Design and Fabrication of a Precision Alignment System and Package for a Two-Photon Fluorescence Imaging Device
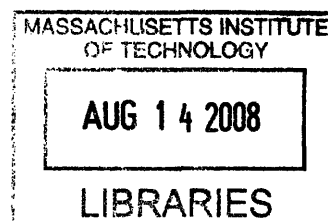
by

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Design and Fabrication of a Precision Alignment System and Package for a Two-Photon Flourescence Imaging Device

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ABSTRACT

A compact, lightweight precision alignment system and package for an endomicroscope was designed and fabricated. The endomicroscope will consist of a millimeter-scale fiber resonator and a two-axis silicon optical bench. The alignment system provided five degrees of freedom and was designed to align the fiber resonator with the microchip with a resolution of one micron.

The alignment system consisted of a system of ultra-fine screws and two compliant mechanisms to deamplify the motion of the screw. Finite element analysis was performed to optimize the compliant mechanisms for the desired transmission ratio of 20:1. The alignment system was fabricated and testing showed that the transmission ratios were lower than expected (18.6 for one compliant mechanism and 2.68 for the other). Testing also showed that the alignment system met the functional requirements for the ranges of motion.

Thesis Supervisor: Martin L. Culpepper
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Chapter 1

Introduction and Background

1.1 Purpose of Research

The primary purpose of this research is to design and fabricate a compact precision alignment package for a two-photon fluorescence microscopy imaging device. The imaging device will allow for *in vivo* cell imaging in deep brain areas in live rats, and will give researchers new insight on the development and activity of neurons in response to environment, training, or experience. Since the device employs two-photon excitation fluorescence microscopy, the device has significant advantages over current imaging methods, including the noninvasive study of live tissues in three dimensions with sub-cellular resolution.

1.2 Two Photon Fluorescence Microscopy

In 2007, Dr. Shih-Chi Chen and Professor Martin L. Culpepper of the Precision and Compliant Systems Lab at the Massachusetts Institute of Technology developed an imaging device that utilized two-photon fluorescence microscopy (TPM). TPM has significant advantages in biological imaging over traditional imaging methods. For example, TPM reduces photodamage, allowing the imaging of live specimens and can image dense specimens with sub-micrometer resolution down to a depth of a few hundred micrometers [1]. TPM is based on the nonlinear excitation of flourophores, which occurs with the simultaneous absorption of two photons whose combined energy matches the energy gap between the ground and excited states [1]. After excitation, the flourophore relaxes to the lowest energy state, emitting a single photon with a wavelength typically in the visible spectrum [1]. The emitted photons can be detected to form a three-dimensional, high-resolution image of the specimen.

Chen and Culpepper developed a three-axis high speed micro-scanner to enable optical raster scanning for two-photon microscopic applications. The micro-scanner utilized a thermomechanical micro-actuator optimized by Chen and Culpepper via geometric contouring and mechanical frequency multiplication [2]. The resulting micro-

scanner was able to scan a volume of 125 x 200 x 200 μm^3 at a frequency of 3500Hz x 100Hz x 30Hz [2]. It also required low voltage to operate (less than 5 V) and is therefore suitable to be used for *in vivo* procedures.

Figure 1-1 shows the two subcomponents that compose the three-axis micro-scanner: a fiber resonator that drives one axis of the scanner, and a silicon optical bench system which contains a small-scale electromechanical scanning system that raster scans the focal point of the optics in the remaining two axes. The fiber resonator contains an optical fiber, a double clad photonic crystal fiber, or DCPCF, that both delivers and collects the photons during imaging. The silicon optical bench system contains a prism and a graded index (GRIN) lens along with an actuation system that focuses the optical elements.

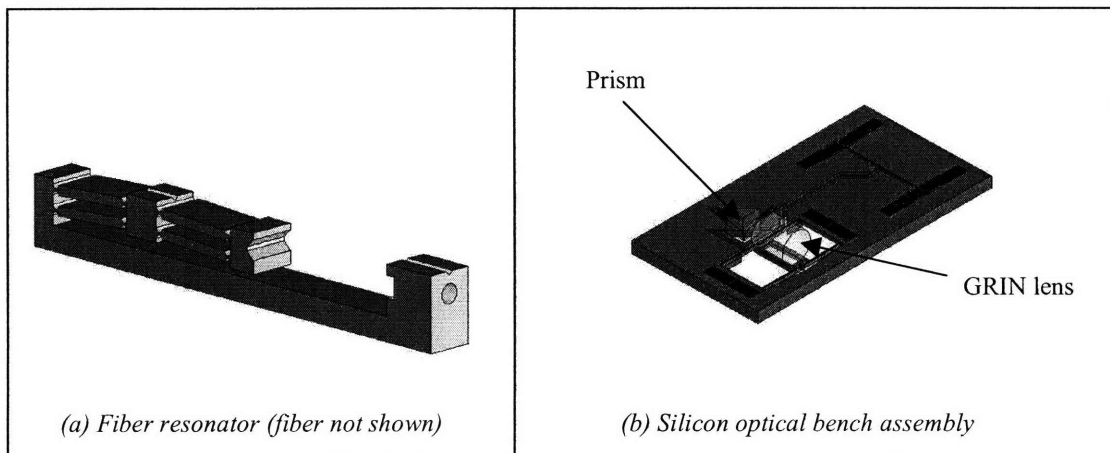| *(a) Fiber resonator (fiber not shown)* | *(b) Silicon optical bench assembly* |

Figure 1-1: 3D models of microscanner components (not to scale)

