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Line-field confocal optical coherence tomography, a novel non-invasive tool for the diagnosis of nail disorders

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3 List of Abbreviations

CLSM: confocal laser scanning microscopy

LC-OCT: line-field confocal optical coherence tomography

OCT: optical coherence tomography

PCR: polymerase chain reaction

PPV: positive predictive value

NPV: negative predictive value

4 Abkürzungsverzeichnis

KLM: konfokale Laserscanmikroskopie

LC-OCT: konfokale line-field optische Kohärenztomographie

OCT: optische Kohärenztomographie

PCR: polymerase-Kettenreaktion

PPV: positiver prädiktiver Wert

NPV: negativer prädiktiver Wert

5 List of Publications

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1. **Main Paper:**
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2. **Appendix:**

   Eijkenboom QL, Daxenberger F, Guertler A, Steckmeier S, French LE, Sattler EC. **Line-field confocal optical coherence tomography (LC-OCT) for the in vivo examination of nails: Analysis of typical features for the differentiation of nail disorders.** *J Eur Acad Dermatol Venereol.* 2024 May;38(5):e413-e416. doi: 10.1111/jdv.19641. Epub 2023 Dec 7. PMID: 38059388.

Further publications:

3. Nutz MC, Deußing M, Hartmann D, Daxenberger F, Eijkenboom QL, Gust C, French LE, Schuh S, Welzel J, Sattler EC. **Line-field confocal optical coherence tomography: Characteristic hints for the diagnosis of scarring alopecia due to lupus erythematodes: A preliminary study.** *Skin Res Technol.* 2024 Aug;30(8):e13859. doi: 10.1111/srt.13859. PMID: 39096179; PMCID: PMC11297418.
4. Deußing M, Eijkenboom QL, Thamm J, Desch A, Fünfer K, Mozaffari M, Wirsching H, Mayer O, Schlingmann S, French LE, Hartmann D, Welzel J, Schuh S, Sattler EC. **Unveiling the hidden boundaries: AI-assisted line-field optical coherence tomography margin mapping for precise excision of basal cell carcinoma - A step-by-step tutorial.** *Skin Res Technol.* 2024 Feb;30(2):e13594. doi: 10.1111/srt.13594. PMID: 38297955; PMCID: PMC10831192.
5. Thamm JR, Daxenberger F, Viel T, Gust C, Eijkenboom Q, French LE, Welzel J, Sattler EC, Schuh S. **Artificial intelligence-based PRO score assessment in actinic keratoses from LC-OCT imaging using Convolutional**

Neural Networks. *J Dtsch Dermatol Ges.* 2023 Nov;21(11):1359-1366. doi: 10.1111/ddg.15194. Epub 2023 Sep 14. PMID: 37707430.

6. Daxenberger F, Deußing M, Eijkenboom Q, Gust C, Thamm J, Hartmann D, French LE, Welzel J, Schuh S, Sattler EC. **Innovation in Actinic Keratosis Assessment: Artificial Intelligence-Based Approach to LC-OCT PRO Score Evaluation.** *Cancers (Basel).* 2023 Sep 7;15(18):4457. doi: 10.3390/cancers15184457. PMID: 37760425; PMCID: PMC10527366.
7. Guertler A, Volsky A, Eijkenboom Q, Fiedler T, French LE, Reinholz M. **Dietary Patterns in Acne and Rosacea Patients-A Controlled Study and Comprehensive Analysis.** *Nutrients.* 2023 Oct 17;15(20):4405. doi: 10.3390/nu15204405. PMID: 37892480; PMCID: PMC10609993.
8. Stanclift CR, Dwight SS, Lee K, Eijkenboom QL, Wilsey M, Wilsey K, Kobayashi ES, Tong S, Bainbridge MN. **NGLY1 deficiency: estimated incidence, clinical features, and genotypic spectrum from the NGLY1 Registry.** *Orphanet J Rare Dis.* 2022 Dec 17;17(1):440. doi: 10.1186/s13023-022-02592-3. PMID: 36528660; PMCID: PMC9759919.
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6 Introduction

6.1 Scientific Background - Non-invasive diagnostic methods in dermatology

In recent years, various non-invasive techniques have emerged to facilitate and improve the diagnosis of skin and nail disorders. In addition to (video) dermoscopy and sonography, these tools include confocal laser-scanning microscopy (CLSM), optical coherence tomography (OCT) and line-field confocal optical coherence tomography (LC-OCT). The latter procedures initially gained recognition for the early detection of melanocytic and non-melanocytic skin tumors.[1] Recent studies have also reported on the advantageous extension of these non-invasive diagnostic methods to non-oncological areas of dermatology, especially for inflammatory and infectious skin and nail disorders. [2-4] The aim of using CLSM, OCT and LC-OCT is to offer high diagnostic accuracy while reducing invasiveness and time expenditure, which each method offers in slightly different ways.

6.1.1 Confocal laser scanning microscopy (CLSM)

Confocal laser scanning microscopy is already widely established as a diagnostic method in dermatology. Given its high resolution, CLSM allows for accurate and detailed evaluation of skin morphology, similar to histology.[5] CLSM can be considered a helpful non-invasive diagnostic procedure in many areas of dermatology, especially for the differentiation between pigmented and melanocytic lesions,[1] but also for the diagnosis of non-melanoma skin cancer,[6, 7] inflammatory skin diseases,[8, 9] and nail disorders, such as onychomycosis.[4, 10, 11] In

our present studies, the commercially available Vivascope® 1500/3000 Multi-wave device (VivaScope GmbH, München) was used, which takes high-resolution horizontal images of the skin using an 830 nm (max. 35 mW) diode laser in reflective mode. The device has two options for use, the standard VivaScope ® 1500 modality, which also incorporates a dermoscopic camera to navigate, or the handheld device VivaScope ® 3000. The light that is generated by the laser reflects off different structures of the skin, which is then recognized and integrated by the detector to generate images with a field of view of 0.75 x 0.75 mm^2. The natural chromophores of the skin, such as melanin, keratin and cell organelles reflect most of the light and hence appear brighter than the cytoplasm consisting mainly of water, thus appearing dark.[1] A pinhole aperture ensures that signals from only one particular horizontal plane are recognized by the detector, which leads to high cellular resolution (1 - 3 µm) but simultaneously reduced penetration depth (approx. 250 µm).[5] CLSM requires the use of immersion oil and ultrasound gel between the device probe and the surface of the skin to improve index matching and reduce light reflection.

6.1.2 Optical coherence tomography (OCT)

Another well-established non-invasive diagnostic method in dermatology is optical coherence tomography. OCT is especially useful for identifying epithelial skin tumors, such as basal cell carcinomas and distinguishing them from other tumors and precancerous lesions.[1] In addition, previous studies have shown the usefulness of OCT in examining nail morphology and disorders, most notably onychomycosis.[4, 11] Contrary to CLSM, OCT has a higher penetration depth to the lower dermis (around 1500 µm), but lower lateral resolution (7.5 – 10 µm). The images are also based on the degree of light reflection from certain structures of

the skin, with denser structures and chromophores appearing brighter than fluid filled structures. OCT also has a dynamic mode to allow the visualization of blood flow within tissue.[1] In our present studies, the commercially available VivoSight Dx™ System (Michelson Diagnostics Ltd, Maidstone, Kent, UK) was used to generate vertical cross-sections of the skin and nail with a laser source that has a central wavelength of 1305 nm. The field of view is 6 x 6 mm^2. OCT also has an integrated dermoscopic camera that allows navigation on the lesion of interest. In focusing on the skin area of interest, OCT requires the use of a suitable plastic spacer on the handheld probe, whereas an index-matching fluid is not required.

6.1.3 Line-field confocal optical coherence tomography (LC-OCT)

Line-field confocal optical coherence tomography is the most recently developed non-invasive technique that combines the advantages of OCT and CLSM with line-field illumination,[12] in offering high cellular resolution (1.1 - 1.3 µm) with a penetration depth to the mid dermis (500 µm).[1] LC-OCT is a promising tool to aid in the diagnosis of various skin diseases. Previous studies have already shown that LC-OCT offers high diagnostic accuracy for the detection and differentiation of precancerous skin lesions, non-melanocytic and melanocytic skin cancer.[12-15] Other studies have shown its usefulness also for mite skin infestations, [16, 17] autoimmune bullous diseases,[18], as well as inflammatory dermatoses.[3] In our present studies, the LC-OCT system *deepLive*™ (DAMAE Medical, Paris, France) was used, allowing the acquisition of real time horizontal “en-face” and vertical “en-coupe” images and integrating these to generate 3D images. LC-OCT comprises a two-beam interference microscope, which uses an 800 nm supercontinuum laser source and a line-scan camera as photodetector to generate images with axial resolution of 1.1 µm and lateral resolution of 1.3

µm.[1, 3, 14] The device works by measuring time delay and amplitude of light reflected from cutaneous microstructures and offers a field of view of 1.2 mm x 0.5 mm x 0.5 mm (length x width x depth). As in OCT and CLSM, the images are in greyscale, with chromophores and denser structures of the skin appearing bright or hyperreflective and fluid filled cytoplasm appearing darker or hyporeflective. LC-OCT also requires immersion oil between the device probe and the skin surface to ensure better index matching.

6.2 Scientific background – Nail diseases

Nail diseases are widespread, can occur at any age and are commonly seen by dermatologists. They can be quite burdensome to patients and lead to a diminished quality of life, [19] as the nail unit plays an important role in aesthetics and is essential for tactual and protective functions.[20, 21]

6.2.1 Differential diagnosis of nail diseases

Nail disorders have many different etiologies: they can result from infections, occur as part of inflammatory dermatoses (for example atopic dermatitis, psoriasis or lichen planus) or other systemic diseases, have neoplastic origins, can be acquired due to trauma or can be drug induced.[22] Given the similarities in clinical presentation, differentiating between various nail disorders can be challenging, even for experienced dermatologists.[4, 10, 20] However, early detection and accurate diagnosis are crucial for effective therapy and thus for improving the patients' outcome and quality of life in a timely manner.

Existing diagnostic methods for nail disorders include clinical inspection, dermoscopy, microbiological or mycological examinations and histopathology, but all

have certain disadvantages in terms of sensitivity or specificity, duration, cost intensity, effort and invasiveness.[4, 20]

6.2.2 Onychomycosis

As onychomycosis accounts for almost 50% of all nail disorders, [23] it is particularly worth investigating as an infectious nail disease. Patients with onychomycosis not only suffer from aesthetic and functional consequences, for example resulting from nail dystrophy, but can also experience pain.[24, 25] In addition, those affected by onychomycosis have a higher risk of developing other infectious skin diseases in the immediate vicinity, such as erysipelas.[26-28] Accurate diagnosis is therefore essential for effective patient treatment.

The current gold standard methods used for the diagnosis of onychomycosis are potassium hydroxide (KOH) preparation, dermatophyte culture, histopathology with periodic acid-Schiff reaction (PAS)-staining and polymerase chain reaction (PCR).[20] However, these methods all differ with regards to sensitivity, specificity, time expenditure and costs.[4, 29] Of these standard methods, only PCR and culture allow for species differentiation, which can be helpful for selecting the appropriate antifungal treatment.[26]

It is important to note that all standard methods require invasive nail sampling, which can be unpleasant for the patient. Moreover, to obtain an accurate diagnosis, enough sample material must be taken precisely from the most affected area.[4, 30, 31]

6.3 Non-invasive diagnostic methods for nail disorders

Accurate diagnosis of most nail disorders currently requires a nail biopsy or sample, which is subsequently examined using histopathology or microscopy. In the past few years, non-invasive techniques have become increasingly popular for examining nails as well. The major advantage of using non-invasive diagnostic methods for nail disorders is a reduction in the need for invasive nail biopsies while maintaining diagnostic accuracy, thus minimizing the risks of surgical intervention and potential consecutive nail growth disturbances or dystrophies.[32]

6.3.1 CLSM and OCT for the diagnosis of nail disorders

Recent studies have identified CLSM and OCT as valuable tools for studying the morphology of healthy nails and facilitating the diagnosis of nail disorders such as onychomycosis.[4, 10, 11] With CLSM the nail plate can be visualized in horizontal stacks with high cellular resolution. Kaufman et al. discuss how the transparent morphology of nails even enables CLSM to reach deeper layers of the nail plate. [33] As OCT has a high penetration depth, but lower resolution, is it particularly helpful in visualizing the entire nail thickness[11] and the transition to the nailbed.

6.3.2 LC-OCT for the diagnosis of nail disorders and research objective

As LC-OCT combines the advantages of CLSM and OCT by offering high cellular resolution in combination with reasonably high penetration depth, the present study assumed that this method could be useful for studying nails and nail disorders as well. Since no literature existed on LC-OCT and nail disorders at the

beginning of our research, the aim was to examine and qualitatively describe typical morphological features of healthy nails and common nail disorders using LC-OCT. [34] In the first stage, we examined the nails of 16 patients; two patients respectively with healthy nails, leukonychia, subungual hemorrhage, psoriasis, lichen planus, longitudinal melanonychia, subungual melanoma (acral lentiginous melanoma) and onychomycosis.

