Development of new and accurate measurement devices (TruSlice and TruSlice Digital) for use in histological dissection: an attempt to improve specimen dissection precision

G. E.ORCHARD*, M. SHAMS*, T. NWOKIE*, C. BULUT*, C. D'AMICO*, J. GABRIEL*, Z. RAMJI*, A. GEORGAKI*, A. NEICHCIAL*, F. SHAMS†, H. NEESAM‡, N. HAINE‡ and C. BREWER‡.

*St. John's Histopathology Department, Viapath, St. Thomas' Hospital, Block 7, South Wing, Westminster Bridge Road, London; †Barts and the London Medical School, University of London, and ‡CellPath, 80 Mochdre Enterprise Park, Newtown, Powys, SY16 4LE, UK.

Accepted: 16 July 2015

ABSTRACT

Histological dissection of human tissue has relied on conventional procedures, which have largely remained unchanged for decades. Practices to determine measurement parameters employed in these procedures have largely relied on the use of rulers and weighing scales. It is well documented in the scientific literature that both fixation and processing of tissue can significantly affect the viability of the of tissue sections both for tinctorial and immunocytochemical investigations. Both of these factors can be compounded in their negative effects by inappropriate sampling of tissue at histological cut up. There are five key factors to ensure good surgical grossing technique, flat uniformly perpendicular specimen cutting face, appropriate immobilisation of the tissue specimen during grossing, good visualisation of the cutting tissue face, sharp cutting knives and the grossing knife action. Meeting these factors implies the devices are fit for purpose. Here we describe an innovative approach to designing cut up devices to improve accuracy and precision, which take these five key requirements into consideration. The devices showed accuracy and precision, enabling tissue slices to be produced in a uniformly perpendicular fashion to within 2 mm in thickness and to enable consistency and reproducibility of performance across a series of tissue types. The application of a digital rule on one of these devices ensures accuracy and also enables quality control issues to be clearly assessed. As cellular pathology laboratories conform to ever increasing standards of compliance and performance in practice, the advent of assured precision and accuracy at cut up is awaited. Recommendations from accreditation bodies such as the United Kingdom Accreditation Service (UKAS) continue to push for improvements in this area of histological investigation. These newly designed devices may give the answers to these requirements and provide the impetus for a new generation of innovative equipment for histological dissection.

KEY WORDS: Design.
Histological dissection.
Microtomy.
Precision.
Reproducibility

Correspondence to: Dr Guy Orchard
Email: guy.orchard@viapath.co.uk

Introduction

Surgical pathology has always relied on the need for uniformity of process and procedures at the surgical grossing bench.[1] Errors incurred at this point in the process often result in more complex and problematic issues with subsequent analytical steps, such as processing, microtomy and section staining. Similarly the advent of microwave tissue fixation and processing has increased the need to ensure consistency of tissue size selection for these procedures. Inaccuracies in this area can have a significant impact on the final tissue quality and the subsequent tinctorial and immunocytochemical staining achieved. Traditionally reliance on ensuring grossing procedures are adequate and fit for purpose has relied on the skill and knowledge of the practitioner and the use of simple cut up equipment to aid accuracy and precision. There are five key factors to ensure good surgical grossing technique:

- Flat, uniformly perpendicular specimen cutting face
- Appropriate immobilisation of the tissue specimen during grossing
- Good visualisation of the cutting tissue face
- Sharp cutting knives
- The grossing knife action

Maintaining consistency across all these variables is complex and can be subjective.

There have been several attempts at developing cut-up equipment that address some or all of the above criteria. Thus we have seen the introduction in conjunction with Xpress automated processor (Sakura Finetek, USA), attempts to utilise a grossing board with an indented well/slotted metal plate and a track to follow for knife blade slicing. A further development from this has been the introduction of

