FABRICATION OF A FIBER SCANNING MULTIPHOTON MICROENDOSCOPE

A Thesis Presented
By
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to
The Department of Bioengineering

in partial fulfillment of the requirements for the degree of

Master of Science
in the field of
Bioengineering

Northeastern University
Boston, Massachusetts
(Dec 2019)
ACKNOWLEDGEMENTS

I would like to thank my Principal Investigator Dr. Bryan Spring whose supervision and important advice has been integral to all the research work for my thesis over the last two years. I would also like to thank Kai Zhang, Taresh Sharan, Eric Kercher and Ryan Lang who have been very supportive and helpful in imparting knowledge and making me get through my research and thesis in the smoothest way possible. Lastly, I would like to thank Dr. Mark Niedre and Dr. Sara Rouhanifard who kindly agreed to be a part of my thesis defense.
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ABSTRACT

Microendoscopy is a technique that involves imaging deep within the body including small volume locations. These devices are essentially miniature microscopes for linear (confocal or wide-field) or nonlinear (multiphoton) imaging applications. The multiphoton excitation technique provides a method to generate nonlinear modes of tissue contrast, including autofluorescence using non-linear near infrared excitation, harmonic generation and long wavelength (~1000 to 1700 nm) generation of visible fluorescence from exogenous probes. This thesis introduces simple methods to fabricate a stable low-cost fiber scanning microendoscope probe that facilitates miniature laser scanning multiphoton microscopy. We introduce enhancements that ease fabrication and improve performance including: (1) the use of 3D printed parts to construct the fiber scanner in place of expensive and labor intensive ceramic parts (in combination with a miniature piezoelectric tube); (2) video rate scan speeds using higher modes of fiber resonance; (3) custom design of an achromatic microlens objective to achieve diffraction limited resolution over a field of view of several hundred microns. We anticipate that these developments will enhance the applicability of fiber scanning multiphoton microendoscopy for in vivo imaging of preclinical models as well as the potential for clinical translation to image human disease.
INTRODUCTION

Imaging the human body has been an important part of understanding anatomy as well as disease etiology. Imaging has been an integral part in detecting diseases and clinical diagnosis disorders clinically with potential to provide feedback regarding responses to therapeutic interventions. Further, there is potential to perform in-situ non-invasive optical biopsy in order to reduce unnecessary tissue biopsies that sometimes miss or incompletely sample smaller lesions. There have been uses of multiple modalities in order to image disease and these various modes could be classified into invasive and non-invasive techniques. Invasive techniques involve methods that involve some form of surgery in order to get access to the parts needed to be imaged in comparison to completely non-invasive techniques where the source and detectors used to image the body are on the exterior and no surgery is required. Non-invasive clinical imaging modalities include X-ray, computed tomography, magnetic resonance imaging (MRI), Near Infrared Spectroscopy (NIRS), ultrasound etc. These non-invasive methods generally have poor resolution compared to microscopy due to intrinsic trade-offs in tissue penetration versus resolution. For this reason, surgical navigation and endoscopy using optics complement these techniques to provide clinicians with access to higher resolution images when necessary.