In the second and major part of our study, we quantitatively evaluated the sensitivity, specificity, positive and negative predictive values, functionality, and efficacy of LC-OCT for the diagnosis of one of the most common nail disorders, onychomycosis, as compared to CLSM, OCT, and current gold standard methods (KOH-preparation, fungal culture, PCR and histopathology).[35] 100 patients with nail dystrophy were recruited, of which 86 were clinically or dermoscopically suspicious for onychomycosis while the remaining 14 suffered from other underlying nail conditions not typical for onychomycosis. The latter patients were included in the control group.

All patients in the study were observed at the Department of Dermatology and Allergy of the Ludwig Maximilian University Hospital in Munich, Germany. Approval for the study was obtained in 2022 from the local ethics committee of the university hospital after submitting an amendment to the original ethics application from 2017 (Project Ref.-Nr. 17-699). In addition, each patient gave written informed consent before inclusion. The study was conducted in accordance with the Declaration of Helsinki and international guidelines for human studies.

7 Summary

7.1 Qualitative Analysis of healthy and pathological nails using LC-OCT

Our studies show that by acquiring vertical, horizontal and 3D images of each nail plate, LC-OCT is a helpful tool for the rapid and non-invasive examination of nail morphology and for the diagnosis of various nail disorders such as onychomycosis, leukonychia, subungual hemorrhage, psoriasis, lichen planus, longitudinal melanonychia and even subungual melanoma.[34, 35] Our findings are summarized in the following.

7.1.1 Healthy nails

Using LC-OCT in vertical mode, healthy nail plates could be characterized by multiple layers, which were differentiated by variations in the intensity of light reflection: a thin hyperreflective surface layer, corresponding to tightly adhered keratinized cells, followed by a slightly thicker homogenous layer of lower reflection, where the corneocytes appeared to be rounder.[34] The third zone from the top was less homogenous, with alternating hyper- and hyporeflective bands and slightly elongated corneocytes. The deepest zone appeared hyporeflective and was followed by the transition to the nail bed (usually around 500 µm) demarcated by a wavelike margin in vertical mode and alternating hypo- and hyperreflective longitudinal corrugated bands, aligned parallel to the digit, in horizontal mode.[34]

We found that the different layers of a healthy nail plate seen with LC-OCT were similar to the zones seen with CLSM.[11] However, earlier reports only distinguished three distinct zones in CLSM, whereas LC-OCT was able to discern the

layers more extensively. Moreover, as LC-OCT has a higher penetration depth, the wavelike transition to the nailbed was clearly delineated, [34] thereby illustrating the distinct longitudinal parallel arrangement of dermal papillae of the nail bed, which is microscopically described as corrugation.[21]

7.1.2 Pathological nails: Leukonychia

Clinically leukonychia was identified as individual white streaks of varying size and shape in the patients' nail plate. A more detailed view with dermoscopy showed that the white streaks were composed of small white dots, corresponding to loose corneocytes merged together. With LC-OCT, leukonychia congruently appeared as hyperreflective bands composed of smaller ovoid structures strung together in vertical mode and bright circular structures in horizontal mode.[34] The disintegrated corneocytes appeared bright in LC-OCT due to stronger light diffraction caused by the switch from a denser substance, in this case dyskeratotic or parakeratotic corneocytes to encompassing air.[34]

The hyperreflective streaks seen in vertical mode could be mistaken for fungus hyphae (as described below), but an important difference was that the streaks were all located on the same level in the nail in leukonychia. The depiction of bright detached round corneocytes in LC-OCT matched the description of leukonychia as seen with CLSM.[11]

7.1.3 Pathological nails: Inflammatory Nail disorders

7.1.3.1 Psoriasis

Clinically, the patients with psoriasis showed dystrophic, rough nails, with slight white to yellow discoloration. Correspondingly, in LC-OCT, clinically mild cases

of psoriasis nails were characterized by a thickened hyperreflective upper layer and an irregular surface with occasional hyporeflective gaps and lamellar splitting.[34] The surface of the nails with more severe clinical presentations did not appear as thickened, but rather showed pitting and trachyonychia, suggesting that the nail matrix had been affected. In these cases, the nail plate appeared highly dystrophic, showing a combination of thin hyper- and hyporeflective ridges, round pinpoint lesions (resembling leukonychia) and gaps. Moreover, the usual nail layering in healthy nails appeared to be disrupted, with dispersed hyperreflective wave-like areas in 3D and vertical mode and bright irregular structures in horizontal mode, most likely corresponding to hyperkeratosis and keratin densification.[34]

7.1.3.2 Lichen planus

Clinically and dermoscopically, the patients with lichen planus showed trachyonychia and longitudinal ridging of the nail plate. Like psoriasis, lichen planus was also characterized by a thickened and rough hyperreflective surface with small hyporeflective spaces resembling a honeycomb pattern.[34] In contrast to psoriasis, the nail plates affected by lichen planus showed alternating hyper- and hyporeflective bands in horizonal mode, embodying the longitudinal ridging that was also observed clinically and is typical for lichen planus.[32, 36] With LC-OCT, the longitudinal ridges could be seen up until a depth of 288 µm. Nail fragility and onychoschizia were also visualized using the 3D mode; the nail surface showed small crater-like pits analogous to the longitudinal ridging in cross-section.[34]

7.1.4 Pathological nails: Onychomycosis

Clinically, onychomycosis presented as thickened, rough nail plates with white to yellow discoloration. Dermoscopically, longitudinal ridging, lamellar splitting, and a brittle nail surface were seen. With LC-OCT, onychomycosis was characterized by hyperreflective filaments corresponding to hyphae of different thickness, size, and degree of branching,[34] which resemble the structures also found using CLSM and in histopathology in fungal nail infections.[4, 37] Horizontal mode in LC-OCT provided a clear depiction of the branching structures, whereas vertical and 3D mode allowed for an overview of the degree of infection and the exact location of the hyphae, suggesting the route of nail invasion (distal lateral subungual onychomycosis, proximal subungual onychomycosis, or white superficial onychomycosis). The hyperreflective filaments typically spanned multiple layers of the nail plate, allowing for the differentiation from leukonychia. Another distinguishing feature of onychomycosis, identified in vertical mode, was a “fuzzy” and irregular looking nail surface compared to the more demarcated and homogenous surface in healthy nails. Moreover, profound cases of onychomycosis characteristically showed clefts surrounded by hyperreflective thready structures in the nail plate, most likely due to the keratinolytic activity of dermatophytes.[34, 37, 38] It appeared troublesome to visualize the nail bed in horizontal mode in patients with onychomycosis, indicating the presence of subungual hyperkeratosis, another characteristic aspect of onychomycosis.

7.1.5 Pathological nails: Differentiating between subungual hemorrhage, melanonychia, and subungual melanoma

An important, but often challenging endeavor is the differentiation between benign longitudinal melanonychia and subungual malignant melanoma.[3, 10, 39] Not uncommonly, subungual hemorrhage can also clinically mimic subungual melanoma due to dark red-brown to black discoloration of the nail plate.[34, 40] However, given the unique characteristics of each one of these diseases visualized by LC-OCT, valuable information can be obtained as to whether the lesion is benign or malignant and if surgery (excision or biopsy) is truly indicated.[34] LC-OCT hence appears of such quality that it can properly support a differential diagnosis.

7.1.5.1 Subungual hemorrhage

In LC-OCT, subungual hemorrhage was visualized as a homogenous hyporeflective band slightly below a hyperreflective surface with lamellar splitting and occasional loose corneocytes.[34]The usual nail layering seen in healthy nails or the transition to the nailbed underneath could not be discerned mostly due to the infiltration of blood.

7.1.5.2 Longitudinal melanonychia vs. subungual malignant melanoma

To differentiate between benign and malignant lesions, LC-OCT was used to image the nail plate and the transition from the nail plate to the proximal nail fold vertically, horizontally and in 3D. As previous studies have shown, typical LC-OCT features for malignant melanomas of the skin include hyperreflective atypical melanocytic cells, irregular honeycomb patterns of the epidermis and

pagetoid spread of suspicious melanocytes.[14] Subungual melanoma and any affected surrounding skin showed comparable features. Pagetoid cells with large irregular nuclei appeared in the pigmented skin surrounding subungual melanoma, whereas the nail plate remnants were characterized by bright globules, which we speculated as melanin nests arising from atypical melanocytes that lost their nuclei in the pagetoid process.[34]

Nails affected by longitudinal melanonychia did not show any of these atypical cells in LC-OCT. Similarly, when scanning the proximal nail fold down to the nail matrix, no pagetoid cells or atypical melanocytes could be identified and the dermal papillae were arranged in a regular pattern surrounded by hyperreflective melanin pigment.[34]

7.2 Quantitative Analysis of LC-OCT for the diagnosis of onychomycosis

7.2.1 Aim and approach

As onychomycosis makes up a large fraction (around 50%) of nail disorders and video dermoscopy, CLSM and OCT have already been identified as useful non-invasive methods for the diagnosis of fungal infections of the nail,[4, 10, 11, 41, 42] our aim was to also evaluate LC-OCT quantitatively for this purpose. Specifically, we calculated the sensitivity, specificity, positive and negative predictive values, functionality, and efficacy of LC-OCT for the diagnosis of onychomycosis, as compared to CLSM, OCT, and current gold standard methods (KOH-preparation, fungal culture, PCR and histopathology).[35]

Our prospective study included a total of 100 patients, of which 86 were clinically suspicious for onychomycosis and 14 with other nail disorders served as controls. The affected nails of each patient were scanned using video dermoscopy, LC-OCT, OCT and CLSM, while the conventional gold-standard methods KOH-preparation, dermatophyte culture, PCR, and histopathology were used as comparative controls. Within our patient collective, different subtypes of onychomycosis[43, 44] were observed clinically and dermoscopically: distal and lateral subungual onychomycosis (DLSO, 57%), totally dystrophic (33%) and mixed pattern (DLSO and superficial, 10%) onychomycosis.[35]

Dermoscopically, the following signs were indicative for fungal infection of the nail: white to yellow discoloration, subungual hyperkeratosis, onychodystrophy, onycholysis, jagged edges and/or longitudinal striae.[35, 41, 42] Nails exhibiting hyperreflective thread-like structures or bright spore-like aggregates in LC-OCT, OCT and CLSM were classified as positive for onychomycosis.[35] Each image was evaluated by two independent examiners proficient in non-invasive imaging and dermatological nail diseases before a consensus about the diagnosis was reached. Following non-invasive imaging, a nail sample was taken and examined using KOH-preparation and direct microscopy, fungal culture, PCR or histopathology (four patients that could not be examined with PCR because of cost constraints). A positive finding in any of these four confirmed the diagnosis of onychomycosis, which was used as the comparative benchmark to calculate the sensitivity, specificity, negative and positive predictive values for each diagnostic method (except histopathology as it was only performed in four patients).

7.2.2 Statistical results: LC-OCT compared to other methods

	Number of tests per-formed	Positive result (%)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
LC-OCT	100	58	92.2	77.6	81	90.5
OCT	100	69	86.3	49.0	63.8	77.4
CLSM	100	49	78.4	81.6	81.6	78.4
PCR	93	46.2	87.8	100	100	88.0
Culture	98	13.3	26	100	100	56
Native/KOH	98	37.8	74	100	100	79

Table 1 *Overview of the results for each diagnostic method including the total number of positive results, the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV).[35]*

We found that LC-OCT had the highest sensitivity (92.2%) of all studied methods, followed by PCR (87.8%), OCT (86.3%), CLSM (78.4%), KOH-preparation (74%), and fungal culture (26%).[35] The sensitivity showed how accurate the study method was in correctly identifying onychomycosis in affected patients.

The specificity indicated how well the study method recognized patients without onychomycosis. As PCR, fungal culture, histopathology, and KOH-preparation were defined as gold standard methods their specificity was naturally set to 100%. LC-OCT had a high specificity of 77.6%, which was slightly lower than that of CLSM with 81.6%, but much higher than OCT (49.0%).[35]

The positive predictive value (PPV) reflected the likelihood that a patient with a positive test result actually had onychomycosis. The gold standard methods inherently had a PPV of 100%, while LC-OCT and CLSM were both similarly high with about 81% and OCT was lower with 63.8%.[35]

LC-OCT also had the highest negative predictive value (NPV) of all methods with 90.5%, which signified a high probability that a patient with a negative test result in LC-OCT had healthy nails.[35] It was closely followed by PCR with 88%, while the other methods had lower NPVs.

