Continuous spectral zooming for in vivo live 4D-OCT with MHz A-scan rates and long coherence

Madita Göb,1 Tom Pfeiffer,1,2 Wolfgang Draxinger,1 Simon Lotz,1 Jan Philip Kolb,1 and Robert Huber1,*

1Institut für Biomedizinische Optik, Universität zu Lübeck, Peter-Monnik-Weg 4, 23562 Lübeck, Germany
2Optores GmbH, Gollierstr. 70, 80339 Munich, Germany
*robert.huber@uni-luebeck.de

Abstract: We present continuous three-dimensional spectral zooming in live 4D-OCT using a home-built FDML based OCT system with 3.28 MHz A-scan rate. Improved coherence characteristics of the FDML laser allow for imaging ranges up to 10 cm. For the axial spectral zoom feature, we switch between high resolution and long imaging range by adjusting the sweep range of our laser. We present a new imaging setup allowing for synchronized adjustments of the imaging range and lateral field of view during live OCT imaging. For this, a novel inline recalibration algorithm was implemented that enables numerical k-linearization of the raw OCT fringes for every frame instead of every volume. This is realized by acquiring recalibration data within the dead time of the raster scan at the turning points of the fast axis scanner. We demonstrate in vivo OCT images of fingers and hands at different resolution modes and show real three-dimensional zooming during live 4D-OCT. A three-dimensional spectral zooming feature for live 4D-OCT is expected to be a useful tool for a wide range of biomedical, scientific and research applications, especially in OCT guided surgery.

1. Introduction

Optical coherence tomography (OCT) was first introduced in 1991 [1] and is a powerful, non-invasive imaging technique to resolve biological microstructure in vivo. With the development of multi-megahertz Fourier Domain Mode Locked (FDML) lasers, the imaging speed of swept-source OCT systems (SS-OCT) was dramatically increased to up to 5.2 million A-scans per second [2–5]. This high-speed swept laser source in combination with the availability of fast analog to digital converters (ADC) and high computational power of consumer grade GPUs enable live 4D-OCT at video volume update rates [6]. Our group recently demonstrated its increasing potential for future applications, such as live 4D-OCT for retinal imaging [7] or for virtual reality guided surgery [8,9]. Considering this technique for a surgical microscope, as well as for various other applications, it is evident that a “zoom out/in” option could be very useful to support navigation on the situs.

In standard surgical microscopes this zoom feature is often achieved by adjustable magnification. For OCT it must be taken into account, that the object under investigation is displayed in 3D. While the lateral field of view (FOV) can be adjusted by simply increasing or decreasing the scanning angle of the galvanometer scanning mirrors, the imaging range and depth resolution is typically fixed in OCT. Thus, modifications of the scan pattern cause a change of aspect ratios in the 3D-rendered OCT images. Also, in standard OCT (without complex-conjugate suppression [10–13]) flipping artifacts are inevitable when non-flat structures are inspected whose height exceeds the axial imaging range. Therefore, a real three-dimensional zoom, scaling all dimensions uniformly, as depicted in Fig. 1, is desired. To perform such zooming now in three
dimensions, the depth imaging range has to be adjusted. This is by far more challenging than increasing the lateral FOV.

Fig. 1. Concept of three-dimensional spectral zooming ("zooming out") in OCT. a) OCT volume with a small FOV but high lateral and axial resolution. b) Lateral zoom out by increased scanning angles, whereas the imaging depth is retained unchanged resulting in a clipped surface structure (left eye). c) Real three-dimensional zooming including adjustments of the axial imaging range. In the upper row the object of investigation is depicted with the scanning window. Drawings in the lower row represent the processed OCT volumes at the corresponding resolution modes in 3D perspective views from front- and side-viewing angle.

In SS-OCT, the imaging range is determined by various parameters related to the characteristics of the laser source and imaging detection system. At fixed sweep bandwidths and rates, long imaging ranges with consistent axial resolution require high detection bandwidths. Furthermore, the coherence length of the laser source is a critical factor limiting the imaging range to a few millimeters for most swept laser sources operating with multiple longitudinal modes. Recently, new swept sources, such as a vertical cavity surface emitting laser (VCSEL) and an akinetic Vernier-tunable laser source with coherence lengths up to meters have been demonstrated [14,15]. Both are characterized by short cavity lengths that allow for single mode operation and long-range 3D-OCT imaging of large volumes at sweep rates of 100 kHz [16,17]. The basic principle of FDML lasers to generate high sweep rates is based on a long fiber cavity matching the inverse tuning frequency of a tunable Fabry-Pérot filter (FFP) [2]. This way an entire sweep can be optically stored within the cavity. To increase phase matching of the longitudinal laser modes and the mode inside the FFP and thus to optimize coherence characteristics of the FDML laser, the dispersion within the laser cavity needs to be well compensated [18–22].

Assuming an FDML laser with sufficient coherence, at a given A-scan rate and spectral sweep range of the laser, then the imaging range of our FDML based OCT system is determined by the analog detection bandwidth. The analog detection bandwidth of the system cannot easily be increased, as digitizer cards are expensive and the ADCs used in our systems already represent the limit of today’s streamable data rates. Also, superfast photo receivers with multi-GHz detection
bandwidth have very low transimpedance gain which may make it impossible to achieve shot noise limited detection. However, narrowing down the sweep range of the laser will increase the imaging range at the cost of axial resolution while the rest of the system, including detection and GPU processing, can remain unchanged. This tradeoff between high axial resolution and enhanced imaging range at a constant number of sample points has been first demonstrated in 2009 by Gora et al. [23]. They used an FDML based OCT system for ophthalmic imaging with an A-scan rate of 200 kHz and two discrete resolution modes. In a high axial resolution mode, a resolution of 9 µm and an imaging range of 2 mm were presented; in an extended imaging range mode 25 µm resolution and 8 mm imaging range.

