Wang *et al.*
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Ultrasonically Assisted Cutting of Histological Sections for Reducing the Environmental and Financial Impact of Microtomy

Dong Wang[1,2*], Daniel De Becker[1] and Anish Roy[1]

Abstract

Modern-day microtomy requires high precision equipment to thinly section biological tissues. The sectioned tissue must be of good quality not showing cutting tracks or so-called artefacts. The quality of these sections is dependent on the blade wear, which is related to the hardness of the tissue sample, cutting angle and cutting speed. A test rig has been designed and manufactured to allow these parameters to be controlled. This has allowed for the blade wear to be analysed and quantified, and this has been completed for both ultrasonically assisted and conventional cutting. The obtained results showed a 25.2% decrease in average blade roughness after 38 cuts when using the ultrasonically assisted cutting regime. The data also showed no adverse effect on the quality of the slides produced when using this cutting methodology. Finally, the cutting force measured for both cutting regimes showed that ultrasonically assisted cutting required less force compared to conventional cutting. With the reduction of surface roughness and force, it is possible to state that ultrasonically assisted cutting reduces the wear of the blade, thereby increasing the life of the blades. An increase of just 10% in blade life would yield a cost saving of approximately 25% thereby reducing the environmental and financial impact of microtomy.

Keywords: Microtome, Ultrasonically assisted cutting, Blade wear

1 Introduction

Unlike other ultrasonically assisted machining methods, ultrasonically assited microtomy has had very little attention and therefore very little literature is availale to reference. Therefore, this work has been generated to understand whether ultrasonically assisted cutting (UAC) within microtomy is a viable alternative of conventional microtomy. With the key outcome being whether UAC can be used to reduce blade wear and therefore increase balde life. As this was found to be true in ultrasonically assited drilling, turning, milling, grinding and etc. [1–6].

*Correspondence: d.wang2@exeter.ac.uk
[1] Wolfson School of Mechanical and Manufacturing Engineering, Loughborough University, Loughborough LE11 3TU, UK
Full list of author information is available at the end of the article

In order to understand UAC within microtomy, it's important to note that the microtome has become a universal tool in the creation of sample slides from tissue embedded paraffin wax blocks for histology. Being a widely used tool, few changes have been made to the operation of the microtome itself over the years. However, incremental changes have been made to the ergonomics and design of the microtome, these changes have not been made to improve the quality of the sections and blade life [7]. Although efforts have been made in increasing the ease of changing the cutting blade, this process is still the most time consuming and costly operation. This is mainly because the time required to "cut in" a new blade as well as the cost of the new blade itself. The most significant change in the industry is the move from reusable blades, which are usually diamond reinforced and could therefore be sharpened after use. The use of reusable blades has become less common mainly due to the significant reduction in the cost of disposable blades and the increased labour cost in the sharpening of reusable blades. Although it was found that the reusable blades tended to have a greater blade life compared to their disposable counterpart, the cost savings in the use of disposable blades strongly outweighed the higher blade life benefits of the reusable blades. Due to the transition to disposable blades, the environmental impact and time associated with changing the blades has also increased. This increase could therefore be offset by increasing the blade life and thus decerase the amount of blades used per histologist per hour. This would in turn decerase the overall environmental impact of single use baldes but would increase the output of each histologist by spending less time changing blades and more time cutting samples.

During conventional microtomy as the blade completes a pass over the sample, brittle fragmentation of the blade edge occurs causing the blade to lose its sharp edge. The fragmentation of the blade causes an increase in the contact area which increases the cutting force required. This effect was also seen in various studies, which showed that the radius of the cutting edge was directly related to the cutting force required and that this effective radius increased with an increase in wear [8]. This increase in blade tip radii has also been shown to increase localised heating of the material as well as induced stress at the blade tip, all these factors can be categorised as directly related to blade wear.