7.2.3 Duration, costs, efforts, species differentiation: LC-OCT compared to other methods

A major advantage of the non-invasive imaging techniques for detecting onychomycosis is the rapid analysis of each nail and short time taken to reach a diagnosis without the need of taking and analyzing a physical sample. LC-OCT only took about 5 minutes to scan the entire nail plate, acquiring horizontal, vertical and 3D images while assessing the images in real time. OCT was equally quick and practical. CLSM took slightly longer with approximately 10 minutes and was somewhat challenging to use on the nail plate without an integrated dermatoscopic camera in the hand-held device.[35] While KOH-preparation with subsequent microscopy only takes a few minutes, it can take days to weeks for PCR, histopathology and culture until a final diagnosis is determined. While the costs are low, these techniques require high levels of staff expertise. A drawback of LC-OCT, CLSM and OCT is that they have high acquisition expenses: an LC-OCT device costs approximately 150,000€, CLSM around 70,000 – 185,000€ and OCT around 85,000€. It should be noted, however, that the main reason for acquiring these devices would be to aid the diagnosis of skin cancer, such as

basal cell carcinoma,[13] and nail analysis would be an add-on advantage.[35] A further drawback of LC-OCT, OCT and CLSM is that they cannot be used to differentiate between different fungal species, which is possible with PCR and culture.

7.3 Conclusion

The present study demonstrates that LC-OCT is a beneficial method for the quick and non-invasive examination of the nail-plate to assist in the differential diagnosis of various nail disorders such as onychomycosis, leukonychia, subungual hemorrhage, psoriasis, lichen planus, longitudinal melanonychia and even subungual melanoma.[34, 35] The in vivo application of LC-OCT is advantageous, as it could prevent unnecessary biopsies since the entire nail unit can be scanned within minutes to look for pathological changes, contrary to just one specific section of the nail, as in histology, PCR and culture. This implies that both the patients' diagnosis and treatment could be determined in just a single consultation, saving time and resources.[35]

Despite the small surface area and slippiness of the nail plate, we found that the LC-OCT hand-held probe allows for good and rapid image acquisition, while the live-images could simultaneously be analyzed on the monitor. The integrated dermoscopic camera also allows navigation to the clinically most affected areas and other regions of interest. The 3D-mode is especially useful for visualizing the nail plate, by simultaneously acquiring vertical and horizontal "stacks" of images that together form a comprehensive 3D-block showing the most pertinent pathological differences versus healthy nails. While CLSM has also been used to describe distinct features of healthy and pathological nails similar to LC-OCT, it only offers

horizontal cross-sections of the nail plate and has limited penetration depth.[4, 10, 11] OCT on the other hand has lower resolution with a high penetration depth, making it slightly more challenging to accurately differentiate between the different nail conditions.

We believe that the possibility of scanning the entire nail plate and acquiring high-resolution horizontal, vertical and 3D images is the reason why LC-OCT offered a higher sensitivity and NPV than the gold-standard and other imaging techniques for diagnosing onychomycosis.[35] KOH-preparation, histopathology, culture and PCR are all greatly dependent on accurate and correct nail sampling of the most affected area, while this is less the case for LC-OCT, OCT and CLSM.[35] Incorrect nail sampling could lead to a higher number of false negatives, resulting in lower sensitivity and NPV.[35]

A drawback of LC-OCT is the high acquisition cost and the inability to differentiate between fungal species for treatment optimization of onychomycosis. Moreover, LC-OCT also requires training and experience prior to its use, also on nails. Future developments of LC-OCT should aim for an even higher penetration depth, which would be especially helpful to better visualize the nail matrix (e.g. in differentiating between subungual melanoma and longitudinal melanonychia) and the nail bed in inflammatory nail diseases (e.g. psoriasis and lichen planus) to identify inflammatory cell infiltration in this region.[10] It could also help to identify fungal hyphae deeper in the nail plate even in hyperkeratotic nails.[35] While this study comprehensively examined LC-OCT for the diagnosis of onychomycosis in a large patient collective, more extensive investigation is needed to further evaluate its sensitivity and specificity against histology of a nail biopsy in the diagnosis of benign vs. malignant melanocytic nail lesions.

Nevertheless, our first qualitative and quantitative findings show that LC-OCT can be a useful addition to the in-vivo diagnosis of nail diseases, onychomycosis in particular, which could help to speed up diagnosis and to avoid unnecessary biopsies bearing the risk of nail dystrophy, and thereby to improve patient care.

8 Zusammenfassung

8.1 Qualitative Analyse von gesunden und pathologischen Nägeln mittels LC-OCT

In unseren Studien konnten wir zeigen, dass LC-OCT mit vertikalen, horizontalen und 3D-Aufnahmen der Nagelplatte ein nützliches Instrument für die schnelle und nicht-invasive Untersuchung der Nagelmorphologie und für die Diagnose verschiedener Nagelerkrankungen wie Onychomykose, Leukonychien, subunguale Blutungen, Psoriasis, Lichen planus, longitudinale Melanonychien und sogar subunguale Melanome ist. [34, 35] Die Ergebnisse sind im Folgenden zusammengefasst.

8.1.1 Gesunde Nägel

Im vertikalen Modus konnten gesunde Nagelplatten in der LC-OCT durch mehrere Schichten charakterisiert werden, die sich durch unterschiedliche Intensität der Lichtreflexion abgrenzten: eine dünne hyperreflektive Oberfläche mit fest anhaftenden keratinisierten Zellen, gefolgt von einer etwas dickeren homogenen Schicht mit geringerer Reflexion, in der die Korneozyten runder erschienen.[34] Die dritte Zone von oben war weniger homogen, mit abwechselnd hyper- und hyporeflektiven Bändern und leicht verlängerten Korneozyten. Die tiefste Zone erschien hyporeflektiv, gefolgt vom Übergang zum Nagelbett (in der Regel bei ca. 500 µm), der vertikal durch einen wellenförmigen Rand und horizontal durch abwechselnd hypo- und hyperreflektive längs geriffelte Bänder, die parallel zum Finger ausgerichtet waren, abgegrenzt war.[34]

Die verschiedenen Schichten der gesunden Nagelplatte, die mit LC-OCT sichtbar waren, ähnelten den Zonen, die in früheren Studien mit KLM beschrieben wurden. [11] Mit KLM konnten jedoch nur drei verschiedene Zonen unterschieden werden, während LC-OCT die Schichten umfassender erkennen konnte. Da die LC-OCT zudem eine höhere Eindringtiefe hat, konnte der wellenförmige Übergang zum Nagelbett deutlich dargestellt werden,[34] der der charakteristischen längsparallelen Anordnung der dermalen Papillen des Nagelbetts entspricht.[21]

8.1.2 Pathologische Nägel: Leukonychie

Leukonychien präsentierten sich klinisch als vereinzelte weiße Streifen unterschiedlicher Größe und Form in der Nagelplatte. Eine genauere Betrachtung mit der Dermatoskopie zeigte, dass die weißen Streifen aus mehreren kleinen weißen Punkten bestanden, die losgelöste jedoch gruppiert stehende Korneozyten entsprachen. Damit übereinstimmend, erschienen die Leukonychien in der LC-OCT als hyperreflektive Bänder, die sich im vertikalen Modus aus aneinandergereihten kleinen ovalen Strukturen und im horizontalen Modus aus hellen kreisförmigen Strukturen zusammensetzten.[34] Die desintegrierten Korneozyten erschienen in der LC-OCT hell, was auf eine stärkere Lichtbrechung zurückzuführen ist, die durch den Wechsel von einer dichteren Substanz, in diesem Fall die dyskeratotischen oder parakeratotischen Korneozyten, zu der sie umgebenden Luft verursacht wird.[34]

Die hyperreflektiven Streifen, die im vertikalen Modus zu sehen waren, könnten mit Pilzhyphen (wie unten beschrieben) verwechselt werden. Ein wichtiger Unterschied ist allerdings, dass sich die Streifen bei Leukonychie alle auf der glei-

chen Ebene des Nagels befanden. Die Darstellung der hellen, abgelösten, runden Korneozyten mit LC-OCT entsprach der Beschreibung der Leukonychie, wie sie auch mit KLM zu sehen war.[11]

8.1.3 Pathologische Nägel: Entzündliche Nagelerkrankungen

8.1.3.1 Psoriasis

Klinisch zeigten die Patienten mit Psoriasis dystrophische, raue Nägel mit einer leichten weißen bis gelben Verfärbung. Dementsprechend waren in der LC-OCT leicht ausgeprägte klinische Fälle durch eine verdickte hyperreflektive und unregelmäßige Oberfläche mit gelegentlichen hyporeflektiven Lücken und lamellärer Aufspaltung gekennzeichnet.[33] Die Nageloberfläche bei schwereren klinischen Erscheinungen wirkte nicht stark verdickt, sondern zeigte Grübchen und Trachyonychie, was auf eine Beteiligung der Nagelmatrix hindeutete. In diesen Fällen erschien die Nagelplatte stark dystrophisch mit einer Kombination aus feinen hyper- und hyporeflektiven Rillen, runden punktförmigen Läsionen (ähnlich wie Leukonychien) und Lücken. Darüber hinaus schien die bei gesunden Nägeln übliche Nagelschichtung gestört zu sein, mit verstreuten hyperreflektiven, wellenförmigen Bereichen im 3D- und vertikalen Modus und hellen, unregelmäßigen Strukturen im horizontalen Modus, was höchstwahrscheinlich auf eine Hyperkeratose und eine Keratinverdichtung zurückzuführen ist.[33]

8.1.3.2 Lichen planus

Klinisch und dermatoskopisch zeigten die Patienten mit Lichen planus eine Trachyonychie und Längsrillen der Nagelplatte. Wie bei Psoriasis war auch Lichen planus durch eine verdickte und raue hyperreflektive Oberfläche mit kleinen

hyporeflektiven Zwischenräumen gekennzeichnet, die einem Wabenmuster ähnelten.[33] Im Gegensatz zur Psoriasis zeigten die Nagelplatten bei Lichen planus im horizontalen Modus abwechselnd hyper- und hyporeflektive Bänder, die der Rillung der Nagelplatte entsprachen. Diese wurde auch klinisch beobachtet und ist für Lichen planus charakteristisch.[32, 35] Mit LC-OCT konnten die Rillen bis zu einer Tiefe von 288 µm beobachtet werden. Nagelbrüchigkeit und Onychoschisis wurden ebenfalls mit dem 3D-Modus beobachtet; die Nageloberfläche zeigte kleine kraterartige Vertiefungen, die den Längsrillen im Querschnitt entsprachen.[33]

8.1.4 Pathologische Nägel: Onychomykose

Klinisch präsentierte sich die Onychomykose mit verdickten, rauen Nagelplatten und weißer bis gelber Verfärbung. Dermatoskopisch waren Längsrillen, lamelläre Risse und eine brüchige Nageloberfläche zu erkennen. In der LC-OCT war die Onychomykose durch hyperreflektive Fäden charakterisiert, die Hyphen unterschiedlicher Dicke, Größe und Verzweigung entsprachen.[33] Diese Strukturen ähneln denen, die mit KLM und in der Histopathologie bei Nagelpilzinfektionen gefunden wurden. [4, 36] Der horizontale Modus der LC-OCT lieferte eine klare Darstellung der Verzweigungsstrukturen, während der vertikale und der 3D-Modus einen Überblick über das Ausmaß der Infektion und die genaue Lage der Hyphen ermöglichten, was auf den Weg der Nagelinvasion schließen lässt (distale laterale subunguale Onychomykose, proximale subunguale Onychomykose oder weiße oberflächliche Onychomykose). Die hyperreflektiven Fäden erstreckten sich typischerweise über mehrere Schichten der Nagelplatte, was eine Abgrenzung zur Leukonychie ermöglicht. Im vertikalen Modus war ein weiteres Un-

terscheidungsmerkmal der Onychomykose eine „verschwommene“ und unregelmäßig aussehende Nageloberfläche im Vergleich zu der besser abgegrenzten und homogenen Oberfläche gesunder Nägel. Darüber hinaus wiesen stärker ausgeprägte Fälle von Onychomykose charakteristischerweise Spalten auf, die von hyperreflektiven, fadenförmigen Strukturen in der Nagelplatte umgeben waren und höchstwahrscheinlich auf die keratinolytische Aktivität der Dermatophyten zurückzuführen waren.[33, 36, 37] Die subunguale Hyperkeratose, ein weiteres charakteristisches Merkmal der Onychomykose, erschwerte die Darstellung des Nagelbetts im horizontalen Modus.

8.1.5 Pathologische Nägel: Unterscheidung zwischen subungualen Hämatomen, Melanonychien und subungualen Melanomen

Eine wichtige, aber oft schwierige Aufgabe ist die Unterscheidung zwischen einer gutartigen longitudinalen Melanonychie und einem subungualen malignen Melanom.[3, 10, 38] Nicht selten kann ein subunguales Hämatom aufgrund der dunkelrot-braunen bis schwarzen Verfärbung der Nagelplatte auch klinisch ein subunguales Melanom vortäuschen.[33, 39] Die LC-OCT kann jedoch einzigartige Merkmale jeder dieser Erkrankungen erkennen und so wertvolle Informationen darüber liefern, ob die Läsion gutartig oder bösartig ist und ob ein chirurgischer Eingriff (Exzision oder Biopsie) wirklich indiziert ist. [33] Die LC-OCT scheint daher in der Lage zu sein eine entsprechende Differentialdiagnose zu unterstützen.

