Original paper

A new laboratory device with mathematically based positioning of a frozen tissue block facilitating precise sectioning of large specimens

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Mohs micrographic surgery (MMS) is a treatment method aiming at thorough, personalized eradication of skin cancers by mean of staged excision of tissues surrounding the tumor with complete (100%) histopathological examination of their margins. In many MMS laboratories, the excised tissue is divided, shaped, frozen in a cryostat with a heat extractor and positioned manually (with the block on the object disc) in an articulated cryostat chuck during cutting. However, these activities may be difficult, time-consuming and associated with the risk of imprecise tissue sectioning.

Development of a laboratory device allowing for processing of large tissue specimens, with the function of mechanical, mathematically steered positioning of the tissue block surface directly to the microtome knife cutting place, eliminating the need for manual adjustment.

The prototype device was designed and manufactured. Its functioning was tested on 513 histological slides produced during 212 operations of skin cancers using MMS. The depth of the first complete sections and the diameter of sections were measured.

Complete sections were obtained at an average depth of 81.60 μm (min. 20 μm, max. 180 μm, SD = 29.15), whereas the average diameter of sections was 18.11 mm (min. 4 mm, max. 42 mm, SD = 9.10).

The histological processing of large specimens with mathematically based positioning of the tissue surface in relation to the cryotome knife cutting plane is precise, fast and easy. The device can be useful in those MMS centers which continue to employ manual setting of the cryostat chuck or share the cryostat with other users, which prevents fixing the chuck position (including large hospital settings). It may also be helpful in centers using a cryostat with a fixed chuck, for the correction of minimal inaccuracies of its preset position.

Key words: Mohs micrographic surgery, skin surgery, dermatologic surgery, oncologic surgery, laboratory device, non-melanoma skin cancer, frozen tissue, fresh tissue, histological processing, whole mount histological sections.

Introduction

Mohs micrographic surgery (MMS) constitutes the method of surgical treatment of skin cancers assuring the highest cure rates with possible preservation of healthy tissue. It aims at thorough, personalized eradication of skin cancers by mean of staged excision of tissues surrounding the tumor with complete (100%) histopathological examination of their margins. Tissue flattening and mounting in a parallel alignment to the surface of the object holder and the microtome knife section plane aims at cutting the most superficial sections of a specimen block, thus enabling the complete histopathological examination of excision margins of skin cancers. Numerous methods and devices have been designed to facilitate these key steps of tissue processing in MMS. Some of these methods are: manual (direct) technique, microscope slide technique, Bard-Parker scalpel, freezing bar technique, plastic "Cryomolds", "Davidson Cryocup" metal molds, Carter's device, Koehn's modification of Carter's device, "Miami Special" clamps, Gormley's device, Motley & Holt's device, Marini's "CryoSystem", Franks' device, and "CryoHist" [1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14]. Some inaccuracies of tissue processing are unavoidable and depend on the unique features of a particular device or technique. The suboptimal specimen orientation is subsequently corrected by the manual adjustment of the object holder in a cryostat [2, 3]. However, it might not only prove difficult and time-consuming but also may be associated with errors: cutting the incomplete sections or cutting the section at considerable depth. This may result in overlooking cancer or unnecessary removal of healthy tissue. Small organs, e.g. eyelids, are particularly prone to such inaccuracies.

Objective

The aim of the study was to design a device which enables exact mechanical adjustment of a specimen block surface of diameter up to 50 mm, to the current position of a microtome knife, as well as flattening, freezing, embedding and mounting of the tissue in MMS.

Material and methods

We designed a prototype device for tissue processing in MMS. Its freezing system consists of a liquid nitrogen container and an outlet hose with a foot-operated magnetic valve. The main body of the device consists of a base (Fig. 1A), an arm supporting a stage (Fig. 1B) moved by an adjustment knob (Fig. 1C), with clamps for microscopic slides (of standard size: 25 × 70 mm or larger: 50 × 70 mm) (Fig. 1D), a mirror (Fig. 1E), a transparent shield (Fig. 1F) and a socket for the specimen holder (Fig. 1G) placed above the gas outlet (Fig. 1H). The socket is surrounded by three threaded mandrels (Fig. 2); the two frontal ones (left and right) are regulated by knurled knobs (Fig. 3). Iron-core specimen holders of 50 mm in diameter are pulled by a magnet fixed in the gas outlet, which provides the necessary stabilization. The mechanical, mathematically based adjustment of a plane of tissue block to the actual position of the knife is carried out in three steps:

Step 1: Preparation of the "sample block" from the frozen OTC medium (without tissue). A specimen holder is inserted into the device socket with the mark indicating 6 o'clock. After the freezing process is released, OTC medium is poured on its surface and pressed with a glass slide mounted on a stage. When the slide is removed, a tissueless sample block is ready.

