In Vivo Microscope Image Stabilization through 3-D Motion Compensation using a Contact-type Sensor

Sungon Lee, Takeshi Ozaki, and Yoshihiko Nakamura

Abstract—This paper presents our microscope image stabilization system for in vivo microscopy. This work is a novel robotic application. In vivo microscopy is very in demand due to its potential impact on biological research [1]. However, it has turned out that in vivo microscopy is significantly disturbed by the motion of the imaged tissue of a living animal. The proposed system virtually removes the unwanted motion by synchronizing the motion of the objective lens with it. In order to realize this idea, we have developed a simple contact-type sensor for estimating the motion of organs, and also made a motion compensator for moving the objective lens. Sensing and compensating have been accomplished for 3-D translational motion. Not only laboratory tests with artificial motion, but also in vivo experiments show the successful motion canceling effect of the proposed system.

I. INTRODUCTION

When we are observing a living subject through microscopes, the subject's inevitable motion due to heartbeat and breathing is one of the difficulties of stable microscopic observation. Recently, observing specific molecules in a living organism has become possible and attracted great attention in the biological researches due to its potential impact on biological research. This technology is called "molecular imaging" [2] [3] [4]. In vivo microscopy is one of powerful tools for molecular imaging. Since it provides highest resolution imaging among all modalities, there is great expectation on this technology. However, high resolution imaging turns out to be not easy because the subject is trembling due to its biological vibration, which naturally and necessarily occurs in cells of living animals.

In our previous work [5], the disturbing motion could be successfully removed through a visual feedback control system. This visual feedback solution uses a high speed camera for sensing the in vivo motion and a 2 DOFs mechanism for moving the objective lens. Even though it was very successful in removing 2-D motions, there are two weak points. One of them is that it can only compensate 2-D motion. We use a high speed camera for detecting the motion. It is inherently only able to detect 2-D motion. Thus, before applying image stabilization, we planarize the motion by strongly pressing the observed tissue with a cover glass. This pressure could have unwanted effect on the tissue. The other weak point is that the high speed camera system is too heavy and expensive a solution.

This paper presents 3-D motion compensation using our contact-type sensor which is able to detect 3-D motion in vivo. The developed sensor utilizes the bending of beam for estimating 3-D motion. It is comprised of three beams. It is a three dimensional cantilever. The estimated motion is compensated by the motion of the objective lens. A developed device driven by piezoelectric actuators moves the lens.

II. IMAGE STABILIZATION SYSTEM WITH A CONTACT-TYPE SENSOR

The image stabilization system consists of a developed contact-type sensor and a 3-D motion compensator. In vivo motion is estimated by the developed contact-type sensor every 1 ms, and this estimated motion becomes input to the 3-D motion compensator.
D motion compensator. The 3-D motion compensator moves the objective lens which is detached from the main body of the microscope. Fig. 1 illustrates the overall system. The main target motion we would like to cancel with our system is the motion caused by the breathing. As shown in Fig. 2 where the motion of a mouse liver which was estimated by a laser-displacement sensor, the biggest motion in tissue is caused by breathing.

III. A CONTACT-TYPE SENSOR

We have developed a contact-type sensor for estimating 3-D micro-motion. It has another important role that it reduces the tissue motion. In fact, the maximum tissue motion without any constraint is more than 1 mm as shown in Fig. 2. It is too big to be compensated with piezoelectric actuators (the maximum stroke of the piezoelectric actuator available in the market is only a few hundred micron). Reducing the motion allows us to use the commercial actuators in designing the compensator.

The contact-type sensor consists of three thin beams. Its structure is shown in Fig. 3. The end tip of the sensor is placed on a subject while the other end is fixed to the microscope. The body of the sensor device is designed to be elastically bent with small force. To keep the contact, the sensor is installed on the subject with a pretension. The tip of the sensor ideally moves together with the tissue under it, leading the bending of the sensor body. Strain gauges attached on the sensor body catch this bending to estimate the motion of the tip.