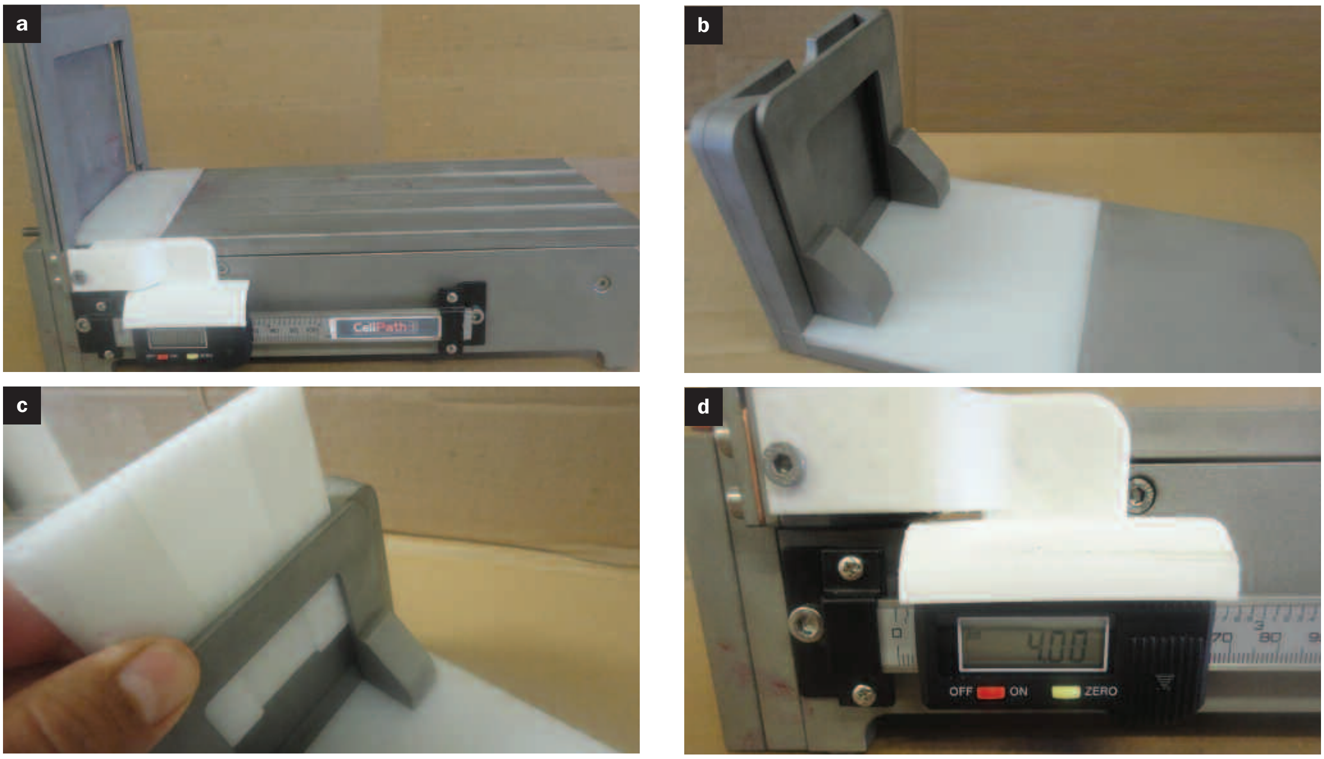

Fig. 1. a) TruSlice Digital; **b)** TruSlice; **c)** TruSlice robust plastic inserts; and **d)** TruSlice Digital electronic micrometre.

a grossing board with numerous adjustable wells (Accu-Edge) of differing depths in an attempt to improve on consistency of tissue slicing thickness again by (Sakura Finetek, USA). More advanced equipment includes the ProCut device (Milestone Medical SRL). This device was designed for dissecting whole tissue organs such as breast and prostate, with accuracy and precision achieved by employing a steel hand-held device, resembling a giant comb with regularly spaced steel teeth at defined 5 mm intervals for accuracy. A scaled down version of this is the CutMATE (Milestone) which relies on the use of innovative forceps with fork like configurations, with defined distances between the prongs of the forks (2, 3 and 4mm). Inserting a scalpel blade in between the prongs of a clamped piece of tissue, would therefore assure the tissue thickness. Following on from this (I.B DIMENSTEIN) developed a device called Biopsy Uniform Section (BUS).[2] This is a simpler device that focuses on factors such as tissue immobilisation, adequate tissue holding options and general visibility of tissue at the cutting edge.[3]

Recent changes to laboratory accreditation across the UK has seen the introduction of UKAS ISO 15189 standards.