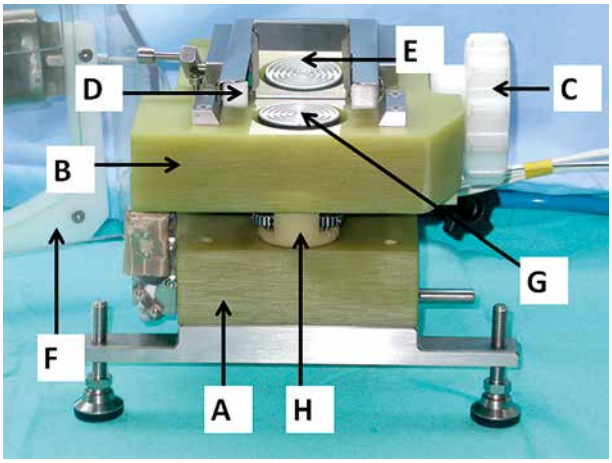

Fig. 1. The prototype device is used on a laboratory table. It consists of a solid base (A), a movable stage (B) with a height adjustment knob (C) clamps for microscopic slides (D), a mirror (E), a heated shield (F), a specimen holder socket (G) and a column with a gas outlet (H)

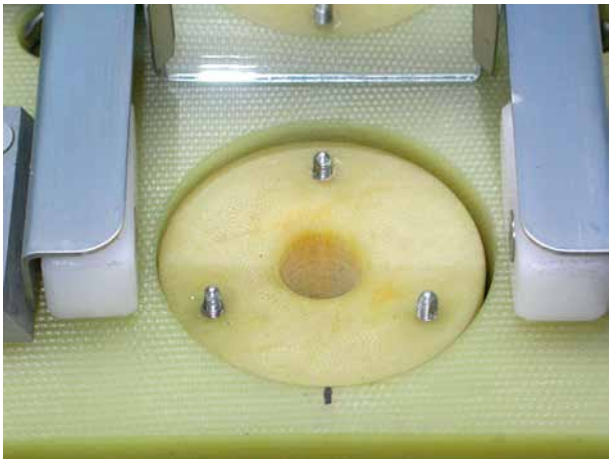

Fig. 2. The socket for a specimen holder is placed above the gas outlet. It consists of a central mounting hole and three circumferential mandrels; the height of two frontal ones is regulated

Step 2: Sectioning the sample block in a cryostat microtome. The sample block is secured in a cryostat socket, with its mark indicating 6 o'clock. Sections of 10 μm are cut. Three parameters should be taken into consideration: I) Position of point on the circumference of the block where the sectioning begins (point A). It should be indicated by the clock – in order to determine it accurately, the special ruler in the form of a dial may be applied. II) Depth of first complete section (in micrometers) – a distance from the block surface which equals the number of the cryostat crank turns multiplied by 10. III) Diameter of the block (in millimeters) between points A and B (point B is situated opposite to A, and constitutes the finally cut fragment of the full face section).

Step 3: Adjusting the position of a specimen holder by means of the rotating mandrels. The results of the described parameters are entered into a computer program (*Block Adjustment Calculator*) which in a fraction of a second provides the necessary shift of mandrels. The adjustment process should be repeated each time when the cryostat chuck position or the settings of the device were presumably altered. To be sure we repeat it every morning before work.

After completing the adjustment, the tissue processing is carried out in the following way: A glass slide is placed on the stage. Tissue (with relaxing cuts if needed) is spread on the slide, flattened, pressed and frozen under visual control (the rear part of the specimen is visible in the mirror) with individually regulated speed. It is also recommended to check if its bottom surface adheres strictly to the slide (it is visible through the glass). After complete freezing of tissue, its edges may be covered with a wreath of medium, which prevents their further detachment from the slide. Then the specimen holder is secured in the device socket with the mark at 6 o'clock and covered with the OTC medium. The glass slide is rotated tissue down and pressed to the OCT medium. When the specimen block is completely frozen, the glass is removed and the object disc is inserted into the cryostat chuck, with the mark indicating 6 o'clock. Then the tissue block is ready for precise sectioning without any additional manual adjustment. The only limitation of the size of sections is the diameter of the object holder, which is actually 50 mm.

The device was used in 212 MMS operations of skin cancer, by which 513 histological slides from tumor margins were prepared. The tissue specimens varied in dimensions and composition, containing skin, fat, muscle fascia or cartilage. The following parameters were measured: the depth of the first complete sections and the largest diameter of specimens.