Our sensor is a three dimensional cantilever. A cantilever is a beam supported on only one end. The beam carries the load to the support where it is resisted by moment and shear stress. Cantilevered beams are now the most ubiquitous structures in the field of micro-electro-mechanical systems (MEMS) specifically as sensors.

We decide the dimension of the cantilever beam. In Fig. 4, the elastic deflection \( v \) with force \( P \) acting on the tip of the beam with length \( l \) can be calculated using:

\[
v = \frac{Pl^3}{3EI}\]

where \( E \) is Young’s modulus and \( I \) is the moment of inertia.

\[
I = \frac{bh^3}{12},
\]

where the section of the sensor body is assumed to be a rectangle with width \( b \) and thickness \( h \). We put limitation on the deflection at the tip point less than 100 \( \mu \)m when \( P, 0.1 \) N is applied. From the deflection of a cantilever with load \( P \),

\[
v = \frac{4Pl^3}{3EI} \leq 0.1mm,
\]

After determining the material, the length and the width, we can find the minimum of \( h \), that is,

\[
h \geq 0.1447mm
\]

The determined size of beam is summarized in Table I

<table>
<thead>
<tr>
<th>Dimension</th>
<th>value (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( h )</td>
<td>0.15</td>
</tr>
<tr>
<td>( b )</td>
<td>12</td>
</tr>
<tr>
<td>( l )</td>
<td>12</td>
</tr>
</tbody>
</table>

Based on this information and FEM (finite element method) analysis, we designed a three dimensional cantilever. Fig. 6 shows the developed contact sensor. Calibration was conducted. Fig. 7 shows the experimental data of displacements vs. strain measured by strain gauges. The displacement was made by using commercial micro-stages. The resolution of the micro-stage is 10 \( \mu \)m. We fixed the tip of the developed contact-sensor on the stage, and then moved the stage with the measurement of sensor signals. From this experiment, we obtained the conversion matrix as follows.

\[
\begin{pmatrix}
\epsilon_x \\
\epsilon_y \\
\epsilon_z
\end{pmatrix} =
\begin{pmatrix}
-1.29 & 0.38 & 0.53 \\
1.41 & -2.48 & -0.53 \\
-0.41 & 1.07 & -0.36
\end{pmatrix}
\begin{pmatrix}
\Delta x \\
\Delta y \\
\Delta z
\end{pmatrix}. (4)
\]
IV. 3-D MOTION COMPENSATOR

3-D motion compensator moves the objective lens to remove the relative motion between the lens and the imaged tissue. We have already developed a 2 DOFs planar mechanism with two piezoelectric actuators as shown in Fig. 8. To this mechanism, we have added one DOF actuator for vertical motion (Physik Instrumente: P-725, travel: 400 μm, see Fig. 9) which is a commercial product for fast auto-focusing.

The 2-D mechanism which we have developed is driven by piezo actuators for high precision [5]. Its shape was designed in order to amplify the insufficient stroke of the actuators. The five-bar like linkage amplifies the strokes more than two times. The joint of two links is connected with a living hinge. A living hinge is a thin section of the material that elastically bends to allow movement. This type of hinge is widely used in the design of MEMS for its lack of any friction and very little wear. Wire EDM (Electrical discharge machining) allowed us to manufacture precise living hinges.

