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Image Stabilization for *In Vivo* Microscopic Imaging

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1. Introduction

Robotics enjoys its growing number of applications in various fields. In this chapter, a robotic system for bio-medical application will be introduced. By adding a robotic system to the conventional microscope, we have solved one of the challenging problems with *in vivo microscopic imaging*. *In vivo microscopic imaging* refers to the imaging technology that visualizes the function of biological process within intact living organism with microscopes. The technology is thought to be a very powerful tool in biological research by enabling biologists to observe what happens inside living organs in a live body, which was impossible before. This useful tool will also play a critical role in many bio-related industries as well. For example, it can greatly enhance the drug discovery process (Bullen, 2008).

However, observing inside a living body with great magnification is not easy. There are some challenges such as insufficient spatial resolution, physical access issues and so on. One of the challenges includes observation problem. Observation itself is significantly disturbed by the physiological motions such as breath, heartbeat, and peristalsis. Even though the animal under observation is usually put under the anesthesia, these motions keep occurring simply because the animal is alive. The motions shake the whole body. So, even very small trembling can happen at any organ. You may not feel it with your own eyes. However, a microscope, the magnifying device, enlarges this trembling as well as organs. As a result, the trembling of the organ sometimes distorts the images from scan-based microscopes such as confocal laser scanning microscopes, or sometimes makes the images totally black by causing out of focus in optical microscopes. All the times, *in vivo* motion brings about observational difficulty.

We tackle this problem. By employing motion canceling robotic technology, we have proposed twp image stabilization methods. After explaining on a fundamental difficulty with *in vivo* microscopy more detail in the next section, two image stabilization systems will be explained with experimental results.

2. *In Vivo* Microscopic Imaging and Its Problem

A fundamental difficulty of *in vivo* microscopic imaging lies in that the microscopy is highly sensitive to motion, which naturally and necessarily occurs at cells of living animals. The causes of this motion include breathing, heartbeat and peristalsis. Since these motions are parts of life processes, they occur even when the subject is put under anesthesia. Those motions significantly disturb the microscopic observation. At worst, they make the observation impossible by causing out-of-focus in the microscope view. Fig. 1 shows an example of unstable observation. Images are from a confocal microscope. Black parts in images are often observed due to out-of-focus by subject's motion. So, continuous observation is impossible being disturbed by the motion.

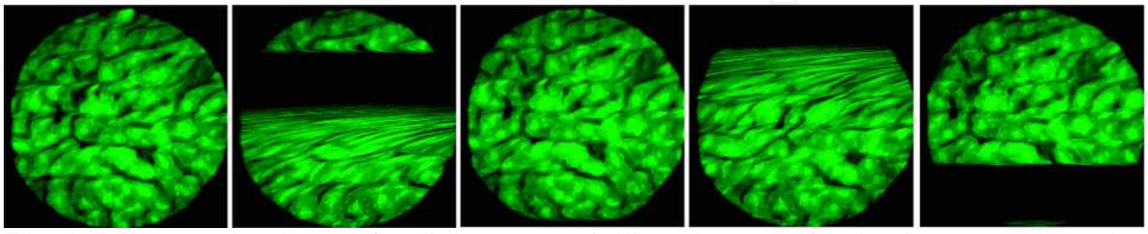

Fig. 1. Microscopic views of a living mouse's liver in a confocal microscope (invasive observation); images are unstable due to body trembling caused by physiological motions such as breathing, heartbeat, and peristalsis.

We have measured this motion. Fig. 2 shows the height of a live mouse liver measured by a laser-displacement sensor. The mouse was under anesthesia. In the graph, the big and periodic impulse-like motion turns out to be caused by breathing. Breathing vibrates the whole body once per one or two seconds. Between the respirations, heartbeat also trembles the body slightly with approximately 10 Hz, which is also periodic. Another low frequency motion, which moves the body slowly, is also observed. This motion is thought to be caused by peristalsis.