Beyond simply switching resolution modes, many applications demand even a live online zooming feature with range adaption on the fly. In principle, FDML based MHz-OCT systems have this capability [24,25], however the high speed poses a series of additional challenges. In this work, we analyze in detail the capability of FDML lasers to enable high quality video rate 4D-OCT with up to ten centimeters imaging range using a home-built FDML laser with a very well dispersion managed cavity and therefore dramatically improved coherence [20]. We show screen recordings of live 4D-OCT at three different resolution modes and discuss the performance and limitations. Further, we implemented a continuous axial zoom, termed “spectral zooming”, that can be adjusted online during OCT imaging operation by simply turning a knob. We demonstrate stepless spectral zooming in live 4D-OCT. A specially designed software facilitates synchronized adjustments of the FDML laser’s spectral bandwidth and scanning angles while the MHz-OCT system is operating. A novel inline recalibration algorithm was implemented that allows for live processing of the 3D-rendered OCT images with different imaging ranges.

2. Methods

2.1. OCT imaging setup

All OCT data presented in this work were acquired using a home-built FDML-based MHz-OCT system running at a center wavelength of 1292 nm. The main components of the FDML laser are a semiconductor optical amplifier (SOA; Thorlabs Inc., BOA1132S, USA) as the gain medium, a home-built, ultra-high speed tunable Fabry-Pérot filter, a custom chirped fiber Bragg grating (CFBG; Teraxion Inc., Canada) and a delay fiber spool [20]. The dispersion compensation fiber mix is composed of Corning HI1060, SMF28e and LEAF fiber. The FFP was operated at 411 kHz tuning frequency and SOA-modulation allows for 8-times optical buffering resulting in a sweep repetition rate of 3.28 MHz [26]. The buffered laser output was amplified using another booster SOA [27]. The spectral bandwidth of the laser output was adjustable up to 120 nm and monitored in parallel using an optical spectrum analyzer (OSA).

The buffered and amplified swept laser output is directed into the OCT system as depicted in Fig. 2. Several fiber couplers with varying coupling ratio and optical circulators are used to generate reference, sample arm and calibration signals. In the sample arm, the output beam from the optical fiber is collimated using an 18.4 mm aspheric lens, directed to a pair of scanners to raster-scan the sample for 3D volumetric OCT imaging. The scanning optics comprise a non-resonant galvanometer mirror scanner (Cambridge Technology, 6215H, USA) for the slow axis and a resonant 2.7 kHz scanner (Electro-Optical Products Corp., SC-30, USA) for the fast axis. For the analysis of three discrete resolution modes, varying focus lenses were used to facilitate the required Rayleigh length for short to long imaging ranges. The imaging parameters of the different setups are listed in Table 1, the scanning optics were adjusted manually for each modality.

The incident optical power on the sample was 40 mW. The interference signal of sample and reference arm was recorded using a 1.6 GHz balanced photodetector (Thorlabs, PDB480C-AC, USA) and a 4 GS/s data acquisition board with a sample depth of 12 bit (AlazarTech, ATS9373, Canada). Real-time processing and visualization of the OCT data was done using NVIDIA
Table 1. Imaging parameters of different imaging modalities

|                      | High-Resolution Mode | Intermediate Mode | Long-Range Mode |
|----------------------|----------------------|-------------------|-----------------|
| Spectral Bandwidth   | 120 nm               | 17 nm             | 4 nm            |
| Imaging Range        | ~3.5 mm              | ~25 mm            | ~100 mm         |
| Beam Diameter\(^a\) | 3.2 mm               | 3.2 mm            | 3.2 mm          |
| Spot FWHM            | 22 µm                | 44 µm             | 440 µm          |
| Lateral FOV          | 85 × 85 mm\(^2\)     | 160 × 160 mm\(^2\) | 1600 × 1600 mm\(^2\) |
| NA Scan Lens         | 0.032                | 0.016             | 0.0016          |
| Rayleigh Length      | 1.6 mm               | 6.5 mm            | 650 mm          |
| Working Distance     | 50 mm                | 100 mm            | 1000 mm         |
| Scanning Mode        | Pre-Objective        | Pre-Objective     | Post-Objective  |

\(^a\)1/e\(^2\)-diameter

GPUs (NVIDIA, GeForce GTX690 and GeForce GTX680, USA). Details concerning the signal processing and large data management of the live 4D-OCT software of our group can be found in previous publications [6].

2.2. Spectral zooming using FDML lasers

Typically, long-range OCT with high axial resolution requires high detection bandwidths, but since the maximum bandwidth of our MHz-OCT system is already utilized for the high A-scan rate, there is no margin for larger imaging depths while maintaining the axial resolution. However, at narrower spectral bandwidths and thus lower axial resolution, the frequency of the acquired fringes is lower resulting in higher imaging ranges. Consequently, a compromise was found between imaging range and axial resolution determined by the spectral bandwidth of the laser. One advantage of FDML laser sources is that the sweep ranges are tunable. By de- or increasing the driving voltage of the FFP filter the spectral bandwidth can easily be changed up to a certain degree restricted by the spectrum of the SOA and CFBG as well as the chromatic dispersion of the cavity. In order to compensate the chromatic dispersion of the laser, the fiber mix within the delay spool and temperature gradient at the CFBG have been optimized as described previously [20]. This ultra-low noise FDML laser was used for the MHz-OCT system in this work, in order to implement a spectral zooming function. The adjustments of the spectral widths and thus zoom levels are realized by manually turning the rotary potentiometer of the filter voltage amplitude. Theoretically MEMS-tunable VCSEL devices could also dynamically adjust the amplitude and thus their laser bandwidth. A recently published Multi-MHz MEMS-VCSEL source [28] exhibits narrow instantaneous linewidths that may support long imaging ranges. However, dynamic adjustments of the lasing bandwidth might be problematic due to electrostatic spring softening, since MEMS devices with 10% relative wavelength tuning range are inherently operated in a regime that exhibits pronounced mechanical non-linearities and to the best of our knowledge no dynamic tuning-range adjustments have been demonstrated for MEMS-tunable VCSEL sources yet.