Table 1. Specification features for the TruSlice and TruSlice Digital devices.

	Specifications				
Device type	**Materials**	**Overall dimensions**	**Cutting face dimensions**	**Weight**	**Features**
TruSlice	Stainless steel	120 mm (W) 260 mm (D) 110 mm (H)	62 mm (W) 71.75 mm (H)	Approx. 5 kg	Colour-coded predefined inserts UKAS calibration set Small footprint Non-slip feet Storage compartment at rear for inserts Immobilisation plates
TruSlice digital	Stainless steel	120 mm (W) 310 mm (D) 195 mm (H)	101 mm (W) 87.5 mm (H)	Approx. 10 kg	Adjustable moving guide Digital rule Formalin umbrella UKAS calibration set Small footprint Non-slip feet Plastic cutting surface Immobilisation plates

W: Width, D: Depth, H: Height

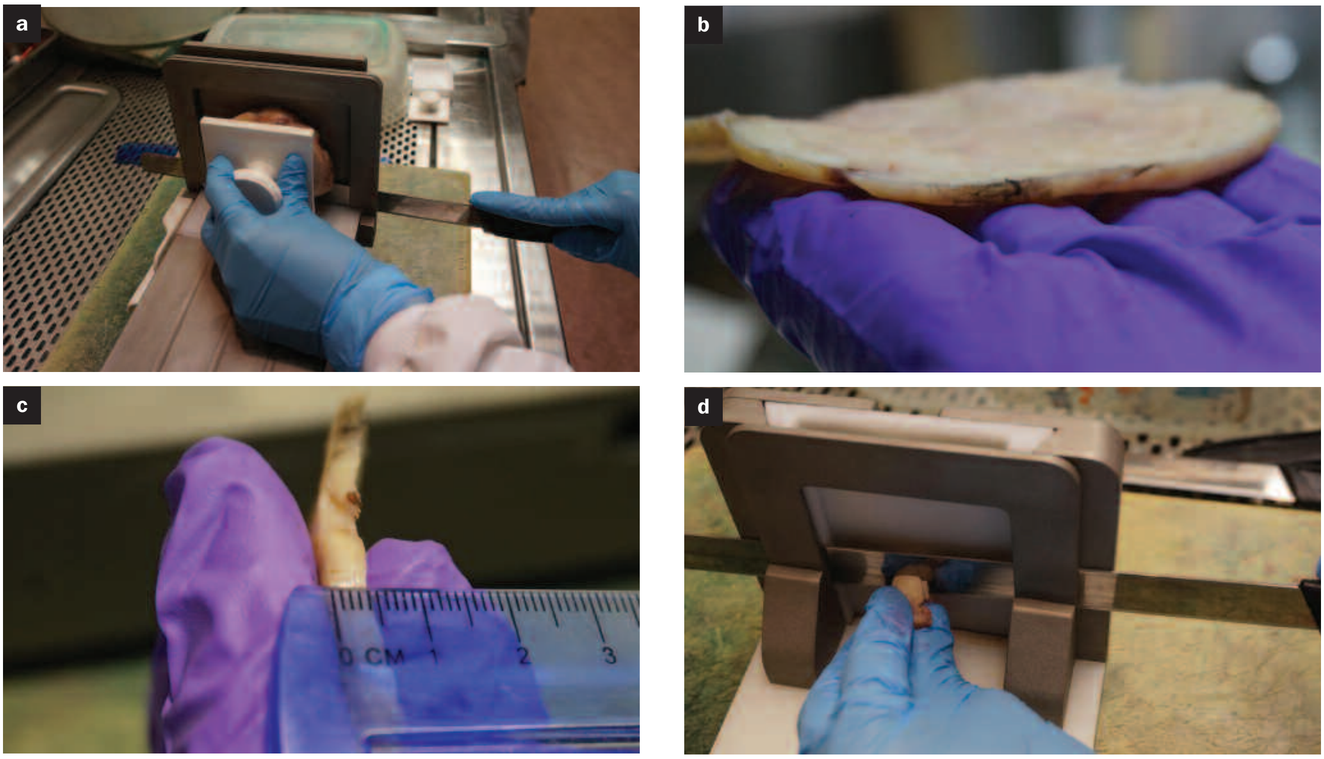

Fig. 2. a) TruSlice Digital grossing of a large lipoma, using an immobilising plate; **b)** Transverse slice of lipoma grossed (as in Fig. 2a), demonstrating the uniformed perpendicular result achieved; **c)** Lipoma slice (as in Fig. 2b), demonstrating the uniform thickness of 4 mm, across the entire length; and **d)** A sebaceous cyst grossed using the TruSlice device

Within these new laboratory accreditation standards the introduction of the requirements to meet: 'The measurement of uncertainty' has been stated. In brief, these new standards require evidence of measurement and consistency of accuracy of performance that is assured across the range of tests offered by any given laboratory.

With regard to equipment used for measurement purposes there are recommendations that state:

- Equipment used is calibrated regularly to a traceable standard
- Traceable calibrations can be sourced in the following ways:
  - Calibration laboratories that have been assessed and accredited by UKAS (or another signatory to the multilateral agreement). In these cases the calibration certificate will carry the UKAS (or international equivalent) symbol.
  - By the medical laboratory performing in house calibrations, which are then assessed by UKAS (to ISO/IEC 17025) as part of their assessment to ISO15189 standards.

Many of the current devices lack a means of electronic adjustment and calibration. Here we report an attempt to improve on precision, accuracy and consistency at the grossing bench by employing a fixed digital rule to a new grossing device, with some additional innovative design features that will enable consistency of tissue processing, microtomy and tissue section staining.