These invasive techniques are further classified into in-vivo and in-vitro methods. In-vitro techniques are those performed in a test-tube or culture dish i.e. outside of the normal biological context. They allow a much more detailed and controlled analysis but many not produce accurate predictions due to important biological cues only found within the living organism e.g. vascular, lymph and immune biology. In-vivo methods are methods where the test is performed within the whole, living organism rather than to a tissue biopsy ex-vivo or in-vitro cultures. In-vivo studies are perhaps best suited for understanding the results of the experiment in a living subject. In-vivo microscopy has been of interest for clinical purposes to observe changes at the cellular and organ level in response to therapy and to diagnose medical conditions that are missed by standard clinical imaging modalities.
In-vivo techniques are challenging and come with their own set of predicaments in the form of inaccessibility of parts inside a living organism and movement of the cells inside the body owing to motions of the hand-held probe and of the body, such as breathing and the heart rate. There is a need to make appropriate techniques in order to get images in-vivo to understand the working of organisms effectively. Endoscopy is one such method which has come into prominence over the last 100 years. It is a technique used to image deep inside the body such as a hollow organ or cavity with the help of an endoscope which is a slender and tubular structure making use of a lighting source and an imaging camera. It is highly effective owing to its uses in image various parts of the body from the alimentary canal and the respiratory tract to the urinary tract etc. Endoscopy makes use of the property of fluorescence and fluorophores[1] i.e. chemical that re-emit light upon excitation, to overcome the lack of light in the interiors of organisms. However, this technique is also restricted by the resolution at, depth into tissue due to light scattering and absorption dependent on the tissue type as well as the intensity and wavelength of the imaging light source and endoscopes generally lack microscopic resolution. A microendoscope is one method to overcome this restriction of the endoscope. A microendoscope is essentially a smaller endoscope but with microscopic resolution which operates similarly to an endoscope but helps in imaging smaller parts of organisms with microscopic resolution. Endoscopy and microendoscopy facilitate navigation deep within the body for surgical procedures as well as tissue diagnostics, with potential to reduce the need for tissue removal and biopsy. Although optical endoscopy and microendoscopy have greatly improved in many respects, there still remains significant development of multiphoton microendoscopy to test the impact and safety of multiphoton excitation for clinical use. Towards this overall goal, this thesis sought to improve the fabrication and performance of fiber scanning multiphoton microendoscopy, which has generally been only demonstrated at slower frame rates (~7 Hz or less) that suffer from frequent motion artifacts.

As an alternative to fiber scanning coherent fiber bundles consisting of multiple optic fibers bunched together have been used extensively in the literature in both wide-field and laser scanning confocal modes of operation, but the resolution of these devices is restricted by the core-core distance of adjoining fibers. Fiber scanning elegantly enables
laser scanning directly at the tissue to facilitate diffraction-limited resolution but requires assembly of a miniature actuator in at least two dimensions. In general, there are two types of laser scanning based on the relative position of the excitation light source from the scanner [2], proximal and distal scanners. Proximal scanners make use of a fiber optic assembly to illuminate an opaque object and transmit an image back from it without a lens system on the distal end [3]. Distal scanning incorporates a scanning apparatus onto the probe where both the laser excitation and returning fluorescent emission is sent and received respectively by the optic fiber in the scanning probe [4]. Resonance fiber optic scanners with microlenses are used to overcome the large MEMS mirrors and control circuits (~1 cm) which limit the miniaturization of the probe [5].

The main principle of microendoscopy similar to microscopy involves light activated excitation of fluorophores and collection of the light re-emitted by the fluorophores. Using multiphoton excitation in this provides us with improvement such as intrinsic optical sectioning, reduced out-of-focus photobleaching and phototoxicity and enhanced depth of penetration making it a suitable tool for in-vivo biomedical imaging applications [6,7]. However, adding this feature in a microendoscope has its own set of challenges. There is use of a ceramic micromachined piece to mount the optical fiber into mechanical contact and coupling with Piezoelectric tube (PZT) as part of the fiber cantilever. Achieving video rate imaging while maintaining spatial image resolution could also prove difficult due to mechanical stress to actuate the fiber at higher rates and because of the data acquisition speed required in a point-by-point image data collection.

Here, we show that higher scanning rates are indeed possible through optimization of the fiber scanner design. To do this, we make use of the first harmonic resonance frequency of the fiber cantilever system over the fundamental resonance frequency, which enables higher frame rates while maintaining the standard microendoscope field-of-view and radial resolution. Moreover, we developed 3D printed parts in place of ceramic parts which eases the fabrication process of the fiber cantilever to achieve stable fiber scanner operation.