To characterize the theoretically maximum possible imaging range, several roll-off measurements were performed. First the fringe visibility / amplitude roll-off was analyzed for increasing optical path length differences using a Mach-Zehnder interferometer as described in [24]. The interferometer has a reflective mirror in the delay path, so all mechanical delay values correspond to a 2x longer optical delay. Hence, the delay values given in this paper correspond to single sided OCT imaging depths. The measurement was performed using a 50 GHz photodiode (Finisar, XPDV2320R, USA) and a fast real time oscilloscope (Teledyne LeCroy, LabMaster 10 Zi-A, USA) with 36 GHz detection bandwidth. Second, point spread function (PSF) roll-off...
measurements were performed using a newer FDML laser. The setup of this laser is similar to the one used for the imaging experiments with a slightly shifted center wavelength at 1300 nm and an FFP tuning frequency of 418 kHz. For the decay measurements, interference fringes were acquired as described above. The interferometer setup includes an adjustable mechanical delay line of 80 cm. In this case, data acquisition was performed using a different real time oscilloscope (Keysight, DSOZ634A Infiniium, USA) with 63 GHz detection bandwidth but the same 50 GHz photodiode. The acquired fringes were numerically recalibrated, linearized and subsequently the PSF was determined at each delay as described by Klein et al. [29].

To analyze how axial resolution and imaging range scale with the spectral bandwidth of our OCT system, we calculated the theoretical maximum values. The theoretical axial resolution $\Delta z_{\text{FWHM}}$ was determined based on the spectral full width at half maximum (FWHM) and center wavelength $\lambda_c$ of the laser source [30]. The imaging range $\Delta z_{\text{max}}$ of SS-OCT is determined by the modulation frequency of the interference signal. The modulation frequency depends on the duration of the sweep $T_{\text{Sweep}}$, the center wavelength and spectral bandwidth $\Delta \lambda$ of the laser source. The maximum detectable frequency is limited by the analog detection bandwidth $B$ of the photodetector and ADC. Considering the Nyquist criterion for the detection rate, the following formula can be used to calculate the maximum imaging range of SS-OCT systems:

$$\Delta z_{\text{max}} \approx \frac{B \lambda_c^2}{2 \Delta \lambda T_{\text{Sweep}}}$$

Note that the output spectrum of the FDML laser is not Gaussian-shaped and the tuning frequency is not entirely linear considering the values of the axial resolution. Also, for high fringe frequencies the recalibration process is challenging, which will also affect the presentable OCT imaging depths. Thus, all theoretically calculated values must be considered as a rough estimate. Furthermore, in tissue both values need to be corrected for the refraction index.

2.3. Inline recalibration and k-linearization

While the modification of the sweep bandwidth of the laser is simple, we faced several challenges concerning live processing of the OCT data with variable laser spectra. The main challenge to enable smooth and continuous zooming was to develop a technique to calibrate the raw OCT fringes for image processing.

As for most high-speed swept laser sources, FDML lasers exhibit nonlinear sweep behavior and thus a recalibration step is required before applying the Fourier transformation to the OCT fringes. An obvious hardware recalibration approach would be direct k-clocking of the digitizer card [31]. However, an implementation of a k-clock is very challenging for MHz-OCT systems since at GHz fringe frequency even picosecond level timing jitter causes substantial noise in the OCT image. Alternatively, we numerically linearize the OCT raw fringes using separately acquired calibration fringes. This linearization process includes phase unwrapping of the calibration signal using the Hilbert transform, phase inversion and subsequent non-uniform resampling using Hermite spline interpolation. In previous applications, the numerical recalibration step was performed prior to the OCT imaging session, because the FDML laser provided sufficiently high phase stability [32]. However, when the spectrum of the laser is changed during the imaging process, this approach is not applicable.

Thus, we extended the imaging setup by a separate calibration interferometer, that allows simultaneous acquisition of calibration and OCT fringes. In order to use the full possible detection bandwidth and for the sake of less computational burden, we did not use a separate channel of the digitizer card for continuous recalibration [23]. Instead, the recalibration signal is acquired within the dead time of the OCT raster scan at the turning points of the fast axis scanner. This novel inline recalibration procedure allows resampling of the OCT signal for every frame during live processing of the 3D-rendered OCT images.
To enable fast switching of the calibration and OCT interferometer signal a radio frequency (RF) switch was inserted in front of the ADC card [33]. We developed a scanner driver board to synchronize the scanner and switch signals to the sweep rate of the FDML laser, since timing errors are very critical for MHz-OCT image processing. The board is also used for amplitude and phase control for bidirectional scanning. Furthermore, the algorithm of the real-time processing software had to be adapted to enable resampling of the OCT signal for every frame. A schematic of the adapted imaging setup and the principle of calibration signal acquisition at the turning points of the scanner are illustrated in Fig. 2.