8.1.5.1 Subunguales Hämatom

In der LC-OCT konnte ein subunguales Hämatom als homogenes hyporeflektives Band knapp unterhalb einer hyperreflektiven Oberfläche mit lamellärer Aufspal-

tung und gelegentlich lockeren Korneozyten erkannt werden.[33] Die bei gesunden Nägeln übliche Nagelschichtung oder der Übergang zum darunter liegenden Nagelbett konnte meist aufgrund der Blutinfiltration nicht erkannt werden.

8.1.5.2 Longitudinale Melanonychie vs. subunguales malignes Melanom

Zur Unterscheidung zwischen benignen und malignen Läsionen wurde die LC-OCT eingesetzt, um die Nagelplatte und den Übergang von der Nagelplatte zur proximalen Nagelfalz vertikal, horizontal und in 3D darzustellen. Wie frühere Studien gezeigt haben, gehören atypische melanozytäre Zellen, unregelmäßige Wabenmuster der Epidermis und eine pagetoide Ausbreitung verdächtiger Melanozyten zu den typischen LC-OCT-Merkmalen für maligne Melanome der Haut.[14] Das subunguale Melanom und die betroffene umgebende Haut zeigten vergleichbare Merkmale. In der pigmentierten Haut, die das subunguale Melanom umgab, traten pagetoide Zellen mit großen, unregelmäßigen Kernen auf, während die Reste der Nagelplatte durch helle runde Strukturen gekennzeichnet waren. Bei diesen Strukturen handelte es sich am ehesten um Melaninnester, die von atypischen Melanozyten stammen, die ihre Kerne im pagetoiden Verlauf verloren haben.[33]

Nägel, die von longitudinaler Melanonychie betroffen waren, wiesen in der LC-OCT keine dieser atypischen Zellen auf. Auch beim Scannen der proximalen Nagelfalz bis hinunter zur Nagelmatrix konnten keine pagetoiden Zellen oder atypischen Melanozyten identifiziert werden und die dermalen Papillen waren in einem regelmäßigen Muster angeordnet, das von hyperreflektivem Melaninpigment umgeben war.[33]

8.2 Quantitative Analyse von LC-OCT für die Diagnose von Onychomykose

8.2.1 Ziel und Vorgehensweise

Da die Onychomykose einen großen Teil (etwa 50%) der Nagelerkrankungen ausmacht und Videodermatoskopie, KLM und OCT bereits als nützliche nichtinvasive Methoden für die Diagnose von Pilzinfektionen des Nagels identifiziert wurden,[4, 10, 11, 40, 41] war es unser Ziel, LC-OCT auch quantitativ für diesen Zweck zu bewerten. Konkret ermittelten wir die Sensitivität und Spezifität, sowie den positiven und negativen Vorhersagewert, Funktionalität und Wirksamkeit der LC-OCT für die Diagnose von Onychomykose im Vergleich zu KLM, OCT und den derzeitigen Goldstandardmethoden (KOH-Präparation, Pilzkultur, PCR und Histopathologie).[34]

Unsere vorliegende Studie umfasste insgesamt 100 Patienten, von denen 86 klinisch verdächtig auf Onychomykose waren und 14 mit anderen Nagelerkrankungen als Kontrollen dienten. Die betroffenen Nägel der Patienten wurden mit Hilfe von Videodermatoskopie, LC-OCT, OCT und KLM gescannt, während die konventionellen Goldstandard-Methoden KOH-Präparation, Dermatophytenkultur, PCR und Histopathologie als Vergleichskontrollen verwendet wurden. Innerhalb unseres Patientenkollektivs wurden klinisch und dermatoskopisch verschiedene Subtypen der Onychomykose, [42, 43] beobachtet: distale und laterale subunguale Onychomykose (DLSO, 57%), vollständig dystrophische (33%) und gemischte Onychomykose (DLSO und oberflächliche, 10%).[34]

Dermatoskopisch wiesen die folgenden Anzeichen auf eine Pilzinfektion des Nagels hin: weiße bis gelbe Verfärbung, subunguale Hyperkeratose, Onychodystrophie, Onycholyse, gezackte Ränder und/oder Längsstreifen. [34, 40, 41] Nägel, die in der LC-OCT, OCT und KLM hyperreflektive fadenförmige Strukturen oder helle sporenähnliche Aggregate aufwiesen, wurden positiv für Onychomykose bewertet.[34] Jedes Bild wurde von zwei unabhängigen Untersuchern analysiert, die sich mit nichtinvasiver Bildgebung und dermatologischen Nagelerkrankungen auskannten, bevor ein Konsens über die Diagnose erreicht wurde. Im Anschluss an die nichtinvasive Bildgebung wurde eine Nagelprobe entnommen und mittels KOH-Präparation und direkter Mikroskopie, Pilzkultur, PCR oder Histopathologie untersucht (vier Patienten konnten aus Kostengründen nicht mit PCR untersucht werden). Ein positives Ergebnis mit einer dieser vier Methoden bestätigte die Diagnose einer Onychomykose und diente als Bezugswert für die Berechnung der Sensitivität, Spezifität, negativen und positiven Vorhersagewerte für jede Diagnosemethode (mit Ausnahme der Histopathologie, da diese nur bei vier Patienten durchgeführt wurde).

8.2.2 Statistische Auswertung: LC-OCT im Vergleich zu anderen Testmethoden

	Anzahl der durchgeführten Tests	Positives Ergebnis (%)	Sensitivität (%)	Spezifität (%)	PPV (%)	NPV (%)
LC-OCT	100	58	92.2	77.6	81	90.5
OCT	100	69	86.3	49.0	63.8	77.4
KLM	100	49	78.4	81.6	81.6	78.4
PCR	93	46.2	87.8	100	100	88.0
Kultur	98	13.3	26	100	100	56
Nativ/KOH	98	37.8	74	100	100	79

***Tabelle 1**: Überblick über die Ergebnisse der einzelnen Diagnosemethoden, einschließlich der Gesamtzahl der positiven Ergebnisse, der Sensitivität, der Spezifität, des positiven prädiktiven Werts (PPV) und des negativen prädiktiven Werts (NPV).[34]*

Wir haben festgestellt, dass die LC-OCT die höchste Sensitivität (92,2 %) aller untersuchten Methoden aufwies, gefolgt von PCR (87,8 %), OCT (86,3 %), KLM (78,4 %), KOH-Präparation (74 %) und Pilzkultur (26 %).[34] Die Sensitivität galt als Maßstab, wie genau die Untersuchungsmethode die Onychomykose bei den betroffenen Patienten korrekt identifizieren konnte.

Die Spezifität zeigte, wie gut die Untersuchungsmethode Patienten ohne Onychomykose erkennen konnte. Da PCR, Pilzkultur, Histopathologie und KOH-Präparation als Goldstandard-Methoden definiert wurden, wurde ihre Spezifität entsprechend auf 100 % festgelegt. Die LC-OCT hatte eine hohe Spezifität von

77,6 %, etwas niedriger als KLM (81,6 %), aber deutlich höher als OCT (49,0 %).[34]

Der positive prädiktive Wert (PPV) spiegelt die Wahrscheinlichkeit wider, dass ein Patient mit einem positiven Testergebnis tatsächlich eine Onychomykose hat. Die Goldstandard-Methoden hatten von Natur aus einen PPV von 100 %, während LC-OCT und KLM mit etwa 81 % einen ähnlich hohen Wert aufwiesen und OCT mit 63,8 % einen deutlich niedrigeren.

Die LC-OCT hatte auch den höchsten negativen prädiktiven Wert (NPV) aller Methoden mit 90,5 %, was eine hohe Wahrscheinlichkeit bedeutet, dass ein Patient mit einem negativen LC-OCT Testergebnis gesunde Nägel hatte,[34] dicht gefolgt von der PCR mit 88 %, während die anderen Methoden niedrigere NPVs hatten.

8.2.3 Dauer, Kosten, Aufwand, Differenzierung der Pilzarten: LC-OCT im Vergleich zu anderen Methoden

Ein großer Vorteil der nicht-invasiven Bildgebungsverfahren für die Erkennung der Onychomykose ist die schnelle Analyse jedes einzelnen Nagels und die kurze Zeit, die für eine Diagnosestellung benötigt wird, ohne dass eine Nagelprobe entnommen und analysiert werden muss. Innerhalb von 5 Minuten konnte mit der LC-OCT die gesamte Nagelplatte gescannt werden, wobei horizontale, vertikale und 3D-Bilder aufgenommen und in Echtzeit ausgewertet wurden. OCT war ebenso schnell und praktisch. KLM dauerte mit 10 Minuten etwas länger und war, ohne eine im Handgerät integrierte dermatoskopische Kamera, etwas schwierig auf der Nagelplatte anzuwenden.[34] Während die KOH-Präparation mit anschließender Mikroskopie nur wenige Minuten in Anspruch nimmt, kann es bei

der PCR, Histopathologie und Kultur Tage bis Wochen dauern, bis eine endgültige Diagnose feststeht. Auch wenn die Kosten gering sind, erfordern diese Techniken ein hohes Maß an Fachwissen des Personals. Ein Nachteil der LC-OCT, KLM und OCT sind die hohen Anschaffungskosten: ein LC-OCT-Gerät kostet etwa 150.000 €, KLM etwa 70.000 - 185.000 € und OCT etwa 85.000 €. Es ist jedoch darauf hinzuweisen, dass der Hauptgrund für die Anschaffung dieser Geräte die Unterstützung der Diagnose von Hautkrebs, z. B. Basalzellkarzinom, ist [13] und dass die Nagelanalyse ein zusätzlicher Nutzen wäre.[34] Ein weiterer Nachteil von LC-OCT, OCT und KLM ist, dass sie nicht zur Unterscheidung zwischen verschiedenen Pilzarten verwendet werden können, was jedoch mit PCR und Kultur schon möglich ist.

8.3 Schlussfolgerung

Die vorliegende Studie zeigt, dass die LC-OCT eine wertvolle Methode für die schnelle und nicht-invasive Untersuchung der Nagelplatte ist, um die Differentialdiagnose verschiedener Nagelerkrankungen wie Onychomykose, Leukonychie, subunguales Hämatom, Psoriasis, Lichen planus, longitudinale Melanonychie und sogar subunguales Melanom zu unterstützen. [33, 34] Durch die vorteilhafte in-vivo-Anwendung der LC-OCT könnten unnötige Biopsien vermieden werden, da im Gegensatz zur Histologie, PCR und Kultur, die nur einen bestimmten Abschnitt des Nagels untersuchen, der gesamte Nagel innerhalb von Minuten auf pathologische Veränderungen gescannt werden kann. Somit könnten sowohl die Diagnose als auch die Behandlung des Patienten in einem einzigen Arztbesuch festgelegt werden, was Zeit und Ressourcen spart.[34]

Trotz der kleinen glatten Oberfläche der Nagelplatte ermöglicht die LC-OCT-Handsonde eine gute und schnelle Bildaufnahme, während die Live-Bilder gleichzeitig auf dem Monitor analysiert werden können. Die integrierte dermatoskopische Kamera ermöglicht außerdem eine Navigation zu den klinisch am stärksten betroffenen Bereichen und anderen interessanten Regionen. Der 3D-Modus ist besonders hilfreich für die Visualisierung der Nagelplatte, da gleichzeitig vertikale und horizontale „Stacks“ von Bildern aufgenommen werden, die zusammen einen umfassenden 3D-Block bilden, der wiederum die wichtigsten pathologischen Unterschiede zu gesunden Nägeln zeigt. Ähnlich wie LC-OCT wurde auch KLM bereits eingesetzt, um die unterschiedlichen Merkmale von gesunden und pathologischen Nägeln darzustellen. KLM liefert allerdings nur horizontale Querschnitte der Nagelplatte und hat eine begrenzte Eindringtiefe. [4, 10, 11] OCT hingegen hat eine geringere Auflösung mit einer hohen Eindringtiefe, was eine genaue Abgrenzung zwischen den verschiedenen Nagelerkrankungen etwas schwieriger macht.

