Determination of the Zinc Concentration in Human Fingernails Using Laser-Induced Breakdown Spectroscopy

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Abstract

The absolute concentration of Zn in human fingernail clippings was determined ex vivo using 1064 nm laser-induced breakdown spectroscopy and confirmed by speciated isotope dilution mass spectrometry. A nail testing protocol that sampled across the nail (perpendicular to the direction of growth) was developed and validated by scanning electron microscopy energy-dispersive X-ray spectrometry. Using this protocol, a partial least squares (PLS) regression model predicted the Zn concentration in the fingernails of five people to within an average of 7 ppm. The variation in the Zn concentration with depth into the nail determined by laser-induced breakdown spectroscopy was studied and showed no systematic variation for up to 15 subsequent laser pulses in one location. The effects of nail hydration (dehydrated and over-hydrated) and nail surface roughness were investigated to explain an anomalously large scatter observed in the measurements. This scatter was attributed to the layered nature and fibrous structure of the fingernails, which resulted in non-uniform ablation as determined by scanning electron microscopy. This work demonstrates that a protocol consisting of low pulse energy (<10 mJ) 1064 nm laser pulses incident on human fingernail clippings in an Ar environment can produce quantifiable Zn emission in the laser-induced plasma and that the measured Zn intensity can be used to accurately predict the Zn concentration in human fingernails.

Introduction

Zinc is one of the most important nutritionally obtained elements in the human body. It has an important structural role in proteins and figures prominently in many important signaling pathways.[1] Zn is a component of over 3000 different mammalian proteins and is found in every organelle of every mammalian cell type, as well as in fluids such as semen, pancreatic juice, saliva, and tears.[2,3] Zn deficiency is a leading cause of death among toddlers worldwide[4,5] and the remediation of Zn deficiency by detection and supplementation has been identified by at least one organization to be the most cost-effective improvement to world health that can be made.[6] Zn is a crucial component in the neurophysiology and pathology of signaling in the brain and the central nervous system.[2,7–9] Evidence indicates that brain Zn is involved in the development of dementia/Alzheimer's disease[10,11] as well as autism,[12] stroke, epilepsy, seizures, and a host of other pathologies.[1]

The diagnosis of Zn deficiency has traditionally been made by drawing 10 mL of venous blood (preferably in the morning after overnight fasting), separating out the serum, and measuring the serum Zn concentration by laboratory instrumental methods such as mass spectrometry or atomic absorption spectrometry.[13,14] These methods are clearly unsuitable for the at-risk populations primarily found in under-served countries and communities. Even though dietary Zn supplements are relatively inexpensive and essentially completely safe (up to 10–20 times the

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minimum daily requirement is routinely used),[15] there is a general reluctance to prescribe or administer Zn supplements without some prior screening to detect deficiencies. The screening of at-risk populations is essential, as indicated in the best practices paper produced in 2008 by the Copenhagen Consensus.[6] Convenient and timely testing to determine the efficacy of supplementation is always desirable.

Serum Zn is known to fluctuate dramatically with circadian rhythms and in response to Zn-rich meals, therefore it is possible that fingernail Zn (like bone Zn)[16,17] could potentially be a superior measure of the longer term nutritional status of Zn. The fingernail concentrations of elements or chemicals such as Se, nicotine, Hg, and ethanol metabolites have been used successfully to determine past oral intakes.[18–21] The concentration of Zn in hair (another keratinous tissue) has also been shown to reflect dietary intake.[22,23] We recently conducted a preliminary study linking fingernail Zn to blood serum in which the Zn measured in the fingernails of 30 elderly residents of a nursing home in Detroit was correlated with an assignment of good Zn nutriture or poor Zn nutriture based on their dietary phytate/Zn ratios obtained from serum.[24] This study also included 17 Pakistani children from a group classified as undernourished by their local clinicians and in this case fingernail Zn was also found to be a good predictor of the children's Zn nutriture.

The concentration of Zn and other trace elements may be quantified in fingernails using traditional analytical techniques such as inductively coupled plasma mass spectrometry[25] or X-ray fluorescence.[26] Each of these methods exhibits insufficiencies for this particular application, including complexity, a lack of portability, the necessity of chemical digestion, the use of ionizing radiation, and the requirement of clipping. It is our contention that the concentration of Zn as measured in fingernails via laser-induced breakdown spectroscopy (LIBS) can serve as a surrogate assay for serum Zn concentrations. A LIBS Zn assay on nails in situ could be carried out safely and almost non invasively, and could provide results immediately so that appropriate Zn intervention could be commenced. By performing point samples on the nail along the direction of nail growth, this method could also be used to monitor changes in Zn nutriture over time due to supplementation. This was recently demonstrated in a year-long study.[24]

LIBS has been utilized previously to elementally assay human fingernails[27] as a potential method for the quick determination of health problems such as hyper- and hypothyroidism,[28] the fungal infection onychomycosis,[29] and the identification of opium-addicted patients.[30] In these studies, the elements Al, C, Ca, Fe, H, K, Mg, N, Na, O, Si, Sr, and Ti were routinely observed, but not Zn.

Rusak et al.[31] utilized LIBS with a 1064 nm laser to study Mg, Ca, and Zn in human fingernails obtained from 11 volunteers. Utilizing the Zn I line at 481 nm, a calibration graph with an R^2 value of 0.9896 was created after the determination of the absolute Zn concentration via atomic absorption spectrometry. The authors noted a large relative standard deviation in these measurements (approx. 15–20%, which was also observed in the current study) due to the imprecise ablation of mass from the highly layered fingernail. They also concluded that pressed keratin pellets doped with Zn make poor test standard samples for the creation of a calibration graph to test a LIBS fingernail apparatus.

In this work, the quantitative measurement of Zn in fingernails from five volunteers was investigated via LIBS. The variation in measured Zn among the fingers and between the left and right hand of each volunteer was investigated, as was the effect of the state of hydration of the clipped nail. Scanning electron microscopy (SEM) energy-dispersive X-ray spectrometry (EDS) was performed to better interpret the LIBS results. The Zn concentrations of nail clippings from the left hands of the volunteers as determined by speciated isotope dilution mass spectrometry were used to prepare Zn calibration graphs utilizing a multivariate partial least squares (PLS) regression. Nail clippings from the right hands of the volunteers were used to test the PLS model, which predicted the actual Zn concentration to within 7 ppm with an average standard deviation of 14 ppm. The fingernail concentration of Zn in volunteers with low Zn nutriture can be as much as 50 ppm lower than that of volunteers with an exemplary Zn intake (unpublished results), therefore a 14 ppm uncertainty would still allow the diagnosis of severely deficient volunteers.

Materials and Methods

Equipment

The apparatus used to perform these LIBS experiments has been described in detail elsewhere.[32,33] A 1064 nm Nd:YAG laser (Spectra Physics LAB-150-10) with 10 ns pulses operating at 10 Hz was used in all experiments. The pulse energy was approximately 5 mJ/pulse. The pulses were focused by a high-damage threshold AR-coated 5× infinite conjugate microscope objective with a long working distance (LMH-5X-1064, OFR). A charge-coupled device (CCD) camera placed in line with this objective allowed observation of the nails as the data were collected. An alignment He–Ne laser was used to visualize the laser beam focus on the sample (Figure 1). Nails were held in the microscope objective focus on a steel sample holder inside a Plexiglas Ar purge chamber mounted on a manual translation stage. During data acquisition, the chamber was flushed with Ar at a flow-rate of 20 SCFH.