Figure 1-2 shows a block diagram outlining the paths that the femtosecond laser and the resulting excited photons take in order to obtain a volumetric image of the sample.
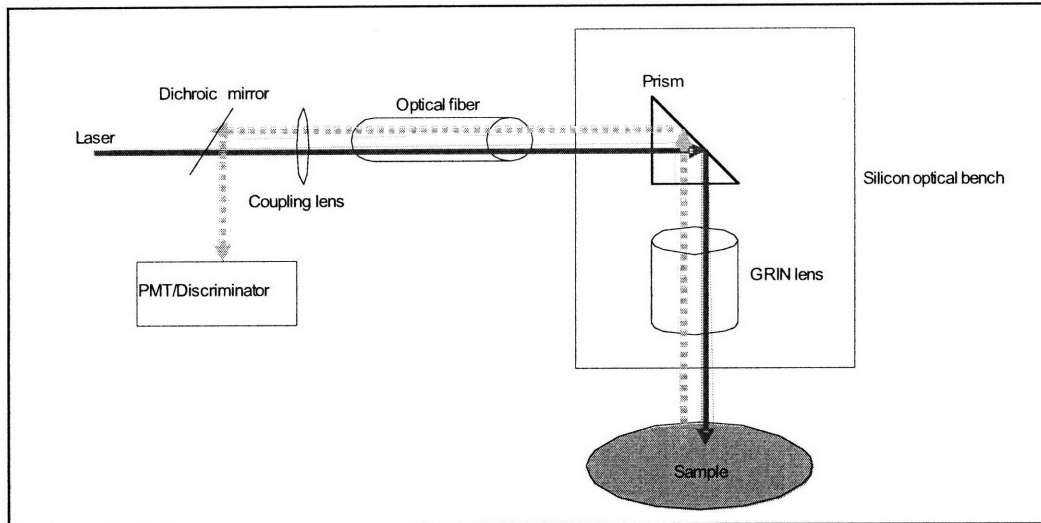
Figure 1-2: Block diagram of laser path and excitation photons during imaging

1.3 Applications in Neurobiology

TPM has many applications to neurobiology, as it resolves many of the challenges that are presented when imaging brain activity. The most useful images are from live tissue, and TPM is much less damaging than other imaging methods. There is a current need to image brain activity in live, awake mammals since the most useful information is from studying neurons in their natural, intact environment [3]. In addition it has been found that anesthesia greatly reduces overall brain activity [4] and alters neural dynamics [5].

Previous efforts to image neural activity in awake mice include a head-mounted microscope developed by Helchem et al. They were able to develop a microscope that was 7.5 cm long and weighed 25 g, and successfully imaged individual capillaries and dendrite processes at a depth of up to 250 μm [6]. Their device, however, was sensitive to moderate animal movement as relative motion between the device and the brain occurred. In addition, the images were achieved at a reduced depth penetration and resolution – a typical two-photon microscope can image at a depth of up to 500 μm [6]. Another group, Flusberg et al., was able to develop an endomicroscope that weighed only 3.9 g, however the device employs two fiber optics: one for light delivery and another for fluorescence collection [7].

Most mammalian brain regions lie deeper than 500 μm within tissue. These areas which are unreachable by conventional optical microscopy methods, can be reached by endomicroscope probes comprised of multiple GRIN micro-lenses [8]. Jung, et al. was able to image individual neurons and dendrites in the CA1 hippocampus area by using a GRIN lens assembly with a two-photon endomicroscope [8]. Chen and Culpepper's fiber resonator and silicon optical bench system will be later coupled with a GRIN lens assembly and developed into a head-mountable endomicroscope to allow for deep-brain imaging. The endomicroscope will be used to study activity in the hippocampus area in live, awake mice. This endomicroscope is unique because it will be able to perform *in vivo* three-dimensional volumetric deep-brain imaging, as opposed to the two dimensional raster scanning two-photon devices that have been previously developed. Its ability to scan at a high frequency (3500 Hz x 100 Hz x 30 Hz) will also be useful in recording neurophysiological processes, which usually occur over milliseconds.

1.4 The Need for a Precise Alignment System

The silicon optical bench and the fiber resonator must be precisely aligned with each other in order to obtain the best image. Precise alignment is critical to this project because the ray tracing results of the optical assembly indicate that misalignment of even 1 micron will degrade the lateral resolution of the imaging device [2]. Computer simulations conducted by Chen have shown that the full width at half maximum of the point spread function (or FWHM, a measure of the best possible spatial resolution that can be achieved by an optical imaging system) degrades from the optimal value of 0.73 micron to 1.5 micron when the imaging components are misaligned by one micron [2].

A six-axis stage, shown in Figure 1-3, for fine position adjustments to align the optical fiber with the silicon optical bench system was used when the microscanner was being developed. However, it is very large and heavy and cannot be used for a head-mountable endomicroscope.
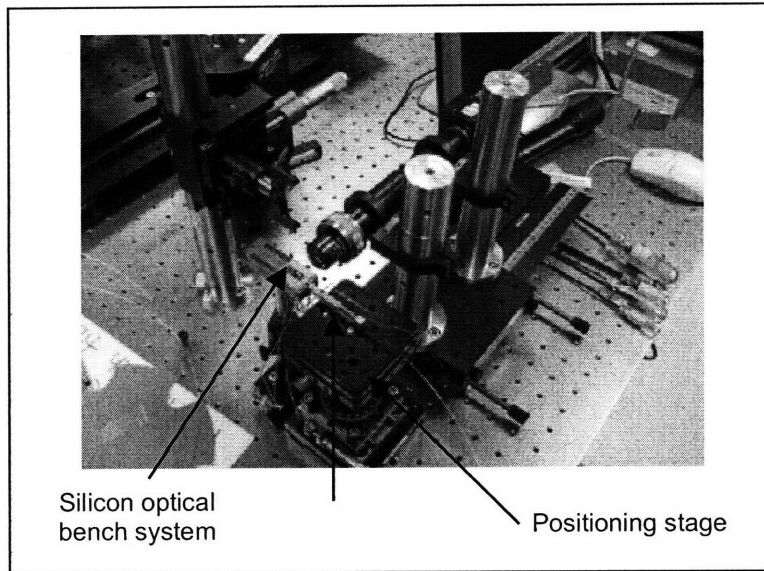
Figure 1-3: Alignment system used during microscanner development

The functional requirements of the alignment system are shown in Table 1-1. The axes conventions that will be used throughout this paper are shown in Figure 1-4.