Die Möglichkeit, mit LC-OCT die gesamte Nagelplatte zu scannen und hochauflösende horizontale, vertikale und 3D-Bilder zu erzeugen, könnte ein Grund dafür sein, dass LC-OCT eine höhere Sensitivität und einen höheren NPV als der Goldstandard und andere bildgebende Verfahren für die Diagnose von Onychomykose bietet.[34] KOH-Präparation, Histopathologie, Kultur und PCR hängen in hohem Maße von der genauen und korrekten Entnahme von Nagelproben aus dem am stärksten betroffenen Bereich ab, während dies bei LC-OCT, OCT und KLM nicht der Fall ist.[34] Eine falsche Entnahme von Nagelproben könnte zu einer höheren Anzahl falsch negativer Ergebnisse führen, was eine geringere Sensitivität und einen niedrigeren NPV zur Folge hätte.[34]

Ein Nachteil der LC-OCT sind die hohen Anschaffungskosten und die Unfähigkeit zwischen Pilzarten zu unterscheiden, um die Behandlung der Onychomykose zu optimieren. Darüber hinaus erfordert die LC-OCT spezialisiertes Training und Erfahrung vor ihrer Anwendung, auch bei Nägeln. Zukünftige Entwicklungen der LC-OCT sollten auf eine noch höhere Eindringtiefe abzielen. Dies wäre besonders hilfreich, um die Nagelmatrix (z. B. bei der Unterscheidung zwischen subungualem Melanom und longitudinaler Melanonychie) und das Nagelbett bei entzündlichen Nagelerkrankungen (z. B. Psoriasis und Lichen planus) zu visualisieren, um so entzündliche Zellinfiltrationen in dieser Region zu erkennen. [10] Es könnte auch dazu beitragen Pilzhyphen tiefer in der Nagelplatte und bei hyperkeratotischen Nägeln zu erkennen.[34] Obwohl die vorliegende Studie die LC-OCT für die Diagnose von Onychomykose in einem großen Patientenkollektiv umfassend untersuchte, sind weitere Untersuchungen erforderlich, um die Sensitivität und Spezifität des Verfahrens im Vergleich zur Histologie einer Nagelbiopsie bei der Diagnose von benignen und malignen melanozytären Nagelläsionen zu evaluieren.

Dennoch zeigen unsere ersten qualitativen und quantitativen Ergebnisse, dass die LC-OCT eine nützliche Ergänzung der In-vivo-Diagnosik von Nagelerkrankungen, insbesondere der Onychomykose, sein kann. Sie könnte die Diagnosestellung beschleunigen und dazu beitragen unnötige Biopsien mit dem Risiko einer Nageldystrophie zu vermeiden und damit die Patientenversorgung zu verbessern.

9 Contribution to the publications

9.1 Contribution to paper 1

My contribution to the paper “Line-field confocal optical coherence tomography, a novel non-invasive tool for the diagnosis of onychomycosis” included planning the study, recruitment of patients with onychomycosis and other nail diseases, the main data collection (i.e. the acquisition and analysis of the nail images using LC-OCT, CLSM, OCT and video dermoscopy), image processing and fine tuning, statistical analysis of the data and writing the manuscript.

To plan the study, I wrote and submitted the study protocol and an amendment to the original ethics application from 2017 (Project number 17-0699), which was subsequently approved by the local ethics committee of the university hospital prior to starting my data collection in 2022. I recruited 100 test subjects at the Department of Dermatology and Allergy at the LMU clinic and examined the affected nails using the four non-invasive diagnostic devices LC-OCT, CLSM, OCT and video dermoscopy.

I evaluated the images together with my supervisor Prof. Dr. med. Elke Sattler to reach a consensus regarding the diagnosis of each patient. Subsequently I carried out the statistical analysis and wrote the manuscript. Following revision from my co-authors I revised the paper and submitted it to the relevant journal.

9.2 Contribution to paper 2 (Letter to the editor, see Appendix)

Both paper 1 and the letter to the editor “Line-field confocal optical coherence tomography (LC-OCT) for the in vivo examination of nails: Analysis of typical features for the differentiation of nail disorders” (attached as appendix) were based on the same clinical study as described above. While the methods were the same, the focus for the letter lay on nail disorders other than onychomycosis.

As for paper 1, my contributions thus included the planning of the study (with study protocol and ethics application), the recruitment of patients at the Clinic for Dermatology and Allergy at LMU, the data collection (i.e. the acquisition and analysis of the images of nails using LC-OCT and video dermoscopy), the processing of the images and the writing of the manuscript. I evaluated the images in collaboration with my supervisor, Prof. Dr. med. Elke Sattler and proceeded to draft the manuscript. I then revised it in accordance with the suggestions provided by the co-authors and submitted it to the relevant journal.

10 Paper I

Eijkenboom QL, Daxenberger F, Gust C, Hartmann D, Guertler A, Steckmeier S, Deussing M, French LE, Welzel J, Schuh S, Sattler EC. **Konfokale Line-Field optische Kohärenztomographie, ein innovatives nicht invasives Instrument zur Diagnose der Onychomykose: Line-field confocal optical coherence tomography, a novel non-invasive tool for the diagnosis of onychomycosis.** *J Dtsch Dermatol Ges.* 2024 Mar;22(3):367-376. doi: 10.1111/ddg.15310_g. PMID: 38450988.

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ORIGINAL ARTICLE

Line-field confocal optical coherence tomography, a novel non-invasive tool for the diagnosis of onychomycosis

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Summary

Background and objectives: Onychomycosis is common and important to distinguish from other nail diseases. Rapid and accurate diagnosis is necessary for optimal patient treatment and outcome. Non-invasive diagnostic tools have increasing potential for nail diseases including onychomycosis. This study evaluated line-field confocal optical coherence tomography (LC-OCT) as a rapid non-invasive tool for diagnosing onychomycosis as compared to confocal laser scanning microscopy (CLSM), optical coherence tomography (OCT), and conventional methods.

Patients and Methods: In this prospective study 86 patients with clinically suspected onychomycosis and 14 controls were examined using LC-OCT, OCT, and CLSM. KOH-preparation, fungal culture, PCR, and histopathology were used as comparative conventional methods.

Results: LC-OCT had the highest sensitivity and negative predictive value of all methods used, closely followed by PCR and OCT. Specificity and positive predictive value of LC-OCT were as high as with CLSM, while OCT scored much lower. The gold standard technique, fungal culture, showed the lowest sensitivity and negative predictive value. Only PCR and culture allowed species differentiation.

Conclusions: LC-OCT enables quick and non-invasive detection of onychomycosis, with advantages over CLSM and OCT, and similar diagnostic accuracy to PCR but lacking species differentiation. For accurate nail examination, LC-OCT requires well-trained and experienced operators.

KEYWORDS
CLSM, LC-OCT, nail disorders, non-invasive diagnostics, OCT, Onychomycosis

INTRODUCTION

Nail diseases are common and often quite troublesome for the patient.[1] Accounting for nearly 50% of all nail diseases,[2] onychomycosis is widespread, but sometimes difficult to distinguish from other nail disorders like psoriasis, lichen planus, eczematous nails, or onychodystrophy.[1,3] Patients with onychomycosis are prone to additional infectious diseases near the affected site (e.g. erysipelas)[4–6] and can suffer from psychological distress due to physical appearance, nail dystrophy, and pain.[7,8] Accurate diagnosis is essential for optimal treatment, improving patient quality of life and minimizing healthcare costs.[9,10]

The last two authors contributed equally to this work.

Potassium hydroxide (KOH) preparation, dermatophyte culture, histopathology with periodic acid-Schiff reaction (PAS)-staining, and polymerase chain reaction (PCR) are the standard methods for diagnosing onychomycosis.[11] However, each method has weaknesses regarding sensitivity, specificity, time exposure, invasiveness, and costs.[1,12]

Non-invasive diagnostic tools such as video dermatoscopy (VDS), confocal laser scanning microscopy (CLSM) and optical coherence tomography (OCT) have become increasingly important in the diagnosis of onychomycosis.[1,13–16] In recent investigations, our study group has found that line-field confocal optical coherence tomography (LC-OCT) also shows potential for the in-vivo diagnosis of nail conditions including onychomycosis. Advantages of imaging tools over conventional methods are the possibility to obtain quick real-time images of the entire nail and avoid nail sampling to support the diagnosis. LC-OCT appears promising for diagnosing onychomycosis as it combines the advantages of CLSM and OCT resulting in high cellular resolution combined with penetration depth and the generation of 3D nail-plate images.[17] This study evaluates the sensitivity, specificity, positive and negative predictive values, functionality, and efficiency of LC-OCT for the diagnosis of onychomycosis as compared to CLSM, OCT, and current gold standard methods (KOH-preparation, fungal culture, and PCR) of diagnosis.

PATIENTS AND METHODS

Patients

The study included 100 patients observed at the Department of Dermatology and Allergy of the Ludwig Maximilian University Hospital in Munich, Germany. Figure 1 shows the patient distribution in each group (onychomycosis vs. control). There were no limitations in age or gender. Inclusion in the study required an ongoing, clinically or dermoscopically apparent nail condition and good quality CLSM, OCT, and LC-OCT images. Patients with systemic or topical antimycotic therapy within 3 months prior to inclusion were excluded.

In the onychomycosis group, 48 patients showed onychomycosis of toenails and three patients had affected fingernails. The observed subtypes of onychomycosis[18,19] were distal and lateral subungual onychomycosis (DLSO, 57%), totally dystrophic (33%) and mixed pattern (DLSO and superficial, 10%) onychomycosis.

Study approval was granted by the local ethics committee of the university hospital (Ref.-Nr. 17–699) and each patient gave written informed consent before inclusion. The study was conducted in accordance with the Declaration of Helsinki and international guidelines for human studies.

Methods

First, dermatoscopic images of the nail plate were collected with video dermatoscopy (FotoFinder®, FotoFinder Systems GmbH, Bad Birnbach, Germany). Macroscopic and microscopic high-resolution images of the nail were taken using optical magnification of 20x to 140x. The images were examined for typical dermatoscopic signs of onychomycosis such as white to yellow discoloration, subungual keratosis, onychodystrophy and onycholysis, jagged edges with spikes at the proximal edge of the lesion and/or longitudinal striae.[13,14]

Following dermatoscopy, the nail plate was imaged using CLSM, OCT, and LC-OCT. Table 1 summarizes the device characteristics (resolution, penetration depth, image sizes, and other details) for each method.

1. CLSM was conducted with the VivaScope® 3000 Multiwave handheld device with 830 nm diode laser in reflection mode. Clinically suspicious nail areas were scanned horizontally to the nailbed using the "VivaStacks" function. The sample was considered positive for onychomycosis when observing bright, hyper-reflective filamentous structures or spore-like aggregates, as seen in histopathology.
2. OCT was conducted with the VivoSight Dx™ System, which generates vertical images of the nail plate. The sample was considered positive for onychomycosis when bright, hyper-reflective filamentous structures or spore-like aggregates were seen.
3. LC-OCT was conducted with the deepLive™ System. Each nail was imaged using the vertical (en-coupe), horizontal (en-face) and 3D modalities of the device. Bright, hyper-reflective filamentous structures with "fuzzy" appearance, which interrupted the normal, homogenous integrity of the nail plate were regarded as fungal hyphae. Such streaks typically extended across multiple levels of the nail plate and were often accompanied by visible clefts due to nail destruction.

All images were analyzed by two examiners, experienced in non-invasive imaging as well as in dermatological nail diseases, who agreed on the outcome for each patient.

After non-invasive imaging, a nail sample was obtained and analyzed using:

1. KOH-preparation and direct microscopy: The nail sample was clarified and stained with a solution comprising 90 mL 7.5% potassium hydroxide (KOH), 10 mL DMSO and 120 mg Chlorazol black E. Incubation time was between 2 and 10 minutes. The sample was subsequently examined under a light microscope for (pseudo-)hyphae using 200x magnification.
2. Fungal culture: Kimmig fungal agar (suitable for dermatophytes and yeast) was used for cultivation of fungi. The sample was placed onto the agar with 50 mg/l

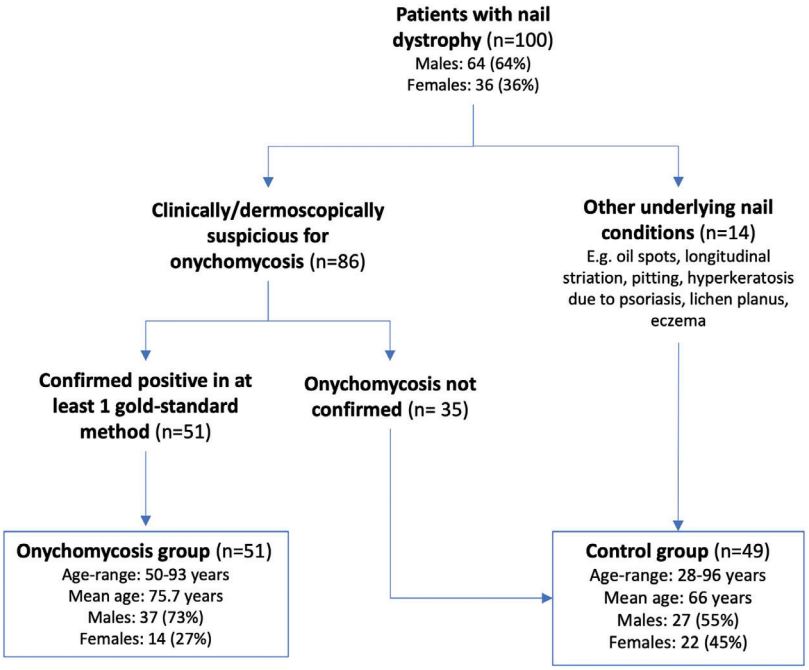


FIGURE 1 Flowchart showing patient distribution in the onychomycosis and control group, including number of patients, age-range, mean age and percentage of males and females in each group.

TABLE 1 Device and measurement characteristics for LC-OCT, OCT and CLSM.