Assessments were conducted on two newly designed devices produced in conjunction with CellPath constructed in a collaborative project with Viapath and Guy's and St. Thomas' NHS Trust. The objective was to determine if consistency of performance across all five grossing variables could be achieved on both animal and human tissue specimens employing the new devices. The devices were designed to ensure accuracy of measurement of tissue grossing thickness and reproducibility and consistency across the entire cut surface. The TruSlice and TruSlice Digital were designed to be accurate to a thickness range of 2--5 mm and 2--10 mm (and beyond), respectively.

Materials and methods

A brief word on design should include a mention of how the concept arose. There were two principle aspects. The first was based on the primary author's observations whilst standing at a delicatessen counter in the local supermarket and observing the action of a bacon/meat slicer being used to cut slices of ham from a large joint of meat. The second aspect came from consideration of historical facts surrounding the accuracy and precision of the guillotine, a device used to behead poor unfortunate souls during the French revolution and introduced as a means to carry out the death penalty in France in 1789 onwards. These two concepts provided the initial impetus to try and make something that would meet the requirements of a modern grossing device.

In collaboration with CellPath a concept design was created fundamentally linked to a guillotine-like structure which we have provisionally called 'TruSlice' and 'TruSlice Digital' (Figs. 1a and 1b). TruSlice is a smaller less complex device without an electronic measurement, but with robust plastic inserts with defined specific dimension to allow sectioning of 2–10 mm (Fig. 1c). 'TruSlice Digital' was designed for more precision-based work and uses a digital rule (Fig. 1d).

Both devices are made of steel predominantly, with the main difference between the two devices being that the 'TruSlice' would have the calibrations set by defined inserts. In contrast the 'TruSlice Digital' device would have an adjustable moving guide, digital rule and be of a standard of accuracy that would meet UKAS calibration requirements (Table 1).

The aim of the design project was to create an accurate and precision dissection device that would be robust, consistent in performance, easy to use, clean and maintain, and would be easy to calibrated to meet ISO 15189 standards for certification.

The five key requirements highlighted above for good grossing technique would also be required to be met by the device's performance in dissecting a cross section both of human and animal tissue types.