In order to increase blade life, the blade could be vibrated ultrasonically. Ultrasonic cutting usually operates between 20 and 100 kHz with a vibration amplitude between 2 and 25 μm. The frequency at which the piezoelectric elements are excited at is dependent on the natural frequency of the blade holder. The ultrasonic vibration causes localised plastic deformation at the blade tip. This localised plastic deformation is caused by the high strain rates [9] along with this the pulsating effect of the vibration causes a reduction in contact time between the blade and the sample [10]. The pulsating effect of the blade causes an accumulation of damage to appear in front of the blade, thus decreasing the cutting force required. Although no study has been completed on the effect of blade life within microtomy, studies have been completed on the effect of tool wear on the diamond cutting of optical glass [11] and turning of low alloy steel [10]. Both these studies showed that the introduction of an ultrasonically assisted cutting tool increased tool life due to the decrease in the cutting force required.

2 Factors Contributing to the Blade Life

There are various factors which can affect the wear and therfore the balde life of the blades. One of these factors is the cutting force and in turn the cutting angles. These cutting angles and their effect on cutting force within microtomy has been extensively studied. When a sample is cut, the force can be split into two independent components, the tangential and radial components. The tangential component is the force in line with the cutting direction and the radial component is orthogonal to this direction. Later research into these two components found that the radial component of the cutting force provides no mechanical advantage in the cutting process, causing unwanted compressive stresses as well as contributing to tool wear [12]. In order to decrease the tangential force, the blade would ideally be parallel to the sample, thus cutting angle becomes very important when measuring cutting force. The key research into varying the cutting angle was completed by Dempster [13], which showed there to be three key angles when expressing a microtomes' cutting angle (Figure 1). These are denoted by Dempster as the bevel angle, clearance angle and the rake angle where the sum of these three angles equals 90°. Suggestions have been made that the clearance angle should be between 3° and 5° [14] while the bevel angle remains between 16° and 20° [13]. Although these suggestions have been made, it should be noted that due to the various blade designs these values are not always achievable.

A further factor that affects cutting force is cutting speed. For conventional cutting, it was known that an increase in cutting speed would dramatically increase the cutting force required as well as affect the surface finish [15]. This is mainly due to the increase in strain rate, which causes an increase in cutting resistance. Through the testing of microtomy forces, Vincent showed that the optimum speed for a good quality surface finish was around 15 mm/s [15].

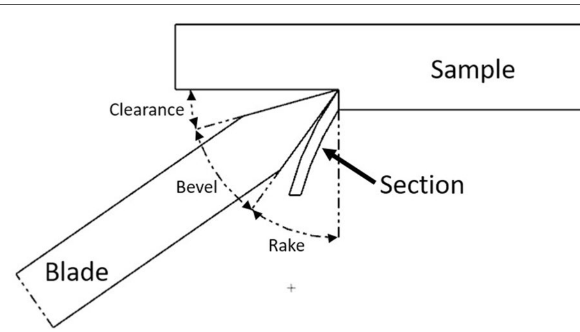

Figure 1 Definition of angels associated with the blade

The final key factor that affects the surface finish and overall cutting force is the temperature. This includes the temperature of the sample as its being cut as well as the temperature increase due to the cutting action itself. As the sample is being cut, the primary and secondary bonds are broken, this breaking of bonds leads to energy dissipation in the form of heat. Due to the paraffin' low thermal conductivity, between 0.21 and 0.24 W/mK [16], localised heating occurs within the sample thus making it more ductile and lowering the surface finish. This localised ductility also increases the cutting resistance as shown in the study by Farag et al. [17]. This study showed that as the blade temperature is lowered the excess heat caused by the breaking of bonds is conducted into the blade. This effect is mainly due to the blade having a higher thermal conductivity as well as the large temperature difference between the cooled blade and the paraffin wax [17]. This thermal energy absorption reduces the likelihood of the paraffin wax becoming ductile. Furthermore, the heating effect is reduced further by decreasing the temperature of the sample block, this can be achieved with the use of cold plates, which has become the standard practice within microtomy.

