Design of a fiber-optic multiphoton microscopy handheld probe

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Abstract: We have developed a fiber-optic multiphoton microscopy (MPM) system with handheld probe using femtosecond fiber laser. Here we present the detailed optical design and analysis of the handheld probe. The optical systems using Lightpath 352140 and 352150 as objective lens were analyzed. A custom objective module that includes Lightpath 355392 and two customized corrective lenses was designed. Their performances were compared by wavefront error, field curvature, astigmatism, F-θ error, and tolerance in Zemax simulation. Tolerance analysis predicted the focal spot size to be 1.13, 1.19 and 0.83 µm, respectively. Lightpath 352140 and 352150 were implemented in experiment and the measured lateral resolution was 1.22 and 1.3 µm, respectively, which matched with the prediction. MPM imaging by the handheld probe were conducted on leaf, fish scale and rat tail tendon. The MPM resolution can potentially be improved by the custom objective module.

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1. Introduction

Multiphoton microscopy (MPM) is an important biological imaging modality. It can be applied to non-invasive study of biological samples to obtain sub-micron resolution imaging in three dimensions. MPM utilizes multiple incident photons to excite one emission photon. This nonlinear imaging modality generates sharp contrast and high resolution images [1–5]. Currently, most MPM systems use bench top setup based on free-space optical elements, resulting in the inconvenience of accessing some tissue sites. Hence, requirement to expand the applications of MPM by handheld probe or endoscope is urgent, where light can be delivered in flexible fiber and images can be captured using a miniaturized probe. To meet the above requirement, many research groups focus on endoscope design and establishment [6–9].

Miniature objective lens is required to make the MPM probe or endoscope compact. The miniature objective lens needs to have high numerical aperture (NA) and low aberrations in order to provide tight focusing of the laser beam for efficient MPM excitation. Gradient index (GRIN) lens that has relatively high NA and very small diameter has been used [9]. However, GRIN lens suffers from high aberration which deteriorates the image quality. Customized miniature objective lenses have been developed by some research groups [8, 10] but they are not commercially available to the public and their specifications were tailored to specific applications. Commercial miniature aspherical lens has small size, relatively high NA, and low spherical aberration. Thus they have the potential to be used as the objective lens in MPM endoscopy. Nevertheless, their performance still needs to be investigated.

For MPM imaging, a tightly focused laser beam is scanned by an XY scanner to acquire 2D images. Normally, a scan lens and a tube lens are inserted between the scanner and the objective lens, where the scanner is imaged to the back focal plane of the objective lens. The scan lens and tube lens act as a relay system and also a beam expander to overfill the back aperture of the objective lens. Therefore, to examine the MPM system performance, the optical system, including the objective lens, scan lens, and tube lens need to be investigated together. Hoy et al. studied several miniature aspherical lenses in their MPM probe design [11]. They found that off-axis aberration deteriorated the image resolution at large scanning angles. They further designed aspheric relay lenses to reduce the aberration. However, their experimentally measured resolution was much lower than the theoretically predicted resolution. Therefore, there is the need to further investigate the performance of the lens system in MPM handheld probe and endoscope.

Scientific studies and clinical application require high optical resolution and large field of view (FOV). MPM prototypes established by off-the-shelf miniature lenses can hardly satisfy this requirement. A full design of all custom lenses is time consuming and expensive. The idea of utilizing a minimum number of customized lenses to replace some commercial lenses in a focusing and scanning module has emerged. Negrean et al. performed optimization on the scan and tube lens design to improve the resolution over a large FOV for a laser scanning microscope [12]. This approach not only took into account the cost, but also improved the optical performance of the prototypes; furthermore, it pointed out to a promising direction of improvement for future prototype industrialization.