The samples included human uterine fibroids, keloids, prostate, lipomas, liver, spleen, cartilaginous tissue and bone decalcified tissue. In addition and for comparison, assessments on animal lamb and pig liver as well as lamb kidney tissue were assessed. For each sample the above the five listed criteria were assessed. The thickness of each slice produced was assessed using a conventional ruler in all cases and checked across the entire length and breadth of each tissue slice. Sample slices were also assessed not only for suitability for conventional System II cassettes but also for SupaMega cassettes.

Results

The results indicated that tissue slices could be produce consistently defined thicknesses of 2–10 mm, with good uniformity of thickness across the cut surface and across the entire range of tissue samples assessed in the study, including both animal and human tissue types. In both cases, appropriate immobilisation of the tissue was achieved during the grossing procedure with good visualisation of the cutting face for the tissues examined. The knives used for both devices were the CellPath disposable macro knives (10″ and 6″ blades). The cutting action employed with both devices was one of drawing the blade through the tissue at the cutting surface in one direction. The tissues were secured at the cutting face by the use of hand-held retaining plates. Both devices could accommodate SupaMega cassette sampling requirements. Both devices were also capable of producing consistency of thickness of slices throughout grossing of entire tissue samples (Figs. 3 and 4a–f).

Fig. 3. Uniformed tissue slices grossed through the same sample at **a)** 2 mm, **b)** 4 mm, and **c)** 6 mm.

Discussion

Human and animal tissue has great variety in terms of tissue composition and this is reflected in the complexity of tissue types present within the organ systems of the body. The first issue to consider in any grossing assessments is tissue composition and complexity, which reflects differences in tissue density, resulting in some tissue types being hard and dense (i.e., cartilaginous/muscle tissue). Other tissue types such as subcutaneous tissue often have high mucinous or water content and are in general softer and more delicate in composition. It is these variations in tissue composition which pose the greatest challenge to constructing a grossing device that can be consistent across all the tissue types and which can produce precision and accuracy in performance in a highly reproducible fashion. The second issue that can affect quality of grossing is the size of the tissue sampled, too thick or an uneven tissue slice will compromise tissue processing and can lead to poor microtomy. Finally the third key issue is the orientation at the cutting face, which is critical to ensure the tissue is not grossed at an oblique angle producing cross cutting effects.

The devices employed in this study were able to meet the key criteria for optimal grossing because the knives used were restricted from moving from an absolute perpendicular cutting face as the metal frame secured the blade in a set plane. In the case of the smaller TruSlice device the plastic inserts proved accurate but offered less versatility of cutting thickness options.

The issue of immobilisation of the tissue to prevent movement when the knife makes contact with the tissue was achieved by the use of immobilisation plates, which would be placed on the tissue to secure its location against the metal/plastic end plate, depending on which device you are using. The key here is to ensure that enough pressure is applied to ensure the tissue is indeed immobilised, but yet not to apply too much pressure which may compromise the composition of the tissue. Of interest was the fact that even relatively unfixed tissue could be sliced effectively and accurately with both devices. However the range of tissue sizes that could be grossed on the TruSlice device were considerably smaller than on TruSlice Digital. One option here is that slicing tissue to sizes that would fit the smaller device could be employed before grossing on the TruSlice device. This would enable more manageable pieces of tissue to be grossed.

Both devices also offered good visualisation of the cutting face when grossing the tissue. This enabled better tissue manipulation and improved the issue of accuracy and precision, as tissue could be clearly seen to be aligned in a perpendicular fashion to the metal/ plastic cutting face plate before grossing.

Having tried both single and double honed blades we found that the use of a double honed blade with these devices proved more successful and accurate than a single honed blade and allowed accuracy and precision to within