Table 1-1: Functional Requirements of Alignment System

Axis	Range	Resolution
x	1 mm	1 μm
y	1 mm	1 μm
z	1 mm	20 μm
θ_x	2°	0.1°
θ_y	1°	0.1°

Figure 1-4: Axes conventions for endomicroscope

In addition to having a precision of 1 micron, the alignment system must have a travel range of up to 1 mm. This is to accommodate the misalignment of the fiber during

the assembly of the fiber resonator. The fiber is secured onto a v-groove on the fiber resonator using a crystal bond.
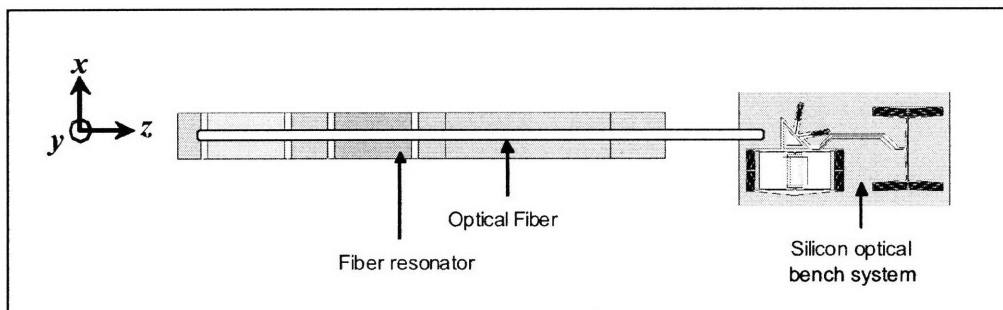
The thesis that follows will focus on the design and the fabrication of the five-axis system that will align the fiber resonator with the silicon optical bench system. It will also focus on the design of the package that will contain the imaging components and the alignment system. The package will be designed to be compact and lightweight so that it can later be developed into a head-mountable package.

Chapter 2

Design of Alignment System and Package

2.1 Concepts for Alignment System

Several concepts were considered during the design of the alignment system, and are shown in the Pugh chart in Table 2-1.

Table 2-1: Concepts for Actuation of Alignment system

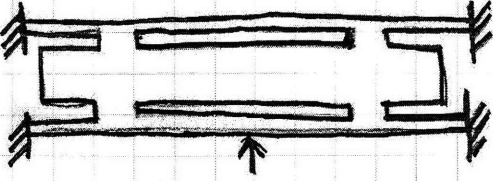| Concept	Metrics				Total
	Manufacturability	Cost	Degrees of freedom	Size	
A: Piezoelectric actuator	1	-1	-1 (1 DOF)	0	-1
B: Differential micrometer drive	1	-1	-1 (1 DOF)	-1	-2
C: Miniature positioner	1	-1	1 (3 DOF)	-1	0
D: Motion driven by set screws coupled with compliant mechanisms	0	1	0 (1-3 DOF depending on design)	1	2
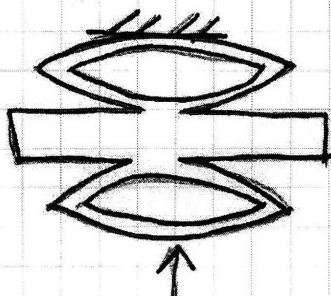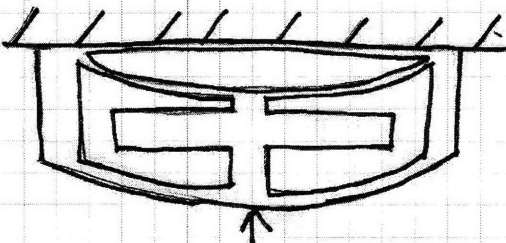
Coupling ultra-fine set screws with compliant mechanisms will give us the desired range of motion and resolution while keeping within the desired size and weight specifications. Concepts A, B, and C are off the shelf components with the required range of motion and resolution, but they are too large and heavy and are therefore unsuitable to be used for the endomicroscope.

Assuming that the smallest amount that the user can control the rotation of the set screw is 25 degrees, a 0-80 set screw will be able to provide fine linear motion down to 20 µm. In order to have linear motion that is controllable down to 1 µm, the compliant

mechanism design selected must deamplify the motion of the set screw with a transmission ratio of 20:1.

After brainstorming different designs for compliant mechanisms, the concepts were evaluated in the following Pugh chart, shown in Table 2-2.

Table 2-2: Concepts for Compliant Mechanism

Concept	Metrics			Total
	Deamplifies motion	Manufacturability	Size	
Concept A	1	1	1	3
Concept B	1	-1	-1	-1
Concept C	1	0	0	1

Concept A was chosen for its small size, easy manufacturability, and ability to deamplify motion. It will be used for the fine adjustment of the fiber resonator. A modified version of Concept A will be used for the fine adjustment of the silicon optical bench assembly.

The positions of the fiber resonator and the silicon optical bench assembly will be adjusted with a system of compliant mechanisms, ultra-fine set screws, and spring steel strips, which will be explained later in Section 2.3.

2.2 Design and Optimization of Compliant Mechanisms

The endomicroscope was designed to have two compliant mechanisms: one contains the fiber resonator while the other contains the silicon optical bench system, or microchip. The fiber resonator compliant mechanism will provide the fine adjustments in translation along the x axis. The microchip compliant mechanism will provide the fine adjustments in translation along the y axis.

