

1540 nm LIBS Investigation of Healthy and Pathological Human Nails

Andrey V. Belikov, Sergey N. Smirnov, Anastasia D. Tavalinskaya*, and Victoria S. Pomazanova

ITMO University, 49 Kronverksky Pr., Saint Petersburg 197101, Russia

*e-mail: ntavalin@gmail.com

Abstract. The paper discusses the possibility of using laser-induced breakdown spectroscopy (LIBS) as a method for the diagnosis of human nail onychomycosis. LIBS spectra obtained with excitation of plasma on the surface of healthy and onychomycotic nails by pulses of Q switched Yb, Er: Glass laser radiation with a wavelength of 1540 nm were compared for the first time. The spectrum of onychomycotic nail contained unique lines additional to characteristic spectral lines of healthy nails. These additional lines disappeared after 90 days of sample storage in air at room temperature 20 ± 3 °C. © 2020 Journal of Biomedical Photonics & Engineering.

Keywords: LIBS; onychomycosis; Yb, Er: Glass laser; nail; diagnostics; spectrum.

Paper #3369 received 18 May 2020; revised manuscript received 19 Jun 2020; accepted for publication 25 Jun 2020; published online 30 Jun 2020. [doi: 10.18287/JBPE20.06.020310](https://doi.org/10.18287/JBPE20.06.020310).

1 Introduction

Laser-induced breakdown spectroscopy (LIBS) is a technology for fast remote chemical analysis based on the excitation of plasma on the surface of a sample by a short laser pulse and the study of its spectral composition. LIBS technology is applicable to many samples, including metals, semiconductors, glasses, biological tissues, and others. The accuracy of LIBS allows one to quantitatively characterize the elemental composition with high spatial and temporal resolution, as well as detect substances at their relatively low concentration – from 2 ppm [1, 2]. The ability to identify chemical elements is limited only by the spectral resolution of the used spectrometer (usually ≈ 0.3 nm). LIBS analysis compares favorably with many other analysis technologies: it is sensitive to elements with a low atomic number, unlike, for example, X-ray Fluorescence analysis, and it is sensitive to trace concentrations of materials, unlike, for example, Prompt Gamma Neutron Activation Analysis [3]. The extremely short time required for sample preparation is also an advantage of LIBS analysis. Of particular interest is the spectral region from 180 to 850 nm, which includes the spectral lines of most of the known elements [4].

Onychomycosis is a widespread fungal disease of the nails. Early rapid diagnosis and treatment of onychomycosis are very important. If treatment is not done on time, the infection can progress penetrating deeper into the nail and destroying it. In addition to

aesthetic discomfort, onychomycosis can be a cause of pain during walking, which limits a person's ability to work. For diabetics, mycoses can provoke the appearance of ulcers or secondary infection by bacteria [5, 6]. When detecting onychomycosis, the remoteness of the analysis performed is very important in order to avoid contact with the affected tissue and to eliminate infection of medical personnel. Traditional diagnosis of onychomycosis requires extraction of the sample and its study in the laboratory, which takes considerable time and does not exclude contact with the affected biological tissue [7]. An optical diagnostic method is also possible. Traditionally, the color of the nail is analyzed [8, 9]. Healthy and onychomycotic nails have different optical properties, as fungi contain unique chromophores. For example, one of these chromophores is a polysaccharide chitin present only in fungal cell walls. Spectral studies are being carried out. In Ref. [10], the absorption spectra of healthy and onychomycotic nails were measured. *Trichophyton rubrum* showed a unique absorption peak at 415 nm. This peak most likely corresponds to mitochondrial cytochrome absorption. However, in medical practice when diagnosis of onychomycosis the spectral methods are not currently widely used. All this necessitates the search for new effective remote sensing methods as one of which LIBS analysis can be considered.