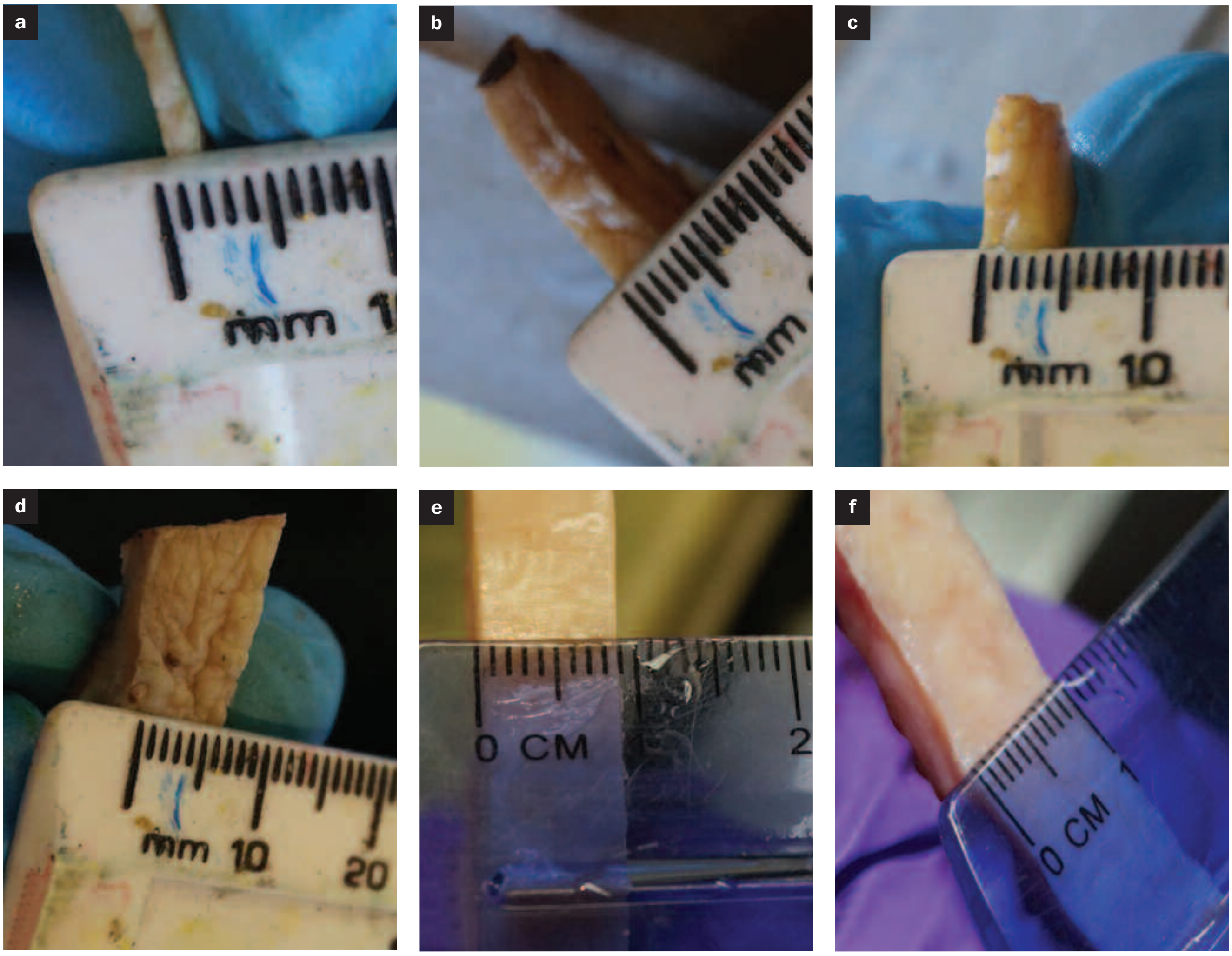

Fig. 4. TruSlice Digital demonstrating the ability to gross consistently through tissue at thicknesses of **a)** 2 mm, **b)** 3 mm, **c)** 4 mm, **d)** 6 mm, **e)** 9 mm, and **f)** 10 mm.