As a result of the curved anterior surface of the nail samples, horizontal translation of the nail sample caused the lens to sample distance (LTSD) to vary, which, if unaccounted for, can add scatter to the LIBS measurements. To compensate for the curvature of the fingernails, a heavily
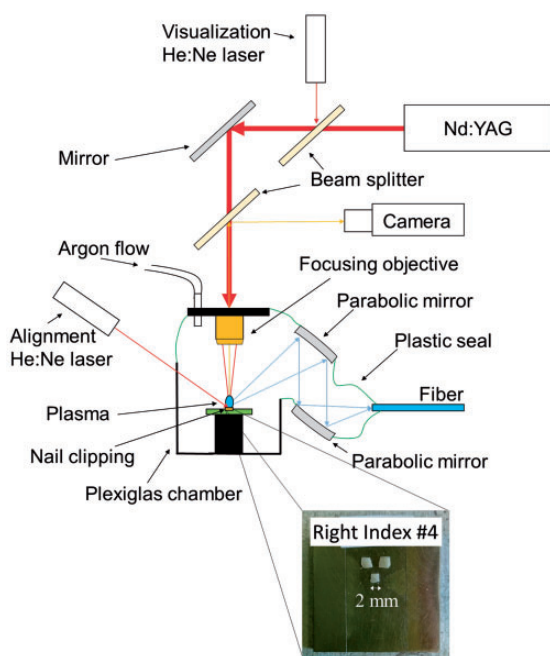
Figure 1. Experimental apparatus utilized in this work.

attenuated secondary He–Ne laser (Uniphase Model 155 A) illuminated the nail sample through the purge chamber wall at an angle of $\sim 45°$. Changes of the LTSD due to the nail curvature caused lateral motion of this secondary visible laser spot. This spot was used to accurately and reproducibly adjust the LTSD to its nominal value, keeping the focal spot size of the LIBS laser constant.

The laser-induced plasma emission was collected and relayed to the spectrometer via two matched off-axis replicated Al parabolic mirrors (3.81 cm diameter, 5.08 cm effective focal length), which focused the light into a 1 m steel-encased multimode optical fiber (core diameter 600 μm, numerical aperture 0.22). The fiber was coupled to an Echelle spectrometer equipped with a 1024×1024 pixel $(24 \, \mu m^2)$ intensified CCD (ICCD) camera (LLA Instruments, Inc., ESA3000). The spectrometer provided spectral coverage in the range 200–840 nm and was controlled by a personal computer running manufacturer-provided software (ESAWIN v3.20), which controlled both the ICCD shuttering as well as the firing of the laser pulses.

Collection of Fingernail Specimens

Nail samples were obtained from volunteers presenting no noticeable trauma or pathology to their nails. All samples were acquired in accordance with the University of Windsor research ethics approval #32272. Nail clippings were taken of the index, middle and ring fingers (both right and left hands) of five volunteers, providing a total of six nail clippings per volunteer. The clippings were cleaned with acetone in an ultrasonic bath for 10 minutes

and allowed to dry for 20–30 minutes. The clippings were then cut into approx. 2×2 mm fragments to provide a flat target (Figure 1). In the buffing study, the nails were smoothed with an extremely fine grit TiO_2 buffing board manufactured for use on fingernails. Prior to clipping, the nails were buffed vigorously for 20 seconds, then clipped and ultrasonically cleaned with acetone to remove any potential residue from the buffing board.

Optimization of LIBS Emission

Data were acquired by collecting the emission from 10 sequential laser pulses in one location prior to a lateral translation of the nail (across the nail perpendicular to the direction of growth). Five such lateral locations were averaged in the software to make one measurement spectrum (50 laser shots per spectrum). In our standard testing protocol and unless otherwise stated, 10 such spectra were averaged and the standard deviation of those 10 measurements (500 laser shots total) was calculated to determine the average emission intensity and uncertainty of the measurements due to shot-to-shot variation. Because the image-intensified camera gain setting was variable, the observed emission intensity was relative and is reported here in arbitrary units (a.u.). All the intensities reported here are background-subtracted integrated areas under the curve as measured by the ESAWIN software. To allow direct comparison of the data, the gain was not changed for the duration of the study once an appropriate gain setting was determined to utilize the maximum dynamic range of the camera without overflowing the CCD array.

In one study, single-shot spectra were acquired from 15 sequential laser pulses without translating the nail to determine whether vertical depth effects due to the striated layered structure of the nails were measurable. Studies were performed to optimize the Zn emission intensity and signal-to-noise ratio. These studies indicated that an ICCD camera delay time of 1 μs and an ICCD camera opening (integration) time of 5 μs provided the optimum experimental conditions for testing in Ar. Spectra were also acquired in air and He, but had decreased intensities and signal-to-noise ratios.

Hydration of Nails

The effect of the presence of liquid water in the nail specimen on the LIBS spectrum was investigated. In an attempt to over-hydrate a nail specimen, clipped nail samples from each of three volunteers were soaked in distilled water for about one week and were then dried under a cool air gun for one hour before being cut into segments and tested. As a control, nail samples from the same group of volunteers were cleaned in acetone before being air dried for one hour and then cut into segments. These segments were then tested in the Ar environment using the standard LIBS
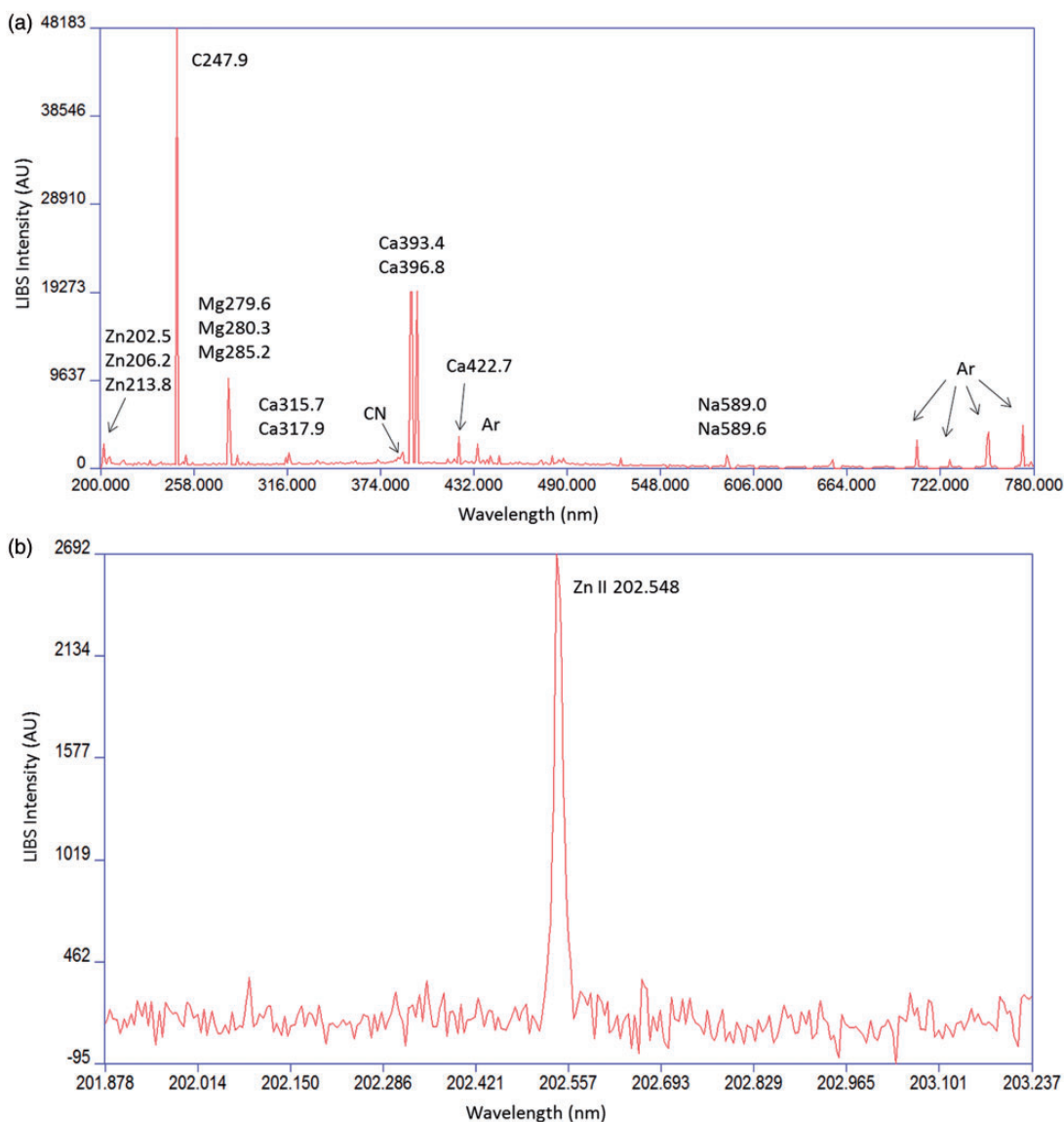
Figure 2. (a) Full LIBS spectrum from a fingernail sample and (b)–(d) the spectral regions of interest.

protocol described earlier. Attempts were also made to intentionally dehydrate the nails. Clipped, cleaned, and segmented nail samples from three volunteers were heated in a 122 °C oven for approximately 48 hours to drive off the water in the nails. Once removed, the nails were kept in a desiccator to prevent any moisture from returning prior to LIBS testing.