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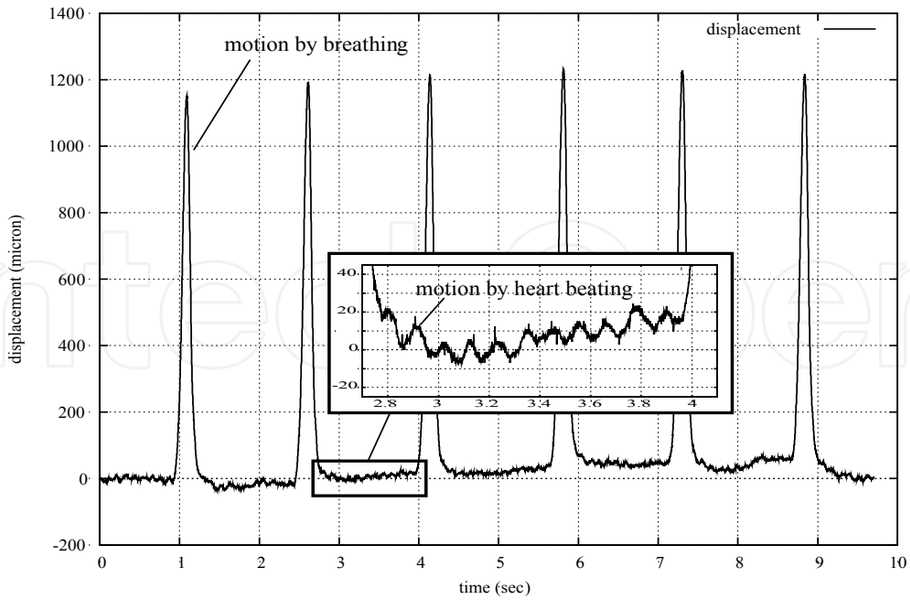

Fig. 2. Motions at a live mouse liver under anesthesia.

In the following sections, we introduce two robotic systems stabilizing observed images through motion synchronization. An objective lens will be controlled to synchronize itself with the subject's motion. This synchronization will virtually remove the relative motion between the lens and the subject, leading to stabilized images.

3. Motion Compensation by Visual Servoing

3.1 System

The first solution is a vision based compensation system (Lee et al. 2008a). We use a high-speed camera for detecting the *in vivo* motion, and move the objective lens to follow the detected motion. To implement this idea, a high-speed camera with 1000 fps is installed into one port of the microscope to measure motion on the image plane, and a robotic closed arm with enough accuracy and power was designed to move the objective lens. In robot technology terms, the system can be classified into an image-based visual servoing system (Hutchinson et al. 1996). In the image-based visual servoing, the motion signal f is defined in the image space. The image Jacobian $\mathbf{J}_{im}$ and the robot Jacobian $\mathbf{J}_{rob}$ map the motion signal f to the joint velocities q as follows:

$$\dot{\mathbf{f}} = \mathbf{J}_{im}\dot{\mathbf{r}} = \mathbf{J}_{im}\mathbf{J}_{rob}\dot{\mathbf{q}} \tag{1}$$

where $\dot{\mathbf{f}}$ is the velocity of the motion, $\dot{\mathbf{r}}$ is the end-effector velocity, and $\dot{\mathbf{q}}$ is the joint velocity. From (1), we design a stable control law based on the resolved motion rate control.

$$\dot{\mathbf{q}} = \mathbf{K}\mathbf{J}^{-1}\Delta\mathbf{f} \tag{2}$$

where the Jacobian matrix $\mathbf{J} = \mathbf{J}_{im}\mathbf{J}_{rob}$, the error vector $\Delta\mathbf{f} = \mathbf{f}_d - \mathbf{f}$ and K is a gain matrix. Then, $\Delta\mathbf{f}$ behaves as follows:

$$\dot{\Delta f} + K\Delta f = 0. \tag{3}$$

Before applying the visual feedback solution to the problem, we need planarize the in vivo motion because a single camera can only detect 2-D motion. If the motion moves the body in the direction of the light, the images becomes blurred by out-of-focus, making no image processing available. In order to prevent the subject from moving in that direction, we employ a simple pressing mechanical device (we call it *mechanical stabilizer*). The stabilizer presses the observed area with a small cover glass. Then, the motion was successfully restricted to the horizontal motion. And, since the translational motion is dominant compared to the rotational motion through the observation, our target motion to be stabilized is set as the 2-D translational motion.

We have developed a piezo-driven robotic closed arm with two DOFs to move the objective lens. It is a five-bar linkage with living hinges. Two accurate piezo-actuators push the mechanism, and then the enlarging mechanism amplifies the insufficient motion of the piezo-actuators. The living hinge, a thin section of the material, is widely used in the design of the MEMS due to its lack of any friction and very little wear.