![Fig. 2. Schematic diagram of the OCT imaging setup with spectral zooming feature. The upper right insert illustrates switching between interferometer signal acquisition while scanning for inline recalibration. A specially designed software interface enables synchronized adjustments of the spectral bandwidth and scanning angles.](image)

### 2.4. Scan control

For a real three-dimensional zoom, all dimensions need to be scaled uniformly, which also affects the span of the scanners. In order to adapt the 2-axis scanning amplitude to the axial zoom levels online, a special software interface and custom scanner driver and trigger generation circuit board was designed. This board includes an STM32F4 microcontroller (STMicroelectronics), a home-built driver for the resonant galvanometer scanner and it is synchronized with the FDML laser. The spectral bandwidth of the laser, which is permanently monitored, is used to calculate the scale factor of the scanner’s amplitude. The calculation is based on the previously shown formula to determine the imaging range and the value is sent to the interface board. The scanner driver board serves as an arbitrary waveform generator transferring waveforms to the galvanometer drivers based on the number of lines and frames combined with the scaled amplitude. Using the STM32F4, the phase of the scanners can be freely adapted. Further, feedback and phase monitoring has been implemented within the fast scanner driver.

The scanner driver board is also used to trigger the RF switch to facilitate inline recalibration. Therefore, a trigger is generated closely before and after the turning point of the fast galvanometer scanner in each direction. This way synchronized scanning and data acquisition is realized with data recalibration for every other B-Scan.

### 3. Results and discussion

#### 3.1. System performance

The general performance of the dispersion compensated FDML laser used in this work has already been published [20]. Figure 3(d) shows the balanced fringe signal of two interfered 120 nm laser sweeps, that was used to measure the fringe amplitude decay for different spectral widths settings. This fringe amplitude decay versus the interferometer delay is shown in Fig. 3(a). As evident from the graph, we were able to detect fringes up to the maximum mechanical delay...
of our interferometer of 735 mm for a spectral width of 15 nm. For all plotted measurements, the fringe amplitude does not drop until the fringe frequencies exceed the 36 GHz detection bandwidth of the oscilloscope, which is indicated by dotted lines for each spectral width.

To further characterize the long-range OCT performance, PSF roll-off measurements can be seen in Fig. 3(b-c). To compare the performance of the proposed inline recalibration with previously demonstrated two-channel solutions [23], the data were evaluated using two different methods.

First, the recalibration steps including the entire processing chain were performed on two identical fringes mimicking zero time delay between recalibration fringe measurement and sample measurement. This corresponds to the situation in most non-FDML swept sources, where recalibration fringe signals are simultaneously acquired on a second ADC channel. The corresponding PSFs are displayed in Fig. 3(b) showing 6 dB decays of ~10 cm for 118 nm, ~25 cm for 60 nm, ~50 cm for 30 nm and ~80 cm for 15 nm spectral width, indicating highly stable coherence behavior of the laser. Compared to the fringe amplitude roll-off data, the OCT signal roll-off using this method appears better but this is mainly caused by the 36 GHz bandwidth oscilloscope used for the fringe visibility measurement and the higher 63 GHz bandwidth oscilloscope used for the PSF roll-off. For a spectral width of 15 nm, we were able to detect a proper OCT signal up to a delay of ~80 cm, which is the maximum delay of our experimental interferometer setup.

Second, the recalibration steps were performed on two fringes with a time delay of approximately the acquisition time of one frame, corresponding to the timing settings used for the proposed inline recalibration algorithm. During this measurement, we faced timing problems in terms of trigger jitter, since for detecting multi-GHz fringe frequencies perfect synchronization with picosecond precision is required for accurate recalibration. Since we used a trigger signal with ~9 ns risetime for the individual fringe measurements, we faced a substantial ~1 ns timing jitter between the acquisitions.

In a phase locked sample clock configuration as used in our OCT system this problem would not occur. Thus, the acquired fringe data were numerically corrected by shifting of several samples in order to compensate trigger jitter errors, followed by the standard recalibration steps. As visualized in Fig. 3(c) the roll-off performance is inferior compared to Fig. 3(a-b). For 15 nm bandwidth settings, there is a 6 dB signal roll-off at 30–40 cm. However, the overall PSF amplitude does not drop below 12 dB at a mechanical mirror delay of 800 mm, corresponding to a 1600 mm optical delay. The individual roll-off measurement results for 30 nm, 60 nm and 118 nm laser bandwidths can be found in Supplement 1 (Fig. S1). As stated above, critical timing errors influence the quality of the roll-off measurement, that could not be fully eliminated by numerical correction. Thus, PSF signal fluctuations were clearly visible (compare PSF at delay of 300 mm / 20 GHz). We expect even better roll-off performance using optimized trigger and phase-locking settings. Moreover, we also expect increased performance using a new FDML laser generation using high-finesse FFP-filters [34].

Nevertheless, comparing the different bandwidths settings, it is obvious that using narrower sweep ranges results in reduced fringe frequencies and allows the detection of long delays, which correlates well with the theory described above. Thus, theoretically imaging ranges up to meter scales should be possible using the dispersion compensated FDML laser with narrow spectral width. Especially for ranging applications for navigation purposes, e.g. in a surgical situs where only the surface needs to be detected, the performance of our setup as demonstrated would already be sufficient.