Scanning Electron Microscopy

Scanning electron microscopy studies were performed using a low-vacuum environmental scanning electron microscope (Quanta 200 FEG, FEI) without C or Au coating. Nail samples and a piece of stainless-steel plate used for the daily spectral calibration of the spectrometer system were imaged to compare the ablation performance and

crater size. The SEM was equipped with an EDS apparatus, which was used to make Zn concentration point measurements and line measurements on nail sections by monitoring the Zn Kα line.

Results and Discussion

LIBS Spectra

Figure 2a shows a typical nail spectrum acquired in Ar using the described experimental parameters. The strongest observed emission features are identified, along with the center wavelength of the emission line. In addition to the Zn analyte lines, the dominant emission was attributable to the C 247 nm line from nail keratin and the spectroscopically intense emission lines from the ions of the metals Ca
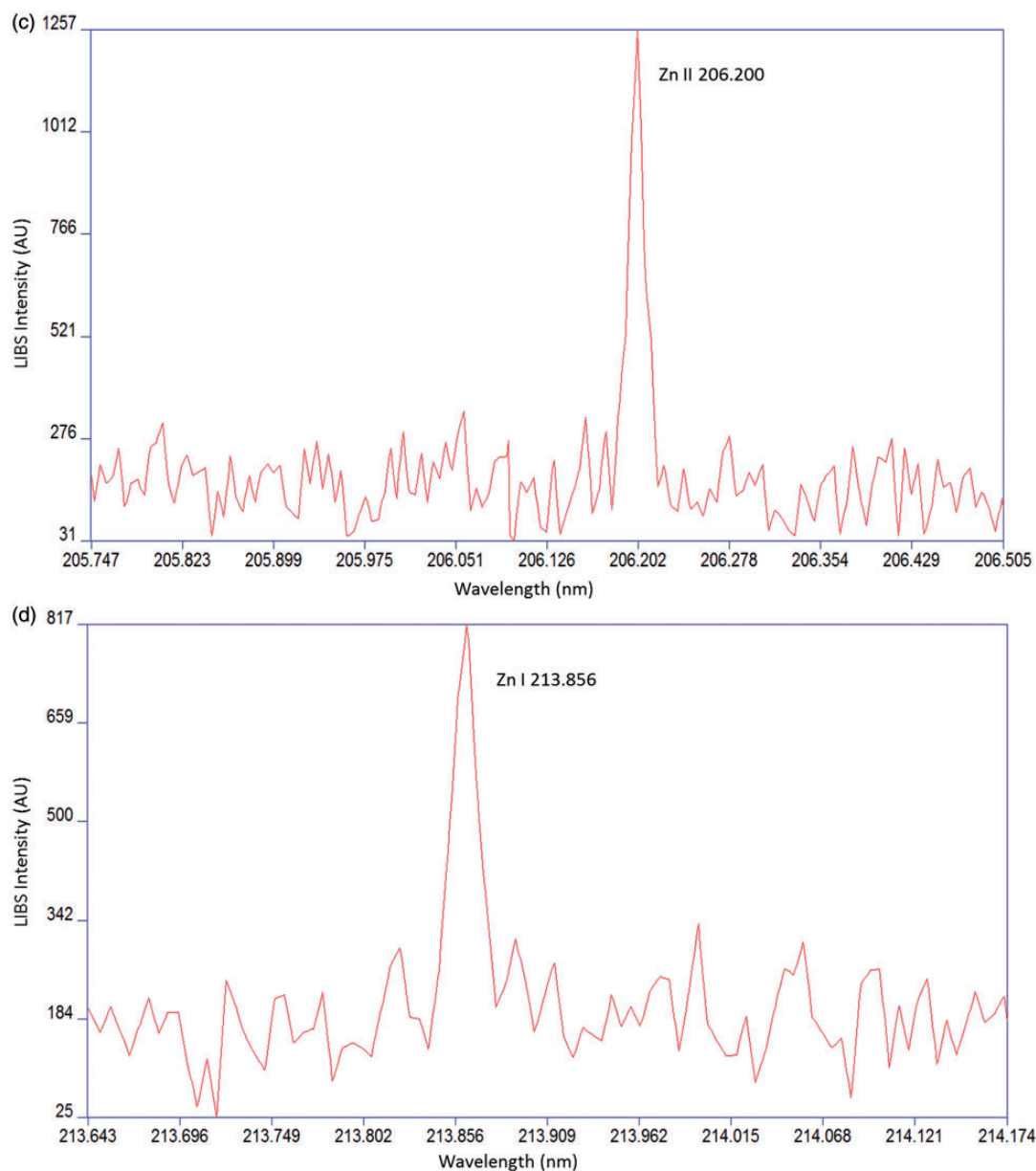
Figure 2. Continued.

and Mg, present in the nail at concentrations of ∼2310 and 570 ppm, respectively.[34] The strong Hα line at 656 nm was unobserved in the spectrum due to the presence of a spectral gap in the Echelle spectrometer's wavelength coverage (no light is recorded in these spectral gaps.) Therefore this line, commonly associated with the presence of water molecules, could not be used to directly monitor the hydration status of the fingernails. Figures 2b, 2c, and 2d show the spectrum in the regions of the strongest Zn emission lines from the singly ionized Zn^+ ion (Zn II 202.547 nm and Zn II 206.200 nm) and the neutral Zn atom (Zn I 213.855 nm). As a result of the intensity and the reduced shot-to-shot variation of the Zn II emission, the sum of the integrated areas under the background-subtracted curve of the two Zn II lines was used in all experiments as the measurement of the LIBS Zn intensity. The use of other emission lines, including the intense C I 247.856 line, for normalization was investigated, but did not result in any improvement in precision or accuracy.

