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Ultrasonically Assisted Cutting of Bio-tissues in Microtomy

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Abstract

Modern-day histology of bio-tissues for supporting stratified medicine diagnoses requires high-precision cutting to ensure high quality extremely thin specimens used in analysis. Additionally, the cutting quality is significantly affected by a wide variety of soft and hard tissues in the samples. This paper deals with development of a next generation of microtome employing introduction of controlled ultrasonic vibration to realise a hybrid cutting process of bio-tissues. The study is based on a combination of advanced experimental and numerical (finite-element) studies of multi-body dynamics of a cutting system. The quality of cut samples produced with the prototype is compared with the state-of-the-art.

Peer-review under responsibility of the Ultrasonic Industry Association.

Keywords: Ultrasonically assisted cutting; Power ultrasonics; Microtome; Experimental study; Surface roughness

1. Introduction

Microtomes have been employed for many years for preparing samples of biological tissue in a form of thin slices suitable for histological examinations with optical microscopy. Although modern microtomes were invented by Wilhelm His in the 19th century (Anonymous (1964)), there are no fundamental changes of microtomes to this day. Typically, a microtome has a blade mounted in a blade holder, and a tissue sample embedded in a wax block is moved relative to the blade along a pre-defined path. A thin section of the sample is sliced from the block, which placed in a pathway of the blade. There are a number of drawbacks (or challenges) with conventional microtomy techniques including high blade wear and damage, poor section quality and difficulty of section transportation. So, it is desirable to improve the cutting process with reduced blade wear, high cutting quality, high precision cutting and easy sectioning of hard tissues. Some or all of these requirements may be met by providing an ultrasonically assisted cutting device in a microtome.

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Ultrasonically assisted cutting is widely used in the food processing and processing of hard-to-cut materials (Astashev and Babitsky (2007)). A superposition of ultrasonic vibration on movement of the cutting blade produces a nearly frictionless surface between the blade and the material. Benefits for structural – including hard-to-machine – materials can be obtained including the reduction of cutting forces and blade wear, improvement in surface finish etc. (Babitsky et al. (2004)). However, an ultrasonically assisted microtome cutting is a novel concept in histology; there is limited research completed in this area. This study aims to fill the gap and develop an ultrasonically assisted microtome capable of sectioning biological tissues, providing thin sections in a controlled and repeatable way with reduced cutting forces and minimal blade wear, thereby improving longevity of blades.

Nomenclature

CC	conventional cutting
FEM	finite-element model
PC	piezoelectric ceramic
UAC	ultrasonically assisted cutting
UACD	ultrasonically assisted cutting device

2. Methods

To the best knowledge of the authors, there is no precise research data on the life of microtome blades but it is widely agreed that even a small increase in their life would allow for lower costs as well as decreasing the environmental impact. The increase in the blade life may be achieved by reduction of cutting forces and blade wear. Larger cutting forces applied in the cutting operation decrease sharpness of the blade; thus, reliability and reproducibility of sections reduces. Furthermore, as the blade deteriorates due to wear or damage through its use, a higher cutting force may be required, which was found by Allison and Vincent using load cells to measure acting forces for conventional cutting in microtomy. Therefore, blade-wear analysis and measurements of cutting forces should be done to compare conventional cutting (CC) and ultrasonically assisted cutting (UAC).

2.1. Blade-wear analysis

A cutting edge of a specialist blade, used for the microtome cutting from Cellpath Ltd. UK, is shown in Fig. 1. There are three bevels on the cutting edge, which made it hard to manufacture. Surface roughness of the third bevel, which is the contacting surface between the sample and the blade, was measured for virgin and used blades using an Alicona InfiniteFocus microscope; area roughness parameters S_a were obtained as a result. The used blades were employed for several conventional microtome cutting of biological tissues. Such blades provided poor section quality as assessed by an experienced histotechnologist in Cellpath Ltd. UK.

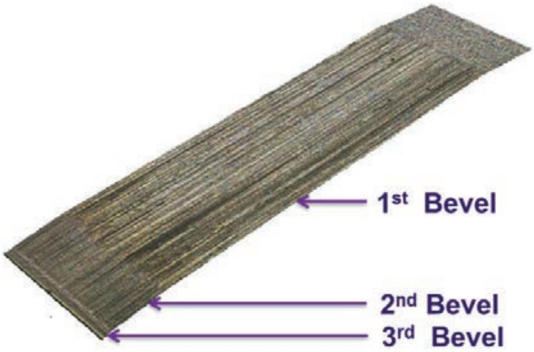


Fig. 1. The cutting edge of a specialist blade

To compare differences in wear for CC and UAC, the same cutting tests were repeated for 2, 5 and 8 cuts for both cutting techniques.