3 Experimental Study

Within this study, the effect of temperature and speed have been taken into account by maintaining a constant room temperate and a constant cutting speed. As well as an optimum cutting angle has been calculated. This cutting angle was kept constant throughout the conventional and ultrasonically assisted cutting, thereby mitigating any variations in the wear caused by blade angle. Finally, the impedance of the blade holder was calculated and thus the natural frequency was found, this allowed the blade and blade holder to vibrate in resonance, thus increasing the efficiency of the cutting action.

To measure the wear of the blade tip the surface quality of the blade was measured. This method of measuring blade wear has been validated and verified in a previous study which showed that an increase in blade tip roughness was directly related to the increase in blade wear [14]. To fully understand the surface roughness the arithmetical mean peak height (S_a) was measured. Therefore, in order to quantify if ultrasonically assisted cutting increases efficiency within microtomy three aspects will be investigated, the roughness of the blades after cutting, the cutting force required to complete the sections and finally the sample quality itself.

This paper hopes to prove that with the introduction of an ultrasonically assisted cutting blade a reduction in the cutting force, and thus blade wear can be achieved. This will in turn increase the blade life which will have both a positive environmental and economic impact.

4 Experimental Test Rig Design

A custom test rig was designed and manufactured which allowed for both conventional and ultrasonically assisted cutting to occur (Figure 2). The test rig was designed using a stepper motor which drives a 2 mm pitch lead screw allowing a constant cutting speed to be achieved. To translate the step frequency of the stepper motor to a translational speed an equation was formulated, where f is the step frequency of the motor, and v is the speed of the blade.

$$v = \frac{f}{200}. \tag{1}$$

The test rig frame was mainly made of aluminium including the two upright posts, the top and bottom

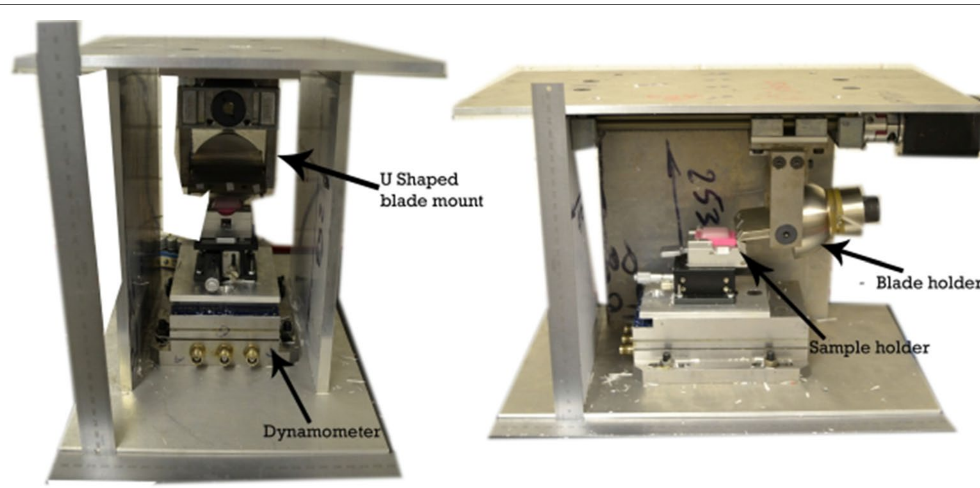

Figure 2 Image of test rig used to measure cutting forces for both conventional and ultrasonically assisted cutting

plates. To enable a stiff blade holder, the U shaped component holding the blade as shown in Figure 2, was made of mild steel. This increased stiffness mitigated any movement of the blade holder during cutting. To raise the sample into the path of the blade, a Sunwin Z-axis precision linear stage was used, this allowed for micron precision sample sections to be cut. However, due to the mechanism of the micrometre lifting stage, the blade had to be mounted in an inverted position. To hold the sample in position, the sample holder used was a cassette clamp. This is an industrial standard sample holder used with a microtome such as the Leica RM2125.

The blade holder itself was designed and modelled in-house, this design incorporated two piezoelectric ceramic rings that were sandwiched between a back mass and blade holder. Where the design of the balde holder was in such a way to cause the applied vibration from the blade holder with an inclination to the sectioning direction, which was detailed in Ref. [18]. Futhermore, the width of the design was also carefully selected to match the industry-standard blades, allowing the blade holder to be easily transposed into an industrial microtome. The blades themselves were held in place using two screws which were mounted on the underside of the blade holder and passed through the slots on the blade.