The basic design for the fiber resonator compliant mechanism is shown in Figure 2-1 below. Blades of blue tempered 1095 spring steel 0.127 mm thick will be used as the flexing elements because the material has the highest elastic limit and fatigue values for common spring steels. The material properties are shown in Table 2-3 below.
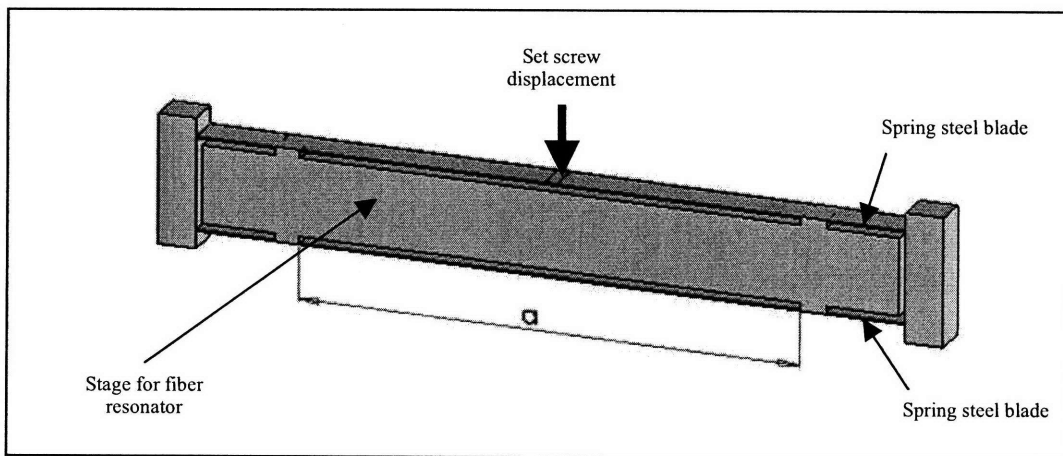
Figure 2-1: Basic design of fiber resonator compliant mechanism

Table 2-3: Mechanical Properties of 1095 Spring Steel [9]

Yield Strength	525 MPa
Tensile Strength	685 MPa
Modulus of Elasticity	205 GPa
Shear Modulus	80.0 GPa
Poisson's Ratio	0.29

The geometry of the compliant mechanism would determine the transmission ratio. In order to ensure that the compliant mechanism had the correct transmission ratio

of 20:1, the flexure was optimized on Cosmosworks. The distance between the two posts, *a*, was varied and the resulting displacement of the fiber resonator stage was determined. The results are shown in Figure 2-2 below.

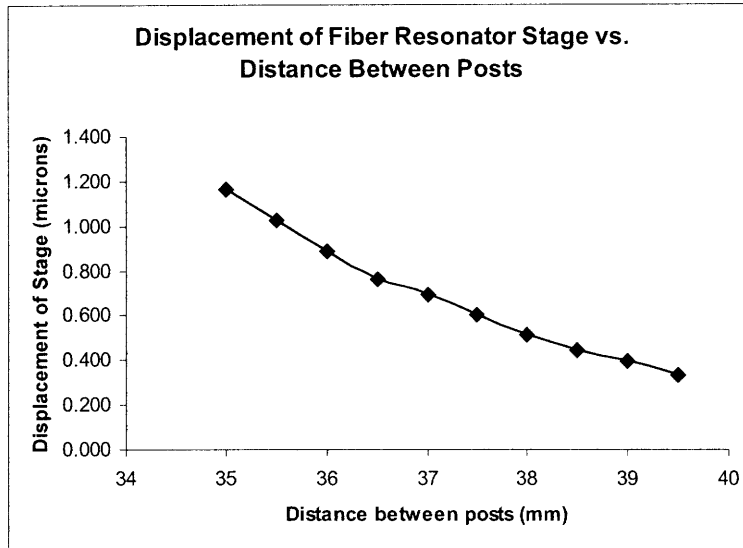**Figure 2-2: Results of Cosmosworks optimization on fiber resonator compliant mechanism**

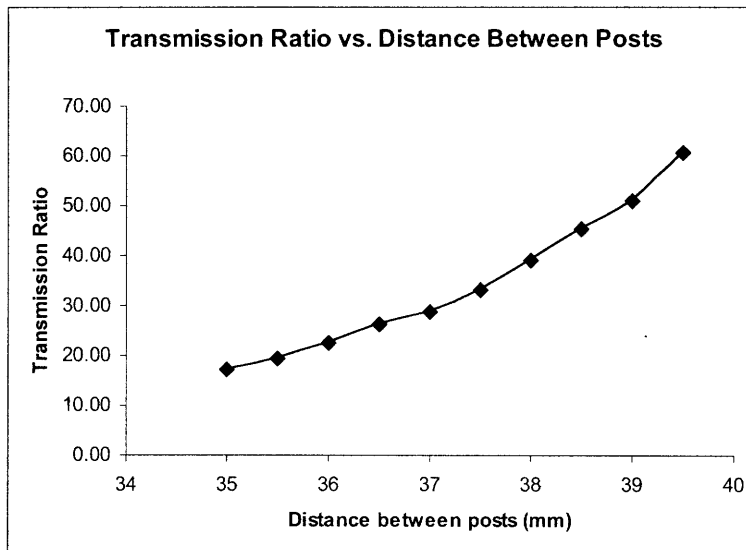The transmission ratios for each design scenario were calculated and are shown in Figure 2-3 below.

Figure 2-3: Transmission ratio of fiber resonator compliant mechanism

Based on the results of the optimization, the distance between the posts will be 36.5 mm, resulting in a transmission ratio of 26.2.

A finite element static analysis was performed for the case of $a = 36.5$ mm to evaluate the stresses on the compliant mechanism. The analysis was performed for the worst case scenario: a set screw displacement of 0.5 mm (the maximum possible displacement based on the geometry of the model).

Model name: flexure6- mod2
Study name: Study 2
Plot type: Static nodal stress Plot1
Deformation scale: 1