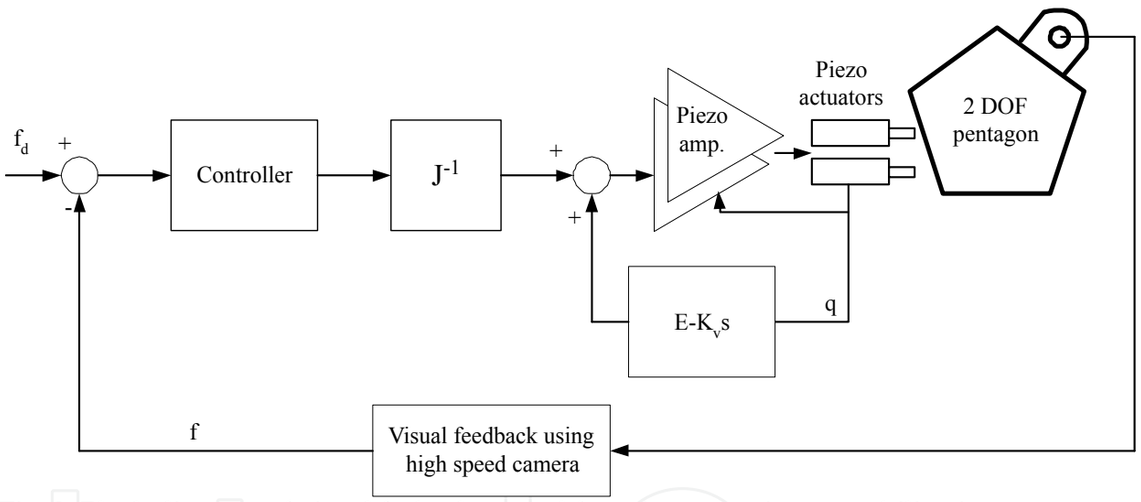

Fig. 3. Block diagram of visual feeback control for microscope image stabilization

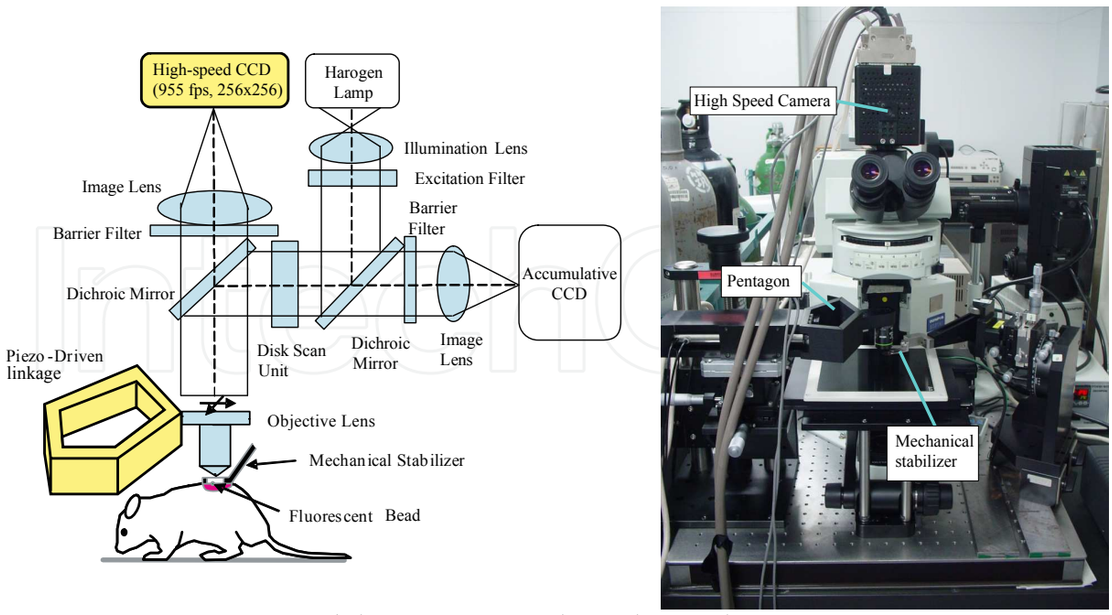

Fig. 4. Microscope image stabilization system through visual servoing

3.2 Result and Discussion

The *in vivo* experimental results show success of the visual servoing based compensation; motions were almost canceled, and as a result, we were able to get stationary image sequences.

Fig. 5 represents the compensated and the remaining motions. The solid line represents the residual motion while the compensated motion is also plotted with the dotted line in X axis (In Y axis, similar result was obtained). The residual motion was less than ±10 μm, while the maximum amplitude of the compensated motion was more than 150 μm. Thus, the image stabilization system removed more than 90% of the motion. The successful motion synchronization consequently generates stable image sequences, as shown in Fig. 7, which would be shown as in Figs. 6, without image stabilization. As we can compare with these image sequences, the vision-based image stabilization system greatly has improved *in vivo* image sequences. The stabilized image sequence is surely much easier to observe. Seamless and stable observation has become possible.