Method	Device	Manufacturer	Resolution (μm)	Penetration depth in nails (μm)	Images/field of view	Other details
CLSM	VivaScope® 3000 Multiwave	VivaScope GmbH, Munich, Germany	1.25–5	400–500	0.75×0.75 mm^2 at 6.5 μm depth intervals	Immersion oil and ultrasound gel application between the device probe and nail surface for better index matching
OCT	VivoSight DX™ System	Michelson Diagnostics Ltd, Maidstone, Kent, UK	7.5–10	1500	6×6 mm^2	No index-matching fluid is required, just an appropriately selected plastic spacer for the handheld probe to ensure that the nail is put into focus.
LC-OCT	deepLive™ System	DAMAE Medical, Paris, France	1.1–1.3	500	1.2×0.5 mm^2 (2D vertical and horizontal mode) 1.2×0.5×0.5 mm^3 (3D mode)	Immersion oil was applied between the glass window of the device and the nail surface for better index matching.

Abbr.: CLSM, confocal laser scanning microscopy; OCT, optical coherence tomography; LC-OCT, line-field confocal optical coherence tomography

chloramphenicol and left to incubate for 3 weeks at 28°C. The plate was scanned weekly for fungus growth. Species differentiation was performed in positive cases, based on the presence of specific morphological features.

3. PCR: DNA extraction from the sample was performed using the QIAamp DNA Mini Kit (ID:51304, Qiagen, Hilden, Germany), yielding DNA sized up to 50 kb. PCR was subsequently performed with EUROArray Dermatomycosis (EUROIMMUN Medizinische Labordiagnostika AG, Lübeck, Germany), which amplified specific gene regions of pathogens in a multiplex procedure. Annealing temperature was 55°C. The PCR products were fluorescently labelled and hybridized to corresponding probes on biochip microarray slides. Detection and evaluation of amplified DNA products was performed with the proprietary software, warranting objective result reliability.[20]

PCR was not done for four patients due to cost restrictions. In these cases, histopathology was performed:

TABLE 2 Overview of the results for each diagnostic method including the total number of positive results, the sensitivity, specificity, PPV, and NPV.

	Positive result (%)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
LC-OCT (n = 100)	58	92.2	77.6	81	90.5
OCT (n = 100)	69	86.3	49	63.8	77.4
CLSM (n = 100)	49	78.4	81.6	81.6	78.4
PCR (n = 93)	46.2	87.8	100	100	88
Culture (n = 98)	13.3	26	100	100	56
Native/KOH (n = 98)	37.8	74	100	100	79

Abbr.: PPV, positive predictive value; NPV, negative predictive value; LC-OCT, line-field confocal optical coherence tomography; CLSM, confocal laser scanning microscopy; OCT, optical coherence tomography; PCR, polymerase chain reaction; KOH, Potassium hydroxide

1. Histopathology with PAS staining: Sampled nail material was embedded in paraffin, stained using PAS reaction and analyzed microscopically.

Statistical Analysis

Sensitivity, specificity, negative and positive predictive values were calculated for each diagnostic method, except for histopathology as it was only performed in four patients.

Sensitivity, defined as the percentage of true positives, demonstrated how well a method could detect onychomycosis in affected patients. Specificity, defined as the percentage of true negatives, determined how accurately the technique could identify patients without fungal infection. The positive predictive value (PPV) indicated the probability that a patient with a positive test result truly had onychomycosis. The negative predictive value (NPV) represented the probability that a patient with a negative test result did not have onychomycosis. KOH-preparation, fungal culture, PCR or histopathology were used as benchmark and gold standard. A positive finding in any of the four confirmed the diagnosis of onychomycosis, as not all methods could be performed for every patient.

RESULTS

Table 2 summarizes the total number of positive results, sensitivity, specificity, PPV, and NPV of each method, excluding histopathology.

Sensitivity

LC-OCT achieved the highest sensitivity (92.2%), followed by PCR (87.8%), OCT (86.3%), CLSM (78.4%), KOH-preparation (74%), and fungal culture (26%) (Table 2).

Specificity

PCR, fungal culture, histopathology, and KOH-preparation were taken as gold standard methods for onychomycosis, which implied a specificity of 100%. Compared to the gold standard techniques, CLSM had the highest specificity (81.6%), followed by LC-OCT (77.6%), and OCT (49%) (Table 2).

The non-invasive imaging techniques LC-OCT, OCT, and CLSM were compared with each other and gold standard methods. Positive and negative correlations between the diagnostic methods for onychomycosis are also discussed.

LC-OCT

LC-OCT showed positive results in 58% of all tests conducted (58/100), higher than the average of all methods (46%). 81% (47/58) of the positive results were found in the onychomycosis, 19% (11/58) in the control group. Figure 2 shows exemplary images in vertical, horizontal, and three-dimensional mode.

LC-OCT offered the highest sensitivity of all tested methods (92.2%) and high specificity (77.6%). PPV and NPV were 81% and 90.5% respectively, resulting from a high level of true positives and negatives. Notably, LC-OCT had better sensitivity (92.2%) and NPV (90.5%) than the gold standard methods, although closely followed by PCR (sensitivity 87.8%, NPV 88.8%). The other gold standard methods had considerably lower sensitivities and NPVs than LC-OCT (Table 2). The sensitivity of LC-OCT was also higher than the other non-invasive techniques but closely followed by OCT (sensitivity 86.3%). CLSM ranked third in sensitivity (78.4%).

The NPV of LC-OCT (90.5%) was considerably higher than OCT (77.4%) and CLSM (78.4%). LC-OCT specificity (77.6%) and PPV (81%) were lower than the gold standard methods,

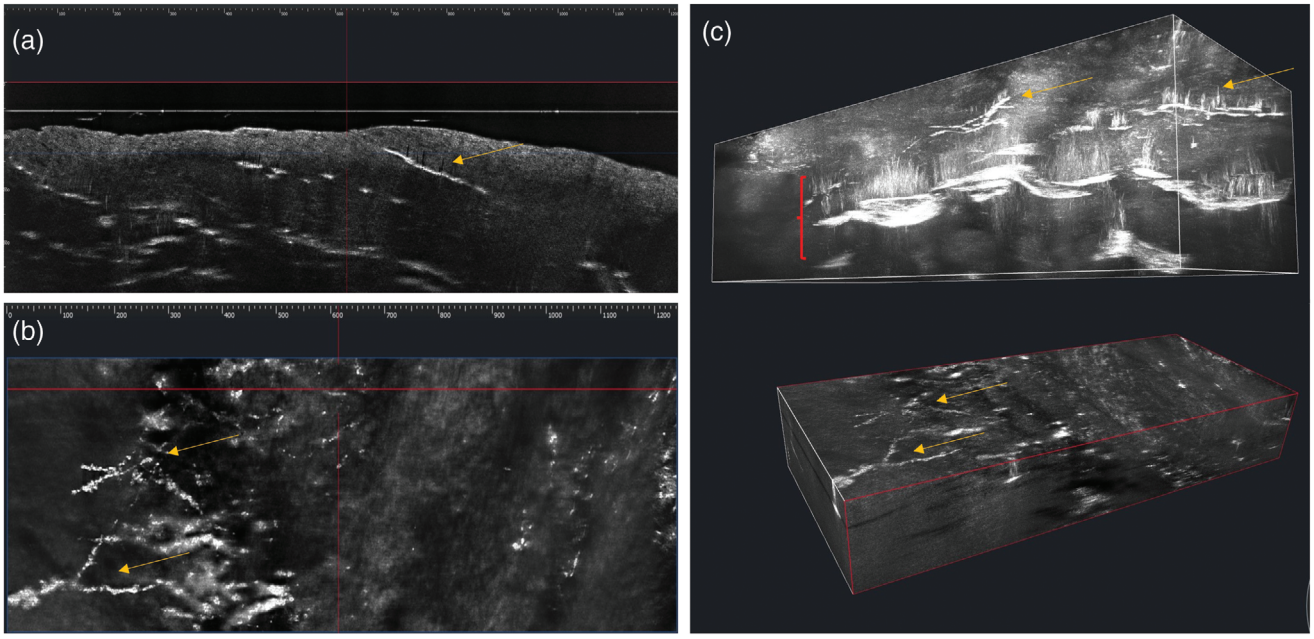
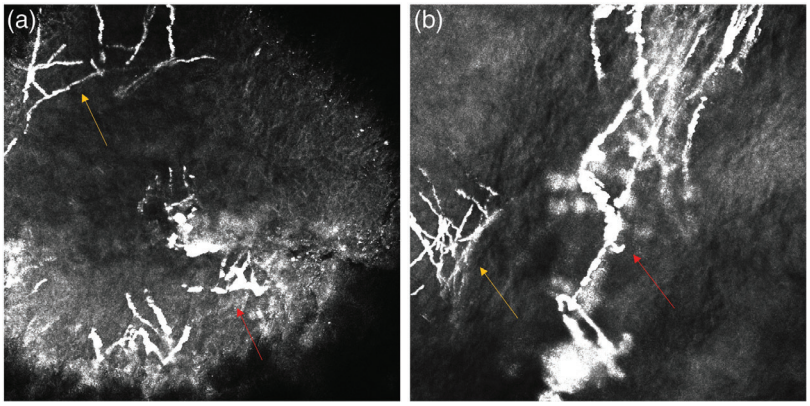


FIGURE 2 Onychomycosis in LC-OCT: (a) vertical (b) horizonal (depth: 250 μm) and (c) 3D LC-OCT images showing a nail plate affected by onychomycosis. The hyphae are visible as hyper-reflective thin, branching structures spanning multiple layers of the nail plate (yellow arrows). Nail destruction and clefting can also be seen surrounding the hyphae (red bracket). (LC-OCT, DAMAE medical, 2D images: 1.2×0.5 mm^2, 3D images: 1.2×0.5×0.5 mm^3)

FIGURE 3 Onychomycosis in CLSM. (a, b) Horizontal CLSM images showing hyper-reflective thin filamentous hyphae (yellow arrows), as well as slightly thicker and rounder spore-like aggregates with a similar presentation as in histopathology (red arrows). (CLSM, VivaScope GmbH, 750×750 μm)



but comparable to CLSM (specificity 81.6%, PPV 81.6%) and much higher than OCT (specificity 49%, PPV 63.8%).

LC-OCT showed the highest positive correlation with OCT (79.5% of patients) and lowest with culture (20.7% of patients). Conversely, the highest negative correlation was seen with culture (92.9% of patients) and lowest with OCT (38% of patients).

CLSM

CLSM showed positive results in 49% of tested patients (49/100), resembling PCR (46.2%) and higher than the average of all methods (46%). 82% (40/49) of the positive results were found in the onychomycosis, 18% (9/49) in the control group. Figure 3 shows exemplary images taken in horizontal mode.

CLSM had a sensitivity of 78.4% and specificity of 81.6%. The PPV and NPV were 81.6% and 78.4% respectively, representing a high number of true positives and negatives. Compared to gold standard methods, the sensitivity of CLSM (78.4%) ranked below PCR (87.8%) but above KOH-preparation (74%) and culture (26%). The NPV of CLSM (78.4%) was below PCR (88.0%) and comparable to KOH-preparation (79%), but clearly above culture (56%).

In specificity, CLSM (81.6%) ranked higher than LC-OCT (77.6%) and OCT (49%). Conversely, CLSM's sensitivity (78.4%) was lower than LC-OCT (92.2%) and OCT (86.3%).

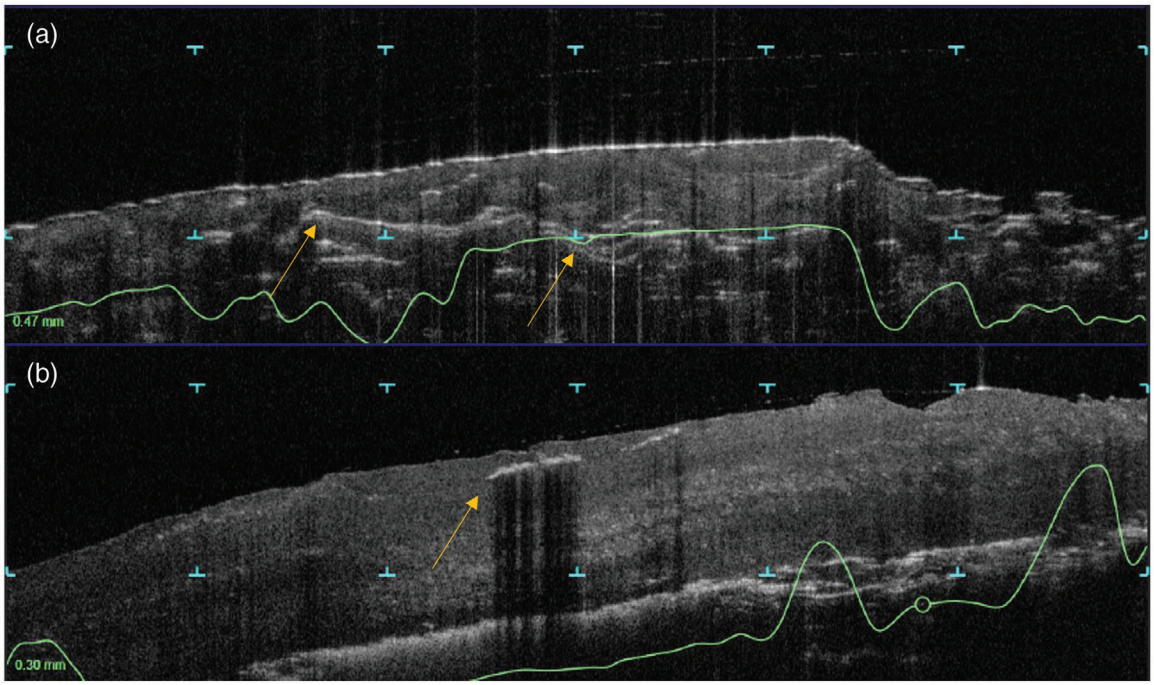


FIGURE 4 Onychomycosis in OCT. (a, b) Vertical OCT images of the nail plate showing hyper-reflective thin filamentous hyphae with a darker hypo-reflective zone underneath (yellow arrows) (OCT, Michelson Diagnostics Ltd, 6×6 mm).