We have recently developed a fiber-based compact MPM system with a handheld probe using femtosecond fiber laser and MEMS scanner [13]. Here we report on the detailed design considerations of the optical system of the handheld probe. The optical systems using off-the-shelf miniature aspherical lens as objective lens were analyzed in details by Zemax simulation. Critical optical parameters, including wavefront error, field curvature, astigmatism, F-θ error, collection efficiency, and tolerance were compared for different lens systems.
Experiments were carried out to demonstrate the image quality obtained by the handheld probe system. Tolerance analysis showed that tolerance factors such as alignment error reduced the resolution, which explained why the experimentally measured resolution was worse than the theoretical diffraction-limited resolution. While off-the-shelf lens suffered from large aberrations, we further designed a custom objective lens module to improve the optical performance. The lens module was designed using a commercial high NA aspherical lens and two customized spherical lenses. With tolerance analysis, the results showed that the customized lens module was able to achieve lateral resolution less than 1 µm even with reasonable tolerance. In the paper, Section 2 presents the configuration of the MPM system and handheld probe. Section 3 presents the detailed analysis of the optical systems by Zemax simulation. Section 4 presents the experimental results obtained by the MPM handheld probe. Section 5 concludes the paper.

2. System configuration

Figure 1 shows the schematic of the compact MPM system based on femtosecond fiber laser. The detailed description of the system can be found in Ref [13]. The femtosecond fiber laser provides a pulsewidth of 250 fs, output power of 180 mW, and wavelength of 1580 nm. A frequency-doubling crystal is used to convert the wavelength to 790 nm in order to excite intrinsic MPM signals from tissues. The crystal is mounted inside the handheld probe. The fiber laser output is delivered to the handheld probe by a single mode fiber. The received MPM signal is sent from the handheld probe to a photomultiplier tube detector through a multimode fiber. The handheld probe measures 165 mm × 45 mm × 40 mm in length, width and height, respectively.

The detailed optical layout of the handheld probe is shown in Fig. 2. The optical system is mainly composed of three parts: the frequency-doubling module, the scanning and focusing module, and the MPM signal collection module. In the frequency-doubling module, the laser beam is focused onto a periodically-poled lithium niobate (PPLN) crystal to achieve high conversion efficiency [14]. After the PPLN, the beam is collimated. The collimated beam is raster scanned by a MEMS mirror, and then reflected by a dichroic mirror (FF670-SDi01, Semrock). In the scanning and focusing module, the beam is relayed by a scan lens and tube lens, and then fills the back aperture of an objective lens. The scanning center of the MEMS is...
imaged to the back focal plane of the objective lens by the relay optics. The MPM signal is backward collected by the same objective lens, and then passes through the dichroic mirror. In the signal collection module, the MPM signal is coupled into a multimode fiber.

3. Optical system simulation by Zemax

The optical resolution of the system is mainly determined by the NA of the objective lens, and the aberrations from the scanning and focusing module. In the next, we will analyze the performance of off-the-shelf aspherical lenses as objective lens. A custom objective lens module is also designed to improve the performance. The different lens systems are compared for their performances.

3.1 Comparison of the optical performance of off-the-shelf lens system

Miniature aspherical lenses are a good candidate of objective lens for MPM handheld probe due to their relatively high NA and small diameter. Table 1 shows the specifications of three off-the-shelf miniature aspherical lenses of relatively high NA. As the incident laser has a relatively narrow bandwidth, the influence of chromatic aberration is not taken into account in this study. The influence of monochromatic aberrations is considered, which includes spherical aberration, coma, astigmatism, field curvature and distortion.

| Model            | Focal length (mm) | Diameter (mm) | Clear aperture (mm) | NA | Working distance (mm) |
|------------------|-------------------|---------------|---------------------|----|-----------------------|
| Lightpath 352140 | 1.45              | 2.4           | 1.6                 | 0.55 | 0.88                  |
| Lightpath 352150 | 2                 | 3.0           | 2.0                 | 0.50 | 1.09                  |
| Lightpath 355392 | 2.75              | 4.0           | 3.6                 | 0.64 | 1.50                  |