The micro-endoscope probe consists of two 3D printed parts that couple a double clad optic fiber to a PZT actuator. The schematic of this structure is shown in Fig 1. The PZT
actuator is made of a certain ceramic material (proprietary) and has a cylindrical geometry which can be fabricated into small dimensions (outer diameter up to 1.5 mm) ideal for miniaturized scanners. The PZT actuator’s outer surface is divided into 4 quadrants to form two pairs of opposing actuation electrodes [8,9]. Ideally, for a sinusoidal actuation voltage with each pair shifted in phase by 90°, the PZT should bend with respect to the quadrants thereby causing the fiber cantilever to move [10]. The PZT can only undergo small displacement (a few micrometers) owing to its small size though comparatively larger scan amplitudes could be attained by oscillating the fiber at its resonance frequency.

We explain the use of 3D printed mounts to couple the optic fiber cantilever to the PZT based actuator in comparison to a ceramic micromachined part. For the ceramic part, the high cost of manufacturing (~$500 per part) and the requirement to be custom made by an experienced engineer makes the lead time to obtain these parts to several weeks while the 3D printed mounts could be obtained for less than a dollar and with negligible lead time. Simulations are done on ANSYS in order to figure out the best possible actuation frequency to get optimal results.

Fig 1: SOLIDWORKS model depicting the fiber cantilever setup comprised of the PZT(red), 3D printed parts (green) and optic fiber.

The results show that the optimal frequencies are those near the resonance frequency[11]. as it leads to minimal stress on the fiber cantilever and simultaneously also maximizes the field of view owing to maximum deflection of the fiber tip. The frequency 4.8 kHz is picked as being the most appropriate frequency to give optimal results for spatial and radial resolution while maintaining the required speed. Typically, a fiber cantilever of 11.3 mm length has a fundamental frequency of around 750 Hz and a first harmonic
frequency of around 4.8 kHz. A faster oscillating fiber cantilever will be able to scan over the field of view at a higher rate thus giving enough data points required over the scan for proper image reconstruction for the same frame rate of the system. We use the first harmonic resonance frequency in place of the fundamental resonance frequency. There are a multiple advantages in using the first harmonic frequency over the fundamental frequency. It ensures lower stress on the fiber cantilever for the same actuation force while also giving much larger displacement for the same stress hence proving to be a doubly better option for actuation of the cantilever.

The quality and reliability of these fiber scanners are largely dependent on two mechanical components. 1) The 3D mount which attaches the PZT to the metal tube and 2) the 3D mount which couples the PZT movement to the fiber (smaller mount) [12]. This smaller mount holds the base of the fiber cantilever thereby directly influencing the resonance frequency of the system. The mount needs to be designed in a way that one end of the fiber is securely held allowing the PZT to vibrate. These mounts are glued which slightly dampen the vibration and thereafter the position and length of the fiber cantilever is fixed along with the resonance frequency of the system.

The experimental setup to test the working of the microendoscope is as follows. The light source from a laser if focused using a lens into an optic fiber of diameter 125 µm with core diameter 10 µm. This is then extended across and finally goes into the microendoscope system which comprises of the aforementioned PZT actuator coupled with the optic fiber cantilever. The fiber cantilever is vibrated in a spiral scanning pattern using an amplified voltage signal controlled with a custom-made LabVIEW control program. The excitation light from the end of the optic fiber will be focused through a microlens setup onto a Position Sensitive Detector (PSD). This is used to determine the exact features of the scanning pattern. When we want to image something clinically, this light from the microendoscope is used to activate a set of fluorophores in a certain cell culture and this fluorescence light is collected back in the outer core of the double clad fiber and this image intensity signal is guided to a photomultiplier tube (PMT).

This light signal is converted to an electrical signal. This electrical signal is digitized and collected by a computer using NI-DAQ 6115. Image reconstruction is done using a
combination of position data of the fiber scanner from the PSD and intensity data from the PMT. An algorithm based on nearest point intensity is used for this purpose. However, the limited space in the microendoscope and sensitivity of the fiber scanner to external forces makes it cumbersome and difficult to build a position sensor into the microendoscope system. A way to get past this problem is to use pre-recorded position data i.e. recording the positions of the scanning pattern. This method will only lead to an appropriate and valid image if the fiber scanner is stable enough and we can consider the scanning pattern to be identical between different frames across a long time period.