To this 2-D mechanism, we added a commercial 1 DOF actuator which have been developed for the purpose of high speed auto-focusing in microscopy. Fig. 10 shows the 3-D motion compensator combined with an objective lens. Table II summaries the maximum working space of the 3-D compensator.
TABLE II
MAXIMUM WORKING RANGE OF THE 3-D COMPENSATOR

<table>
<thead>
<tr>
<th>Direction</th>
<th>Range (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>563</td>
</tr>
<tr>
<td>Y</td>
<td>462</td>
</tr>
<tr>
<td>Z</td>
<td>400</td>
</tr>
</tbody>
</table>

Contact-type sensor

Fig. 11. Experimental setup for 3-D motion compensation; artificial motion is generated by two micro-stages

V. EXPERIMENTS

A. Laboratory Tests

Laboratory tests were performed with respect to artificial motion instead of the motion of living tissues. Instead of the motion of a living animal’s tissue, we used two micro-stages for generating motion (Sigma koki: SGAM20-35). The two stages generate horizontal and vertical motion. This motion is estimated by the developed contact-type sensor and compensated by the 3-D compensator. Fig. 11 shows our experimental setup for image stabilization.

We put the sample tissue of a mouse liver on the micro-stage. Sine waves with various frequencies were generated to test the frequency response of the system (They were not exact sine waves due to low sampling points). The amplitude of the wave is 100 μm both in vertical and horizontal directions.

Fig. 12, 13, 14 show the image sequence and the error, with and without the motion compensation at the frequency of 0.5, 1, 5 Hz, respectively. The images were captured by a cooled CCD installed in the microscope, and the error represents the difference between the estimation of the contact-type sensor and the movement of the 3-D compensator. The improvement with the compensation is prominent both on error plane and image plane when 0.5 and 1 Hz. For 5 Hz, we can also observe quite improvement on error plane, but the improvement on the image plane was not as marked as on the error plane. This is because such a small error in z as 10 μm could severely blur the image, which is due to the low depth of field of the microscope. In all cases, the error in z was bigger than any other error. The cause of the relatively poor performance in the vertical direction comes from the insufficient performance of the corresponding actuator.

B. In Vivo Tests

We conducted in vivo experiments with a mouse as shown in Fig. 15. Although it turned out that with the 3-D compensator, we cannot expect a stabilized image sequence with respect to the motion of higher frequency than 3 Hz, the in vivo test was worth doing with two reasons. One is that at
least we can test the performance of the developed contact-type sensor. The other is that the \textit{in vivo} motion caused by breathing under the contact-type sensor could have lower frequency than 3 Hz. In this case, we can expect a stabilized image sequence.

A molecular probe (Invitrogen: Alexa Fluor 594) which is fluorescent dye for the observation of blood flow was injected into the mouse’s vein. The mouse was put under the 3\% anesthesia. The mouse kidney was imaged after incision.

The motion of the organ was estimated by the developed contact-type sensor. Fig. 16 shows the estimated motion. It shows that the sensor is successful in estimating the \textit{in vivo} motion. The motion caused by breathing and heart beat is clearly shown in it. Then, the 3-D motion compensator moves the objective lens following the estimation. Fig. 17 shows the remained error after motion compensation. Horizontal motion in x and y directions was so successfully compensated.
that maximum value reduced from more than 100 μm to less than 10 μm. Approximately 90% of the horizontal motion decreased. For vertical motion in z the motion became from approximately 100 μm to 30 μm. More than 65% of the vertical motion was removed. In Fig. 18, the microscope image sequences before and after the stabilization were presented. We can notice that the blurring in the images has quite decreased. However, there still remains blurring in the image sequence which is mostly caused by the error in z.

VI. DISCUSSION

We stabilize microscope images with respect to a vibrating subject. Stabilized image sequence could be obtained by removing the relative motion between the objective lens and the imaged tissue. As a sensor for estimating the in vivo motion, a simple contact-type sensor has been developed with using a 3-D cantilever. The 3-D motion compensator driven by three piezoelectric actuators moves the objective lens following the motion estimation by the contact-type sensor.

Both laboratory test and in vivo experiment showed that the proposed system significantly compensates the motion. However, we also noticed the fact that a small remaining error in z direction such as 10 μm could severely blur the image sequence. This gives us a very useful indication for improvement that vertical motion compensation is more important than horizontal one.

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REFERENCES