The optical system using Lightpath 352140 as the objective lens is analyzed. Figure 3(a) shows the optical layout of the scan lens (AC080-020-A, f = 20.0 mm, Thorlabs), tube lens (AC080-020-A, f = 20.0 mm, Thorlabs) and objective lens. Figure 3(b) is a zoom in of the Lightpath 352140 layout. The RMS wavefront error versus FOV is shown in Fig. 3(c). The RMS wavefront error increases as the FOV increases. Only in the central FOV of ± 30 µm it satisfies the diffraction-limited criterion of 0.0745 wave RMS wavefront error. The RMS waveform error increases to 0.25 wave at the FOV of ± 70 µm, and to 0.55 wave at the FOV of ± 100 µm, respectively. The field curvature and astigmatism are shown in Fig. 3(d). At the edge of the FOV of ± 100 µm, the sagittal and tangential rays come to focus around 5.2 µm and 13 µm away from the focal plane, respectively, resulting in a separation between the focus of the sagittal and tangential rays of ~7.8 µm. This large separation will reduce the resolution and
signal intensity at the edge of the FOV. F-θ error is another important factor which determines whether the images are distorted [15]. In raster scan, the focus position is assumed to be proportional to the angle of the scan mirror. At small angles this assumption holds well but it deviates from the linear relation at large scan angles. The F-θ error is shown in Fig. 3(e). It can be found that the F-θ error is around 1.2% at the edge of the FOV of ± 100 µm.

Fig. 3. Simulated focusing and scanning module composed of Lightpath 352140 aspherical lens. (a) optical layout by Zemax (b) objective lens Lightpath 352140 layout by Zemax (c) RMS wavefront error versus field of view (d) Field curvature and astigmatism versus field of view (e) F-θ error versus field of view.

The optical system using the Lightpath 352150 as the objective lens is also analyzed. Figure 4(a) is the optical layout of the scan lens (AC080-020-A, f = 20.0 mm, Thorlabs), tube lens (AC080-020-A, f = 20.0 mm, Thorlabs) and objective lens. Figure 4(b) is the zoom-in Lightpath 352150 layout. The RMS wavefront error versus FOV is shown in Fig. 4(c). In the central FOV of ± 60 µm it satisfies the diffraction-limited criterion. At the FOV of ± 140 µm, the RMS wavefront error is still within 0.25 wave. The field curvature and astigmatism are shown in Fig. 4(d). At the edge of the FOV of ± 140 µm, the sagittal and tangential rays come to focus around 7.5 µm and 13 µm away from the focal plane, respectively, resulting in a separation between the focus of the sagittal and tangential rays of ~5.5 µm. Figure 4(e) shows the F-θ error. It can be found that the F-θ error is around 0.4% at the edge of the FOV of ± 140 µm. In comparison, the achievable FOV by Lightpath 352150 is much larger than that by
Lightpath 352140 under the same RMS wavefront error. The astigmatism and F-\(\theta\) error for the Lightpath 352150 module are less than that of Lightpath 352140 module, respectively.

### 3.2 Design of custom objective lens module

The Lightpath 355392 aspherical lens has an NA of 0.64 which is higher than 352140 and 352150. Thus the Lightpath 355392 has the potential to provide higher resolution than the other two lenses. Here, the Lightpath 355392 aspherical lens used as the objective lens is also analyzed. Figure 5(a) shows the scan lens (AC050-008-A, \(f = 8.0\) mm, Thorlabs), tube lens (AC080-020-A, \(f = 20.0\) mm, Thorlabs) and objective lens optical layout of Lightpath 355392 module. When no other correction lens is used, the RMS wavefront error versus FOV is shown in Fig. 5(c). Only a very small FOV of \(\pm 10\) \(\mu\)m is within the 0.25 wave RMS wavefront error. The RMS wavefront error dramatically escalates with the increase of the FOV, even reaching 3.0 waves at the FOV of \(\pm 100\) \(\mu\)m due to significant off-axis aberrations. From the above results, we can see that the off-axis aberrations are much more significant for higher NA objective. In this case, using only the off-the-shelf lenses is not sufficient to compensate for the aberrations and achieve high resolution over a reasonable FOV.