2.2. Ultrasonically assisted cutting device design

A design of an ultrasonically assisted cutting device (UACD) was carried out to meet requirements of controlled vibration transmission. To ensure a reliable performance of the UACD, several performance criteria should be satisfied in its design including an operating frequency at close to a resonant one, a high vibration amplitude at feed direction and separation of the operating frequency from close non-tuned modes. Furthermore, to fit the UACD in a commercial microtome, several dimensional factors were considered in the design including a width of the cutting edge for industrial-standard blades, and a cutting length over a stroke length of the microtome.

Unlike a conventional power ultrasonic transducer, the UACD has a non-symmetrical blade as the front mass. Two piezoelectric ceramic (PC) rings were sandwiched between a front mass and a backing mass using a central bolt. The front mass was made of stainless steel to match the industrial-standard blade, provide great acoustic properties and acceptable wear characteristics. A slot for clamping the blade was designed at the cutting edge. The nodal points of the device were fixed at the test rig with bolts.

A finite-element model (FEM) was developed for the design, which was optimized to transfer the radial movement of the piezoelectric discs to the longitudinal movement at the edge along the cutting direction (z axis). The modal shape of the operating vibration mode is shown in Fig. 2. The normalized contour plot predicted the displacement along z axis (U3) at the mode of vibration.

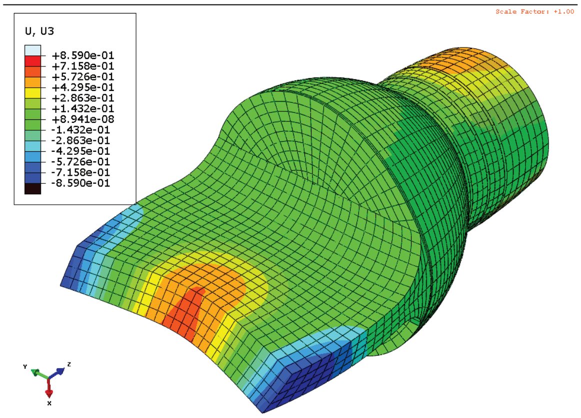


Fig. 2. FEM predicted the normalized displacement along z axis at the operating mode of UACD

2.3. Characterization of the UACD

Based on the FEA results, the device was manufactured and assembled (Fig. 3). The numerical results were compared with the experimental analyses, carried out using a laser Doppler vibrometer (LDV) and an impedance analyzer. The vibration amplitudes along the z axis were measured using the LDV on the blade edge at three different positions – A, B and C (Fig. 3). An impedance analyzer was used for measuring the resonant frequency and the maximum output amplitude when the device was in harmonic excitation. The experimental impedance-versus-frequency diagram could be obtained.

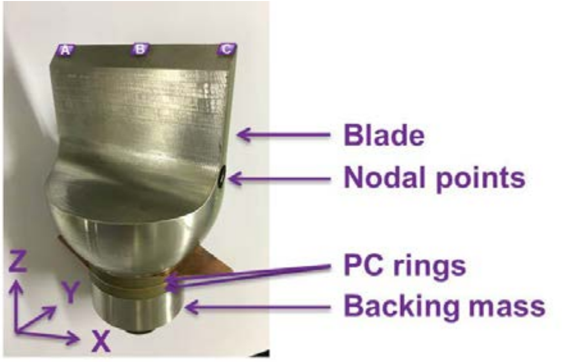


Fig. 3. Manufactured and assembled UACD

2.4. Design of test rig

To measure the level of cutting forces accurately, a test rig was designed with a mounted dynamometer and the UACD. The test rig was capable of making multiple cuts with consistent section thickness and at a constant feed rate with two cutting techniques –ultrasonically assisted and conventional. The tasks of design were achieved by using a stepper motor and an accurate lead screw in a slider. A sample was attached to the dynamometer, which was used for force measurement. The sample and the dynamometer could be lowered and raised using a 1 mm-pitch threaded screw. The design is illustrated in Fig. 4.