However, to numerically linearize these fringes for OCT imaging, even higher detection bandwidths are required. The overall detection bandwidth of the OCT system used in this work is limited to 1.6 GHz by the photodetector, resulting in shorter imaging ranges. In Fig. 3(e) the theoretically calculated imaging range is plotted versus the spectral bandwidth. It reveals that the
Fig. 3. Characteristics of FDML lasers and OCT system. a) Fringe amplitude decay for different spectral widths. The dotted lines represent the 36 GHz detection bandwidth limit of the real-time oscilloscope [24]. b) PSF signal decay using identical interference fringes with different spectral widths settings. c) PSF signal decay using interference fringes with ~ 100 µs time delay at a laser bandwidth of 15 nm. Each spectral width is color-coded (red: 120/118 nm, blue: 60 nm, yellow: 30 nm, green: 15 nm). d) The balanced fringe signal of two interfered 120 nm laser sweeps acquired with 36 GHz. e) The calculated correlation between spectral bandwidth, axial resolution and imaging range. The vertical dotted lines correspond to three measurements at 4 nm, 17 nm and 120 nm spectral bandwidth, whose spectra are displayed in f) – h). All delay values directly correspond to single sided OCT imaging depth or a 2x longer optical delay.
actual imaging range of the current system will not exceed 15 cm. The curve was interpolated based on three calculations at spectral widths of 4 nm, 17 nm and 120 nm which are depicted in Fig. 3(f-h). Please note, that the spectral resolution of the OSA was adjusted during the spectral measurements. For the 4 nm spectrum, the resolution was increased which is the reason why the power of the spectrum appears to be lower in Fig. 3(f). The graph in Fig. 3(e) shows the correlation between axial resolution and imaging range.

Surprisingly, it was not possible to linearize sweeps for imaging ranges beyond 10 cm using the current detection setup, even though the OCT roll-off measurement results indicate much longer coherence lengths. The fringe amplitude does not drop until 36 GHz which is much larger than the 1.6 GHz bandwidth limit of the used photo detector. We assume, that the FDML laser source exhibits a repetitive, but non-uniform phase tuning behavior [35]. However, due to the extreme tuning parameters this is not straightforward to directly measure and more investigations are required to evaluate this effect. We also noticed that for high delays the numerical recalibration process is very challenging as the point spread function starts to widen. Thus, in the long-range mode we will not achieve the theoretically calculated values for axial resolution and imaging range. However, this is not a problem since the long-range mode will only be used for navigation purposes and the display of superficial layers is sufficient.

3.2. Live 4D-OCT at three discrete resolution modes

To test the feasibility of spectral zooming during live 4D-OCT and its impact on the image quality, we initially defined three discrete resolution modes: a high-resolution mode at 120 nm spectral bandwidth, which should theoretically exhibit 6.3 μm axial resolution but only 3.5 mm imaging range; an intermediate mode at 17 nm spectral width with approximately 44 μm axial resolution and 25 mm imaging range and a long-range mode at 4 nm spectral width with approximately 190 μm axial resolution and 10 cm imaging range.

To compare the modalities, different samples, such as a finger or hand, were imaged using our live 4D-OCT system. Screen shots of the live rendered 3D volumes are displayed in Fig. 4. The corresponding screen capture videos of the live 3D views can be found within the supplementary materials. All in vivo experiments were conducted on voluntary basis by experts of our group and approved by the Ethics Committee of the University of Lübeck. Please note, that the scalebars displayed in Fig. 4, Fig. 5, and Fig. 6 are estimated values to provide better orientation for the observer (precise scalebars are not possible due to the perspective view of the OCT datasets). The exact imaging parameters and dimensions of each imaging mode are provided in Table 1.

3.2.1. High-resolution mode

A fingertip and fingernail were investigated using the high-resolution mode. The high axial resolution of this imaging mode allows to identify different superficial skin layers and subsequently to draw conclusions about the health condition of human skin. The data were processed in real-time providing live 3D views at volume update rates of 22 volumes per second at a volume size of 240 × 300 A-scans. As visible in Fig. 4(a-b), this imaging mode provides only a small lateral FOV and imaging range. However, detailed tissue structures of the superficial skin layers, such as spiral sweat ducts can be observed (Fig. 4(a)). In Fig. 4(b) different layers of the fingernail are visible.

3.2.2. Intermediate mode

Using the intermediate mode setup, the surface of the entire fingertip (Fig. 4(c)) or fingernail (Fig. 4(d)) can be displayed. The surface structures, such as the friction ridges of the fingertip appear very distinct. Due to the decreased axial resolution, small depth-features, such as the sweat ducts in the epidermis cannot be differentiated as compared to the high-resolution mode. However, the advantage of a larger overview of the investigated structure is obvious. 3D OCT
Fig. 4. Three discrete resolution modes: Live 4D-OCT using the high-resolution mode with 120 nm bandwidth (the standard imaging mode of our system [20,24,25] (a-b), intermediate mode with 17 nm bandwidth (c-f) and long-range mode with 4 nm bandwidth (g-k). 3D views of a fingertip and a canula (a, c), of a fingernail (b, d), a caterpillar on a leaf (e) and a snail (f). 3D views of the researcher’s face wearing laser protection glasses (g), shaking hands (h), and holding a cup (i). The corresponding 2D view (j) and en face view (k) of the cup scene. The displayed images are taken from screen recordings of the live 4D-OCT software; the corresponding videos can be found within the supplementary materials (Visualization 1, Visualization 2, Visualization 3). Estimated scalebars.
videos of different moving samples, such as a caterpillar (Fig. 4(e)) and snail (Fig. 4(f)) proof the overall good image quality at live video update rates. In the live renderings only minor specular reflex artifacts are present and the different objects of investigation are clearly visible with well-defined surface features. Compared to previous publications, the image quality of the live rendered OCT data is very good despite the high A-scan rate and live processing without averaging. As in the high-resolution mode, the data were processed in real-time providing live 3D views at volume update rates of 22 volumes per second at a volume size of 240 × 300 A-scans.