METHODS AND COMPONENTS

The fiber scanner consists of a PZT, optic fiber cantilever and 3D printed mounts to couple the fiber cantilever to the PZT and the metallic tube. The actuation voltage is supplied to the PZT using soldered leads. This takes the form of a modulated sine wave and is controlled by a custom written LabVIEW code. A continuous diode laser module of power 4.5 mW and wavelength 635 nm is used as an excitation light source for testing this setup. A PSD is used to track the movement of the fiber tip whose signal is collected with a LabVIEW code and is studied to determine the stability of the scanning pattern.

One of the quantitative measures to analyze stability is to monitor the quality factor which is measured by changing the actuation voltage signal frequency around the resonance frequency while measuring the oscillation amplitude on the PSD. The position of the fiber scanner at the same phase is measured over different frames. Ideally, this difference should be negligible as a stable fiber scanner will have the same scanning pattern. This allows the use of the pre-recorded position data in image reconstruction.

Finally, in order to verify the long-term stability of the probe, position data of 50 randomly selected points is recorded and the variation of these points is plotted to detect any supposed drift over time.

PIEZOELECTRIC TUBE

Piezoelectric materials have the ability to generate electrical charge from mechanical stress or move in certain direction when under a certain voltage. This is used in the form of a PZT in order to vibrate in a certain defined pattern for a specific voltage. PZTs (Physik Instrumente GmbH & Co., Germany) can produce a small positional
change (~ nm) for a minimal change in operating voltage. These are majorly used in scanning fiber microendoscopic probes to deliver a dynamic scan to the fiber cantilever. The PZT actuator used in the microendoscope setup is made of a monolithic PZT with the outer surface divided equally into four conductive quadrants. A double clad optic fiber is passed through it with the help of 3D printed mounts. A differential voltage signal controlled by a custom LabVIEW program and generated with a NI DAQ device and amplified by the Physik Instrumente driver circuits. At lower frequencies, the PZT impedance is very high which reduces the signal current (≪120 mA) therefore making it safe to use for endoscopic applications [9]. When a periodic voltage signal is applied, the PZT actuator vibrates accordingly. This positional change of the actuator is transferred to the fiber cantilever to vibrate in a similar pattern. Hence, the fiber can be vibrated in a desired pattern when driven by a designated modulated signal.

![Figure 1: AutoCAD model](image)

3D printed mounts used in this setup is fabricated using Stratasys Eden 260v printer in the PMMA material. There are 2 different parts used which are attached to opposite ends of the PZT actuator. Figure 1(b) shows the larger mount in which a hole is made using a 250 µm drill to get the optic fiber through it. It is used to fix the PZT-fiber assembly to a metal tube which had grooves made to accommodate PZT driving signal wires. The smaller mount in Fig 1(c) is attached to the other end of the PZT and is used to couple the
PZT to the fiber cantilever. These parts are glued on both ends to the PZT using 3M super glue in order to keep the consistency of the vibrational energy transfer between the PZT and the fiber. These mounts are of the dimensions of 0.5mm length, 1.5mm diameter and 5mm length, 2mm diameter. Multiple such mounts are printed together in order to maintain repeatability of features in the microendoscope.

**FIBER CANTILEVER**

The fiber cantilever is the final part of the optic fiber that acts as the delivery medium of the excitation light. The field of view (FOV) of the probe depends on the deflection of the probe and the distance of the object from the probe. This deflection of the probe is maximum when the system is driven at the resonance frequency. The speed of image acquisition is dependent on the time duration taken by the fiber cantilever to complete one scan i.e. the time taken to go from the initial position to the end of the scanning pattern and coming back to the initial central position. In order to improve the radial resolution and avoid excessive stress on the fiber cantilever, a higher order resonance frequency preferably the first harmonic frequency is used over the fundamental resonance frequency.