Max: 1.495e+008

von Mises (N/m^2)
1.495e+008
1.370e+008
1.245e+008
1.121e+008
9.964e+007
6.718e+007
7.473e+007
6.227e+007
4.982e+007
3.736e+007
2.491e+007
1.246e+007
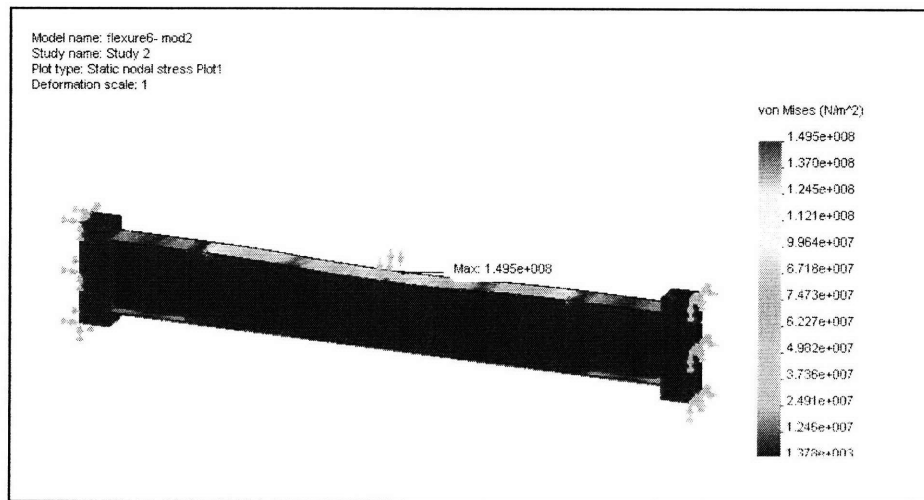1.373e+003

Figure 2-4: Finite element static stress analysis on fiber resonator compliant mechanism

The maximum von Mises stress on the spring steel elements occurs at the middle of the spring steel blade and is 149.5 MPa, which is well below the yield strength of 525 MPa.

A similar design was selected for the compliant mechanism that contains the microchip, and is shown in Figure 2-5.
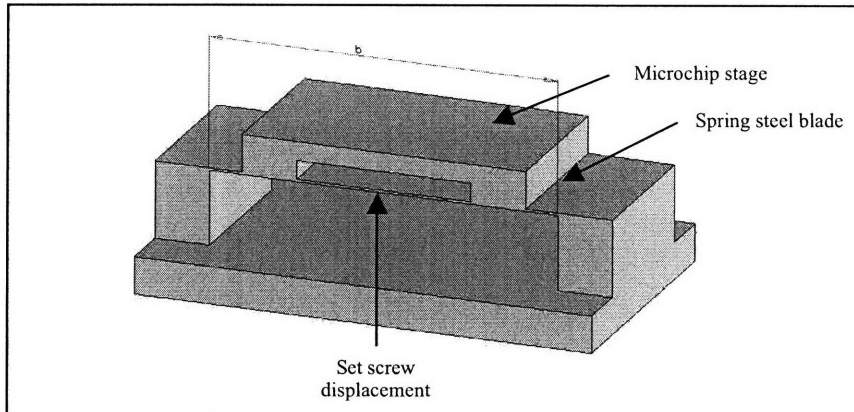
Figure 2-5: Basic design of microchip compliant mechanism

Again, the distance between the posts, *b*, will determine the transmission ratio. The device was optimized on Cosmosworks to have the desired transmission ratio of 20:1, and the results are shown in Figures 2-6 and 2-7 below.
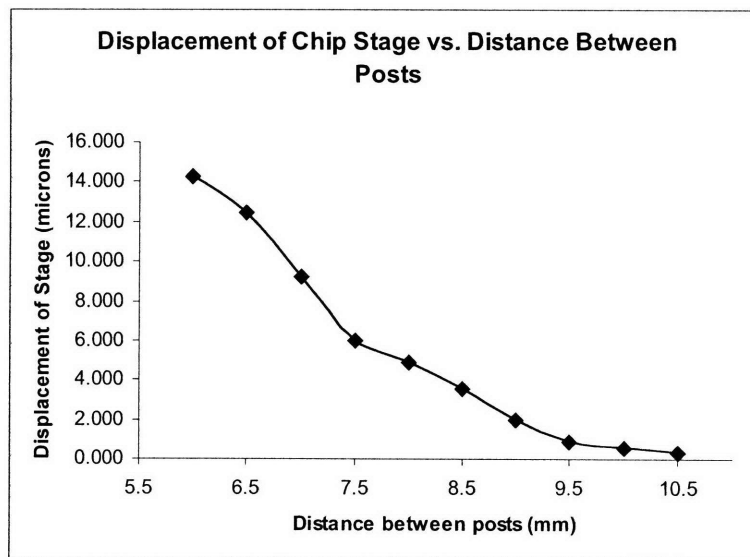
Figure 2-6: Results of Cosmosworks optimization on microchip compliant mechanism
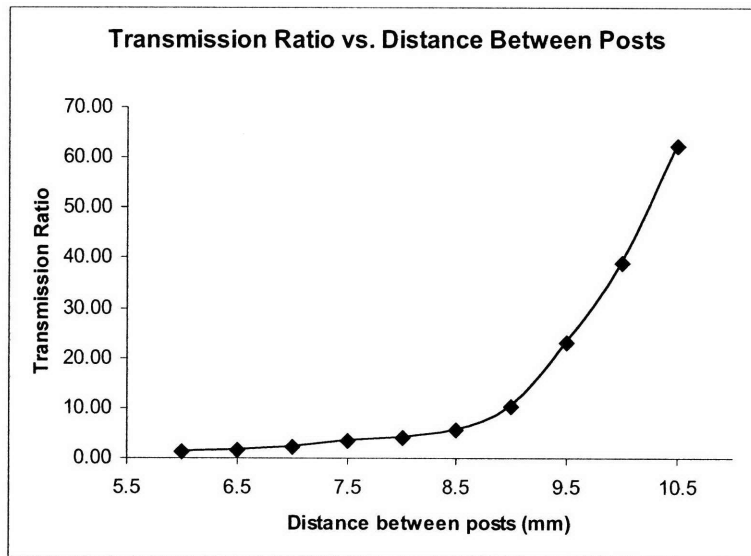
Figure 2-7: Transmission ratio of microchip compliant mechanism

The distance between the posts was chosen to be $b = 9.5$ mm, resulting in a transmission ratio of 23.0. Finite element analysis was performed to ensure that the maximum von Mises stress on the spring steel blade did not exceed the yield strength of the material. The analysis was performed with a set screw displacement of 0.75 mm. The results are shown below in Figure 2-8.

Figure 2-8: Finite element static stress analysis on microchip compliant mechanism

The analysis shows that the maximum stress occurs near the posts and is 471 MPa, which is below the yield stress of 525 MPa.