The nail consists of a large number of chemical elements, 26 of which relate to essential and structural

micro- and macroelements (Cl, I, B, V, Fe, K, Ca, Co, Si, S, Li, Mg, Mn, Cu, Mo, Na, Ni, Se, P, Cr, Zn, O, N, C, H, F), the rest are toxic [10]. The 82 emission lines belonging to the atomic and ionic lines of 13 elements – Al, C, Ca, Fe, H, K, Mg, N, Na, O, Si, Sr, and Ti – can be identified in the LIBS spectrum of the nail [11–14]. LIBS analysis does not require sample extraction, as it can be performed *in vivo*. Elemental analysis of nails can be used not only for the diagnosis of nail diseases, but also for detecting an imbalance of elements and the presence of pathological processes in the human body as a whole [14, 15]. In the latter case, an analysis of the intensities ratios of emission lines in the LIBS spectra can serve as an indicator of the level of a pathology. Using the LIBS technology, it is possible to identify various types of fungi and bacteria [16, 17]. For example, the *Candida* LIBS spectrum reveals the elements C, N, H, O, and CN [17]. Thus, depending on the type of pathogen, the spectrum of the onychomycotic nail can probably change.

A difference was found [14] in the LIBS spectra of normal and pathological nails when analyzing the intensities of calcium, sodium, and potassium lines. To excite the plasma, the radiation of a frequency-doubled Nd: YAG laser ($\lambda = 532$ nm) was used, which is the main drawback, because does not exclude damage to the eyes of the doctor and patient during LIBS excitation at this wavelength. The radiation of a Yb, Er: Glass laser ($\lambda = 1540$ nm), in contrast to the radiation of frequency-doubled Nd: YAG laser ($\lambda = 532$ nm), belongs to the eye-safe spectral range, since the radiation of this laser is absorbed by hydrated tissues of the anterior segment of the eye and does not reach the more sensitive retina [18–20]. In this regard, 1540 nm LIBS analysis can be an effective, remote and safe method for diagnosing a wide range of diseases, including the diagnosis of onychomycosis.

The main goal of this work was to study the possibility of using the radiation of a compact Q-switched Yb, Er: Glass laser ($\lambda = 1540$ nm) for LIBS detection of the nail region affected by onychomycosis.

2 Materials and Methods

The *in vitro* study involved one sample of healthy fingernail and one sample of onychomycotic human fingernail obtained from the same volunteer in order to avoid the influence of physiological, genetic and other factors when comparing the spectra obtained from a healthy and diseased nail. The 30 healthy human fingernail samples obtained from 5 other volunteers were also investigated. After separation from the nail, samples were stored in a dark place at a temperature of 20 ± 3 °C for no more than 24 h for the spectral measurements just after extraction and 90 days for the measurements after longer storage. Photos of the healthy fingernail and the onychomycotic fingernail of the same volunteer before their separation from the nail are shown in Fig. 1.

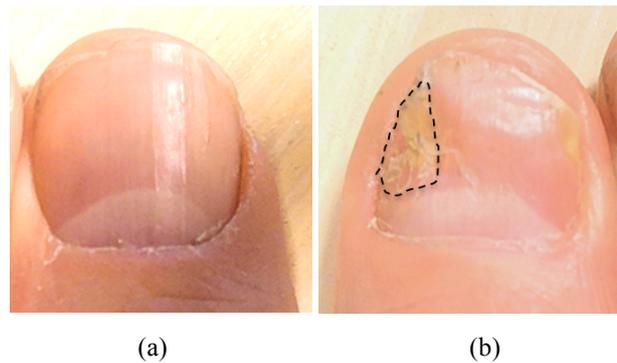


Fig. 1 Photos of healthy (a) and onychomycotic (b) fingernail of the same volunteer (dashed line marks the border of onychomycotic area)

During the LIBS investigation, the laser beam was focused on the dorsal layer of the nail plate. The change of the nail plate color, its delamination and crumbling are typical signs of onychomycosis. The area of the nail affected by onychomycosis usually becomes white or yellowish [8, 9]. Thus, the color serves as an indicator of the presence of infection in the nail. For present LIBS analysis, a sample of a nail affected by onychomycosis was taken from a region having a yellow color (this region is indicated by a dashed line in Fig. 1b).

The scheme of experimental setup for LIBS analysis of nail samples is shown in Fig. 2a.