Effect of Lateral Translation and Depth Measurements

To determine whether the data collection procedure was appropriate, studies were undertaken to search for any indication of a dependence of the measured LIBS Zn
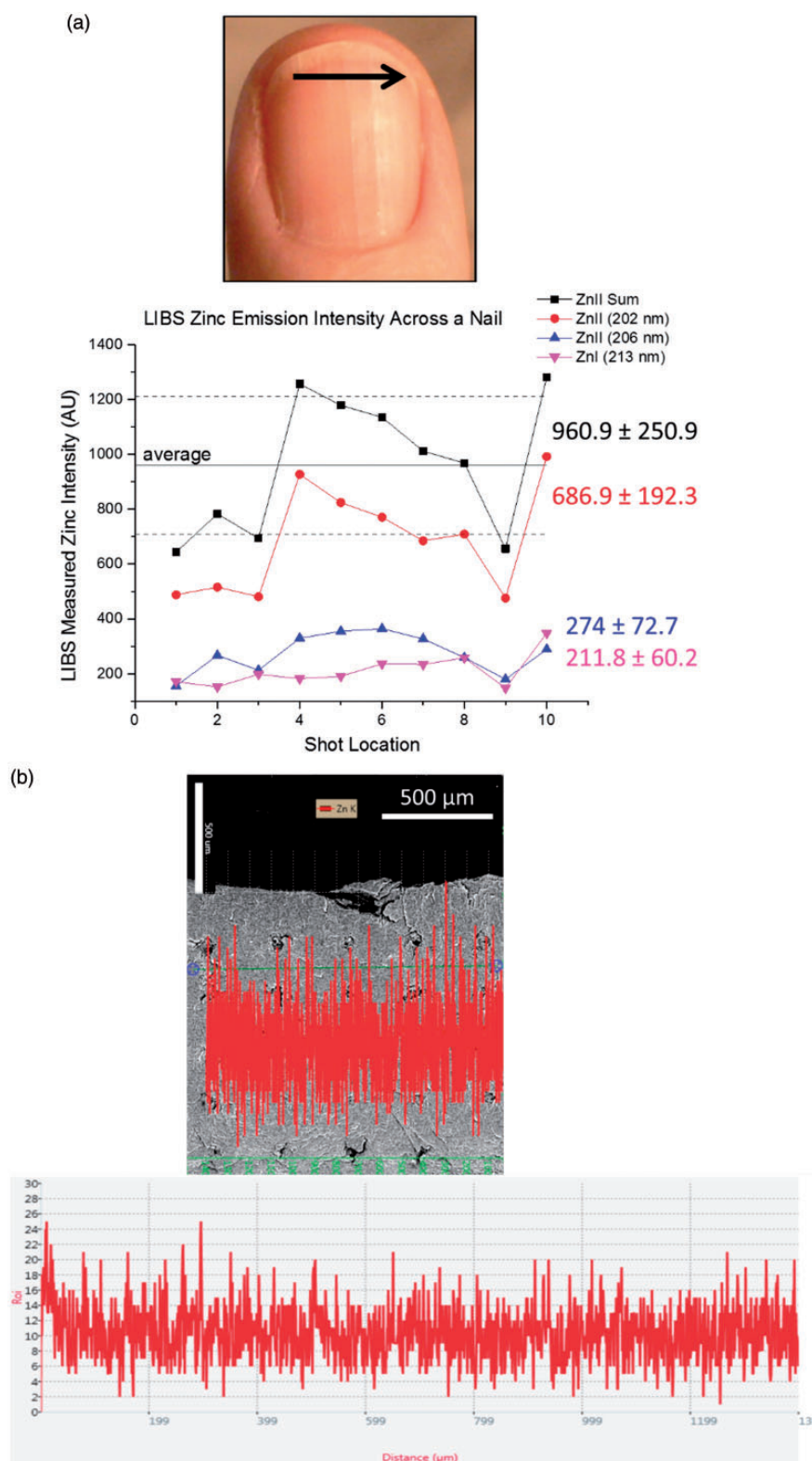
Figure 3. (a) Measured LIBS zinc intensity as a function of lateral distance across the fingernail. (b) Energy-dispersive X-ray measurement of the Zn Kα line strength superimposed on an SEM image of the nail with LIBS craters clearly visible.
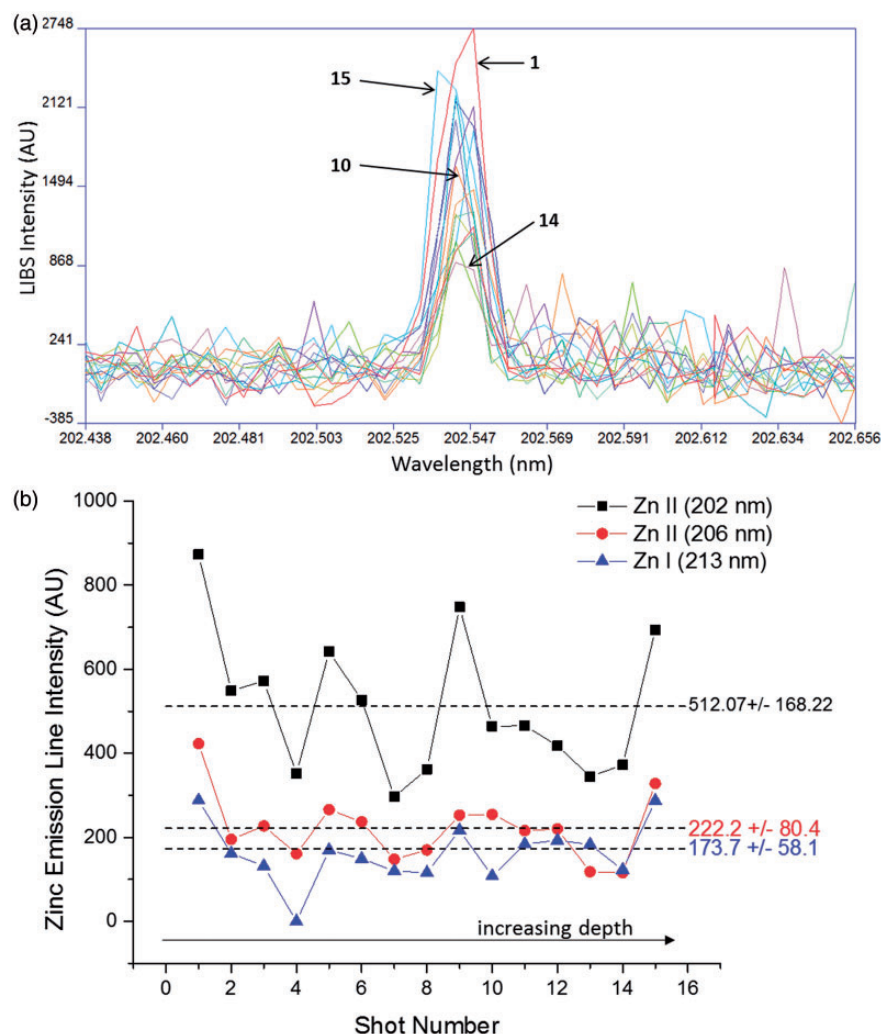
Figure 4. (a) Overlapped spectra from 15 sequential single-shot LIBS spectra acquired in a single location. No consistent trend as a function of depth in the nail was observed. (b) Measured Zn emission intensities for the 15 laser pulses.

emission intensity on the lateral position across the nail or depth in the nail. Figure 3a shows the lateral scan direction and the result of 10 sequential measurements across the nail (perpendicular to the direction of growth) with 10 laser pulses at each location. None of the three observed Zn analyte emission lines demonstrated any systematic trend or variation across the nail, but all showed significant scatter. The average and standard deviation of the 10 measurements are shown to the right of the intensities of each emission line. The sum of the strong Zn II lines had a fractional standard deviation (defined as the 1σ standard deviation divided by the average) of 0.26. The SEM-EDS measurements confirmed this lateral uniformity. Figure 3b shows a 1.4 mm line scan of the Kα Zn signal consisting of 1400 measurements spanning five LIBS craters (visible above the superimposed Kα signal in the image). Below the SEM image is the Kα data, which showed no systematic lateral change in Zn concentration.