The resonance frequency \( F_r \) of a cylindrical structure is given by

\[
F_r = \frac{\beta_n}{4\pi L^2} \sqrt{\frac{Y R^2}{\rho}}
\]

Where \( L \) is the length of the fiber cantilever, \( R \) is the radius of the fiber cantilever, \( Y \) and \( \rho \) are the Young’s modulus and the mass density respectively of the cylinder [13]. \( \beta_n \) is a constant determined by the vibration mode number. For our microendoscope setup, \( \beta \) for the fundamental resonance frequency and the first harmonic frequency is 3.52 and 22.4 respectively [14]. Also, the fiber cantilever is modeled in ANSYS as a cylinder of outer diameter 125 μm and a length of 11.3 mm and 4.5mm in ANSYS. The length was selected in order to get the required fundamental resonance frequency and first harmonic resonance frequency near 4.8kHz. One end of the fiber cantilever is fixed to study the natural response. The model is meshed using the program control and a total of 495 elements and 2594 nodes for fiber length 4.5 mm and a total of 1221 elements and 6422 nodes for fiber length 11.3 mm are created which is sufficient to produce a statistically acceptable result.
The material used for simulation is pure silica glass with density of 2700 kg/m$^3$, Young’s modulus of 73 GPa and Poisson’s ratio of 0.17. Modal analysis is used to obtain the natural frequencies and mode shapes of the structure. Figure 2(a) shows the completed probe with wires to deliver the excitation voltage to PZT. Figure 2(b) depicts the 3-D printed parts used to couple the fiber cantilever with PZT.

![Image](image_url)

Figure 2 (a) Fabricated probe showing its different parts. (b) 3D printed mounts

**LABVIEW CONTROL PROGRAM**

A custom LabVIEW program is written to control the differential voltage signal to drive the PZT. This program is capable of generating and mixing modulated sinusoidal signal from two different channels. The signal drives the fiber cantilever to oscillate at any given frequency. A modulated sine wave signal along one principal axis makes the tip of the fiber vibrate in a linear pattern and a brake signal is applied to stop the oscillation at the end of one frame. To get a spiral pattern, two modulated sine wave signals along the two principal axes are mixed and applied similarly at the resonance in order to maximize field of view of the microendoscope.

The imperfections in the fiber cantilever, 3D printed mounts and the non-ideal nature of the PZT leads to the resonance frequency to be different along two different directions. Hence, actuation voltage of modulated sine waves with a 90° phase shift in 2 perpendicular directions yields uncontrollable and undesired scanning patterns. To overcome this problem and apply effective brake signal, driving signal has to be applied along the principal axis. The principal axis is the direction along which the displacement of the fiber tip is parallel to the driving force. When the sine wave signal is not along the principal axis, the oscillation will not be a line but rather form a 2D elliptical pattern. To achieve any desired scanning pattern, the program calculates the components of the driving signal along the principal axis in the x and y directions according to user defined angles and basic trigonometry principles.
The following 3 steps are to be followed to drive the fiber scanner to vibrate in a spiral pattern. 1) Find the resonance frequency and direction of the principal axes, 2) apply the driving signal to the fiber and 3) adjust the braking signal to make the fiber stop at the end of the frame. The data points of the spiral scan are ensured to have an equal distribution across the changing radii for appropriate image reconstruction. These can be achieved by varying the different parameters in the wave shape control part of the program. It consists of six identical modules which can set the amplitude, frequency and initial phase of a sinusoidal wave and its modulation parameters such as linearly modulated, exponentially modulated etc. These 6 modules are arranged in a 2x3 matrix form where the two rows represent the two channels used to apply the actuation signal along the two principal axes and the three columns are for the three tiers of the modulated wave. Settings such as “angle”
and “duty length” exist for each row and column which determine the angle i.e. direction of the signal and the length percentage of each tier of the wave signal.