A dynamometer was attached to the sample holder which allowed for the force in both the tangential and radial directions to be measured. Both force components were measured and recorded to validate the wear measurements. Finally, the cutting angle was measured using an accelerometer connected to a microcontroller. This allowed the angle to be measured relative to the ground plane and thus knowing the geometry of the blade and blade holder the bevel, rake and clearance angle could be measured and kept constant between cuts. This approach is very similar to how a digital inclinometer works however as it has been purpose-built the design envelope has been dramatically reduced. Throughout all the tests the rake and clearance angles were kept constant at 65° and 5° respectively. The bevel angle was kept constant by maintaining the same blade-type throughout all tests, in this case, blades from Cellpath Ltd. UK were used which have a cutting edge bevel angle of 20°.

The setup has been proved to generate continuous paraffin wax sections in ultrasonically assisted cutting (UAC), which is demonstrated in Figure 3.

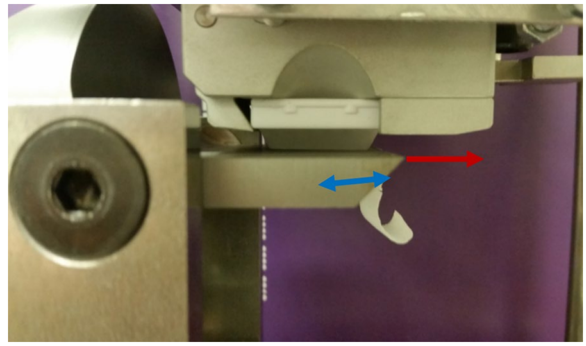

Figure 3 A continuous paraffin wax section was obtained using the designed rig with an inclined ultrasonically assisted cutting (the blue arrow represents vibration directions and the red arrow represents the sectioning direction)

5 Experimental Methodology

Testing was completed at 15 mm/s and cut at a constant sample thickness of 5 μm. The overall dimension of the paraffin blocks used was 28 mm wide by 40 mm long and 5 mm depth however the depth varied considerably between the sample blocks. Once the sample block was inserted into its relevant holder on the test rig the blade holder was advanced and the block was raised until the block and blade made contact. The point of contact was depicted by the point at which the dynamometer registered a radial force. Once this zero position was known the blade was retracted and the sample holder could be raised 5 μm thus allowing the first cut to be completed. Although this will not lead to an initial cut of 5 μm due to the thickness of the blade, the first cut thickness will be consistent across all tests and thus eliminating this variation of blade wear.

Each block was cooled before the cutting process was completed as per the standard microtomy practice. To reduce the block from increasing in temperature during the tests the block was cooled for five minutes after every five consecutive cuts. During ultrasonically assisted cutting the rig was vibrated at 23.5 kHz, as this was found to be the natural frequency of the test rig. The piezoelectric elements were vibrated with a voltage amplitude of 1 V peak-to-peak. Using a laser vibrometer (Polytec OFV-3001), it was found that these two parameters generated a vibrational amplitude of 3.7 V on the oscilloscope. The velocity decoding of the vibrometer is set at 1000 mm/s/V. The obtained velocity V_{pp} is 3.7 m/s. Using the equation below [14], this was converted to a physical vibrational amplitude of 12.5 μm with peak-to-peak amplitude A_{pp} at 25.0 μm. Both the vibrational frequency (f) and amplitude (A) are well within the limits described by Madou [19], as well as this initial testing showed that these specific parameters gave good quality cutting samples as well as consistent sections.

$$A_{pp} = \frac{V_{pp}}{2\pi f}. \tag{2}$$

After 38 cuts were completed the surface roughness of the blade was measured on an Infinite Focus Alicona which has a minimum measurable roughness of 0.03 µm. The surface roughness parameters S_a was measured in three locations, one in the centre of the blade and then one measurement 5 mm on either side of the centre focussing on the blade tip.

