of interest. Heat propagation to the depth is also of importance. Model calculations might further help to develop advanced laser systems.

5 Conclusion

Recently, a new generation of large-area linear scanning hair removal laser operating at 808 and 980 nm has been introduced and extensively studied with regard to safety and efficacy. On top of this, its suitability to treat common pathogens of OM in vitro has been established. Here, we show for the first time by real-time thermographic video recording a contact-free stepwise homogeneous heating of the human nail, most likely hot enough and acting long enough to eradicate pathogens with high efficacy. However, the latter assumption must be confirmed clinically. Once the concept is proven, this approach might be extended to fungal infections of hair-free areas of the human skin, i.e., the soles and palms, which are the sources of nail infections.

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References


Uwe Paasch received his academic degree (thesis, Dr. med.) at the Leipzig University, Germany, in 1996 followed by PhD thesis (Dr. med. habil., habilitation) in 2001. Since 2008, he serves as professor supervising clinical and experimental andrology, dermatopathology as well as lasers and aesthetics. He has published more than 135 peer-reviewed papers and a series of standard laser text books.

Pietro Nenoff received his degree as medical doctor (thesis) from the Department of Dermatology of the Leipzig University, Germany, followed by PhD thesis (habilitation) in 2000. Since 2002, he is directing his own lab together with the microbiologist Dr. Constanze Krüger. As an associate professor, he lectures in dermatology at the Leipzig University and at the Department of Dermatology, Mbarara University of Science and Health, Uganda, East Africa. Special fields of scientific interests are medical fungi, e.g., dermatophytes, and, modern diagnostic methods.

Anna-Theresa Seitz studied medicine at Carl-Gustav-Carus University Dresden in Germany until 2010. She has conferred a doctorate in August 2010 at the Carl-Gustav-Carus University Dresden, Germany. Currently, she is serving as a junior house officer at the Department of Dermatology, University of Leipzig, while being involved in a number of clinical and experimental studies investigating skin laser treatments.

Justinus A. Wagner studied medicine at Graz University until 2006. Hi is a board certified dermatologist, who received his academic degree (thesis, Dr. med. univ.) at the University Graz, Austria, in 2006. His specific interests are dermatologic surgery and aesthetic dermatology. Besides this, he initiated, performed, and published clinical trials, to improve laser scar treatments. To date, eight peer-reviewed papers are published.

Michael Kendler received his degree as medical doctor at the University of Vienna, Austria. Since 2010, he is serving as a senior physician at the Department of Dermatology of the University of Leipzig, Germany. Special fields of clinical and scientific interests are dermatologic surgery and phlebology.

Jan C. Simon received his academic degree (thesis, Dr. med.) at the Department of Dermatology, Freiburg University Medical Center, Freiburg, in 1988 followed by PhD thesis (habilitation) in 1994. Since 2003, he is the professor and chairman of the Department of Dermatology, Venerology, and Allergology. He has published more than 400 papers.

Sonja Grunewald received her doctorate degree at the Leipzig University in 2003 followed by postdoctoral lecture qualification (habilitation) in 2009. Since 2013, she serves as a senior physician in dermatological surgery as well as in lasers and aesthetics. She has published more than 70 peer-reviewed papers and a series of standard laser text books.