To determine whether it was appropriate to average 10 sequential laser shots fired in one location on the nail, the depth dependence of the LIBS Zn emission was studied. Spectra were acquired from single LIBS laser pulses and 15 pulses were fired in one location. Figure 4a shows the region around the Zn II emission line at 202 nm for the 15 spectra with four specific spectra denoted. Figure 4b plots the measurements of LIBS Zn intensity of the three analyte lines and gives the average and standard deviation of the 15 measurements. No systematic behavior with depth was observed in this specimen and the emission from shot 15 (taken last) was essentially as strong as the emission from shot 1 (taken first), while the emission from shot 14 was the weakest. This should be interpreted as typical shot-to-shot variation, not a true variation of LIBS intensity with depth. The actual depth of the laser shots is unknown because SEM was unable to yield accurate depth information. Multiple nail specimens were studied in this way and
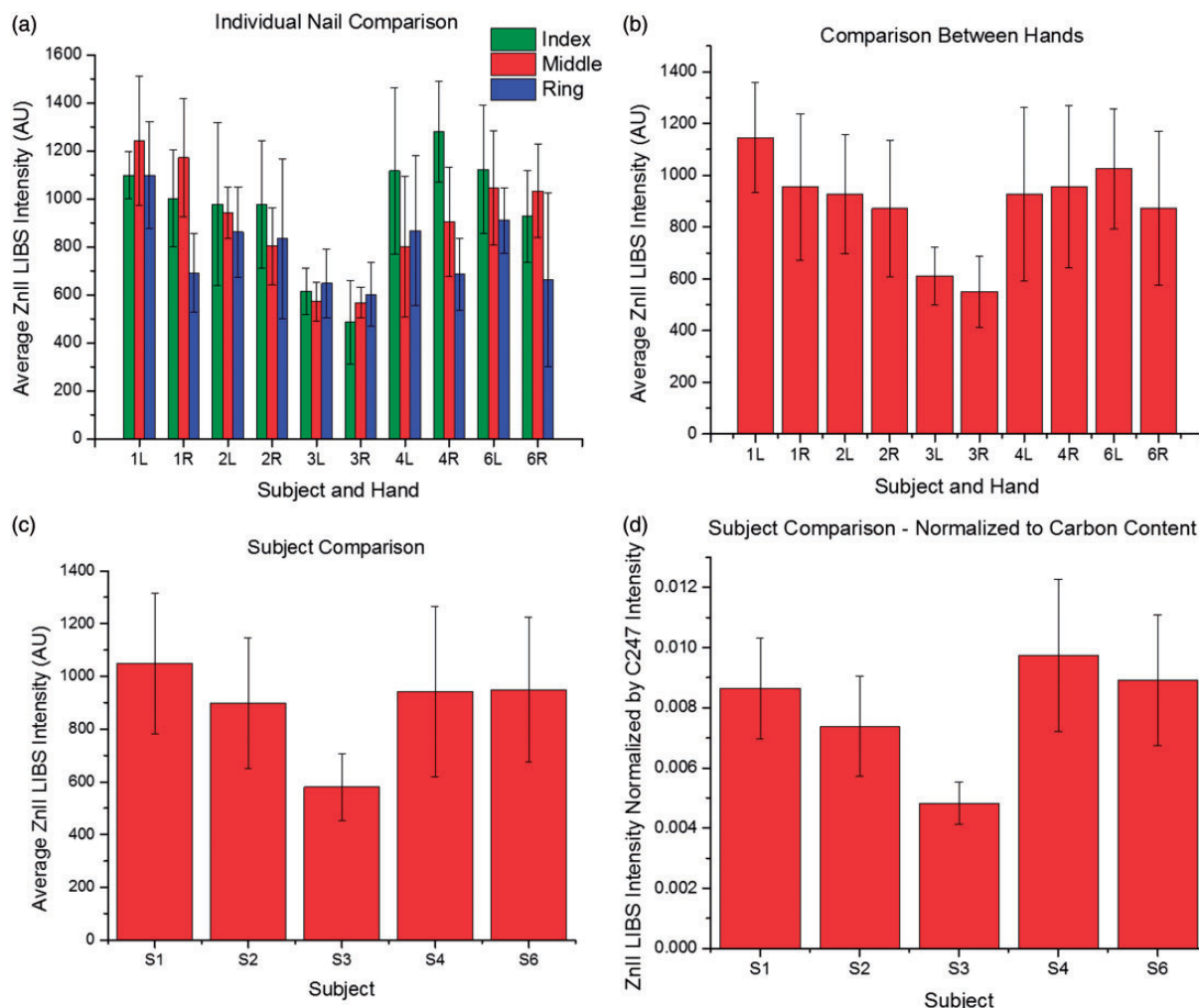
Figure 5. (a) Averaged Zn intensity from 10 measurements on each finger (middle, ring, index only) from both hands of five volunteers. (b) Measurements of all fingers combined per hand and (c) combined per volunteer. (d) Measurements per volunteer after normalization by the C 247 nm line.

no consistent correlation with depth was observed in any of the samples tested; however, no more than 15 laser shots were ever used in any one location. The data shown in Figure 4 were chosen as representative.

Comparison of Fingers and Hands

The LIBS Zn intensity was obtained from clippings from the index, middle, and ring fingers of both hands of five volunteers: S1, S2, S3, S4, and S6. Samples from volunteer 5 were excluded due to the lack of a complete set of nails from all fingers. Figure 5a shows the average measured LIBS Zn intensity from 10 measurements on each finger. The error bars are the 1σ standard deviations of these averages. There was no observed systematic trend in the nails obtained from any specific finger and, due to the large shot-to-shot variation in the LIBS spectra, there was no statistical difference between nails obtained from the three fingers of each volunteer.

Therefore the three fingers were averaged to obtain a single average value for each volunteer's left (L) and right (R) hand (Figure 5b). Each value is an average of 30 spectra or 1500 total laser shots and the error bars are the 1σ standard deviation of those 30 spectra.

The values for left and right hands were also consistent within their uncertainty (Figure 5b) and therefore it was deemed to be appropriate to combine the left and right hand data into a single measurement of Zn intensity for the volunteer. This is shown in Figure 5c, which is the average of 60 spectra or 3000 total laser shots. Despite the large number of laser shots, the scatter of the measurements was still large, with an average fractional uncertainty of approximately 0.28. To reduce this measurement shot noise, the Zn intensity of each spectrum was normalized to the carbon emission intensity. The result of this is shown in Figure 5d, which shows that the normalization was effective in slightly improving the precision of the Zn quantification, reducing

the fractional uncertainty to ~ 0.21, while not distorting the qualitative performance of the assay.

Effect of Hydration

A fractional uncertainty >0.20 (20% noise on the measurement) is not intrinsic to the LIBS method. The fractional uncertainty of a flat stainless-steel calibration standard measured daily using this same apparatus over many months was approximately 0.05 (5%), although it can be larger for less intense emission lines. It is known that the presence of water in a sample can have an impact on the formation of the LIBS plasma and therefore its temperature and emission intensity. A study was conducted to see whether the influence of the state of hydration of the nail sample was responsible for the observed shot-to-shot-variation. Nails were soaked in water to over-hydrate them prior to testing. Three soaked samples (denoted "wet" A–C in Figure 6 and plotted with a filled column) and three control samples (denoted "control" A–C in Figure 6 and plotted with an open column) were tested using the standard protocol.

Figure 6a shows no systematic effect of the soaking on the LIBS Zn emission. The presence of liquid water in the nail sample should serve to reduce the overall LIBS emission intensity and specifically reduce the emission intensity from the Zn ions due to a substantially lower plasma temperature. In fact, soaking increased the emission intensity in two of the cases (A and B) and decreased the emission intensity in one case (C), although none of these changes was significant within the uncertainty of the measurement. If the water was stored in small heterogeneous microscopic pockets within the nail structure, it was hoped that alternation of the amount of water content would impact the shot-to-shot variability of the LIBS measurements as well as the overall intensity. Figure 6b shows no systematic increase or decrease in the fractional standard deviation of the nail measurements. In one case (B), the soaked sample showed a significantly greater scatter of the measurements; in one case (C) the soaked sample exhibited significantly reduced scatter; and in one case (A) almost no difference was observed. These results are consistent with no reproducible systematic effect due to the attempt to over-hydrate the nail samples via extended soaking.

To investigate the effect of extreme drying, nail samples obtained from the same three volunteers (A, B, and C) were dehydrated (denoted "dried" A–C in Figure 7 and plotted with a filled column). Standard nail samples were also tested as a control (denoted "control" A–C in Figure 7 and plotted with an open column). This attempt to dehydrate the nail samples did not systematically alter the LIBS Zn emission (Figure 7a), with one sample (B) showing significantly decreased intensity; one sample (C) showing a slightly greater intensity; and one sample (A) showing almost no change. Again, any observed shift was not statistically significant within the uncertainty of the measurements. Similarly, dehydration did not reduce the shot-to-shot variation of the measurements as anticipated (Figure 7b). One sample (B) showed a significant decrease in the scatter of the measurements, but the other two showed slight increases.

The results of these experiments imply that the state of hydration of the nails was not the most significant factor in determining the overall Zn emission intensity in the plasma or the observed variation in measured intensity. This is fortuitous, because nails tested in any future in situ LIBS nail analysis device used for clinical screening will not be controlled as rigorously as the controls in these experiments. However, these nails will also not be exposed to the extreme conditions of hydration or dehydration utilized in these experiments, which appeared to have little reproducible effect.

Effect of Surface Roughness

To determine whether the intrinsic surface roughness of the nail contributed significantly to the observed shot-to-shot scatter, nails were mechanically buffed smooth. Figure 8 shows SEM images of the control (Figure 8a) and smoothed (Figure 8b) nails, as well as the average Zn II LIBS emission intensity from each of these two samples (Figure 8c). Four ablation craters on a square grid are visible in both Figure 8a and 8b and the difference in surface roughness due to the buffing is appreciable, although it was not quantified. Both samples yielded identical LIBS Zn II emission intensity within the uncertainty (Figure 8c), but the buffed nail had significantly reduced scatter in the measurements. The control nail had an average Zn II emission intensity of 1097.9 ± 221.2 a.u., whereas the buffed nail had an average Zn II emission intensity of 1076 ± 122.3 a.u. Buffing reduced the fractional standard deviation by almost 50% from 0.20 to 0.11. As this test was not performed until near the end of the investigation, buffing was not added to the standard preparation protocol of the nails, but will be in future studies. To determine whether buffing left any residue on the nails that could be detected by LIBS, a qualitative analysis of the nail buffer was performed. Consistent Ti emission was observed in the nail buffer spectra, but not in the nail spectra, indicating that if the nail buffer left any residue on the nails, it was removed during the ultrasonic cleaning process.

