DESIGN OF A PRECISION FLEXURE-BASED VIBRATION MICROTOME FOR WHOLE ORGAN IMAGING

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In this paper, we present the modeling, design, and dynamic characterization of a flexure-based vibrating blade microtome that achieves submicron precision with minimized parasitic motions in multiple axes. The completed microtome is then integrated with a multifocal multiphoton microscope (MMM) for whole organ imaging. The automated system can obtain 3-D organ image at the speed of 10 GB/hr with subcellular resolution.

MICROTOME
A microtome is a sectioning instrument that enables cutting of thin slices of fresh tissue for microscopy studies or pathological analyses. Oscillating blade microtomes, i.e. vibrating blade microtomes, are a variation of the basic microtome, and are widely recognized as superior for cutting thick sections from non-embedded or fresh tissue samples. Important design parameters for optimizing the cutting process include the vibration amplitude, the vibrating speed, the angle of the blade, the feed rate (of fresh tissue samples). Conventional oscillating blade microtomes fail to achieve the desired precision, i.e. precise control of tissue flatness, e.g. 1 µm, and section thickness, for the following reasons: (1) imprecise oscillatory motion caused by the out-of-plane parasitic motion, (2) backlash generated in the conventional mechanical joints, and (3) friction limited frequency and magnitude for leaf spring type microtomes [1, 2]. The error motions in these systems are typically on the order of tens of microns in different axes.

DESIGN CONCEPT
We have been developing a flexure-based vibrating blade microtome device that can address the aforementioned problems and achieve submicron cutting precision. When a vibrating blade microtome is in operation, the sample is fed to the blade in a direction, i.e. cut direction, transverse to the oscillating direction of the blade. To achieve high cut quality, the blade must oscillate in one plane, generating repeatable linear or arcuate motions. The transverse blade motion is typically generated by a motor or electromagnetic actuator coupled with an eccentric cam. A mechanical filter is required to isolate the non-transverse motions from the blade. The block diagram in Fig 1 illustrates how this can be achieved by arranging two flexures in series, where flexure 1 is stiff in the cut direction and flexure 2 is stiff in the transverse direction. The blade assembly is mounted on flexure 1.

Fig 2 shows the final design of the vibrating blade microtome, where flexure 1 guides the motion of the blade and is coupled with flexure 2 that significantly reduce the unwanted mechanical coupling between the motor and flexure 1. The optimized stiffness ratio of flexure 1 and 2, i.e. k_y1/k_x1 and k_x2/k_y2 in Fig 1, are 49 and 3500 respectively. The flexures were fabricated from a 1” thick 7075 aluminum bar using wire-EDM. The 1” thickness was selected to ensure the microtome is stiff in the out-of-plane direction during cutting processes.
CHARACTERIZATION
An ideal cutting operation would generate only horizontal translations of the blade, with no motion in any other axes. In practice the blade will undergo slight parasitic motions in other axes during operations due to loading from the actuators, finite stiffness of the structure and dynamic effects. As shown in Fig 3, a metrology stage was assembled around the cutting stage in order to study the motions in the axes considered most critical for cut quality. The measurement was carried out using five capacitive sensors, sampling at 2 kHz, each aligned to a 1.2 x 1.2 cm² grounded metal target which had been adhered to the moving stage. The data were collected when operated at a vibration amplitude of 0.8 mm. The results show 2 types of parasitic motions of the blade assembly: (1) coupled Δz and θₓ rotation occurring on the cutting edge at 40Hz, and (2) θᵧ rotation occurring with a 5° phase delay, suggesting a rotational resonance that lies at higher frequencies than that of the θₓ rotation. The total error motion of the blade is the superposition of both of the dynamic modes described above. The net parasitic motions at the center of the blade and at the extreme tips of the blade are 0.5 μm and -1/+2 μm respectively.

IMAGE ACQUISITION
The completed vibrating blade microtome system was integrated with an MMM system, and an automated xyz stage for 3-D image capturing, shown in Fig. 4. Figure 5 shows a cross-sectional image of a mouse brain, collected by our system.

CONCLUSION
We have developed a flexure-based vibrating blade microtome and fully characterized its dynamic performance. The microtome shows superior precision and dynamic characteristics than existing commercial models. The improved cut quality and surface flatness significantly improve the 3-D image quality of the integrated whole organ imaging system.

REFERENCES