The experimental results have been very satisfactory, meeting our expectation. For improvement and broader applications, the following points should be considered in the next design.

1) *Coping with more complex motions*: Current design can only compensate 2-D translational rigid-body motion. Motion in the direction light axis can cause out-of-focus blurring in the images, and nonrigid-body motions or rotational motions still remain even though these are small compared to the 2-D translational motion.

2) *Observing a subject as intact as possible*: The pressure from the cover glass of the mechanical stabilizer may have unwanted effects on tissues or the living subjects.

3) *No artificial fiducials for image processing*: The fluorescent beads, the artificial fiducials, restrict the observation. It is difficult to locate them at a specific spot, and the beads themselves block the viewing below them.

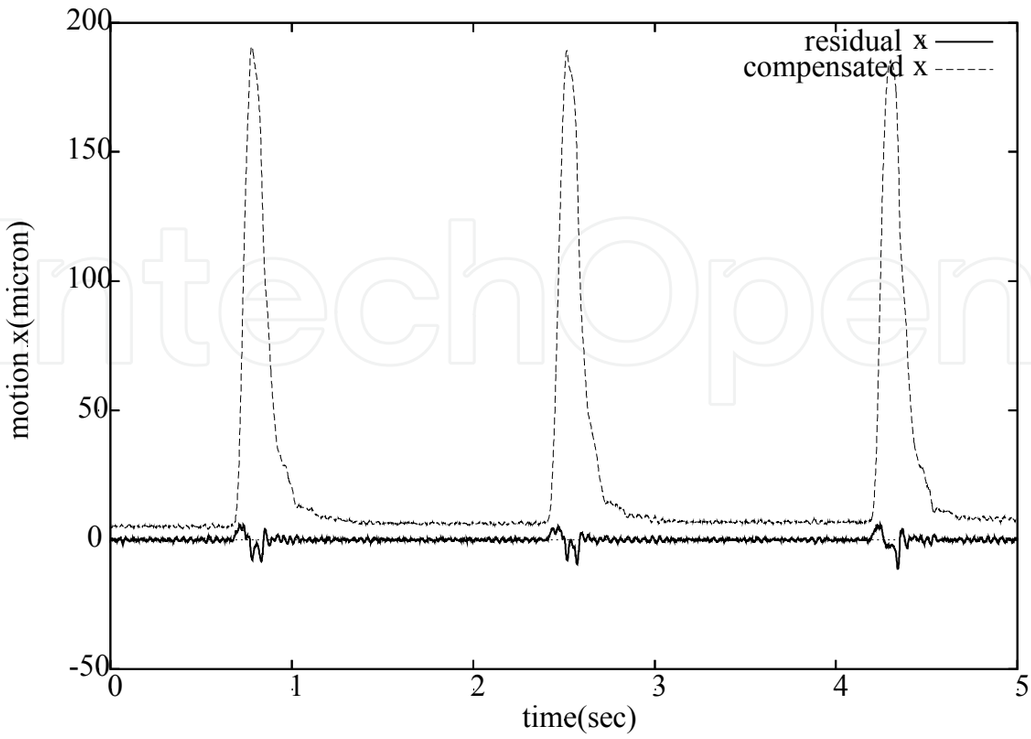

Fig. 5. Solid line: residual motion detected by a high-speed camera, and dotted line: compensated motion caculated from control inputs.