2.3 Design of Endomicroscope Package

The microchip has eight electrical contacts which must be connected to an external controls system which will control the thermomechanical micro-actuators on the microchip. The endomicroscope package therefore needed electrical contacts that could easily be 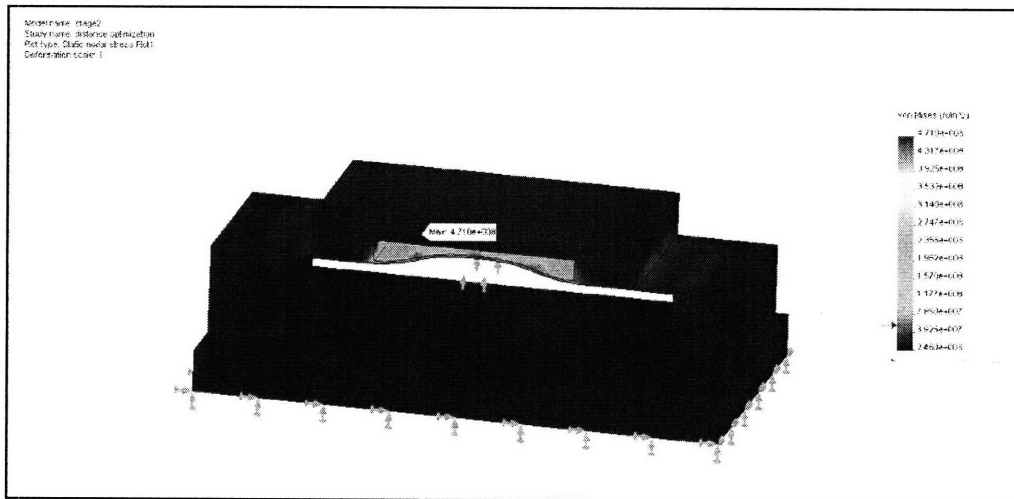connected to the contact pads on the microchip. Three concepts were explored. The first consisted of soldering bent jumper wire to a separate PCB. The second concept consisted of using spring-loaded electrical contact pins, or pogo pins, in a custom built pin holder. The third concept consisted of fabricating a custom PCB which would contain electrical contact pins. It was determined that the most reliable and robust concept was Concept 2. Concept 2 consisted mostly of off-the-shelf components, and required only two custom-made parts. Fabrication and assembly would also be much simpler and cheaper with Concept 2.

As discussed in Section 2.1, the coarse adjustments of the alignment system will be provided by 0-80 screws. These screws will adjust plastic housings which contain the components that need to be aligned. The material of these housings was chosen to be ABS, which is lightweight, easy to machine, and has a low coefficient of friction, eliminating the need for bearings. Figure 2-9 shows the CAD model of the assembly. A platform on the outer package was included to contain the GRIN lens assembly, which will later be added. The pogo pins are not shown in this model.
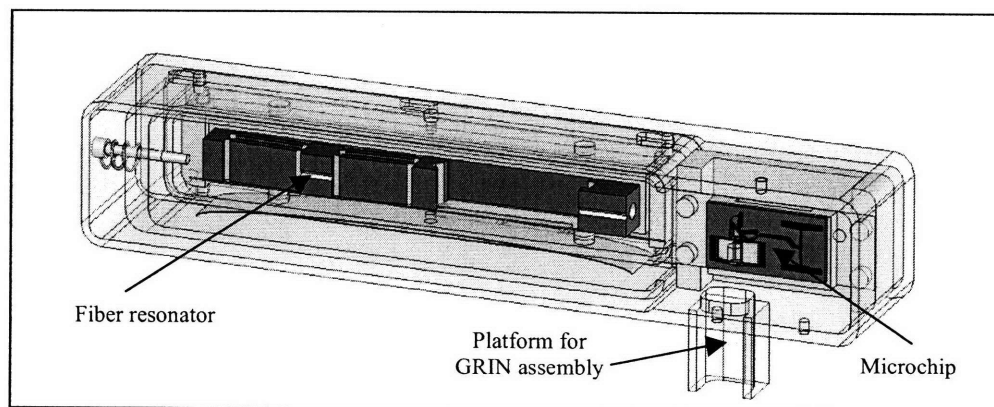

Figure 2-9: Isometric view of alignment system and package

Figure 2-10 shows the side view of the assembly. The microchip compliant mechanism can be coarsely adjusted in the y-direction by two 0-80 screws which are preloaded with springs. It can also be adjusted to rotate about the x-axis by adjusting the two screws a different amount. The screws were set 13 mm apart, and assuming that the largest difference that one screw can travel relative to the other is four revolutions, the maximum rotation of the microchip is 5.6 degrees. The housing which contains the fiber resonator can be coarsely adjusted along the z-direction by a 0-80 screw which is preloaded with a spring.
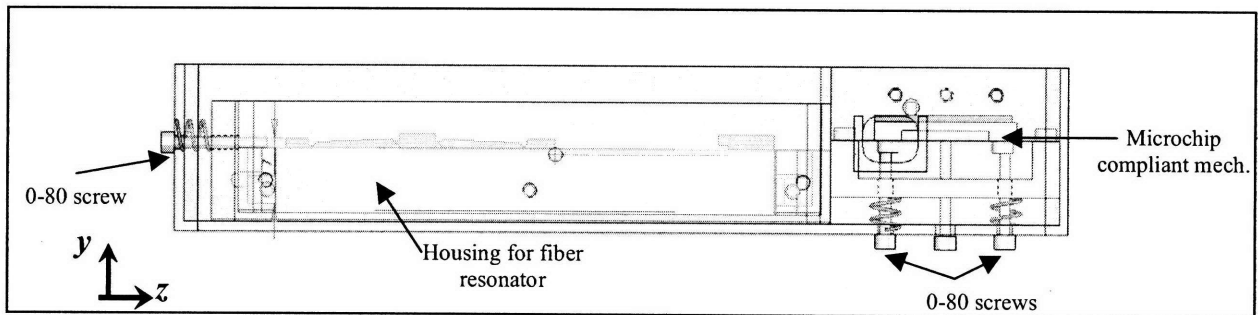
Figure 2-10: Side view of alignment system and package

Figure 2-11 shows the top view of the endomicroscope. A housing which contains the fiber resonator compliant mechanism can be adjusted coarsely along the x-axis by two 0-80 set screws which are pushing against a bent blade of spring steel. The three point contact also allows for rotation about the y-axis by adjusting each of the set screws a different amount. The set screws are 59 cm apart, allowing the maximum rotation of the fiber resonator to be 1 degree.