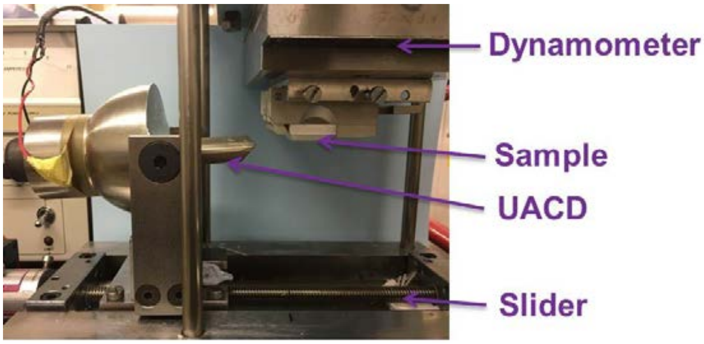


Fig. 4. Test rig used in experiments

2.5. Experimental study of CC and UAC

Experimental tests were completed on wax blocks to quantify the difference between ultrasonically assisted cutting and conventional cutting. The test rig was set up for one wax sample to be cut multiple times. After completing the cuts, the sample and blade were replaced. This process was repeated for the same number of cuts with UAC and CC. Comparisons of cutting forces and blade wear were made for cuts produced with the two studied techniques. Then, this test was repeated for a different number of cuts for UAC and CC. The cutting speed in the tests was 10 mm/s, which is similar to that used by a histotechnologist. A quarter revolution on the lead screw corresponded to thickness of 0.25 mm, which is easily measured on the test rig. The ultrasonic part of the test rig was set up in the way to provide an ultrasonic vibration at 37.6 kHz with the amplitude of the blade edge around 2 μm; these parameters were used in all the tests, to allow their comparability.

3. Results

3.1. Wear of virgin and used blades

Surface-roughness results determined by parameter S_a are shown in Table 1; apparently, the blade wear increased with the cutting operations when compared between virgin and used ones.

Table 1. Area roughness parameter S_a (in µm) for virgin and used blades

Test	Virgin blade	Used blade
1	0.221	0.300
2	0.220	0.358
3	0.230	0.337
Ave.	0.224	0.332

3.2. Vibration characteristics of manufactured device

In Table 2, the results for vibration amplitudes are tabulated as obtained in vibration tests using LDV. The average vibration amplitude along the z direction was 1.87 µm at operating frequency of 37.6 kHz.

In FEA, the resonance frequency was 35.9 kHz and the separation of the operating frequency from the closest non-tuned mode was 1.2 kHz. In comparison, the resonance frequency of 37.53 kHz and frequency separation of 2.9 kHz was measured by the impedance analyser (Fig. 5), when the UACD was clamped at its nodal points with the bolts.

The measured resonant frequency and vibration amplitudes were found to be in agreement with the FEA. The fixation at the nodal points was considered to make the frequency separation larger than the FEA predictions to avoid the coupling of different modes at the operation frequency.

Table 2. Vibration amplitudes measured with LDV for various points of blade (Fig. 2)

	A	B	C
	Amplitude (µm)	Amplitude (µm)	Amplitude (µm)
Z	2.0	1.6	2.0

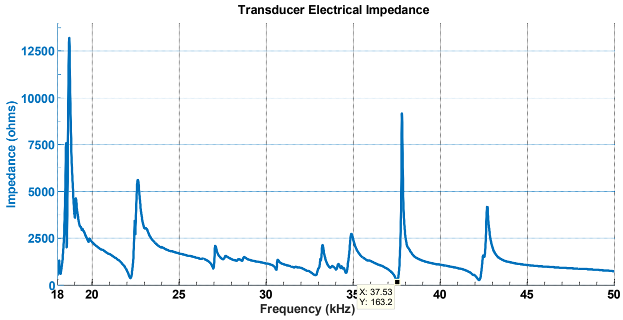


Fig. 5. Electrical impedance of the UACD

3.3. Experimental results for CC and UAC

The cutting force data for all tests are presented in Fig. 6 and Table 3; they show a considerable decrease in the cutting forces for UAC when compared with CC. All three cuts in CC and UAC showed similar cutting forces with some minor deviations. All the cuts had similar cutting trends in the cutting direction. The maximum compressive forces measured for CC and UAC were around 11.5 N and 8.5 N, respectively. The average force reduction in UAC over 3 samples was over 25% compared with that in CC.