The larger than expected deviation of the LIBS measurements made on a single nail specimen can be explained by non-uniform ablation by the 1064 nm Nd:YAG laser due to the structure of the fingernail. Figure 9a shows a $60\times$ magnification SEM image of an array of single-shot LIBS craters formed in a nitrocellulose filter medium by our apparatus; Figure 9b is a magnified view of one ablation crater in this filter medium showing a diameter of $\sim 65\,\mu m$.

(a)

Effect of Hydration on LIBS Zinc Emission Intensity

(b)

Effect of Hydration on Shot-to-Shot Variation in LIBS Zinc Emission Intensity
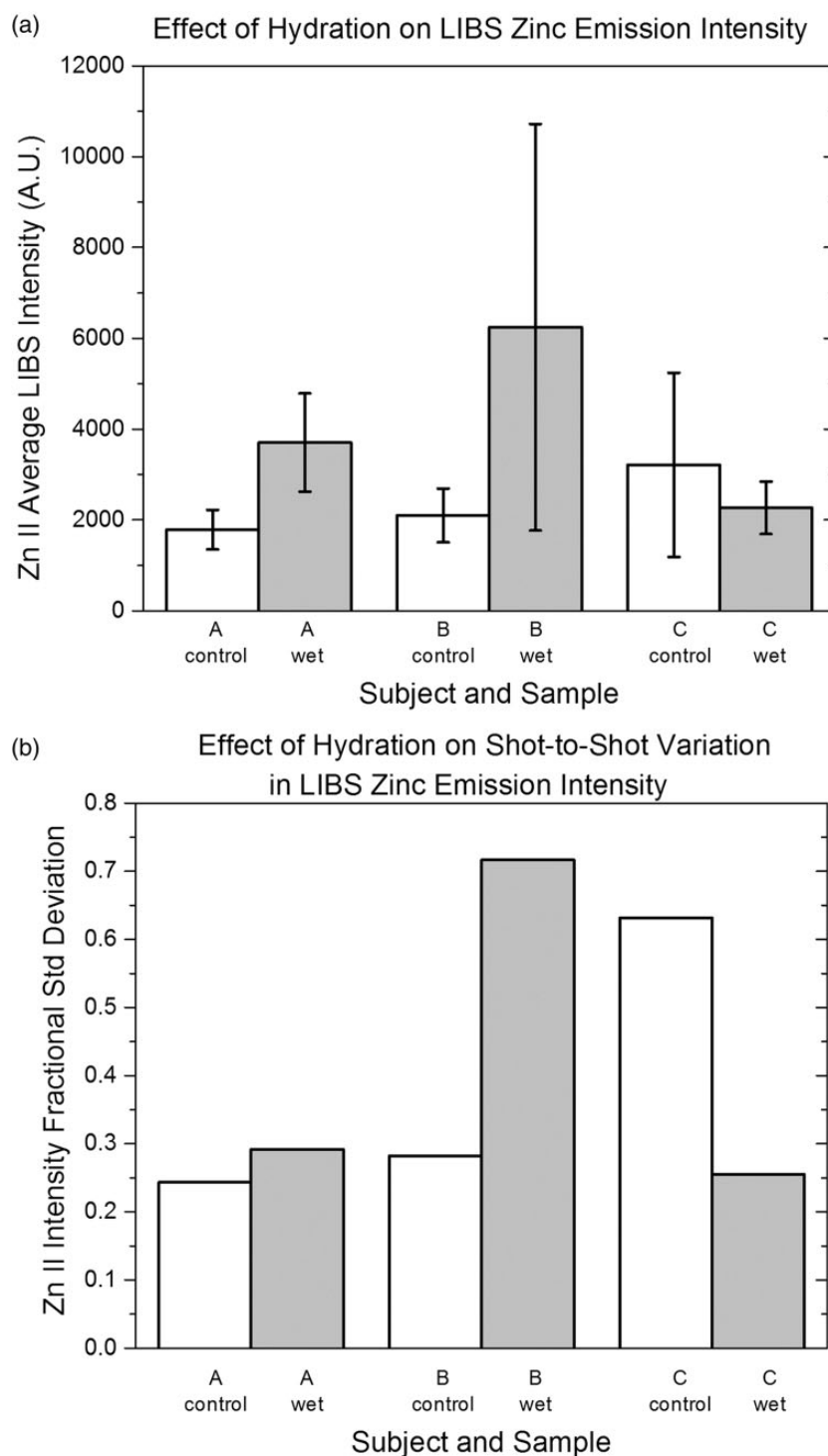

Figure 6. (a) Effect of over-hydration of a fingernail specimen prior to LIBS testing on the measured LIBS zinc emission intensity and (b) standard deviation of the intensity measurements.

For comparison, Figure 9c shows a single-shot LIBS crater in our stainless-steel calibration plate under 250 magnification, whereas Figure 9d shows the same type of ablation on the fingernail at the same 250× magnification. The differences in size and uniformity due solely to the laser–material interaction are immediately apparent. Figure 9e shows a 70× magnification SEM image of a 5 × 5 grid of LIBS craters in a fingernail and Figure 9f is a magnified view of the ablation structure outlined by the square in Figure 9e. Each crater was created by 10 laser

(a)

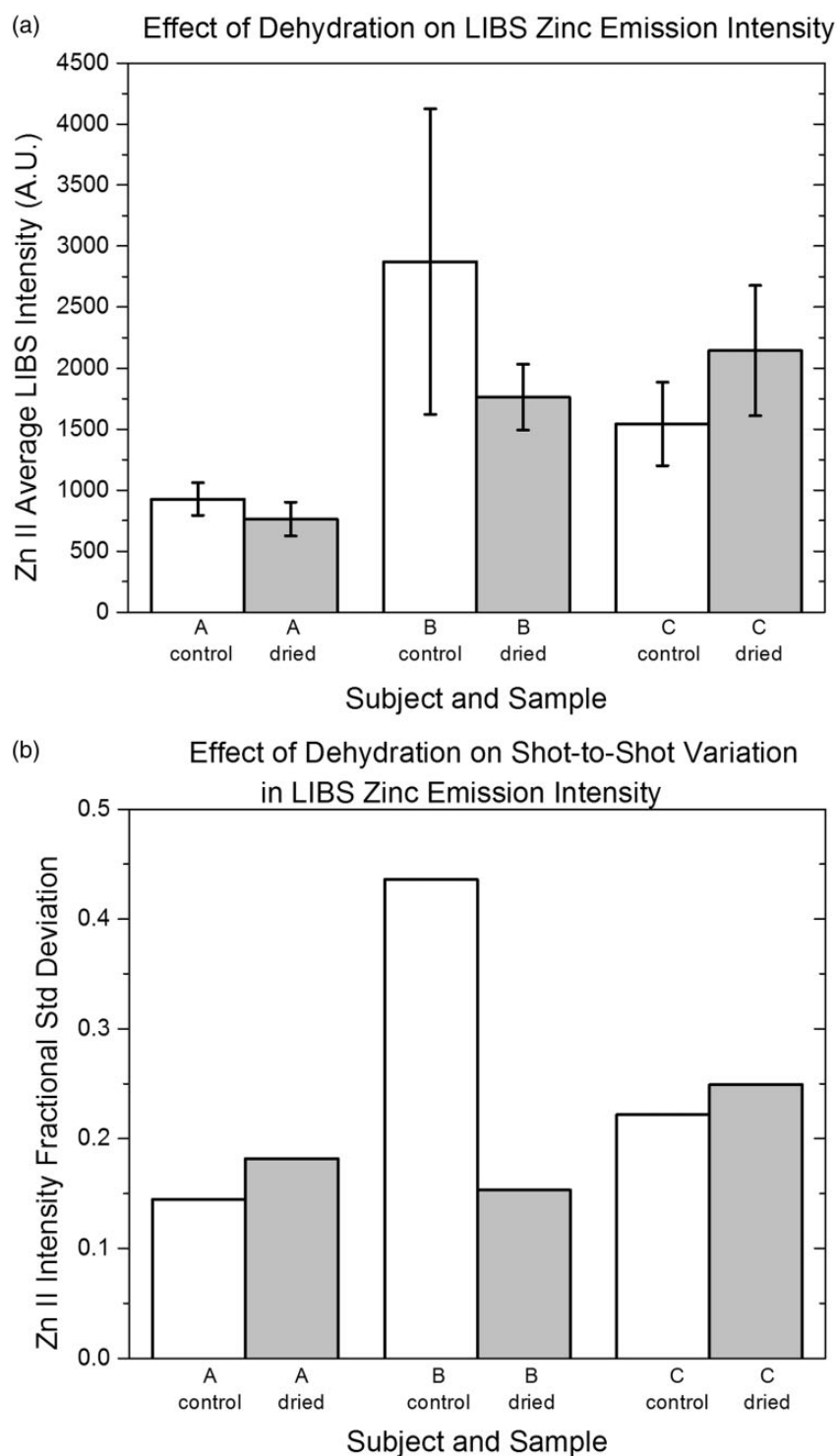## Effect of Dehydration on LIBS Zinc Emission Intensity