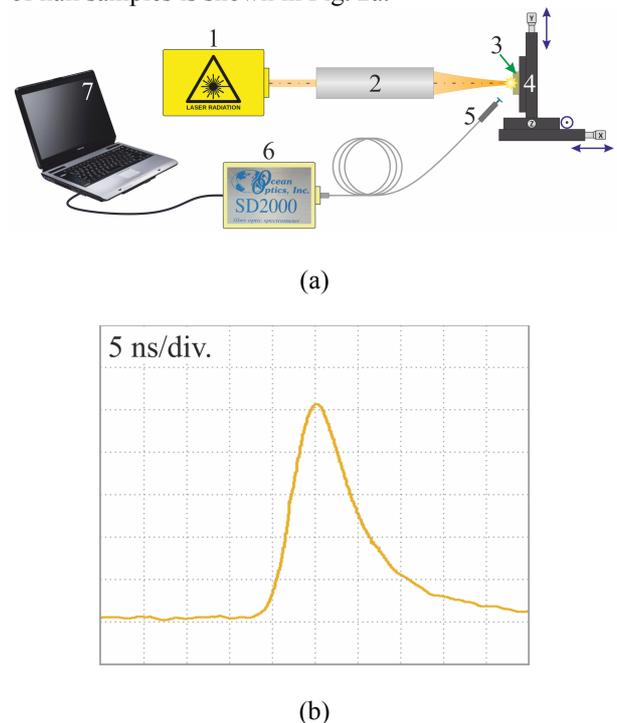


Fig. 2 Scheme of the experimental setup (a): 1 – Yb, Er: Glass-laser, 2 – focusing system, 3 – nail sample, 4 – sample positioning system, 5 – receiving fiber of spectrometer, 6 – fiber-optic spectrometer, 7 – computer; (b) oscillogram of Yb, Er: Glass-laser pulse.

Plasma was excited on the surface of human nail plate samples by pulses of passively Q-switched Yb, Er: Glass laser (Nela Ltd., Russia) with a wavelength of 1540 nm. The laser pulse energy was $E = 2.40 \pm 0.05$ mJ, the pulse duration was $\tau = 8$ ns (FWHM) (Fig. 2b). The radiation intensity on the surface of the nail plate reached 1.4×10^{10} W/cm² and was close to the radiation intensity of neodymium lasers commonly used for LIBS analysis [15]. The emission spectra of laser-induced plasma were recorded using a “USB-2000” (OceanOptics, Inc., USA) fiber-optic spectrometer with spectral resolution of 1.5 nm. This model has the ability to external trigger by a TTL signal. The start of the spectrometer was synchronized with a laser pulse exciting the plasma. The laser pulse was detected by a photodiode placed in the laser housing, which triggered the pulse generator. The signal from the pulse generator with duration of 2 μ s and a delay of 10 μ s relative to the laser pulse was used as an external hardware trigger. The “SpectraSuite” (OceanOptics, Inc., USA) software package was used to control the spectrometer. The integration time 10 ms was set in this program and the spectral signal was recorded within this period after laser exposure.

Radiation from the plasma plume was captured by the receiving fiber of a spectrometer with a core diameter of 125 μ m. The input end of the receiving fiber was located at ~ 10 mm from the sample surface at an angle of $\sim 45^\circ$ to its normal line. A thin (~ 200 μ m) silica glass plate was placed in front of the input fiber end preventing it from being contaminated by particles of an erosion plume leaving the nail surface. Spectra collection was performed by averaging the spectra obtained as a result of exposure to ten consecutive laser pulses.

The emission spectra of laser-induced plasma excited on the surface of healthy nail samples were recorded just after extraction. The spectra of the nail sample affected by onychomycosis were recorded both just after extraction and after 90 days of longer storage. The intensity of the obtained spectra was normalized (normalized intensity) to the line of air N+O at a wavelength of 500 nm, which was chosen as the reference, since air is always present during the experiment, in addition, it does not change significantly its composition under laboratory conditions.