PPV of CLSM (81.6%) was comparable with LC-OCT (81%), but above OCT (63.8%).

In NPV (78.4%), CLSM corresponded with OCT (77.4%) but ranked below LC-OCT (90.5%).

CLSM showed the highest positive correlation with LC-OCT (72.3% of patients) and lowest with culture (20.4% of patients). The negative correlation was highest with culture (92.2% of patients) and lowest with OCT (40% of patients).

OCT

OCT showed positive results in 69% of tested cases (69/100), the highest percentage of all techniques. 64% (44/69) of the positive results were found in the onychomycosis, 36% (25/69) in the control group. Figure 4 shows exemplary OCT images taken in vertical mode.

OCT had high sensitivity (86.3%), but comparably low specificity (49%). PPV (63.8%) and NPV (77.4%) signified a higher proportion of true negatives than true positives. OCT sensitivity (86.3%) was like PCR (87.8%) but higher than KOH-preparation (74%) and fungal culture (26%).

NPV of OCT (77.4%) was lower than PCR (88.0%) and KOH-preparation (79%), but higher than culture (56%).

OCT had the lowest specificity (49%) of all methods tested and lowest PPV (63.8%).

Comparing the non-invasive imaging techniques, OCT had a lower sensitivity (86.3%) than LC-OCT (92.2%), but a higher sensitivity than CLSM (78.4%). NPV of OCT (77.4%) was comparable to CLSM (78.4%), but clearly below the NPV of LC-OCT (90.5%).

OCT showed the highest positive correlation with LC-OCT (79.5% of patients) and lowest with culture (13% of patients). The highest negative correlation was seen with culture (87.1% of patients) and lowest with LC-OCT (38% of patients).

Differentiation of species

Differentiation of fungal species was possible with PCR and culture in 47 cases. Four different pathogens were identified: *Trichophyton rubrum (TR)*, *Trichophyton interdigitale (TI)*, *Candida parapsilosis (CP), and Candida guilliermondii (CG)*.

Reliable species differentiation was possible in 41 of 43 positive PCR tests. The two cases without species determination were reported to have an infection with dermatophytes but lacked sufficient material for exact classification. *Trichophyton rubrum* was found in 73.2% (30/41), *Trichophyton interdigitale* in 17.1% (7/41), *Candida parapsilosis* in 9.8% (4/41), and *Candida guilliermondii* in 4.9% (2/41) of the positive PCR cases. One patient was identified with both *TR and CG* and one with both *TR and CP*. Fungal culture was positive in only 13 cases. The growth of *TR* was seen in 69.2% (9/13) and *CP* in 30.8% (4/13). LC-OCT detected fungal infection in 32/33 cases of TR, 7/7 cases of TI, 4/5 cases of CP and 2/2 cases of CG.

TABLE 3 Comparison of seven diagnostic methods with regards to the duration until final diagnosis, costs per examination (based on the German medical fee schedule, GOÄ), costs of device acquisition and other resources (such as material and staff) and the possibility of species differentiation.

	Time until final diagnosis	Costs per examination (€)	Device acquisition costs and material/staff	Species Differentiation
KOH preparation	Approx. 30 min	8.04	Low costs, but high staff expertise	No
Culture	3 weeks	16.08	Low costs, but high staff expertise	Yes
Histopathology (PAS staining)	1–2 days	46.92	Low costs, but high staff expertise	No
PCR	1–2 days	113.96	High (material, devices, staff)	Yes
LC-OCT	Approx. 5 min	140	High (device approx. 150,000 €)	No
CLSM	Approx. 10 min	140	High (device 70,000–185,000 €, depending on exact configuration)	No
OCT	Approx. 5 min	80	High (device approx. 85,000 €)	No

Abbr.: KOH, Potassium hydroxide; PCR, polymerase chain reaction; LC-OCT, line-field confocal optical coherence tomography; CLSM, confocal laser scanning microscopy; OCT, optical coherence tomography

Duration, costs, efforts

Table 3 gives an overview of the duration until final diagnosis, costs per examination (based on the German medical schedule of fees, GOÄ) and device acquisition, other resources (such as material and staff), and the ability to differentiate between species for each of the seven diagnostic methods used in this study.

DISCUSSION

This study examined the efficacy of LC-OCT for diagnosing onychomycosis and compared it with existing diagnostic procedures. The typical features of onychomycosis observed with LC-OCT correspond to those reported by Hobelsberger et al. in their pilot case report,[21] while the obtained quantitative results are comparable with the findings reported by Rothmund et al.[1]

Our results confirm previous reports that PCR is an accurate method for diagnosing onychomycosis, even when other gold standard results are negative.[1,22,23] However, all gold standard methods for diagnosing onychomycosis are highly dependent on correct nail sample acquisition (e.g. prior disinfection, obtaining enough material) and expertise in choosing the correct and most affected area.[1,24,25] Nail clippings from unaffected areas or too little material could result in a higher number of false negatives, giving lower sensitivity and NPV. This is not an issue for LC-OCT, OCT and CLSM.

Non-invasive imaging techniques advantageously allow scanning of the entire nail plate. Our findings show, however, that only LC-OCT had a higher sensitivity and NPV than the gold standard methods. This could be because LC-OCT offers three high-resolution imaging modalities (horizontal, vertical and 3D) that allow the most comprehensive real-time analysis of the entire nail to identify fungal hyphae. No reliable statement could be made regarding the sensitivity and NPV of histopathology, as it was only performed in four cases. This limitation can be overcome by incorporating more histopathology examinations to properly compare LC-OCT with histopathology in future research.

LC-OCT specificity and PPV was comparable to that of CLSM, which suggests that both methods are equally effective in correctly identifying negative cases and minimizing false positives. Despite its high sensitivity and NPV, OCT had a rather low specificity and PPV. The OCT device has lower resolution (7.5–10 μm) than both LC-OCT (1.1–1.3 μm) and CLSM (1–3 μm),[17] which makes it more challenging to discern hyphae from other nail conditions and deformations (such as leukonychia) and resulted in a higher number of false positives. The higher penetration depth (1.5 mm) of OCT allowed visualization of the entire nail plate and commonly the transition to the nail bed, even in hyperkeratotic nails. LC-OCT and CLSM were limited by their penetration depths (respectively 500 μm and 250 μm). Future developments of LC-OCT should aim at increasing penetration depth while maintaining the same cellular resolution. This would allow for identification of hyphae deeper in the nail plate even in the presence of hyperkeratosis and further reduce the number of false negatives.

The advantages and disadvantages of the gold standard methods for diagnosing onychomycosis can be found elsewhere.[1,12,14,26,27] In practice, conventional techniques, such as KOH-preparation and fungal culture are often combined in diagnosing onychomycosis. However, LC-OCT, CLSM and OCT are advantageous as they do not require prior nail sampling and the diagnosis can be made by trained medical personnel within only 5–10 minutes, compared to days or even weeks, as for PCR, histopathology, or fungal culture (Table 3). This suggests that patients could receive diagnosis and treatment during a single consultation, saving time and resources. We found that LC-OCT and OCT offer the easiest and quickest handling for scanning nail plates, whereas CLSM took longer and was slightly more challenging without an integrated dermatoscopic camera in the hand-held device for exact navigation on the nail. While some authors state that in vivo CLSM is too

complicated for routine use,[28] Krammer et al. found that *ex vivo* CLSM allows for rapid and accurate detection of onychomycosis, with a sensitivity (91.67%) similar to that of PCR and LC-OCT.[5] However, *ex vivo* CLSM requires prior nail sampling.

A drawback of LC-OCT, CLSM, and OCT is that they, in contrast to PCR and fungal culture, do not enable species identification. The differentiation between species is helpful in choosing correct antimycotic treatment.[4] Culture also indicates pathogen vitality, an important factor to monitor in therapy-resistant cases and recurring disease.[1] This advantage is, however, offset by the relatively low sensitivity (26%). Past studies have confirmed the low sensitivity of culture in diagnosing onychomycosis, although with significant study-to-study variability; Rothmund et al. found a comparable sensitivity (20.5%), whereas other studies range between 30% and 60%.[1,27] It remains unclear as to why the sensitivity found in our study falls in the lower range. Possible explanations could be limited viability of fungal cells, too little sample material for growth in culture or suboptimal sampling locations.[25]

LC-OCT was equally capable of identifying infection caused by *Trichophyton* and *Candida* species, but further studies with a higher number of nails affected by *Candida* should be performed.

Another drawback is the high acquisition cost of the imaging devices (Table 3), also true for LC-OCT. However, the primary reason for acquiring the LC-OCT device is for diagnosing skin tumors such as basal cell carcinoma, for which it has shown high diagnostic accuracy and interobserver agreement.[29] Onychomycosis is an additional indication for which the device could be used concomitantly.

The handling of OCT, CLSM and LC-OCT requires specialized training and experience on nails. However, the gold standard techniques and laboratory analysis equally require qualified personnel for acquiring correct nail samples (Table 3). Artificial intelligence could potentially be used in the systematic analysis of collected images to support the diagnosis of onychomycosis in the future.

The high number of false negatives and the fact that traditional gold standard methods require accurate nail clippings, create a need for suitable in-vivo approaches for correctly diagnosing onychomycosis to properly treat this common and life-quality impairing nail condition. LC-OCT allows quick and accurate detection of hyperreflective fungal hyphae in nails without the need for nail sampling and should be considered for standard clinical practice. Further studies should investigate how LC-OCT can be used for tracking clinical progress under therapy.

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CONFLICT OF INTEREST

None.

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12 Appendix: Paper II

Eijkenboom QL, Daxenberger F, Guertler A, Steckmeier S, French LE, Sattler EC. **Line-field confocal optical coherence tomography (LC-OCT) for the in vivo examination of nails: Analysis of typical features for the differentiation of nail disorders.** *J Eur Acad Dermatol Venereol.* 2024 May;38(5):e413-e416. doi: 10.1111/jdv.19641. Epub 2023 Dec 7. PMID: 38059388.

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LETTER TO THE EDITOR

Line-field confocal optical coherence tomography (LC-OCT) for the in vivo examination of nails: Analysis of typical features for the differentiation of nail disorders

Dear Editor,

Given the similarities in clinical presentation, differentiating nail disorders can be challenging even for experienced dermatologists.[1,2] However, accurate and early diagnosis is essential for effective therapy. Non-invasive diagnostic tools, such as confocal laser-scanning microscopy (CLSM) and optical coherence tomography (OCT), were shown to work well in the diagnosis of nail disorders thereby reducing the need for invasive biopsies.[1–4] This study evaluated the latest non-invasive diagnostic tool, line-field optical coherence tomography (LC-OCT) for the assessment of nail disorders.