There is a certain defined procedure used to determine the values for the control signal to get a stable spiral scanning pattern. To find the resonance frequency and the direction of the principal axes, the user initially needs to set the duty length of the first column to a value between 0.7 to 0.9 and set the other duty cycles to zero (0). The frequency and angle of one channel is varied while setting the amplitude of the other channel to zero i.e. turning it off, till a linear vibration with maximum amplitude is observed on the “PSD raw” display on the right bottom corner. This is the point of resonance along one of the principal axis. The changes in the frequency while determining the resonance frequency should be made in jumps of the ‘frames per second (fps)’ to ensure the components of the input wave signal are in phase. Similar process is to be followed while determining the resonance around the second principal axis where the amplitude of the first channel is to be set to zero. It needs to be ensured that the angles of the two principal axes are different. These two angles of the principal axes and the resonance frequency will be used in all the further steps. The “driving signal” display in the bottom left is to be used to examine the shape of the driving signal. The phase offset can be manipulated in order to make the scanning pattern visible on the “PSD Raw” as circular as possible.

**OBSERVATIONS AND RESULTS**

The microendoscope scanner is characterized using a double clad optic fiber of core numerical aperture (NA) of 0.15 with core 6±0.5 μm mode field diameter, 125±2 μm inner cladding diameter and 245±15 μm outer cladding diameter. The PZT actuator is driven by a custom-built four channel amplifier setup controlled through a logical control from NI DAQ USB6363 analog/digital input output(I/O) card to give a gain of 25 i.e. an actuation voltage of ±100V for a logical signal of ±4V. The interior and the exterior surface of the PZT is divided into the ground and four quadrants. A custom-made LabVIEW code controls the actuation of the PZT and is optimized when the opposite pairs of quadrants are driven by a differential voltage. It enables us to control the actuation voltage shape, amplitude, frequency and relative phase.
The fiber cantilever is vibrated at either at fundamental resonance frequency or the first harmonic resonance frequency. The values of these frequencies are dependent on many factors such as the gluing of the 3D printed parts to PZT actuator which leads to damping and also the length of the fiber cantilever. This inverse relationship between the resonance frequency and length can be seen and understood from Fig 4. The probe built for the microendoscope has a fiber cantilever length of 11.3 mm (approx.) for which the fundamental resonance frequency is about 750 Hz and the first harmonic resonance frequency is about 4800 Hz.

![Graph showing variation in resonance frequency as length of fiber cantilever is changed](image)

**Figure 4:** Variation in resonance frequency as length of fiber cantilever is changed.

The scanner characteristics is determined by evaluating the amplitude of oscillation along the principal axes as a function of resonance frequency. This can be seen in Fig 5(c) where both the principal axes show a resonance frequency of around 4800 Hz with a sharp resonance. The two axes show a slight difference in their resonance properties owing to anisotropy i.e. non idealistic features of the PZT which can be seen in the difference in both amplitude and resonance frequency. This difference in frequency is around 20-30 Hz. The quality can be determined by calculating the quality factor (Q-factor) which is the ratio of the resonance frequency to its bandwidth i.e. the full width at half maximum (FWHM). The Q-factor is calculated to be 245 and 286 about the two principal axes. The differences
Figure 5: Simulation results of a) stress distribution for fiber (length=11.3 mm) along first harmonic frequency  b) stress distribution for fiber (length=4.5 mm) for fundamental frequency c) Variation of displacement for these two fibers about their corresponding resonance frequencies
in these values of Q-factor show the non-idealness of the PZT. The high Q-factor shows that the scanner is of good mechanical quality and has minimal damping [15].

The vibration of the fiber cantilever varies as we change the frequency which can be seen and understood from Fig 5(c). The actual resonance frequencies are different to the simulation values because of design based constraints and non-idealness which cannot considered in the simulation. The higher resonance frequency values in the experimental cantilever over the simulation data is owing to the glue used to keep the fiber and 3D printed parts in place which causes supposed reduction in fiber length. The simulation helps us in determining the frequency range where the cantilever will vibrate in resonance.