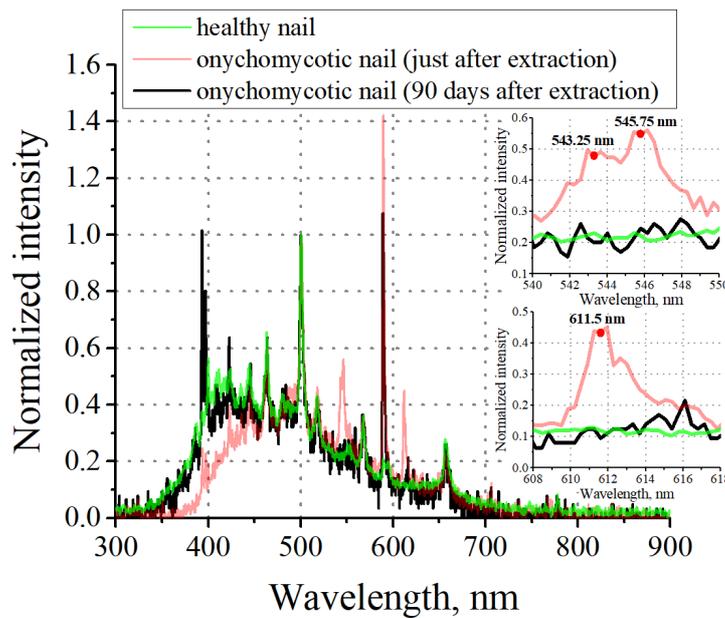
3 Results and Discussion

The emission spectra of the plasma induced by the radiation Q-switched Yb, Er: Glass laser with a wavelength of 1540 nm on the surface of healthy nail samples just after extraction, as well as the nail affected by onychomycosis, just after extraction and after 90 days of longer storage are presented in Fig. 3a. The LIBS spectrum of a healthy nail shown in Fig. 3a was obtained by averaging the spectra of healthy nails of all volunteers studied just after extraction. It should be noted that the LIBS spectra of healthy nails of all volunteers did not have any significant differences between themselves.

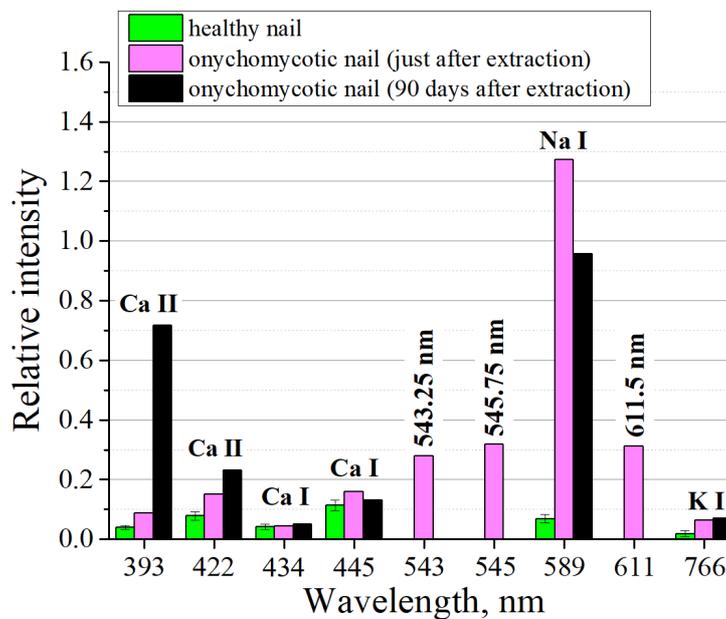
It can be seen that the LIBS spectra of human nail samples contain a continuous component of the emission of plasma electrons, as well as lines of individual chemical elements against its background. The presence of a continuous component is due to the fact that the spectrum was recorded immediately after laser irradiation. The contribution of the continuous component to the recorded spectrum may be reduced by introducing a time delay between the moment the laser exposure begins and the moment the spectrum is recorded [21]. This is relevant in the case when the continuous component of the spectrum does not allow resolving the lines of elements. In our case, the lines of the elements were well resolved against the background of the continuous spectrum.

It can be seen that the main elements in the LIBS spectra of healthy nails are Ca II (393 nm), Ca I (423 nm, 434 nm, 445 nm), Na (589 nm) and K (766 nm). The intensities of the lines of these elements in the spectra of healthy and onychomycotic nails differ from each other. We have not observed the shift of these spectral lines in transition from healthy to onychomycotic nail. In Ref. [22], the LIBS spectra of nails of people of different age and sex were studied. The results show that the line intensities of Ca, Al, Ti, P and K elements are higher among women, and Mg and Na – for men. It was found that the intensity of the lines of Ca and K decreases with increasing age for both sexes. As in our study, in Ref. [22] authors no shift of spectral lines is observed.