Figure 7. (a) Effect of dehydration of a fingernail specimen prior to LIBS testing on the measured LIBS Zn emission intensity and (b) standard deviation of the intensity measurements.

pulses in each location. It is readily apparent that the uniformity of the ablation process in the filter medium and steel is high, whereas in the fingernail no two ablation events appear identical or even similar.

In the center of the nail on the left of Figure 9e, the laser appears to have ablated almost no material at all, resulting in no obvious crater, whereas on the right-hand side of the image in Figure 9e the laser appears to have caused the
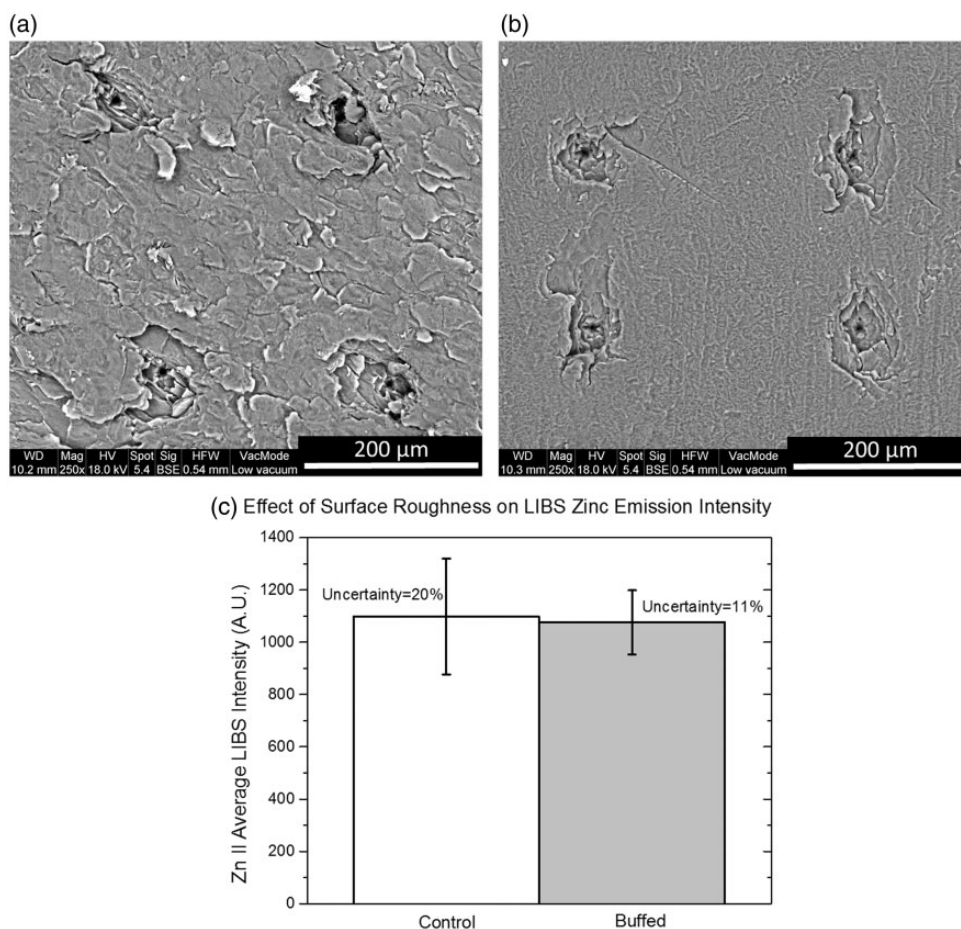
Figure 8. Scanning electron micrographs of (a) a control fingernail and (b) a nail smoothed by buffing. The LIBS Zn intensity measurements were similar for both nails, but the buffed nail showed smaller scatter in the measurements (c).

layered nail structure to peel back (shown in a magnified view in Figure 9f or flake away in a highly uncontrolled and irreproducible way. The damage to the nail material also exhibited a directional inhomogeneity, as evidenced by the tendency of ablation features to stretch diagonally from the upper left-hand corner of Figure 9e to the lower right-hand corner. No such directional asymmetry is present in the apparatus, as evidence by the highly circular ablation shown in Figure 9a, 9b, and 9c. Similar ablation was observed in all the nail samples tested in this study. Thus the layered nature and fibrous structure of the fingernails were responsible for the non-uniform ablation observed in Figure 9, which explains the scatter observed in the measurements reported here.

This behavior may also explain the observation by Rusak et al.[31] that pressed keratin pellets were not an effective LIBS calibration standard for an LIBS fingernail assay using a 1064 nm laser. Pressed keratin pellets created from a uniform homogenous powder would have no such asymmetrical striations or vertical layering, yielding more reproducible results than ablation, but not effectively modeling the behavior of a true fingernail.

It is believed that the weak interaction of the 1064 nm Nd:YAG with the keratin nail is responsible for this effect, allowing the ablation to occur or initiate at depth rather than in a more controlled manner on or near the surface of the nail. Experiments are underway to replace the 1064 nm laser with the quadrupled and tripled Nd:YAG harmonics at 266 and 355 nm, respectively. The absorption coefficient of bulk keratin is greater at both of these wavelengths than at 1064 nm and the absorption at 266 nm is approximately 10 times greater than at 355 nm.[35] As shown, however, the ablation properties of bulk keratin and human fingernails may not correlate well, and we know of no published report of ultraviolet LIBS performed on fingernails.