2mm consistently. In the case of skin tumour grossing, it is general essential to ensure absolute perpendicular slices of tissue are taken, so that the full cut face is assessed and embedded properly. This is particularly important in the assessment of margin clearance.[4,5] In the assessment of sentinel lymph node, ensuring consistency of perpendicular slicing at the cutting face results in improved embedding and a reduction of the amount of material that needs to be trimmed in the subsequent paraffin block before a full face can be exposed. This can be an important consideration as detecting tumour burden within such tissue can be akin to trying to find a needle in a haystack.[6]

Similarly in cases of assessing Breslow depth of tumour invasion in malignant melanoma cases, if the grossed slices are not perpendicular to the cutting face, then tangential slices may be produced. This would make the accurate measurement of the deepest invasive cells from the granular layer within the epidermis to the deepest deposit within the dermis, difficult to determine.[7]

When slicing small skin biopsies the necessity to ensure perpendicular slicing can be an essential requirement particularly in relation to the assessment of alopecia biopsies. In such cases horizontal sectioning is required of a small punch biopsy at the point just below the dermal/epidermal junction.[8,9] There is very little margin for error here and if the grossing is not perpendicular to the epidermis then embedding and microtomy will be subsequently compromised.

Of merit with both devices is the ease of which they can be dismantled and then reconstructed for cleaning purposes, allowing for access with disinfectants and general cleaning agents. This improves the health and safety benefits with the use of these devices in general.

The TruSlice Digital device offers an assured accuracy and precision performance that can be reproduced effectively across an assortment of tissue types. The use of a digital rule to set tissue slice thickness is an innovative development that should also comply with the new ISO 15189 requirements and alleviate concerns of how to tackle issues such as the 'measurement of uncertainty' at the grossing bench. Both devices, TruSlice and TruSlice Digital, are able to be UKAS calibrated by gauge blocks. The conventional use of the human eye assessments of tissue slice thickness is not an accurate measure and one which will also be prone to variation due to issues surrounding perpendicular slicing at the cut surface. As standards of laboratory proficiency and accreditation are growing in complexity, the requirements for evidence of measurement of uncertainty remain a pressing issue. Devices that enable this process to be proven and assured will have an increasing role to play in the development of anatomical grossing procedures.

This study represents the first assessment of the use of these new devices. Multicentre trails across the country are now underway to determine the effectiveness of these devices and to determine if further design alterations are required. This preliminary study satisfactorily demonstrated the accuracy and precision of the devices. Consideration of feedback from these trials will determine more precisely how effective these devices are across a wide range of tissue types.

"Measurement is the first step that leads to control and eventually to improvement. If you can't measure something, you can't understand it. If you can't understand it, you can't control it. If you can't control it, you can't improve it." – *H. James Harrington.* □

References

1 Romaguera R, Nassiri M, Morales AR. Tools to facilitate and standardize grossing. *Histologic* 2003; **36**: 17–21.

2 Dimenstein IB. The concept of adjustable immobilization to aid grossing and facilitate uniform biopsy slices. *J Histotechnol* 2013; **36**: 106–9.

3 Dimenstein IB. Grossing biopsies: an introduction to general principles and techniques. *Ann Diagn Athol* 2009; **13**; 106–13.

4 Orchard GE, Shams M. Dermatofibrosarcoma protuberans: dealing with slow Mohs procedures employing formalin-fixed, paraffin wax-embedded tissue in a busy diagnostic laboratory. *Br J Biomed Sci* 2012; **69** (2): 56–61.

5 Gross K, Howard K. Steinman eds. *Mohs Surgery and Histopathology: Beyond the Fundamentals*. Cambridge: Cambridge University Press, 2009: 86 (ISBN-10: 0521888042).

6 Orchard G. Evaluation of melanocytic neoplasms: application of a pan-melanoma antibody cocktail. *Br J Biomed Sci* 2002; **59** (4): 196–202.

7 Breslow A. Thickness cross-sectional areas and depth of invasion in the prognosis of cutaneous melanoma. *Ann Surg* 1970; **172** (5): 902–8.

8 Elston DM, Ferringer T, Dalton S, Fillman E, Tyler W. A comparison of vertical versus transverse sections in the evaluation of alopecia biopsy specimens. *J Am Acad Dermatol* 2005; **53** (2): 267–72.

9 Stefanato C, Asher R, Craig P, Orchard G. The multiteam approach in the newly introduced alopecia protocol: the St.John's experience. *J Cutan Pathol* 2008; **35**: 137–8.