In addition, the unique lines at 543.25, 545.75, and 611.5 nm (see Fig. 3b) may be found in the spectrum of just extracted onychomycotic nail, while the intensity of these lines in the LIBS spectrum of just extracted healthy nails is at the level of the continuous component of the emission of plasma electrons. Taking into account the resolution of the “USB-2000” (OceanOptics, Inc., USA) fiber-optic spectrometer, these three lines can be correlated with the following elements: 543.25 ± 0.75 nm – S, Mg, Fe, Cu, Mn, I, V, **Br**, P; 545.75 ± 0.75 nm – S, Mn, Fe, Cu, N, Mg, Ca, Cl, **As**, I, V, P, and 611.5 ± 0.75 nm – S, Mn, Cu, Cl, S, Zn, Fe, Cu, N, Al, Ca, **Br**, **Cd**, **As**, V [23]. Elements missing in a healthy nail are highlighted in bold. Fungi can collect elements that are nutrients for them, as it was shown for *Candida* [24]. These elements include S, Cu, Zn, ammonium, Al, Ca, Mg, Fe, Br, Cd, As, I, Mn, Se, V, sulfates, phosphates, and chlorides in small amounts [25–27]. In Ref. [28], samples of healthy and onychomycotic toenails were analyzed by using an inductively coupled plasma-optical emission spectrophotometer to detect the difference in concentrations of such trace elements as Cr, Cu, Fe, Mg, Mn, Se, and Zn. The study showed that Mg, Mn, and Zn levels were significantly changed in toenails with onychomycosis compared to healthy controls, but the difference in Mg levels was the only element independent of age, sex, and smoking [28]. Thus, the unique lines found in our study in LIBS spectrum of onychomycotic nails are difficult to final identify. This



(a)



(b)

Fig. 3 Typical 1540 nm LIBS spectra of samples of a healthy nail, a nail affected by onychomycosis just after extraction and the same nail after 90 days of longer storage (a), and a histogram of the relative intensity of the spectral lines of the elements containing in these samples (b).

identification may be performed in the future using a higher-resolution spectrometer and more amount of onychomycotic nail samples.

Fig. 3b shows a histogram of the relative intensity of the spectral lines of the elements contained in the samples of a healthy nail, a just extracted nail affected by onychomycosis and the same nail after 90 days of longer storage. Relative intensity was determined by subtracting the value of normalized intensity of the continuous component of the spectrum at the wavelength of the element's line from the value of the

normalized intensity of this line. There are no standard deviation bars in Fig. 3b for onychomycotic nail because only one sample of onychomycotic nail was used in the study, and based on the results of measuring the spectrum from one sample, it is impossible to conduct a standard statistical study on the effect of possible differences between samples on the measurement results. In the experiment with this one sample, we did not register statistical differences in the LIBS spectra obtained in different regions of the sample surface.

LIBS spectra obtained in different regions of the sample surface.

It is seen that for the just extracted nail affected by onychomycosis relative intensity of the Ca II (393 nm), Ca I (423 nm), Ca I (445 nm) lines are 2.2, 1.9 and 1.4 times higher than the relative intensity of these lines in LIBS spectrum of healthy nails, respectively. The intensity of the Ca I line (434 nm) in the spectrum of a healthy nail is equal to the intensity of this line in the spectrum of the onychomycotic nail just after extraction. The intensity of the Na (589 nm) and K (766 nm) lines in the spectrum of the just extracted onychomycotic nail is higher than the intensity of this line in the LIBS spectrum of a just extracted healthy nail by 18.6 times and 3.3 times, respectively. The unique lines in the spectrum of onychomycotic nail just after extraction have the same relative intensity equal to 0.31 a.u. The presence of the unique lines in the spectrum may indicate the presence of the vital products of the fungus in the sample.

After 90 days of longer storage the unique lines were not detected in the LIBS spectrum of the sample affected by onychomycosis. This may be due to the death of the fungus as a result of sample storage, since in the extracted nail plate it does not receive enough nutrients to support vital activity. The intensity of Ca I lines at wavelengths of 423 nm and 434 nm increased as a result of longer storage, but not much – by 1.54 and 1.16 times, respectively, and at a wavelength of 445 nm – decreased by 1.2 times. The intensity of K line (766 nm) remained practically unchanged. The intensity of the Na line (589 nm) decreased by 1.3 times as a result of longer storage. It is also worth noting that the Ca II line's intensity (393 nm) increased by 8 times

in the spectrum of the onychomycotic sample after 90 days of longer storage, which may be due to dehydration of the nail plate.