3.2.3. Long-range mode

The long-range mode allows for OCT imaging of an entire hand or face (Fig. 4(g-k)). The image quality of this mode suffers from low signal and subsurface structures are barely visible or blurred. At great distances from the zero delay the numerical calibration of the OCT data deteriorates. Thus, at great imaging depth it is hard to distinguish between distinct layers, which is most prominent in the cross-sectional 2D view in Fig. 4(j). While the cup in the foreground is displayed sharply, the contours of the hand at higher delays are blurred. Nevertheless, the surface structure of the displayed objects is clearly visible and due to the visualization of three-dimensional structures in different perspectives the observer receives a good impression of the depicted 3D object and its orientation. Especially considering the en face projection of the OCT data the above mentioned issues are not relevant, since all superficial features are clearly visualized (Fig. 4(k)). In the long-range mode the OCT data were processed in real-time providing live 3D views at volume update rates of 11 volumes per second at a volume size of 480 × 300 A-scans. The long-range mode reveals the potential benefit for navigation on the situs for future OCT applications.

3.3. Live 4D-OCT with axial zooming

To proof the function of the inline recalibration, initially only one-dimensional axial spectral zooming was performed during live 4D-OCT without adjustments of the scanning angles. The scanning optics of the high-resolution mode was used. OCT images of a wild rose were acquired while varying the spectral bandwidth of the laser. The live rendered OCT volumes at different axial zoom levels are displayed in Fig. 5. In the “zoomed in” image in Fig. 5(a) the carpels and stamina of the rose are clearly differentiable, while the “zoomed out” image in Fig. 5(c) provides a larger imaging range and thus more stamina are visible but less detailed. The images are extracted from a continuous screen capture video of our live 4D-OCT-system (supplementary material, Visualization 4). In fact, in this video also a little beetle is crawling in and around the stamina of the rose. When zooming in, defined features of the insect are visible. This highlights the need for variable range imaging and demonstrates its potential application for navigation.

As apparent in the video, the axial zoom is smooth without any stutter during the live updated display of the 3D volume. However, an increase in laser intensity noise can be observed while modifying the spectral width of the FDML laser. In each zoom level, the rendered OCT images appear very distinct and exhibit less background noise than during the zoom modification process. The zooming speed and imaging range is adjusted dynamically according to the manually adjusted potentiometer turns. This is the reason why especially at the beginning of the video (Visualization 4) some distinct jumps between different zoom levels are present. In the next generation of the scan control board, we aim to control the bandwidth of the laser in software by directly controlling the digital amplitude waveform of the FDML filter. Further, another benefit of axial zooming can be observed. Figure 5(b) shows the center of a blossom that exceeds the axial imaging range of approximately 3.5 mm creating zero delay flipping artifacts. After reducing the spectral width of the laser, the blossom nicely fits into the imaging range (Fig. 5(c)). Thus, flipping artifacts can be avoided using our comparatively simple imaging setup instead of implementing complex demodulation techniques for full-range OCT. Suppressing the mirror
The artefact by zooming out is a useful extra feature, especially since in multi-MHz OCT systems the implementation of techniques to resolve the complex conjugate is challenging. For example, applying a $3 \times 3$ coupler [11] requires an additional expensive high-speed analogue to digital converter channel. The application of frequency shifting techniques [12] is challenging because frequency shifters in the multi GHz range are hardly available and at the same time twice the analogue to digital sampling rate is required.

All demonstrated results prove the feasibility of variable range imaging for spectral zooming in OCT and emphasize the need for a real three-dimensional zooming feature.

![Fig. 5. Axial zooming: Screenshots taken from a live rendered 4D-OCT of a wild rose at different axial zoom levels. Images a) and b) are acquired in a high axial zoom level but at different focus depths. In c) the axial view was “zoomed out” displaying a larger imaging range without flipping artifacts. The corresponding video can be found within the supplementary materials (Visualization 4). Estimated scalebars.](image_url)

3.4. Live 4D-OCT with continuous 3D spectral zooming

With the development of a special software interface, we were able to align the scanning angles with the axial spectral zoom level. To test the three-dimensional spectral zooming feature, an alignment disc with a 1.5 mm hole was imaged as depicted in Fig. 6. The scanning optics of the high-resolution mode was used. Similar to the previously shown OCT data, the images are extracts from a live 4D-OCT screen record. For better understanding of the zooming process in three dimensions, we recommend watching the video attached in the supplementary materials (Visualization 5). Here also the corresponding B-Scan and en face views are displayed along with the 3D-view, which highlights how both, the axial imaging range and lateral FOV scale as a function of the adjusted laser bandwidth.

Due to synchronized adjustments of the amplitude of the galvanometer scanners, the axial imaging range fits to the lateral FOV to give a real three-dimensional zooming impression. As obvious at timepoints 00:02 min and 00:03 min in Fig. 6, the synchronization of the fast and slow axis of the scanners still needs to be improved. While zooming into the hole, distortion artifacts appear due to maladjusted angles of each mirror scanner. Especially concerning the control of the resonant scanner, we faced challenges that included phase shifts of the driving signal. In the attached video, the cross-sectional 2D view of the OCT data is also displayed along with the 3D and en face view. The 2D view displays several averaged frames at the center location of the acquired volume and reveals clearly bidirectional scanning issues. The bidirectionally scanned frames are slightly shifted to each other, resulting in blurred image margins.

Further, we noticed an increase in laser noise at spectral widths exceeding 120 nm, where the physical limitation, the maximal FFP amplitude of the FDML laser is reached. We assume that other streak artifacts visible in the video may arise from phase jumps within the FFP when the amplitude is adjusted. Nevertheless, the experiment clearly shows the feasibility of
three-dimensional zooming in live 4D-OCT, which we believe will open up new possibilities for OCT imaging applications, especially in OCT guided surgery.

Fig. 6. Full 3D zooming: Continuous 3D spectral zooming during live 4D-OCT. The images are extracts from screen records of the live 4D-OCT software at different time points, displaying an alignment disc with a 1.5 mm hole (Thorlabs, VRC2SM1, USA). Lateral and axial zooming was performed simultaneously enabling full 3D zooming. The corresponding video can be found in the supplementary materials (Visualization 5). Estimated scalebars.