We assessed the typical features seen in LC-OCT in healthy nails and nails affected by leukonychia, subungual haemorrhage, psoriasis, lichen planus, longitudinal melanonychia, subungual melanoma (acral lentiginous melanoma) and onychomycosis (two patients respectively) of 16

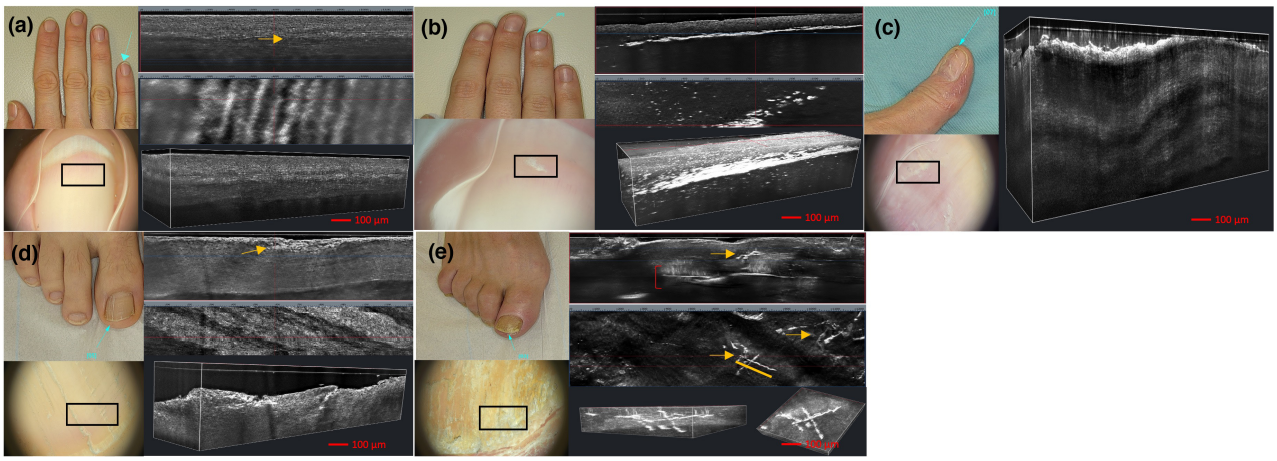

FIGURE 1 Clinical, dermoscopic and LC-OCT vertical (top), horizontal (middle) and 3D images (bottom) of healthy nails versus nails affected by common nail disorders. The affected nail and the scanned position on the nail plate are indicated by the arrows and boxes. (a) Healthy nail plate characterized by different zones of reflected light intensity. Vertical LC-OCT section shows a bright surface, followed by a darker homogenous layer with round corneocytes, a less uniform third layer with varying hyper- and hypo-reflective bands and elongated corneocytes, a hypo-reflective lower zone and a wave-like margin (yellow arrow) defining the transition to the underlying nail bed. Horizontal LC-OCT section (depth: 493 µm) of the nail bed showing alternating hypo- and hyper-reflective longitudinal bands parallel to the digit. (b) Features of a nail plate with leukonychia. Vertical LC-OCT section showing hyper-reflective streaks consisting of ovoid structures grouped together and vertical hypo-reflective bands appearing underneath as shadows. Horizontal LC-OCT section (depth: 110 µm) showing hyper-reflective roundish structures representing loose corneocytes with hypo-reflective surrounding areas. 3D LC-OCT block showing hyper-reflective ovoid streaks corresponding to detached corneocytes. (c) Features of a nail plate affected by psoriasis. 3D LC-OCT block shows a thickened hyper-reflective uneven surface with lamellar splitting, followed by a hypo-reflective area underneath. The nail plate is characterized by diffuse wave-like zones of varying intensity, representing areas of more and less keratin densification. (d) Features of a nail plate affected by lichen planus. Vertical section showing a thickened hyper-reflective surface with dispersed hypo-reflective gaps resembling a honeycomb pattern (yellow arrow), followed by a hypo-reflective zone underneath. Horizontal LC-OCT section (depth: 125 µm) showing alternating hypo- and hyper-reflective longitudinal bands. 3D LC-OCT block showing onychoschizia, crater like depressions corresponding to longitudinal ridging in cross-section, an irregular dystrophic surface and lamellar splitting. (e) Features of a nail plate affected by onychomycosis. Vertical LC-OCT section of the nail plate showing an uneven 'fuzzy' looking surface and hyper-reflective thin filamentous branching structures spanning multiple layers of the nail plate, corresponding to fungal hyphae (yellow arrow). Hypo-reflective clefts surrounded by 'fuzzy' hyper-reflective structures can be identified at a depth of around 300 µm (red bracket). Horizontal LC-OCT section (depth: 150 µm) of the nail plate showing various hyper-reflective thin hyphae (yellow arrows). As reference, the hyphae marked by the yellow line has a size of 180 µm. 3D LC-OCT blocks displaying the same hyper-reflective branching hyphae in more detail and showing that they span multiple layers and cross over at different depths.

exemplary patients (mean age: 58 years, range: 9–84 years) out of 100 patients observed at the Department of Dermatology and Allergy at the Ludwig Maximilian University Hospital in Munich, Germany.

After clinical examination, the affected region was captured using video dermoscopy (FotoFinder®, FotoFinder Systems GmbH, Bad Birnbach) and LC-OCT (deepLive™, DAMAE Medical, Paris). In cases of histopathological confirmation (onychomycosis and subungual melanoma), patients were imaged before biopsy. Diagnoses of psoriasis and lichen planus were confirmed histologically prior to LC-OCT while leukonychia, subungual haemorrhage and melanonychia were verified clinically and dermoscopically.

The different layers of a healthy nail plate (Figure 1a) seen with LC-OCT are comparable with CLSM.[3] However, LC-OCT with its deeper penetration depth additionally demarcated the transition to the nailbed as wave-like structures representing the nailbed's distinct longitudinal parallel arrangement, microscopically described as corrugation.

Detachment of single hyper-reflective round corneocytes in leukonychia seen in LC-OCT (Figure 1b) corresponded well to earlier CLSM-findings.[3] The hyper-reflective structures of leukonychia resulted from a stronger light diffraction because of the transition from denser substance, here the dyskeratotic or parakeratotic corneocyte, to air that infiltrated its surroundings. Lamellar splitting, hyperkeratosis and hyper-reflective keratin densification in the nail plate are typical features of psoriasis nails in LC-OCT (Figure 1c). LC-OCT images of the nail plate affected by lichen planus clearly showed alternating hyper- and hypo-reflective bands, representing longitudinal ridging (Figure 1d).[4,5]

Onychomycosis was identified with LC-OCT by the presence of hyper-reflective filaments (hyphae) of varying thickness, size and degree of branching (Figure 1e). These findings corresponded to the bright thready structures in CLSM and histopathology indicating a fungal infection.[1,6] Prolonged onychomycosis typically showed clefts surrounded by bright

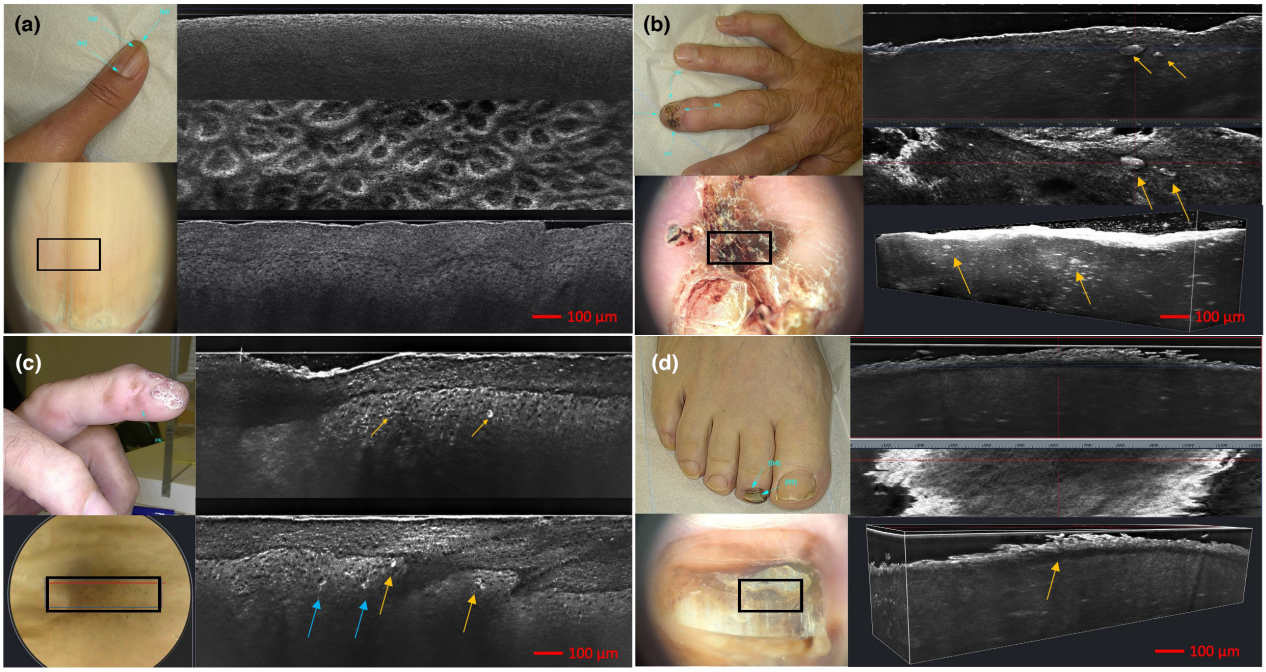

FIGURE 2 Clinical, dermoscopic and LC-OCT vertical, horizontal and 3D images of nails affected by benign and malignant nail disorders. The affected nail and the scanned position on the nail plate are indicated using arrows and boxes. (a) Features of a nail plate affected by melanonychia striata. Vertical LC-OCT section showing an intact nail plate with a homogenous hyper-reflective surface followed by a hypo-reflective zone underneath. Horizontal LC-OCT section (depth: 300 µm) of the proximal nail fold showing a consistent arrangement of the dermal papillae, surrounded by hyper-reflective melanin pigment. Atypical cells are missing. Vertical LC-OCT section of the proximal nail fold showing a regular honeycomb pattern of the epidermis, a well demarcated dermo-epidermal junction, and hypo-reflective dermal papillae in depth. No atypical melanocytes or pagetoid cells are visible. (b) Features of a nail plate affected by subungual melanoma. Vertical LC-OCT section showing a dystrophic hyper-reflective surface and various hyper-reflective melanin globules distributed throughout the nail plate remnants (yellow arrows). Horizontal LC-OCT section (depth: 130 µm) showing an irregular nail plate with hyper-reflective melanin globules and surrounding areas of varying intensity. 3D LC-OCT section showing a hyperkeratotic bright surface and multiple hyper-reflective round structures and globules spread throughout the entire nail area. (c) Features of the surrounding skin of a nail plate affected by subungual melanoma. Vertical LC-OCT sections showing a thicker stratum corneum, due to the palmar location of the hand followed by an irregular honeycomb pattern of the stratum granulosum and spinosum of the epidermis with hyper-reflective pagetoid cells containing hypo-reflective nuclei (yellow arrows) and hyper-reflective branching dendritic cells (blue arrows). The dermo-epidermal junction is not well demarcated, and the hypo-reflective dermal papillae are irregular and not clearly identifiable. (d) Features of a nail plate with subungual haemorrhage. Vertical LC-OCT section showing an incoherent hyper-reflective surface with lamellar splitting and a hypo-reflective homogenous band, representing the subungual haemorrhage underneath. Horizontal LC-OCT section (depth: 80 µm) showing a hypo-reflective round area corresponding to the subungual haemorrhage with a hyper-reflective surrounding border. 3D LC-OCT block clearly showing the hypo-reflective band representing the subungual haemorrhage (yellow arrow).

filamentous structures in the nail plate, caused by the keratinolytic activity of dermatophytes.[6,7]

Differentiating between benign longitudinal melanonychia (Figure 2a) and subungual malignant melanoma (Figure 2b,c) is crucial but often challenging.[2,5,8] Harmless subungual haemorrhaging can also clinically resemble subungual melanoma due to red or black discoloration of the nail plate,[9] but appeared as a homogenous dark band in LC-OCT (Figure 2d). Though biopsy is the common choice for suspicious pigmentation,[4] LC-OCT provides useful clues whether an operation is indicated. LC-OCT is already used for diagnosing skin melanomas with bright atypical melanocytic cells, irregular honeycomb patterns of the epidermis and pagetoid spread of suspicious melanocytes as main features.[10] Our results show that subungual melanoma and any invaded surrounding skin also exhibited these specific features and could clearly be differentiated from longitudinal melanonychia and subungual haemorrhaging. While pagetoid cells with prominent nuclei were characteristic in the pigmented skin surrounding subungual melanoma, the nail plate remnants showed interspersed bright globules, probably melanin nests from atypical melanocytes that lost their nuclei in the pagetoid process.

Further developments of LC-OCT should aim for an even higher penetration depth to better visualize the melanotic nail matrix and to identify inflammatory cell infiltration.[2]

In conclusion, LC-OCT allows for a rapid, non-invasive examination of the entire nail plate, by creating vertical, horizontal and 3D images, compared to just partial sampling as for culture or histopathology.

In this study, 16 patients were examined using LC-OCT to identify preliminary distinguishing features of various nail diseases, serving as a starting point to assist differential diagnosis. Follow-up investigations should include a larger patient collective to corroborate these first findings and evaluate sensitivity and specificity of LC-OCT. Nevertheless, LC-OCT has great potential for accelerating the in vivo diagnosis of nail diseases, may prevent superfluous biopsies with the risk of nail dystrophy and thus improve patients' quality of life.

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CONFLICT OF INTEREST STATEMENT

The LC-OCT device was provided to the Department of Dermatology and Allergy at the LMU university hospital by DAMAE Medical for study purposes. Otherwise, the authors have no conflicts of interest to report.

ETHICS STATEMENT

Approval of study was granted by the local ethics committee of the LMU hospital (Ref.-Nr. 17-699) and each patient included in the study and this manuscript has given written informed consent to publication of their case details. The study was conducted in accordance with the Declaration of Helsinki and international guidelines for human studies.

DATA AVAILABILITY STATEMENT

The data generated and analysed during the current study are available from the corresponding author on request.

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