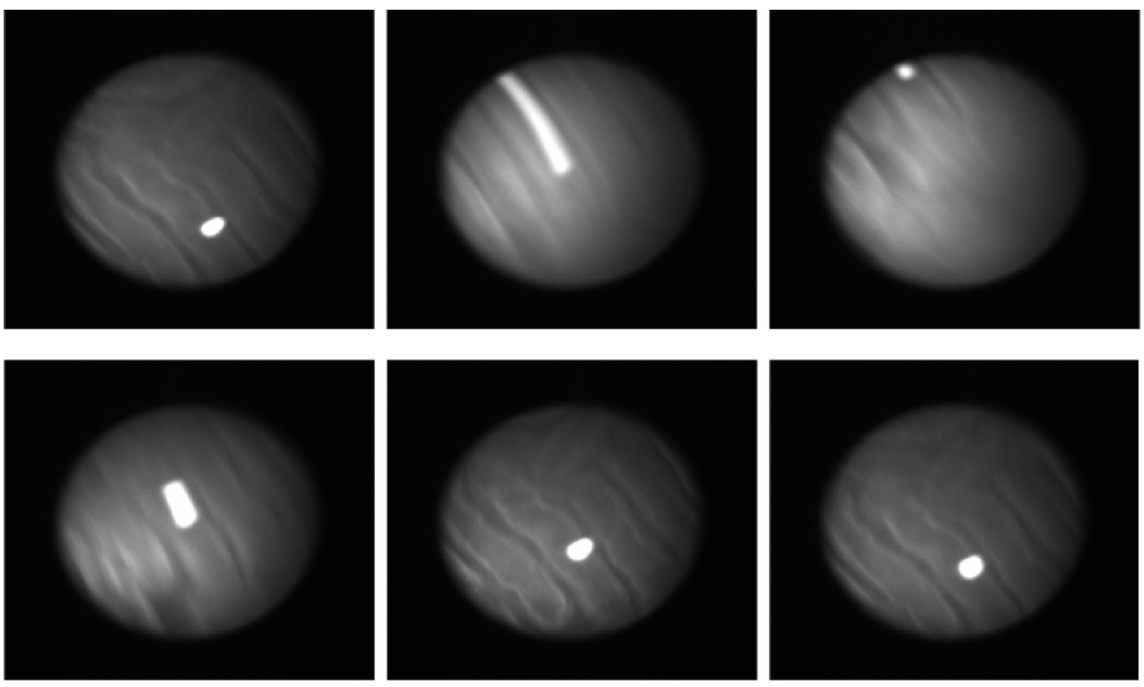

Fig. 6. Microscope image sequence of a mouse kidey (field of view is 200 μm, image sequence was captured by a cooled CCD camera with 37 fps).

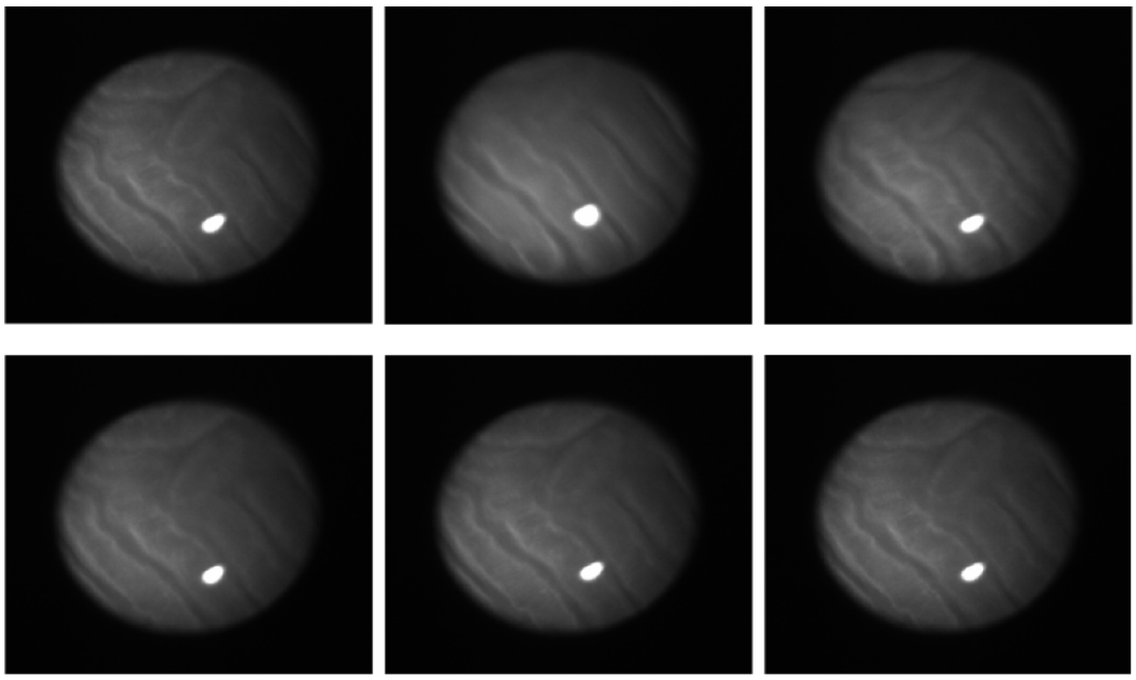

Fig. 7. Motion-compensated microscope image sequence of a mouse kidey (field of view is 200 μm, image sequence was captured by a cooled CCD camera with 37 fps).