Figure 2-11: Top view of alignment system and package

The following chart, Table 2-4, summarizes the expected capabilities of the alignment system. The coarse adjustment systems in the x and y translation directions allows the design to meet the functional requirements of range, while the fine adjustments systems address the required resolutions.

Table 2-4: Expected Capabilities of Alignment System

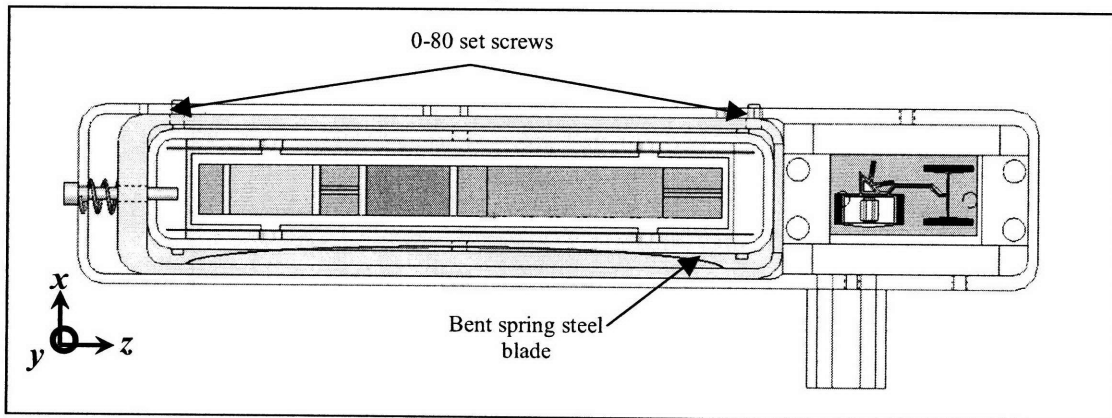| Axis	Adjustment method	Range	Resolution	Function Requirement of Range	Function Requirement of Resolution
x	Coarse: 0-80 set screws moving housing	1 mm	.02 mm	1 mm	.001 mm
	Fine: compliant mechanism	.5 mm	.001 mm		
y	Coarse: 0-80 screws moving compliant mechanism	3 mm	.02 mm	1 mm	.001 mm
	Fine: compliant mechanism	.75 mm	.001 mm		
z	0-80 screw	2 mm	.02 mm	1 mm	.02 mm
θ_x	0-80 screws moving compliant mechanism	5.6°	.088°	2°	.1°
θ_z	0-80 set screws moving housing	1°	.02°	1°	.1°

Chapter 3

Device Fabrication

3.1 Fabrication Methods and Design for Manufacturing

The analysis conducted on the compliant mechanisms in Chapter 2 assumed that they would be constructed out of one piece of spring steel. However, it is unnecessary to fabrication the entire piece except for the flexing sections (the blades) out of spring steel. Each design was modified to be manufacturable. The fiber resonator compliant mechanism was split into five pieces: two end pieces, one middle piece, and two blades of spring steel as shown in Figure 3-1. To save space and reduce the weight of the overall device, the middle piece was designed so that the fiber resonator will be clamped in the middle of the "stage." Since the fiber resonator is much thicker and stiffer compared to the spring steel blades, it can be assumed that the fiber resonator as well as

the stage will act as one rigid body. The same reasoning is valid for the end pieces, since they are much thicker and stiffer than the spring steel blades. The pieces will be assembled together with micro screws. There will be some loss of precision at the joints, but it will only serve to further increase the transmission ratio (further deamplify the motion of the actuating set screw), since there will be some slack in the blades.

Similarly, the microchip compliant mechanism was split into a base piece, a stage piece, and a blade of spring steel, shown below in Figure 3-2. These pieces were assembled together with micro screws and had some loss of precision at the joints. The spring steel blades and the parts for the chip stage were machined using the mill. The parts for the fiber resonator compliant mechanism were sent to N.E.T.W. LLC in New Britain, CT to be machined by wire EDM. A working fiber resonator was unavailable at the time the assembly of the alignment system and package so a dummy fiber resonator was machined on the mill.
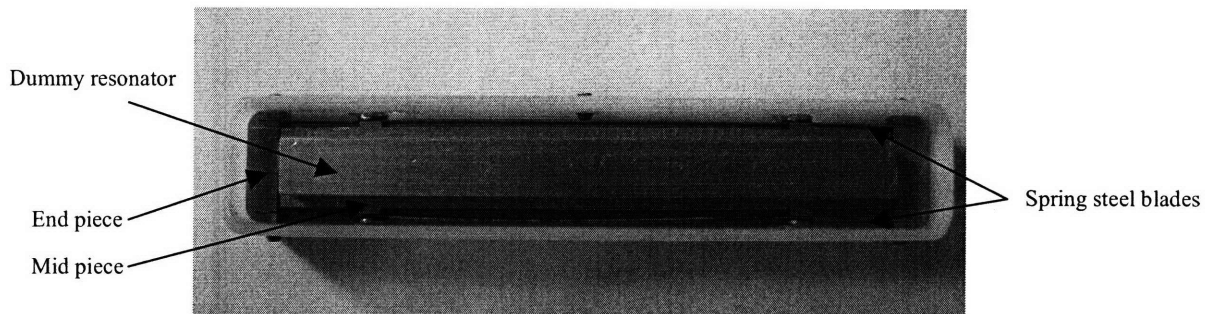