Tissue has varying toughness and therefore causes increase wear of the blade, the amount of wear can also change depending on the section level and type of tissue and therefore the blocks were left blank with no tissue embedded into it. Keeping the paraffin blocks blank with no tissue allowed for consistent and constant cutting material and thus allowed for the results to be comparable.

Testing was also completed with a more conventional microtome, this microtome system was adapted to allow the blade holder to be vibrated ultrasonically however due to the packaging constraints this did not allow for the cutting forces to be measured but instead, this system was used to inspect the quality of the sample slides after both ultrasonically assisted and conventional cutting [18].

6 Results and Discussion

Two significant results were measured after 38 cuts these included the roughness values of the blade as well as the average cutting force during each consecutive cut. Due to design constraints of the test rig it was only possible to complete 38 cuts for a single paraffin block, however, this is greater than the typical use of disposable blade and therefore it was deemmeed to be acceptable as an upper limit.

Furtemore, the roughness of the blades were measured after 38 cuts in order to determine the surface roughness. The roughness values were characterised using the S_a values as seen in Table 1. Where S_a is the average height difference to the surface. These values were taken at three distinct points along the blade, one in the centre of the blade and then two 5 mm on either side.

The results showed that after 38 cuts there is a significant reduction of surface roughness with the application of ultrasonically assisted cutting. This is particularly shown in the average blade roughness value (S_a) which decreases by an average of 25.2% over the span of the cutting area. With this decrease in roughness between UAC and CC and the findings of Wang et al. [[14]], it is possible to state that this decrease in surface roughness is due to the decrease in blade wear. This reduction in blade wear can be seen visually in Figure 4 which shows a contour plot of the two blades after 38 cuts.

Table 1 Blade roughness values of S_a after 38 cuts for both ultrasonically assisted and conventional cutting styles

	S_a(nm)		
	Left	Centre	Right
UAC	157.57	186.55	176.79
CC	224.05	213.04	210.01

This reduction in blade wear is further reiterated in the comparison of cutting force between ultrasonically assisted and conventional cutting as shown in Figure 5. Although it shows a reasonably large variation of cutting forces, the general trend of the cutting forces can be seen. It also shows that as the number of cuts increases the cutting force for the conventional regime also increases, while during the UAC cutting style the horizontal or tangential cutting force stays reasonably consistent with no clear signs of increasing. This increase in cutting force in the conventional cutting style can be attributed to the increase in wear as observed by Reilly et al. [8] who stated that as the wear of the tool increases so does the required cutting force.

To identify the effect on section quality with the addition of ultrasonically assisted cutting, slides were created at various cut numbers. These testing results were reported in Ref. [18] showing that as the number of cuts increase, the tears or damages within the conventional cutting samples become greater and more frequent than those in the ultrasonically assisted cutting samples. The increase can be attributed to the increased wear as previously proven and therefore it can be stated that a greater amount of wear occurs for the conventional cut sections compared to the ultrasonically assisted cutting samples. By reducing the wear, UAC improves the disposable blade life thus allowing for lower costs as well as reducing the environmental impact of the microtome industry.

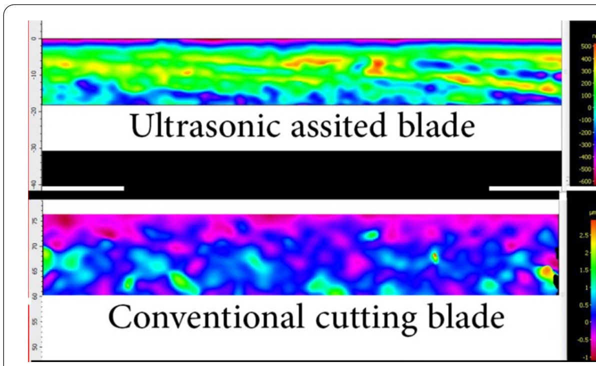

Figure 4 Images showing contour plots for blades after completing 38 cuts ultrasonically and conventionally