To address this problem, we propose a custom design of objective lens module. A commercial lens Lightpath 355392 is used as the primary focusing lens. Two custom correction lenses are designed and inserted between the tube lens and the Lightpath 355392, to compensate for the aberrations. The specific optical parameters of the two customized correction lenses are shown in Table 2. The approach of combining off-the-shelf lens and some customized correction lens components can achieve a higher image quality while also maintain a relatively low cost.

| Name | Surface | Radius (mm) | Thickness (mm) | Glass       | Semi-Diameter (mm) |
|------|---------|-------------|----------------|-------------|---------------------|
| Lens1| spherical | -18.615     | 2.0            | H-K51       | 4                   |
|      | spherical | Infinity    |                | H-LAK53A    |                     |
|      | spherical | 7.085       | 4.0            |             |                     |
| Lens2| spherical | -5.482      | 4.0            | H-ZF88      | 4                   |
|      | spherical | 11.515      |                |             |                     |

The optical performance of Lightpath 355392 with the correction lenses is analyzed. Figure 5(b) shows the optical layout of the scan lens (AC050-008-A, \(f = 8.0\) mm, Thorlabs), tube lens (AC080-020-A, \(f = 20.0\) mm, Thorlabs) and objective lens of Lightpath 355392 with correction lenses. The FOV that satisfies the 0.25 wave RMS wavefront error is significantly increased to \(\pm 100\) \(\mu\)m, as shown in Fig. 5(c). Figure 5(d) shows the field curvature and astigmatism. Near the edge of the FOV of \(\pm 100\) \(\mu\)m, the sagittal and tangential rays come to focus around 3.25 \(\mu\)m and 5.75 \(\mu\)m away from the focal plane, respectively, resulting in a separation between the focus of sagittal and tangential rays of \(\sim 2.5\) \(\mu\)m, as shown in Fig. 5(e). The increase of field curvature and astigmatism is not significant, which indicates reduced off-axis aberration. Besides, the F-\(\theta\) error being around 0.4\% satisfies the requirement of linear scanning, as shown in Fig. 5(f).
To more precisely analyze the optical performance of the new scanning and focusing module, Seidel coefficients are provided [16]. For the scanning and focusing modules without and with the corrective lens, the values of the Seidel coefficients are compared in Fig. 6. Here, S1, S2, S3 and S4 are the Seidel coefficients for coma, astigmatism, field curvature and distortion, respectively. The results show that the field curvature and astigmatism of the focusing and scanning module without corrective lens are considerably large at the focal plane. This is the main factor influencing the resolution over the FOV. To reduce the field curvature and astigmatism, separate positive and negative lenses are required to reduce the aberration in the whole FOV. Therefore, a spherical negative lens and a spherical positive doublet lens are designed and supplied before the Lightpath 355392 objective lens as the corrective lenses. It demonstrates that the field curvature and astigmatism of the new module with corrective lens are significantly reduced, and the coma and distortion are nearly unchanged. When there is no corrective lens in the scanning and focusing module, the aberrations caused by the relay optical path at large scanning angle utterly rely on the aspherical objective lens to balance. However, as
a single aspherical lens has limited ability to balance the aberrations, not all aberrations can be removed sufficiently, resulting in rapid increase of aberrations with the increase of FOV. From the aforementioned analysis and simulation, we can see that the optical module of Lightpath 355392 objective lens performances far better with the corrective lenses than without the corrective lenses.

Fig. 6. Seidel coefficients comparison between the focusing and scanning module composed of Lightpath 355392 with corrective lens and without corrective lens.

3.3 Comparison of resolution

To characterize the theoretical lateral resolution, the intensity profile of the focal spot in the central FOV is simulated and the result is shown in Fig. 7. The lateral resolution is determined as the full width at half maximum (FWHM) of the intensity profile after fitting to a Gaussian function. For the focusing and scanning module composed of Lightpath 352150, 352140, and 355392 with corrective lenses, the theoretical predicted lateral resolution is gradually improved from 0.96 µm, 0.88 µm, to 0.75 µm, respectively. The Lightpath 355392 with corrective lenses module not only achieves the highest resolution among the aforementioned three modules but also provides a reasonable FOV of ± 100 µm. It is mainly because the NA of Lightpath 355392 with corrective lenses module is slightly larger than Lightpath 352140, 352150, and the corrective lenses reduce the aberrations.