4. Motion Compensation by Contact-sensing

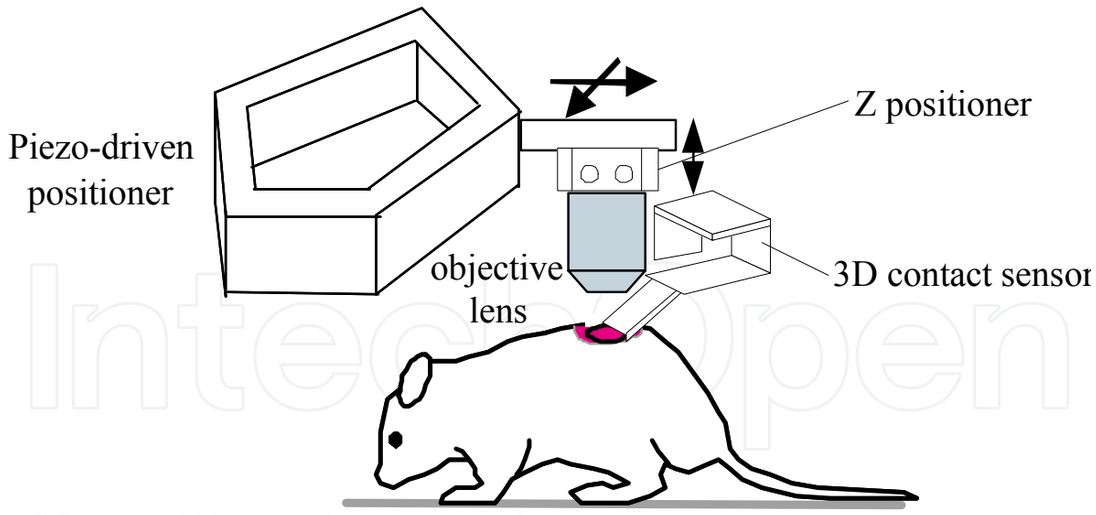

Fig. 8. Image stabilization with contact-sensing

Although the previous solution using a visual servoing system was very successful in removing 2-D motions, there are two weak points. One is that it can only compensate 2-D motion and the other weak point is that the high speed camera system and image processing is too a heavy and expensive solution. This section presents 3-D motion compensation using a developed simple contact-type sensor which is able to detect 3-D motion *in vivo* (Lee et al., 2008b).

4.1 System

The 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, and this estimated motion becomes input to the 3-D motion compensator. The 3-D motion compensator moves the objective lens. Fig. 8 illustrates the overall system. The contact-type sensor consists of three thin beams. The end tip of the sensor is placed on a subject while the other end is fixed to the microscope. The body of the tangible sensor is designed to be elastically bent with small force. To keep the contact, the sensor is initially 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. It is a three dimensional cantilever. Cantilevered beams are now the most ubiquitous structures in the field of micro-electro-mechanical systems (MEMS) specifically as sensors. The signals from three strain gauges in the sensor body are used to estimate the displacement of its tip. We assume that the change of the signals and the displacement has linear relation as

$$\Delta r = C\Delta s \tag{4}$$

where r and s are a 3 × 1 displacement vector and a 3 × 1 signal vector, respectively. C, a 3 × 3 matrix, describes the relation of the two. It is determined experimentally.
For 3-D motion of the objective lens, we use the same actuator developed in visual servoing system in the previous section. To this mechanism, we have added one DOF actuator for vertical which is a commercial product for fast auto-focusing.

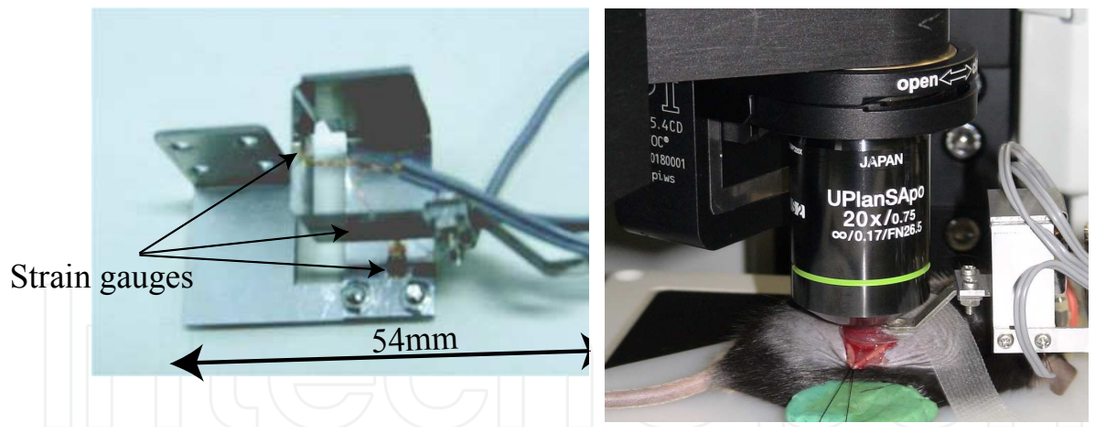

Fig. 9. The developed contact sensor (left) and a contact-sensing based *in vivo* motion compensation (right)