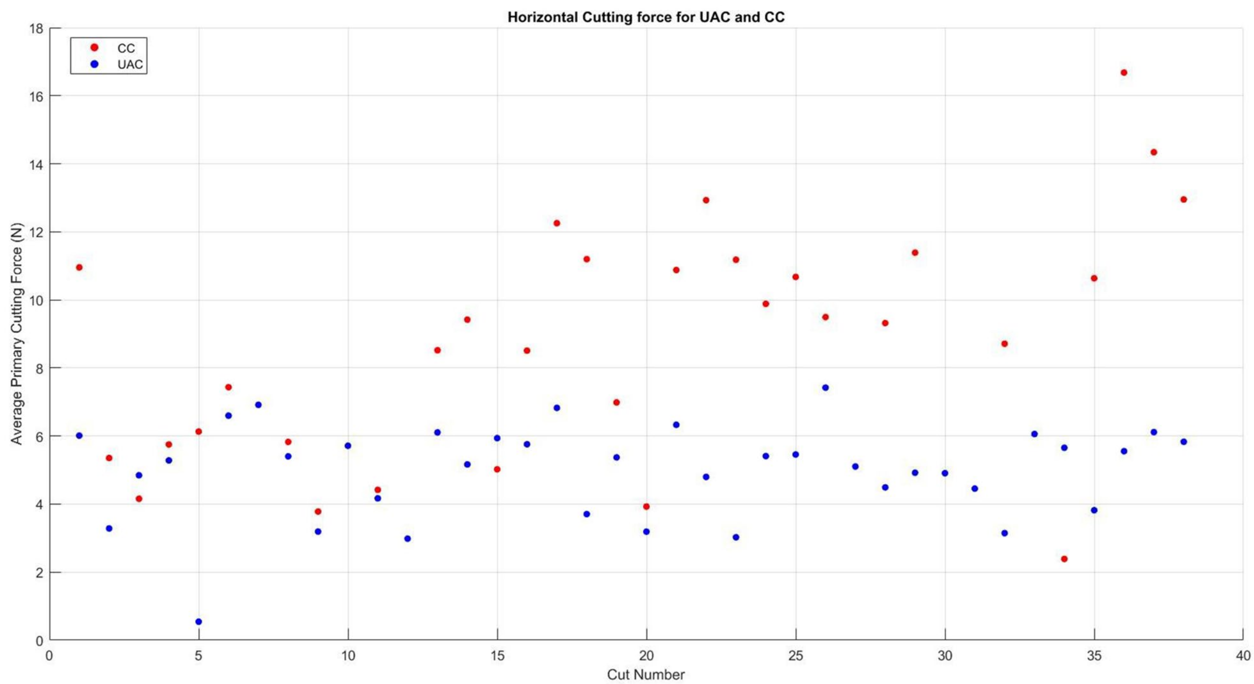

Figure 5 Plot showing magnitude of cutting force against the cut number for ultrasonically assisted (UAC) and conventional cutting (CC)

7 Conclusions

Based on the previous testing, a test rig was designed which could consistently cut sections at 5 μm thick. The results for the surface roughness of the blades after 38 cuts and the cutting force showed that the introduction of ultrasonically assisted cutting tool lead to a 25.2% reduction in surface roughness and a reduction in the required cutting force by 53.8% after 38 cuts. Both of these parameters are evidence of blade wear reduction and blade life increase.