Fig. 7. Zemax simulation of the intensity profile of the focal spot at the central FOV for the focusing and scanning module composed of Lightpath 352140, Lightpath 352150 or Lightpath 355392 with corrective lens.

3.4 Tolerance analysis

The Zemax simulation indicates that the theoretical lateral resolution of the above three systems can reach below 1 µm. However, the systems are established based on the commercial lens category and general precision mechanical process. Those factors will inevitably influence the
system optical performance. To predictably point out the trend in the performance, tolerance analysis is performed. Tolerance analysis considers the effects of manufacturing defects and alignment errors. For example, the axial placement of a lens is affected by both the lens thickness tolerance and the axial alignment tolerance. Considering the manufacturing tolerance of lenses and the alignment tolerance, tolerance analysis is performed to further analyze the resolution. The lenses are chosen from commercial lens category from Thorlabs and Lightpath. According to their specifications, the lenses have fabrication tolerance of lens surface tolerance of 3 fringes, lens thickness tolerance and lens diameter tolerance of $\pm 0.15$ mm and $\pm 0.0/-0.1$ mm respectively for achromatic lens, and the lens thickness tolerance and lens diameter tolerance of $\pm 0.04$ mm and $\pm 0.015$ mm respectively for aspheric lens. In addition, the lens surface irregular tolerance is 1 fringe.

The assembly of the custom objective module of Lightpath 355392 and the corrective lenses is considered “precision” where the three pieces should be assembled into a “sleeve” structure at professional optic-mechanical workshop. The alignment sensitivity of the other lenses is assumed “commercial” level alignment crafts. For “commercial” level lens alignment, the tilt tolerance is $\pm 0.5^\circ$, and the lateral and axial position tolerances are $\pm 80 \mu$m and $\pm 50 \mu$m, respectively [17].

To predict the optical design outcome accurately, Monte Carlo simulation is performed for tolerance analysis. The Monte Carlo simulation randomly generates a series of optical system trials where the tolerance parameters are perturbed within their respective range. Here 2,000 randomly perturbed system trials are generated and the statistical outcome obeys normal distribution. Figure 8 shows the lateral resolution at the central FOV for the three focusing and scanning module at 50% probability. It is found that 50% of the trials exhibit spot size within 1.19 $\mu$m and 1.13 $\mu$m, for the scanning and focusing module composed of Lightpath 352150 and Lightpath 352140, respectively. For Lightpath 355392 with corrective lenses module, it is obtained that 50% of the trials exhibit spot size within 0.83 $\mu$m. Later, we will show that these Monte Carlo simulation predictions are in good agreement with the experimental results.

![Fig. 8. Monte Carlo simulation results on the spot size at the central FOV for the three different scanning and focusing modules.](image)

In MPM handheld probe or endoscope, the lens system needs to have a small diameter and the objective lens needs to have an NA higher than 0.5. Such optical system usually suffers from large off-axis aberrations. While the center of the FOV can satisfy diffraction-limited criterion (0.0745 wave) RMS wavefront error for almost aberration-free performance, the edge of the FOV has much higher wavefront error. The effect of aberration on the point-source resolution is complicated and depends on the specific type of aberrations. For certain types of aberrations, such as spherical aberration, the point-source resolution doesn’t change much even when the RMS wavefront error is increased to 0.25 wave. The magnitudes of the tolerances for different qualities of optical instrument has been described by Shannon, where he defined 0.25 wave RMS wavefront error as the tolerance parameter for commercial-level lens manufacture.
All the lenses used in our handheld MPM probe have the commercial-level lens precision. Therefore, 0.25 wave RMS wavefront error has been used as a practical tolerance for obtaining the FOV with reasonable optical performance.