4.2 Result and Discussion

Tests were performed with respect to artificial motion. We used motorized micro-stages for artificial motion generation. Two stages produce horizontal and vertical motion. This motion is estimated by the developed contact-type sensor and compensated by the 3-D compensator. We put a sample tissue of a mouse liver on the micro-stage. Sine wave at the frequency of 1 Hz was generated. The amplitude of the wave is 100 μm both in vertical and

horizontal directions. Fig. 10 plots the remaining motion without and with the motion compensation. Fig. 11 and 12 are the image sequences at those times, respectively.

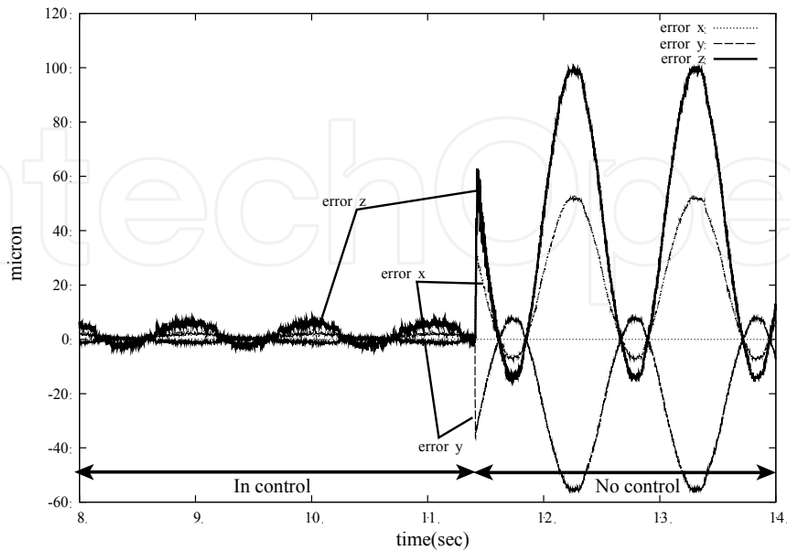

Fig. 10. The remaining motion without (front part) and with (latter part) motion compensation

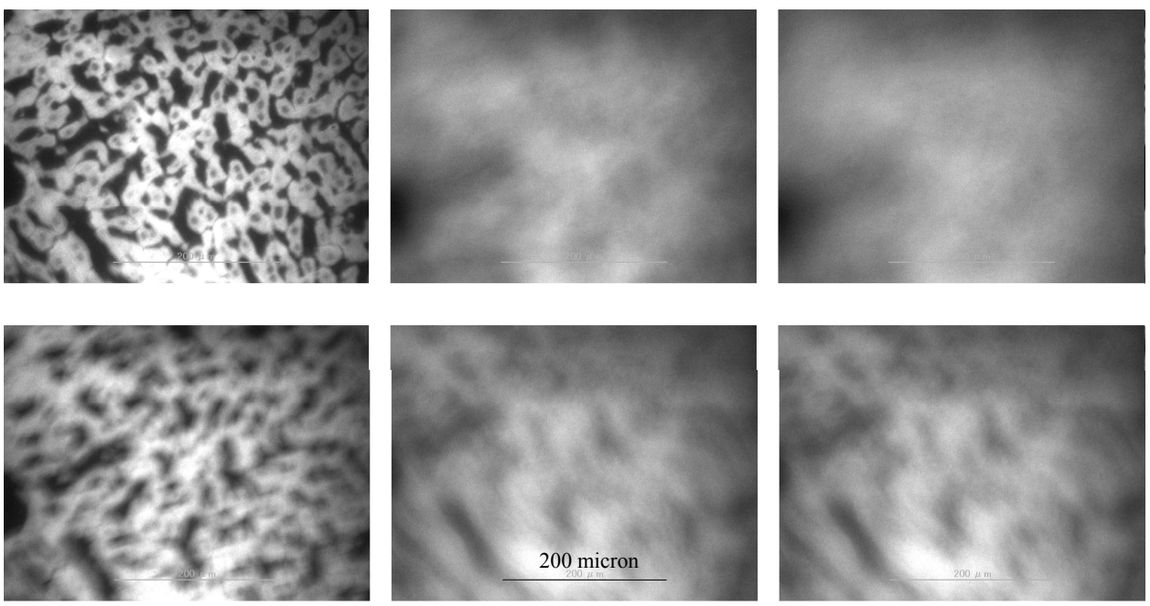

Fig. 11. Microscope image sequence of a mouse liver sample; Artificial sine motion with 1 Hz frequency was generated both in vertical and horizontal directions.