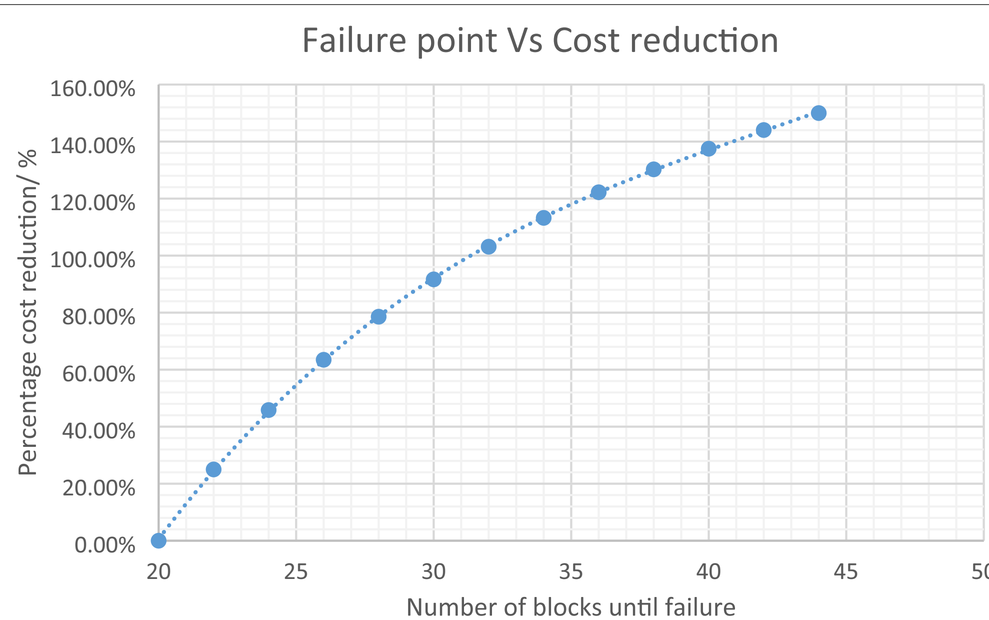

Figure 6 Number of blocks until failure vs cost reduction

Although there is no conclusive evidence of the length of time a disposable blade will last, one study showed that an average blade can cut for around 20 blocks, however, this is highly dependent on the type of blade as well as the type of sample being cut and the skill of the histotechnologist [20]. With 20 blocks per blade being the key assumption, 2.5 blades are used per histotechnologist every working day. The average cost of a disposable blade is around £1.10 per blade [21], thus it is easy to see the significance of the saving that can be made with just small increases in blade life, as shown in Figure 6. The figure shows that an increase of just 10% in blade life (20 to 22 blocks) would yield a cost saving of approximately 25%, this is staggering and is one of the key justification of this project. As the cutting force halved in UAC compared to CC, it could be assumed that the life of the blade could be doubled in UAC compared to CC, which means a cost saving of approximately 140% could be obtained.

From the points made above, it is clear to see that this research has both an economic and environmental justification. The reduction in cutting force and wear would consequently lead to an increase in blade life which would, in turn, lead to both a reduction in cost associated with microtomy as well as a reduction in the environmental impact of the disposable blades.

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Author contributions
DW made the research plan, conducted experiements, analysed results and finalised the manuscript. DDB conducted experiements and wrote experiemtnal report. AR supervised the project, provided research guidance and revised the manuscript. All authors have read and approved the final manuscript.

Authors' information
Dong Wang is currently a lecturer in engineering and entrepreneurship at *University of Exeter, UK*. He obtained his PhD degree from *Univeristy of Glasgow, UK* and worked as research associates at *Imperial College London and Loughborough University, UK*. His research focuses on three core themes crossing over medical instrumentation, biomechanics and advanced manufacturing. His multi-disciplinary research was developed through collaborations with consultants/surgeons, academics and industrial partners, involving developing laboratory prototypes and taking them through commercialisation, clinical validation and human testing to make a real-world impact.
Daniel De Becker is currently a PhD student at *Loughborough University, UK*.
Anish Roy is currently a professor of mechanics of materials and processes at *Loughborough University, UK*. His research interests and activities include dislocation mechanics, meso-scale plasticity in crystals, ultrasonically assisted machining and modelling, mechanics of polymers, composites and finite element modelling and analysis.

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Competing Interests
The authors declare no competing financial interests

Author Details
[1]Wolfson School of Mechanical and Manufacturing Engineering, Loughborough University, Loughborough LE11 3TU, UK. [2]Department of Engineering, College of Engineering, Mathematics and Physical Sciences, University of Exeter, Exeter EX4 4QF, UK.

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