### 3.5 Fiber collection efficiency analysis

The collection efficiency of the optical system is also analyzed by Zemax simulation. The collection optical path is shown in Figs. 9(a) and 9(b) for the system using Lightpath 352140 and 352150, respectively. It is consisted of the source of the excited fluorescence signal, objective lens (Lightpath 352140 or 352150), tube lens and scan lens (AC080-020-A, f = 20.0mm), dichromic mirror, coupling lens (AC050-008-A, f = 8.0 mm), and multimode fiber (FT1500UMT, Ø1500 μm, NA = 0.39, Thorlabs). Here we consider the fluorescence signal which is assumed to be a point source with isotropic direction. Only the fluorescence signal that is within the collection angle of the objective lens will enter the optical system. Our simulation then analyzes the relative collection efficiency by calculating the number of rays that are finally coupled into the fiber divided by the number of emitted rays entered the optical system. At the edge of the FOV, the excited fluorescent signal is harder to be collected by the fiber than those at the center of the FOV. Therefore, the collection efficiency at the edge of the FOV is simulated. The FOVs for the Lightpath module 352140 and 352150 are ± 100 μm and ± 140 μm, respectively. The point source is modeled to emit 500,000 rays within the collection angle of the objective lens. Those rays are distributed at 350 nm, 550 nm, and 650 nm wavelengths to represent the wide range of fluorescence spectrum.

The result shows that the incident rays to the multimode fiber tip fall within a solid angle that corresponds to an NA of 0.13 and 0.12 for the system containing the Lightpath 352140 and 352150, respectively. Those numbers are less than the NA of the multimode fiber. Therefore, the relative collection efficiency is 100% when the fiber is positioned at the focus of the coupling lens. The condition when the fiber is offset from the focal point is also simulated. Figure 9(c) shows how the relative collection efficiency varies with the defocus distance of the fiber. When the axial defocus distance is within ~0.4 mm, the collection efficiency can reach 100%. When the defocus distance is larger, the collection efficiency decreases. The result
shows that the fiber position has sufficient axial tolerance redundancy to achieve high collection efficiency during the probe alignment.

### 4. Experimental results

Based on the off-the-shelf lenses of Lightpath 352140 and 352150, the handheld MPM probe prototype was assembled and tested. The mechanical housing of the probe was fabricated through 3D printing. The custom objective module with Lightpath 355392 requires two customized lenses which requires a long fabrication period and thus it will not be included in the experimental testing in this paper.

The lateral and axial resolution of the image probe is characterized by measuring the point spread functions (PSFs). Two-photon excited fluorescence (TPEF) images of 0.1-μm diameter fluorescent beads (Fluoresbrite Calibration Grade Size Range Kit, Polysciences, Inc.) are acquired. 2D image is acquired by scanning the MEMS mirror in X and Y directions. Axial scan is achieved by scanning the sample with a high precision motorized actuator (LTA-HS, Newport). The axial scanning step size is 1 μm. At each axial step position, a 2D image of the beads is acquired. PSFs are obtained from the 2D image stack. After Gaussian fitting, the FWHMs of the PSFs are obtained, which are shown in Fig. 10. For the focusing module composed of Lightpath 352140 objective lens and Lightpath 352150 objective lens, the lateral resolution is measured as 1.22 μm and 1.3 μm, respectively; and the axial resolution is measured as 13.0 μm and 16.2 μm, respectively.

![Fig. 10. Experimentally measured lateral and axial PSFs for the focusing and scanning module composed of Lightpath 352140, (a) lateral and (c) axial resolution. PSFs for the module composed of Lightpath 352150, (b) lateral and (d) axial resolution.](image)

The FOV of the MPM imaging is limited by the scan angle of the MEMS mirror and the size of the objective lens. The optical system design ensures that the full scanning angle of the MEMS can be utilized which is achieved when the scanning voltage is 10 V for both the X-axis and Y-axis. The voltage can be reduced if a smaller FOV is needed. Figure 11 shows the TPEF...
images of 6 μm diameter fluorescence beads, when the scanning voltages of X-axis and Y-axis are (10 V, 10 V). The beads are clearly observed in both images, where the image obtained by the Lightpath 352150 objective lens shows a larger FOV than that by the Lightpath352140 objective lens. At the corners of the FOV, slight blurring and reduction in intensity is observed due to the off-axis aberrations. The image size is slightly reduced in the X axis where a sinusoidal scanning waveform is used for the fast X axis.