4 Conclusion

Pilot LIBS detection of onychomycosis with excitation of plasma on the surface of nail with eye-safe radiation of compact Q-switched Yb, Er: Glass laser with a wavelength of 1540 nm was performed. The difference was found in the LIBS spectra of a healthy and onychomycosis-affected nail plate *in vitro*. Based on the data obtained, an original method and apparatus for the early rapid diagnosis of fungal diseases of the nail may be developed. During the transition to *in vivo* studies, the main limitations may be associated with the contribution of the nail surface – its relief, curvature and purity (the presence of foreign substances, including residues of solvents, drugs, cosmetics, etc.) will affect the measurement result. In addition, it will be possible to encounter the variance in measurement results when scanning the surface of the nail. All these features can influence the result of the LIBS spectrum registration. Under *in vivo* conditions, these features are difficult to control, which will most likely require additional research and improvement of the measurement technique.

Disclosures

All authors declare that there is no conflict of interests in this paper.

References

1. R. C. Wiens, S. K. Sharma, J. Thompson, A. Misra, and P. G. Lucey, “[Joint analyses by laser-induced breakdown spectroscopy \(LIBS\) and Raman spectroscopy at stand-off distances](#),” *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy* 61(10), 2324–2334 (2005).
2. J. Thompson, R. C. Wiens, D. A. Cremers, J. Barefield, and C. Wetteland, “The suitability of Laser Induced Breakdown Spectroscopy for determining the compositions of extraterrestrial material,” *Lunar and Planetary Science XXXV* (2004).
3. D. Body, B. L. Chadwick, “[Optimization of the spectral data processing in a LIBS simultaneous elemental analysis system](#),” *Spectrochimica Acta Part B: Atomic Spectroscopy* 56(6), 725–736 (2001).
4. R. C. Wiens, S. K. Sharma, J. R. Thompson, A. K. Misra, and P. G. Lucey, “[Joint analyses by laser-induced breakdown spectroscopy \(LIBS\) and Raman spectroscopy at stand-off distances](#),” *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy* 61(10), 2324–2334 (2005).
5. B. E. Elewski, “[Onychomycosis](#),” *American journal of clinical dermatology* 1(1), 19–26 (2000).
6. P. Rodgers, M. Bassler, “[Treating onychomycosis](#),” *American family physician* 63(4), 663–673 (2001).
7. R. K. Scher, A. Tavakkol, B. Sigurgeirsson, D. G. Armstrong, B. C. Markinson, and B. E. Elewski, “[Onychomycosis: diagnosis and definition of cure](#),” *Journal of the American Academy of Dermatology* 56(6), 939–944 (2007).
8. Yu. V. Sergeev, A. Yu. Sergeev, *Onychomycosis. Fungal infections of the nails*, Geotar-medicine, Moscow (1998). ISBN 5-88816-040-7 [In Russian].
9. C. Carney, A. Tosti, R. C. Daniel, R. Scher, P. A. Rich, J. DeCoster, and B. E. Elewski, “[A new classification system for grading the severity of onychomycosis: Onychomycosis Severity Index](#),” *Archives of dermatology* 147(11), 1277–1282 (2011).