4. Conclusion and outlook

In this work, we demonstrated long-range imaging using a home-build FDML based 3.28 MHz-OCT system. Improved coherence characteristics of the FDML laser allowed the acquisition of OCT data sets with different resolution modes and imaging ranges up to 10 cm at real-time update rates of more than 1 GVoxel/s. The high-speed live data processing of our live 4D-OCT software is a significant advantage compared to similar long-range OCT results [16,17]. Especially when considering in vivo imaging applications, motion artifacts may be completely avoided.

Furthermore, continuous 3D spectral zooming during live 4D-OCT has been demonstrated. For this, the numerical recalibration procedure used in our previous systems had to be changed. We showed that a novel frame-by-frame inline recalibration method allows for numerical k-linearization of the raw OCT signal for every frame to enable stepless axial zooming. This is realized by acquiring recalibration fringes at the turning points of the fast axis scanner instead of prior to the imaging session. A separate calibration interferometer and special software interface have been inserted into the detection setup to synchronize lateral and axial zooming during the imaging process. The operating principle of spectral zooming is based on the tradeoff
between high resolution and long imaging range [23]. As visualized in several images and videos, the typical strength of OCT to depth resolve tissue structures gets lost when zooming out. However, at increased imaging ranges and lateral FOVs the observer benefits from better orientation. Continuous 3D zooming may be a useful additional tool to enable navigation on complex structures, especially when considering the live 4D-OCT for a surgical microscope.

One limitation of the current system is the use of simple scan optics without autofocus. Even though the overall OCT-system setup allows for continuous adaptation of the imaging range, the distance to the observed object and lateral FOV is determined by the numerical aperture of the used scan lens. Thus, when “zooming out” for navigation purposes, some parts of the object under investigation may not be in focus resulting in undefined, blurred boundaries within the OCT images. For better performance and resolution in all zoom levels, the use of autofocus optics should be considered. Moreover, the use of a resonant scanners is always subject to complexities concerning phase stability. In future experiments, we want to analyze the use of a non-resonant galvanometer scanners for the fast axis, since additional OCT setups have already proven that a galvanometer scanner is sufficient for live 4D-OCT as well [8]. Furthermore, we would like to transfer the inline recalibration algorithm also for rotational-scanning, endoscopic OCT applications. Since in most micro-motor driven endoscopic scanners used for high-speed imaging shadows cast by the motor supply wiring are present, those regions would allow for the same dead-time recalibration as in the proposed method.

Funding. European Research Council (646669); State of Schleswig Holstein (Excellence Chair Programme); Deutsche Forschungsgemeinschaft (EXC 2167-390884018); Bundesministerium für Bildung und Forschung (15GW0227B).

Disclosures. T. Pfeiffer: University of Lübeck (P), Optores GmbH (E); W. Draxinger: Optores GmbH (I); R. Huber: University of Lübeck (P), Optores GmbH (LPR), Optovue Inc. (I.R), Abott (I.R).

Data availability. Raw data underlying the results presented in this paper are not publicly available due to excessive size but can be obtained from the authors upon reasonable request.

Supplemental document. See Supplement 1 for supporting content.

References
1. D. Huang, E. Swanson, C. Lin, J. Schuman, W. Stinson, W. Chang, M. Hee, T. Flotte, K. Gregory, and C. Puliafito, “Optical coherence tomography,” Science 254(5055), 1178–1181 (1991).
2. R. Huber, M. Wojtkowski, and J. G. Fujimoto, “Fourier Domain Mode Locking (FDML): A new laser operating regime and applications for optical coherence tomography,” Opt. Express 14(8), 3225–3237 (2006).
3. B. Biedermann, W. Wieser, C. Eigenwillig, and R. Huber, “Recent developments in Fourier Domain Mode Locked lasers for optical coherence tomography: Imaging at 1310 nm vs. 1550 nm wavelength,” J. Biophotonics 2(6-7), 357–363 (2009).
4. W. Wieser, B. Biedermann, T. Klein, C. Eigenwillig, and R. Huber, “Multi-Megahertz OCT: High quality 3D imaging at 20 million A-scans and 4.5 GVoxels per second,” Opt. Express 18(14), 14685–14704 (2010).
5. T. Klein, W. Wieser, R. André, T. Pfeiffer, C. Eigenwillig, and R. Huber, “Multi-MHz FDML OCT: snapshot retinal imaging at 6.7 million axial-scans per second,” SPIE BIOS (SPIE, 2012), Vol. 8213.
6. W. Wieser, W. Draxinger, T. Klein, S. Karpf, T. Pfeiffer, and R. Huber, “High definition live 3D-OCT in vivo: design and evaluation of a 4D OCT engine with 1 GVoxel/s,” Biomed. Opt. Express 5(9), 2963–2977 (2014).
7. J. P. Kolb, W. Draxinger, J. Klee, T. Pfeiffer, M. Eibl, T. Klein, W. Wieser, and R. Huber, “Live video rate volumetric OCT imaging of the retina with multi-MHz A-scan rates,” PLoS ONE 14(3), e0213144 (2019).
8. W. Draxinger, Y. Miura, C. Grill, T. Pfeiffer, and R. Huber, “A real-time video-rate 4D MHz-OCT microscope with high definition and low latency virtual reality display,” in Clinical and Preclinical Optical Diagnostics II, SPIE Proceedings (Optical Society of America, 2019), 11078–11071.
9. Y. Miura, W. Draxinger, C. Grill, T. Pfeiffer, S. Grisanti, and R. Huber, “MHz-OCT for low latency virtual reality guided surgery: First wet lab experiments on ex-vivo porcine eye,” in Clinical and Preclinical Optical Diagnostics II, SPIE Proceedings (Optical Society of America, 2019), 11078, 11013.
10. Z. Wang, H.-C. Lee, D. Vermeulen, L. Chen, T. Nielsen, S. Y. Park, A. Ghaemi, E. Swanson, C. Doerr, and J. Fujimoto, “Silicon photonic integrated circuit swept-source optical coherence tomography receiver with dual polarization, dual balanced, in-phase and quadrature detection,” Biomed. Opt. Express 6(7), 2562–2574 (2015).
11. M. V. Sarunic, M. A. Choma, C. Yang, and J. A. Izatt, “Instantaneous complex conjugate resolved spectral domain and swept-source OCT using 3x3 fiber couplers,” Opt. Express 13(3), 957–967 (2005).
12. S. Yun, G. Tearney, J. de Boer, and B. Bouma, “Removing the depth-degeneracy in optical frequency domain imaging with frequency shifting,” Opt. Express 12(20), 4822–4828 (2004).
13. M. Siddiqui, S. Tozburun, E. Z. Zhang, and B. J. Vakoc, “Compensation of spectral and RF errors in swept-source OCT for high extinction complex demodulation,” Opt. Express 23(5), 5508–5520 (2015).