![TPEF images of 6-μm-diameter fluorescence beads](image1)

**Fig. 11.** TPEF images of 6-μm-diameter fluorescence beads acquired by the MPM probe system, under scanning voltages of (10 V, 10 V), (a) acquired by Lightpath 352140 objective lens module and (b) acquired by Lightpath 352150 objective lens module. Scale bar is 50 μm.

Figures 12(a)-12(d) show the TPEF images of fresh leaf sample obtained with Lightpath 352140 and 352150 at scanning voltages of (10 V, 10 V) and (8 V, 8 V), respectively. High resolution images of stomata (holes in a leaf) and papillae on the leaf surface are observed as a well-organized pattern.

![TPEF images of leaf sample](image2)

**Fig. 12.** TPEF images of leaf sample acquired by Lightpath 352140 objective lens module (a) and (b), acquired by Lightpath 352150 objective lens module (c) and (d), under scanning voltages of (10 V, 10 V) and (8 V, 8 V), respectively. The scale bar is 50 μm.

By comparing the above images, the module containing the Lightpath 352140 objective lens shows a higher magnification, while its FOV is smaller than that of the Lightpath 352150 module at the same scanning voltage. The Lightpath 352150 module is more applicable to the imaging of a larger FOV. Also, the resolution of the Lightpath 352150 module looks more uniform in the whole FOV.
The image performance of the handheld probe with the Lightpath 352150 objective lens is demonstrated on fish scale and rat tail tendon. Figures 13(a)-13(b) show the second harmonic generation (SHG) images of fish scale and Figs. 13(c)-13(d) shows the SHG images of rat tail tendon, at the scanning voltages of (10 V, 10 V) and (8 V, 8 V), respectively. The SHG contrast shows the collagen fiber organization. In the rat tendon images, highly organized collagen fiber bundles oriented in the same direction can be identified.

![SHG images of fish scale and rat tail tendon](image)

Fig. 13. (a) (b) SHG images of fish scale, (c) (d) SHG images of rat tail tendon, acquired by Lightpath 352150 objective lens module under scanning voltages of (10 V, 10 V) and (8 V, 8 V), respectively. The scale bar is 50 µm.

The experimentally measured lateral resolution of the MPM probe with Lightpath 352140 and Lightpath 352150 is 1.22 µm and 1.3 µm respectively, which are worse than the theoretical diffraction-limited resolution. This is mainly caused by the manufacturing tolerance and alignment tolerance of the lenses. Our tolerance analysis has predicted such effects, where the predicted spot size with tolerance is 1.12 µm and 1.19 µm, respectively. From the tolerance analysis, it is predicted that the spot size will be 0.83 µm for Lightpath 355392 with corrective lenses module. Assuming similar manufacturing tolerance of lenses and alignment crafts, it is predicted that Lightpath 355392 with corrective lenses module can provide a higher lateral resolution than Lightpath 352140 and 352150. Improving the resolution will enhance the imaging capability to resolve smaller features. It will also improve the MPM signal intensity and imaging speed by focusing the laser beam into a tighter spot.

5. Conclusion

In summary, the scanning and focusing optical system for a handheld MPM probe has been analyzed in details. Off-the-shelf miniature aspherical lenses have been investigated as objective lens from wavefront error, field curvature, astigmatism, F-θ error, and tolerance. Lightpath 352150 is shown to provide a broader FOV with a slight reduction in the resolution compared with Lightpath 352140. The experimentally measured lateral resolution matches well with the theoretical prediction by tolerance analysis. A custom objective lens module is also designed which has the potential to further improve the resolution.

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