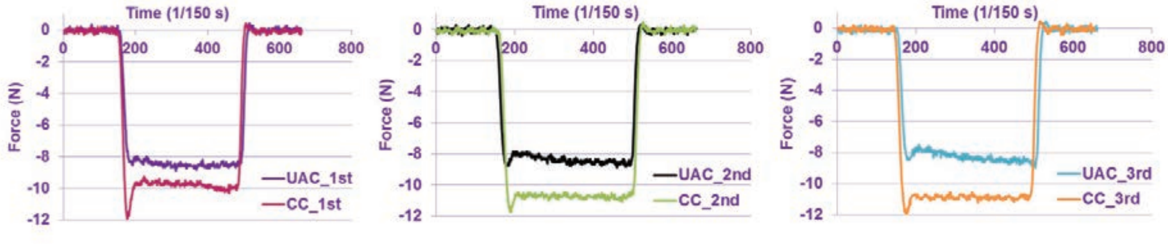


Fig. 6. Cutting force data for CC and UAC

Table 3. Maximum forces recorded in CC and UAC

Set	Maximum force (N)		Force reduction
	CC	UAC	
1	11.7	8.6	26.5%
2	11.5	8.8	23.4%
3	11.7	8.7	25.6%
Ave.	11.63	8.7	25.2%

The sections showed no great difference between CC and UAC. Both were curled up and in one continuous long piece. In general, they were considered as a good-quality section, as can be seen in Fig. 7.

The experimental results give confidence in the ultrasonically assisted cutting's ability to give good consecutive and reliable results for microtomy.

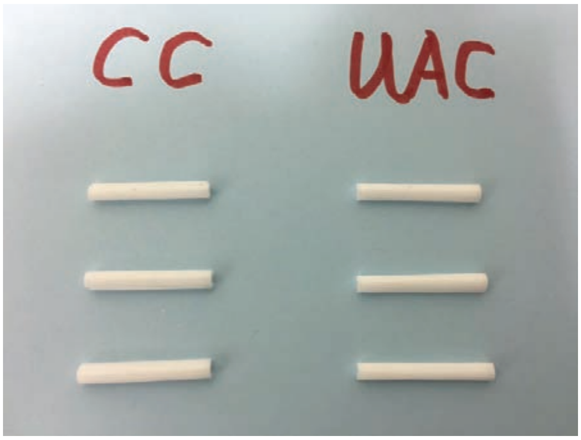
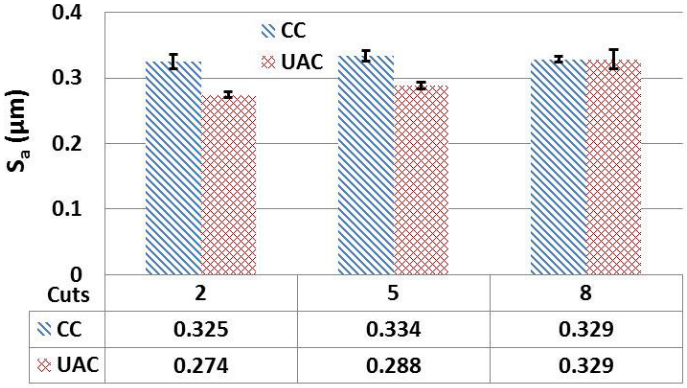


Fig. 7. Sections obtained in CC and UAC

3.4. Blade-wear analyses for CC and UAC

The roughness measurements in Fig. 8 show the decreased levels of wear in UAC for 2 and 5 cuts when compared to CC. There is no difference in the average value of S_a for 8 cuts for the both studied cutting techniques. It is clear that the increased number of cuts led to increased wear in UAC; this trend was not found in CC. The average magnitude of S_a for the used blades is 0.332 µm, which is similar with the average S_a for 2, 5 and 8 cuts in CC. It could be assumed that the blade was blunted already after 2 cuts of sections of 0.25 mm at cutting speed of 10 mm/s in conventional cutting. However, the blades used in UAC could survive at least 5 cuts. Although these results give a good indication of what is happening in CC and UAC, more tests in the further work are needed for statistical analysis.



Cuts	2	5	8
▨ CC	0.325	0.334	0.329
⊠ UAC	0.274	0.288	0.329

Fig. 8. Surface roughness parameter S_a (in µm) for different numbers of cuts with CC and UAC

4. Conclusion

Based on the FE model, an ultrasonically assisted cutting device was developed for cutting thin sections of biological tissues with microtomy. Results for both the cutting force and surface roughness show that employment of ultrasonically assisted cutting resulted in reduction of cuttings forces and blade wear. Good quality of sections was obtained with UAC using the designed test rig.

The results of this work could be used to help the design and manufacture of a microtome with an ultrasonically assisted cutting device. This work showed that UAC can provide good-quality sections of paraffin wax samples, reducing the overall force required to cut the samples and blade wear, therefore, decreasing the costs associated with frequent changing of blades. A design was suggested for attaching the ultrasonically assisted device to the existing microtome, avoiding a design of a new microtome. Instead, a special module can be bolted on to the existing system, thus leading to an increase in the blade life while reducing the cost of purchasing a new microtome. This would be a promising development for the microtomy.

Acknowledgements

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