10. A. K. Gupta, K. A. Foley, and S. G. Versteeg, “Lasers for onychomycosis: current status,” *Journal of cutaneous medicine and surgery* 21(2), 114–116 (2017).
11. W. W. Harrison, G. G. Clemena, “Survey analysis of trace elements in human fingernails by spark source mass spectrometry,” *Clinica Chimica Acta* 36(2), 485–492 (1972).
12. M. Garland, J. S. Morris, B. A. Rosner, M. J. Stampfer, V. L. Spate, C. J. Baskett, W. C. Willett, and D. J. Hunter, “Toenail trace element levels as biomarkers: reproducibility over a 6-year period,” *Cancer Epidemiology and Prevention Biomarkers* 2(5), 493–497 (1993).
13. D. E. Vance, W. D. Ehmann, and W. R. Markesbery, “Trace element content in fingernails and hair of a nonindustrialized US control population,” *Biological trace element research* 17(1), 109–121 (1988).
14. M. Bahreini, B. Ashrafkhani, and S. H. Tavassoli, “Discrimination of patients with diabetes mellitus and healthy subjects based on laser-induced breakdown spectroscopy of their fingernails,” *Journal of biomedical optics* 18(10), 107006 (2013).
15. S. Hamzaoui, R. Khleifia, N. Jaïdane, and Z. B. Lakhdar, “Quantitative analysis of pathological nails using laser-induced breakdown spectroscopy (LIBS) technique,” *Lasers in medical science* 26(1), 79–83 (2011).
16. A. P. Flores, A. K. F. Sanchez, A. Villarreal, F. G. R. Sauz, L. P. Cabrera, and T. F. Reyes, “The potential of compact LIBS system with multi-pulse Nd:YAG laser for bacteria identification,” *Journal of Biomedical Science and Engineering* 8(03), 207 (2015).
17. S. Manzoor, L. Ugena, J. Tornero-López, H. Martín, M. Molina, J. J. Camacho, and J. O. Cáceres, “Laser induced breakdown spectroscopy for the discrimination of *Candida* strains,” *Talanta* 155, 101–106 (2016).
18. M. J. Myers, J. D. Myers, B. Guo, C. Yang, C. R. Hardy, J. A. Myers, A. G. Myers, and S. M. Christian, “Non-invasive in-situ detection of malignant skin tissue and other abnormalities using portable LIBS system with fiber spectrometer and eye-safe erbium glass laser,” *Proceeding of SPIE* 6863, 68630W (2008).
19. J. Taboada, J. M. Taboada, D. J. Stolarski, J. J. Zohner, L. J. Chavey, H. M. Hodnett, G. D. Noojin, R. J. Thomas, S. S. Kumru, and C. P. Cain, “100-megawatt power Q-switched Er-glass laser,” *Proceeding of SPIE* 6100, 61000B (2006).
20. D. De Luca, I. Delfino, and M. Lepore, “Laser safety standards and measurements of hazard parameters for medical lasers,” *International Journal of Optics and Applications* 2(6), 80–86 (2012).
21. M. J. Myers, J. D. Myers, J. T. Sarracino, C. R. Hardy, B. Guo, S. M. Christian, J. A. Myers, F. Roth, and A. G. Myers, “LIBS system with compact fiber spectrometer, head mounted spectra display and hand held eye-safe erbium glass laser gun,” *Proceeding of SPIE* 7578, 75782G (2010).
22. H. A. Harun, R. Zainal, and Y. M. Daud, “Analysing human nails composition by using laser induced breakdown spectroscopy,” *Sains Malaysiana* 46(1), 75–82 (2017).
23. NIST Atomic Spectra Database Lines Form.
24. M. Kieliszek, A. M. Kot, A. Bzducha-Wróbel, S. Błażejczak, I. Gientka, and A. Kurcz, “Biotechnological use of *Candida* yeasts in the food industry: a review,” *Fungal Biology Reviews* 31(4), 185–198 (2017).
25. W. A. Mosher, D. H. Saunders, L. B. Kingery, and R. J. Williams, “Nutritional requirements of the pathogenic mold *Trichophyton interdigitale*,” *Plant Physiology* 11(4), 795 (1936).
26. A. J. Pirzada, P. Iqbal, W. Shaikh, T. Kazi, and U. Ghani, “Studies on the elemental composition and anti fungal activity of medicinal plant *Lippia nodiflora* L against skin mycosis,” *Journal of Pakistan Association of Dermatology* 15(2), 113–118 (2016).
27. G. A. Marzluf, “Regulation of sulfur and nitrogen metabolism in filamentous fungi,” *Annual review of microbiology* 47(1), 31–55 (1993).
28. E. Kilinc, B. Buturak, and F. A. Alkan, “Level of Trace Elements in Serum and Toenail Samples of Patients with Onychocryptosis (ingrown toenail) and Onychomycosis,” *Journal of Trace Elements in Medicine and Biology* 61, 126509 (2020).