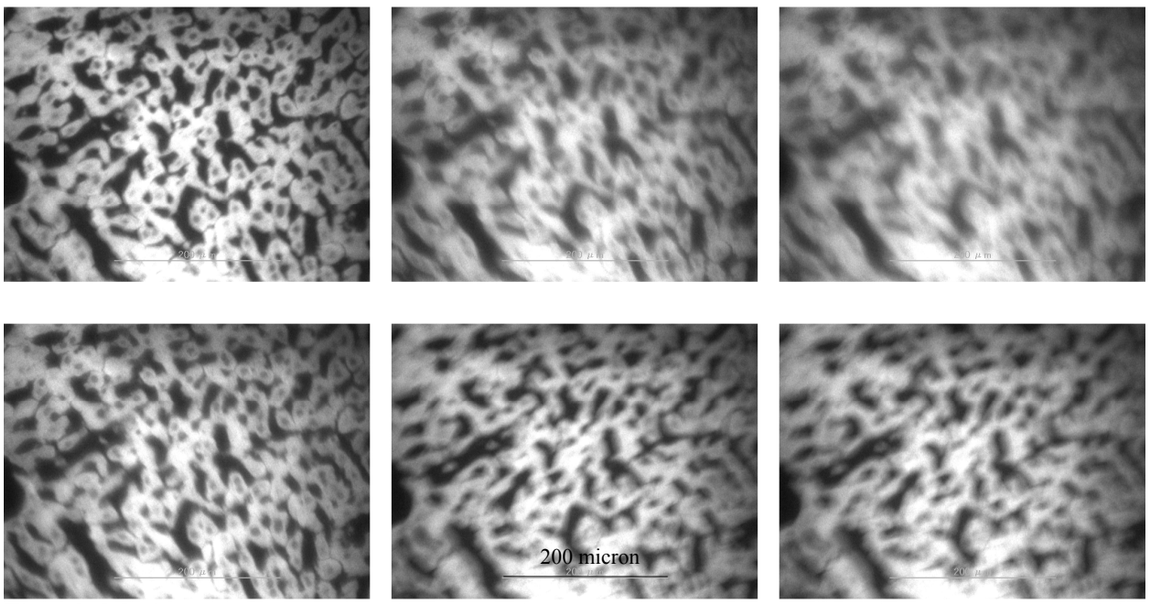

Fig. 12. Motion compensated microscope image sequence of a mouse liver sample; Artificial sine motion with 1 Hz frequency was generated both in vertical and horizontal directions

5. Conclusion

In this chapter, we have introduced microscope image stabilization methods. Two configurations were examined being based on the idea of synchronization. The developed systems mechanically compensate *in vivo* motion by moving objective lens to follow the subject's motion. By removing the relative motion between the objective lens and its subject, we could obtain more stabilized images than ones without compensation. Depending on the sensing methods, we examined two systems: a visual feedback system and a contact-sensing based system. The visual feedback system employs a high-speed camera for detecting fast *in vivo* motion, and the contact-sensing based system uses our developed contact-type sensor which can estimate 3-D motion. In both systems, experimental results show that the image stabilization markedly reduces the effect of the *in vivo* motion, stabilizing the image from the microscope.

The examples introduced in this chapter have shown that the robotic technology can make significant contribution to biological research which is thought to be not directly related to the robotics. Like this, the robotic technology will have great impact on the various fields in the future.

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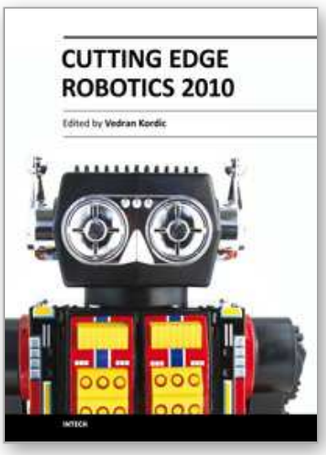

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