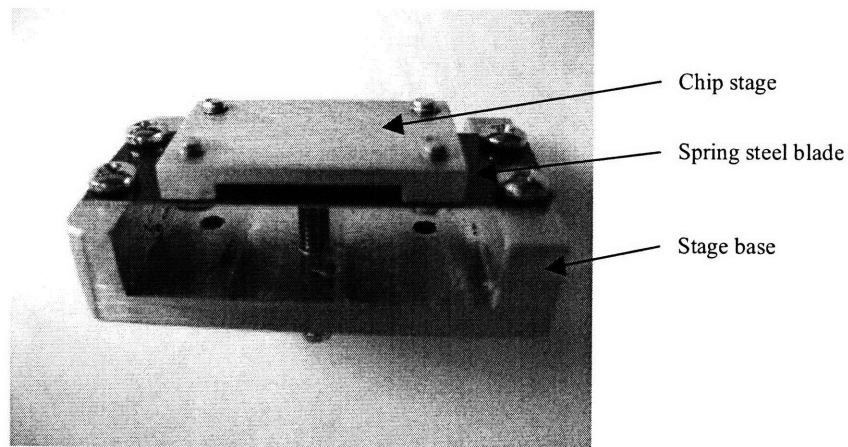**Figure 3-1: Fiber resonator compliant mechanism**

Figure 3-2: Microchip compliant mechanism

The plastic housings and the pogo pin holder were machined using the CNC mill. The assembled alignment system and package is shown in Figure 3-3 below.
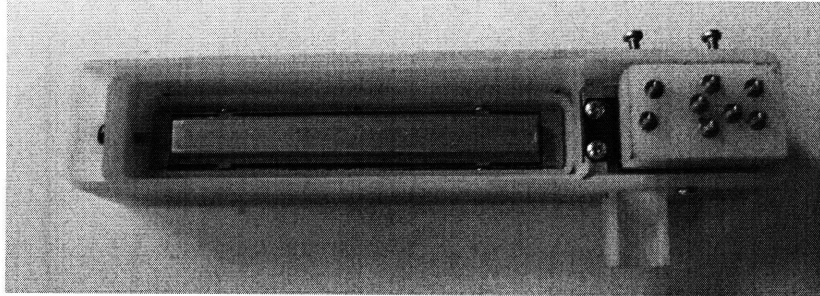

Figure 3-3: Assembled alignment system and package

The final package was 18 mm x 18 mm x 98 mm (not including the platform for the GRIN lens assembly) and weighs 22.851 g, not including the fiber resonator and the microchip, which weigh approximately 6 g. The heaviest components were the outer plastic housing, the microchip compliant mechanism, and the pogo pin array (pins plus pin holder). Their masses are shown in Table 3-1. The mass of the entire system can be reduced by adding weight-reducing holes to each of the plastic housings and the base of the microchip compliant mechanism. The mass of the system can also be reduced by using smaller, custom-designed pogo pins instead of off-the-shelf pins, which would allow the pin holder to be of a lesser size and weight.

Table 3-1: Mass of each component

Part	Mass
Outer plastic housing	6.328 g
Microchip compliant mechanism	4.344 g
Pogo pin array	3.929 g

Chapter 4

Testing

4.1 Results

The real test of the alignment system would be to see if a clear image with the optimal FHWM value of 0.73 microns can be obtained. However, a working fiber resonator and microchip was not available at the time of testing, so an optical comparator

was used to measure the range of motion of the alignment system and the transmission ratios of the compliant mechanisms. The resolution of the alignment system (the minimum amount of controllable motion) was not measured since it depends on the user and the type of tool that is used. The results are shown in Table 4-1. The alignment system meets all of the functional requirements for the ranges of motion.

Table 4-1: Measured Ranges of Motion

Axis	Measured Range of Motion	Functional Requirement
x	1.467 mm	1 mm
y	3.278 mm	1 mm
z	1.429 mm	1 mm
θ_x	4.02°	2°
θ_y	1.355°	1°

The transmission ratio of the fiber resonator compliant mechanism was measured to be 18.7 while the transmission ratio of the microchip compliant mechanism was measured to be 2.68. The transmission ratio of the microchip compliant mechanism was much lower than predicted. This may have been because the assumption that the bolted interfaces between the spring steel blade and the stage and base pieces was not valid.

The transmission ratio of the fiber resonator compliant mechanism was slightly lower than expected. It is possible that the transmission ratio should have been close to that of the microchip compliant mechanism because the same assumptions were made in the analysis of both. However, the fiber resonator compliant mechanism had a much higher transmission ratio because the spring steel blades had some slack (were not taut).

There are several factors that may have contributed to the lower than expected transmission ratios. The spring steel blades were cut on the mill which is less precise than wire EDM. In addition, the clearance holes that were cut in the spring steel to allow them to be attached to the rest of the structure may have caused the blades to be improperly positioned. Finally, there may have been loss of precision at the bolted joints. A more precise way of fabricating these parts is to machine them using wire EDM entirely out of one piece (no bolted joints). This is an area that needs to be explored for future generations of this device.

Chapter 5

Summary

A compact alignment system that aligns a fiber resonator and a microchip for a two-photon fluorescence imaging device was designed and fabricated. Further work should focus on reducing the weight and volume of the package and creating a mouse-to-package interface to allow for *in vivo* brain imaging. The package must also be tamper-resistant so that the rat does not damage the imaging components.

The package can also be developed into a non-invasive cancer screening tool. Work is currently being done by Heejin Choi and Peter So in the Bioinstrumentation Engineering and Microanalysis group at Massachusetts Institute of Technology to develop the fiber resonator and silicon optical bench system into a nonlinear optical endomicroscope for the non-invasive diagnosis of cancer. It will be capable of imaging cellular and extracellular matrix structures in thick tissues. In particular, this device can be used in the diagnosis of epithelial cancers at organ sites such as the cervix, esophagus, colon, and skin. It can be used to complement tradition excisional biopsies in three areas: (1) more precise selection of the correct sites for excisional biopsies, (2) avoiding missed diagnosis/random sampling, and (3) ensuring the complete removal of diseased tissues [10]. In order for the package to be developed into a cancer screening tool, the materials must meet FDA regulations and the package must be watertight.

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