14. B. Potsaid, V. Jayaraman, J. Fujimoto, J. Jiang, P. J. Heim, and A. Cable, “MEMS tunable VCSEL light source for ultrahigh speed 60kHz - 1 MHz axial scan rate and long range centimeter class OCT imaging,” SPIE BIOS (SPIE, 2012), Vol. 8213.

15. M. Bonesi, M. P. Minneman, J. Ensher, B. Zabihian, H. Sattmann, P. Boschert, E. Hoover, R. A. Leitgeb, M. Crawford, and W. Drexler, “Akinetic all-semiconductor programmable swept-source at 1550 nm and 1310 nm with centimeters coherence length,” Opt. Express 22(3), 2632–2655 (2014).

16. S. Song, J. Xu, and R. K. Wang, “Long-range and wide field of view optical coherence tomography for in vivo 3D imaging of large volume object based on akinetic programmable swept source,” Biomed. Opt. Express 7(11), 4734–4748 (2016).

17. Z. Wang, B. Potsaid, L. Chen, C. Doerr, H.-C. Lee, T. Nielson, V. Jayaraman, A. E. Cable, E. Swanson, and J. G. Fujimoto, “Cubic meter volume optical coherence tomography,” Optica 3(12), 1496–1503 (2016).

18. D. Adler, W. Wieser, F. Trépanier, J. Schmitt, and R. Huber, “Extended coherence length Fourier domain mode locked lasers at 1310 nm,” Opt. Express 19(21), 20930–20939 (2011).

19. W. Wieser, T. Klein, D. Adler, F. Trépanier, C. Eigenwillig, S. Karpf, J. Schmitt, and R. Huber, “Extended coherence length megahertz FDML and its application for anterior segment imaging,” Biomed. Opt. Express 3(10), 2647–2657 (2012).

20. T. Pfeiffer, M. Petermann, W. Draxinger, C. Jirauschek, and R. Huber, “Ultra low noise Fourier domain locked laser for high quality megahertz optical coherence tomography,” Biomed. Opt. Express 9(9), 4130–4148 (2018).

21. T. Pfeiffer, M. Göb, W. Draxinger, S. Karpf, J. P. Kolb, and R. Huber, “Flexible A-scan rate MHz-OCT: efficient computational downscaling by coherent averaging,” Biomed. Opt. Express 11(11), 6799–6811 (2020).

22. R. Huber, M. Wojtkowski, K. Taira, J. G. Fujimoto, and K. Hsu, “Amplified, frequency swept lasers for frequency domain reflectometry and OCT imaging: design and scaling principles,” Opt. Express 13(9), 3513–3528 (2005).

23. J. Zhang, T. Nguyen, B. Potsaid, V. Jayaraman, C. Burgner, S. Chen, J. Li, K. Liang, A. Cable, G. Traverso, H. Mashimo, and J. G. Fujimoto, “Multi-MHz MEMS-VCSEL swept-source optical coherence tomography for endoscopic structural and angiographic imaging with miniaturized brushless motor probes,” Biomed. Opt. Express 12(4), 2384–2403 (2021).

24. T. Klein and R. Huber, “High-speed OCT light sources and systems [Invited],” Biomed. Opt. Express 8(2), 828–859 (2017).

25. Optical Coherence Tomography: Technology and Applications, 2nd ed. (Springer, Cham, 2015).

26. J. Xi, L. Huo, J. Li, and X. Li, “Generic real-time uniform K-space sampling method for high-speed swept-Source optical coherence tomography,” Opt. Express 18(9), 9511–9517 (2010).

27. T. Klein, W. Wieser, C. M. Eigenwillig, B. R. Biedermann, and R. Huber, “Megahertz OCT for ultrawide-field retinal imaging with a 1050 nm Fourier domain mode-locked laser,” Opt. Express 19(4), 3044–3062 (2011).

28. R. Huber, W. Draxinger, and T. Pfeiffer, “Verfahren zum Monitoring von zeitabhängigen Eigenschaften des Lichts bei der scansenden Swept-Source Optischen Kohärenztomographie “ Patent, DE102018212100B3 (2018).

29. C. Grill, S. Lotz, T. Blömker, M. Schmidt, W. Draxinger, J. P. Kolb, C. Jirauschek, and R. Huber, “Superposition of two independent FDML lasers,” in 2021 Conference on Lasers and Electro-Optics Europe and European Quantum Electronics Conference, OSA Technical Digest (Optical Society of America, 2021), cf_9_6.