![Graph showing Displacement vs frequency data from the fiber cantilever generated by vibrating the fiber along the two-principal axis](image)

*Figure 6: Displacement vs frequency data from the fiber cantilever generated by vibrating the fiber along the two-principal axis*

The high mechanical Q-factor and the variation in amplitude of scan pattern similar to the simulation proves the sensitivity of the PZT, the efficiency of the 3D printed mounts and how it is a viable replacement for the micromachined ceramic parts and the ability to transfer parametric changes to the fiber cantilever with sustaining efficiency. This is effectively shown in figure 6 where the amplitude variation of the scan pattern i.e. displacement of the probe along the principal axes across the resonance frequency is as expected.
Figure 7: (a) circular pattern obtained when no amplitude modulation is applied to PZT signal. (b) complete spiral scan pattern obtained.

Ideally, a circular pattern should be obtained when the actuation signal is a non-modulated along the two principal axes are of the same frequency and same amplitude as in figure 7(a). However, in an experimental setup the anisotropy of the probe leads to different values of frequency and amplitude required along the principal axis for the appropriate spiral scan. This is obtained by modulating the aforementioned actuation signal as a linear increasing wave. The final pattern as in figure 7(b) is made with a combination of the modulated actuation signal and a killing wave to get the probe back to its initial position.

The stability and repeatability of the scanner is a crucial factor required for image development for the microendoscope. This is determined by recording position data of the scan pattern in multiple 12 hour runs on a PSD. Comparison of positions of data points from adjacent frames gives an understanding of how the probe repeats the same path. This distribution data is compared to similar data collected when the fiber scanner stays still ("no scan") i.e. when there is zero actuation signal in order to determine the reasons behind any instability we see. This is statistically represented in Fig 8, where the average standard deviation of position of fifty randomly selected data points of a frame is plotted for multiple twelve hour runs across a three-and-a-half-day period. These values of standard deviation for the "no scan" and "scan" data are statistically similar to each other proves the stability of the scanning probe. The non-zero values of the standard deviation in both types of scans are owing to the electrical noise in the position detection by the PSD. Also, an important observation is that the standard deviation along the y-axis
is higher than the x-axis because of the experimental setup alignment done to get past the reflections in the light path because of the multiple lenses in the optical path. The consistency in the values of standard deviation for long term runs compared to the ‘no scan’ data confirms the stability of the fiber scanning microendoscope and its ability to be used for image generation in a clinical setup.

Fig 8: The average standard deviation of positions of 50 randomly selected points in x and y direction compared to “no scan” positions.

CONCLUSION

This thesis outlines two major contributions to enhancing the potential clinical impact of fiber scanning multiphoton microendoscopy. First, we show that the first harmonic resonance frequency can be applied successfully to obtain scan rates beyond the fundamental frequency in order to obtain video rates. Second, the development of 3D printed parts to replace a ceramic micromachined part reduces the fabrication cost and complexity of video fiber scanning probes. The scan pattern is controlled by a custom written LabVIEW code and varies with the change in the driving signal parameters in the program as expected which proves the efficient coupling of the 3D printed part with the fiber cantilever and the PZT. The amplitude of the vibration of the fiber tip is highest
around the resonance frequency (both fundamental and first harmonic) which will give the maximum field of view for a scan. An ANSYS simulation is done on a cylinder of similar dimensions to the PZT and stress analysis is done which shows that the first harmonic resonance frequency (~4800 Hz) does not impart undue stress on the fiber compared to the fundamental resonance frequency (~750 Hz). The first harmonic resonance frequency also decreases the scan duration per frame allowing image acquisition at video-rate (~20 fps) while retaining stable scanning patterns. All these results lead to the conclusion that a stable low-cost 3D printed mount-based fiber scanning microendoscope actuated at the first harmonic frequency can be made and used successfully for video-rate image acquisition. Future work will integrate the custom achromatic micro-objective to perform cancer imaging in mouse models, and this new technology will enable research to develop pretreatment for optical biopsy of dynamic drug-resistant cancer cell populations in order to guide precision photomedicine (photodynamic therapy).
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