Calibration via Multivariate Analysis

The absolute Zn concentrations for the five tested nail samples were obtained by speciated isotope dilution mass spectrometry (SIDMS). A PLS regression (PLS_Toolbox, Eigenvector, Inc.) was performed on the data using the 30 measurements from the left hand of each volunteer to
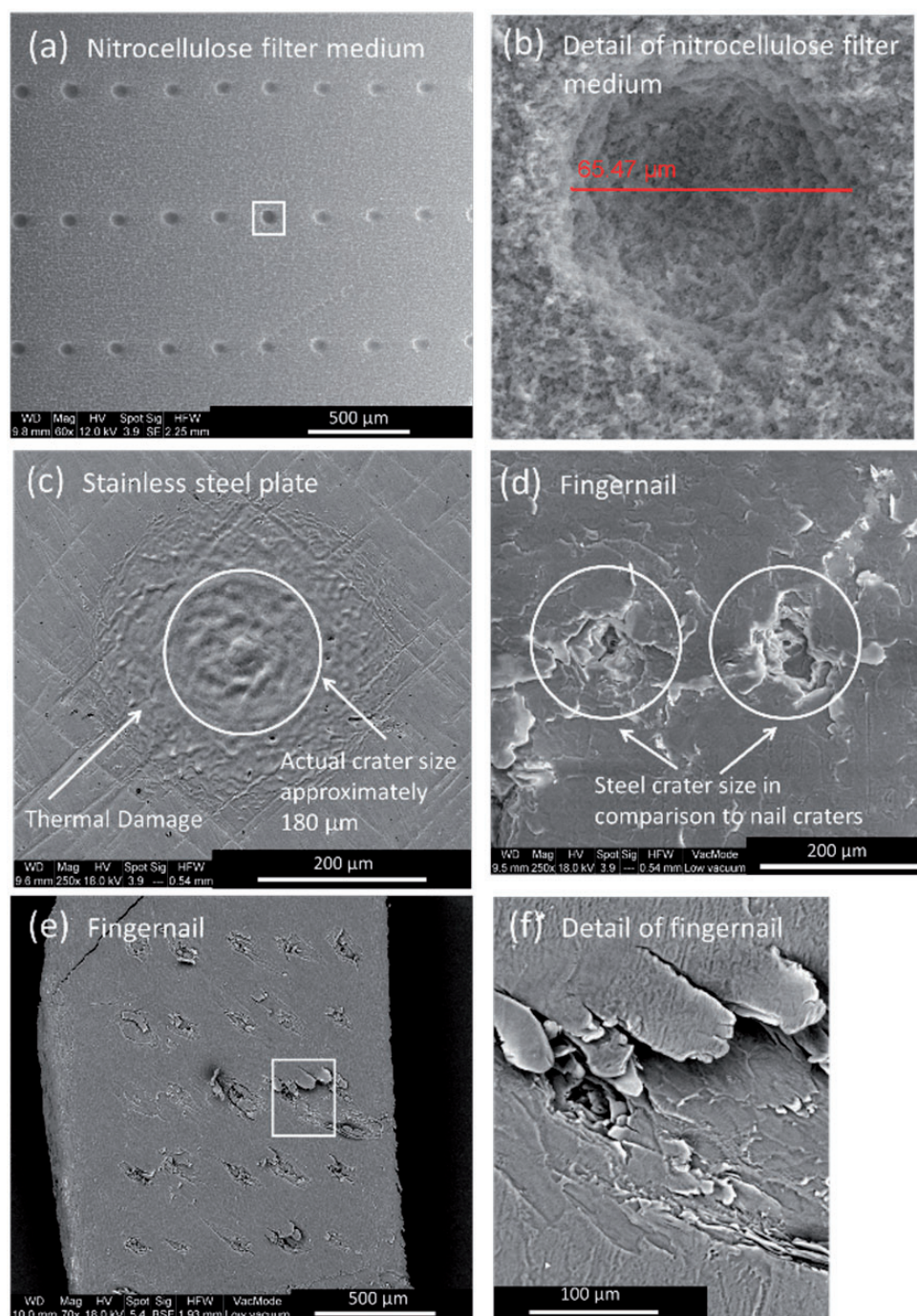
Figure 9. Scanning electron micrographs of LIBS craters formed in (a), (b) a nitrocellulose filter medium, (c) a stainless-steel calibration plate, and (d)–(f) fingernail. Part (b) is a magnified view of the box shown in part (a). Part (f) is a magnified view of the box shown in part (e).

create the regression model and using the 30 measurements on the right hand to test the model. It was assumed that all the fingers had the same Zn concentration, although only the nails from one finger were tested with SIDMS for each volunteer. The results of the analysis are shown in Table 1 and Figure 10. Figure 10 is a plot of the average predicted PLS concentration versus the actual SIDMS concentrations. The error bars are the 1σ standard deviations of the 30 predictions (Table 1) and the line is a linear fit to the predictions with a Pearson's R of 0.945 and an adjusted R^2 of 0.858. Taken in toto, the five predictions differed from the actual concentrations by an average of 6.8 ppm

Table 1. Results of a PLS regression of Zn measured in fingernail LIBS spectra.

Actual Zn concentration (ppm) measured by SIDMS	Average concentration predicted by PLS regression (ppm)	Standard deviation of 30 predictions (ppm)	Difference (SIDMS − PLS regression) (ppm)	Difference (%)
102	102.39	11.69	−0.39	0.3
106	106.23	10.11	−0.23	0.2
114	128.27	20.65	−14.27	12.5
136	136.44	5.99	−0.44	0.3
141	122.42	21.36	18.58	13.2

PLS: partial least squares; SIDMS: speciated isotope dilution mass spectrometry.
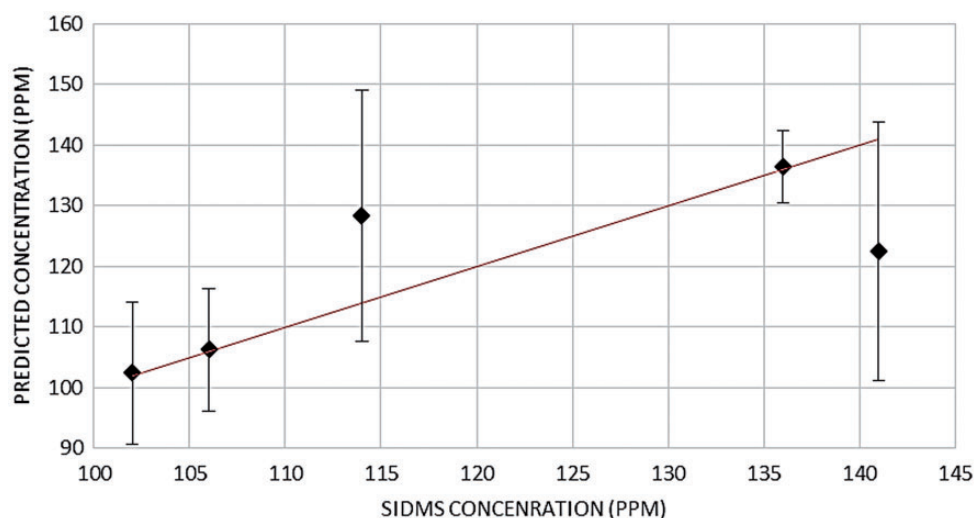
Figure 10. Predicted Zn concentration from five fingernail samples as a function of the actual Zn concentration as determined by SIDMS. The line is a fit to the predictions to show the linearity of the model performance (Pearson's *R* 0.945).

(an average uncertainty of 5%), well below the 1σ uncertainty. The average standard deviation exhibited by all the predictions was 14 ppm, with an average fractional uncertainty on the predictions of 12%.

Conclusions

Nutritional Zn present in human fingernails is quantifiable using the very rapid assay of LIBS. LIBS measurements performed on human nail clippings with a 1064 nm laser exhibited a high degree of variability and intrinsic measurement scatter that was found to not depend on the state of hydration of the nail, the lateral position sampled across the nail, or the depth within the nail at which the plasma was formed (up to a depth achieved by 15 laser pulses). Surface roughness was found to be a contributing factor and buffing the nail smooth prior to testing reduced the standard deviation of the measurements by a factor of almost two. Normalization of the LIBS Zn intensity by other LIBS spectra emission features, specifically the intense C emission at 247 nm, was not found to significantly improve the accuracy or precision of measurement in this protocol. Non-uniform ablation due to the layered nature and the fibrous structure of the fingernails was observed by SEM in all the samples tested in this study and was the dominant source of measurement uncertainty. In spite of this, a PLS regression model built from Zn measurements on the left hand of five volunteers was constructed and used to test the Zn measurements on their right hand, yielding predictions that differed from the actual concentration by an average of 6.8 ppm and a standard deviation of 14 ppm, or 12% fractional uncertainty.

A study with a greater number of overall participants, including a pool of "Zn deficient," participants now needs to be conducted. These preliminary results are highly encouraging for future in situ screening measurements because the fingernail content of volunteers with low Zn nutriture are expected to be as much as 50 ppm lower than

that of volunteers with exemplary Zn intake. Therefore although the uncertainty of the measurements shown in Figure 10 is relatively large and may preclude precise Zn quantification in fingernails, the uncertainty obtained in these experiments would still allow a rapid determination of deficiency or non-deficiency on the basis of the LIBS measurements alone, which was the intention of this study.

These LIBS measurements are not only more convenient and simpler to perform than traditional blood serum measurements, but are also expected to be more robust and indicative of the Zn nutritional status. Serum measurements are unreliable and simply not indicative of Zn nutritional status because serum Zn varies strongly with the time of day and in response to diet (the time of eating as well as the glucose content) and also other physiological conditions such as intestinal disease, pregnancy, infection and even strenuous physical exercise.[36]

It is believed that performing the LIBS measurements with ultraviolet wavelength lasers that are attenuated much more readily by the nail material, yielding more intense plasmas and more controlled laser ablation, will provide an even greater amount of measurement stability, which is necessary to reduce the observed shot-to-shot variation even further. In addition, the increased LIBS intensity and reproducibility would allow a decrease in the number of laser shots or accumulated spectra required to accurately quantify fingernail Zn in a rapid screening diagnostic.

Conflict of Interest

The authors report there are no conflicts of interest.

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