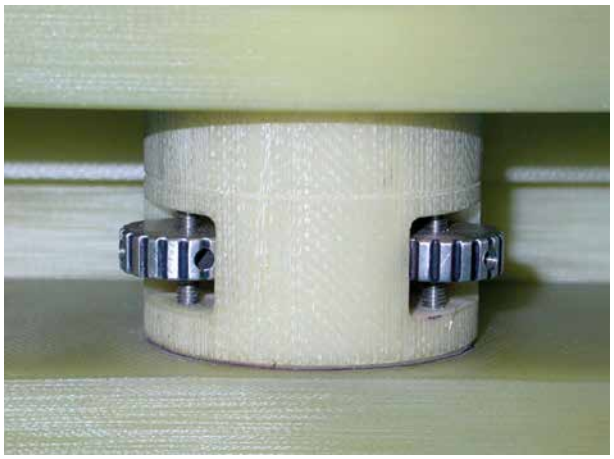

Fig. 3. Two knurled knobs [left (L) and right (R)] adjusting the position of the two frontal mandrels

Results

The full face sections were obtained at average depth of 81.60 μm (min. 20, max. 180, SD = 29.15), whereas the average diameter of sections was 18.11 mm (min. 4 mm, max. 42 mm, SD = 9.10).

Discussion

We have performed the "fresh tissue" Mohs micrographic surgery since 1999. Initially, we used the "direct method" of tissue processing using a heat extractor and cryostat equipped with an articulated chuck. However, this method in our practice proved difficult to control and was burdened with considerable imperfections. Great improvements followed the implementation of a new device – Cryosystem [12]. However, major drawbacks continued to occur with the use of the cryostat together with another laboratory, which forced us to perform manual adjustment of the block surface in relation to the plane of the blade. In this situation, we started research on the mechanical alignment of the tissue block, which after many attempts led to an efficient device. Its usage proved to be a considerable relief, helping us to markedly facilitate the procedure and increase its accuracy. Later our laboratory was equipped with a cryostat with a fixed chuck, which is intended exclusively for MMS. We noticed that such a mechanism is more stable than an articulated chuck, which positively affected the quality of sectioning. However, its factory settings were not set in an ideal position in relation to the plane of the microtome knife. Hence we continued to use the mechanical adjustment with our device.

We have used the device in 212 MMS operations, in which two kinds of cryostats – with a fixed or articulated chuck – were employed. It proved that complete sections were obtained close to the surface of the tissue block, at an average depth of 81.60 μm.

What is important, such results were obtained despite the relatively large size of specimens. (The average diameter was as high as 18.11 mm. The diameter of histological specimens was smaller than the tissue *in vivo* by 20-30%, because of their shrinkage.)

It is thought that the applied method is not as crucial in MMS processing as the experience and scrupulousness of a histotechnician [15]. However, a majority of tissue processing methods do not provide the highest expected precision, even in the most experienced hands. The best accuracy is delivered by the Franks' device [13] and the CryoHist machine [6]. Both of them facilitate obtaining full-face sections at an average depth not exceeding 100 μm, on condition only that the position of the cryostat chuck is constantly fixed. Our device assures similar precision, but is applicable also in cryostats in which the position of the articulated chuck is being changed, for instance by other users. Nevertheless, the degree of precision of most methods is far from that and equals several hundred micrometers. For example, the commonly used direct method may cause inaccuracies of even 2 mm [9]. These drawbacks are well known to specialists – inaccuracies in histological processing are not only troublesome but also may result in producing histological slides which may be ineligible for diagnostic purposes [16, 17]. Our measurements and observations proved that our own designed prototype device is comparable to the most precise methods described so far, and is far more exact than most of the commonly used procedures.

Moreover, the histological processing in MMS is in the majority of its present forms complicated. In consequence, it can be performed only by highly specialized personnel trained for a long time in departments which have a cryostat for their exclusive use. Our device allows one to obtain high quality sections by performing simple, clearly defined actions. Brief training is sufficient – it could be ascertained that after 1 hour technicians were able to operate the device effortlessly with good results. This ease causes that tissue may be processed in a short time; the adjustment usually takes less than 4 minutes, whereas mounting and sectioning takes around 2 minutes.

Especially useful in large tumors is the possibility of creating large histological specimens, with the use of oversized object discs and slides enabled by the device (Fig. 4). This allows one to avoid failures associated with tissue division, such as pushing malignant structures toward the undersurface plane (false positive specimens), as well as orientation errors [18, 19, 20, 21]. This feature may be helpful not only in the treatment of skin cancer using Mohs surgery, but also during the surgical treatment of neoplasms located within other organs, where "whole mount histological specimens" are in use, namely head and neck, breast, prostate, brain, etc.

In summary, the device may prove especially useful in Mohs centers using the direct technique or other techniques in tissue processing, requiring manual adjustment of the cryostat chuck position. It can also be useful in laboratories equipped with a cryostat with a fixed position chuck, aiming at correction of its minimal inaccuracies. The device may also enhance the performance of those Mohs surgery departments which share a cryostat with other units. Especially, it could facilitate MMS in traditional hospital histopathology laboratories without acquiring a separate cryostat.

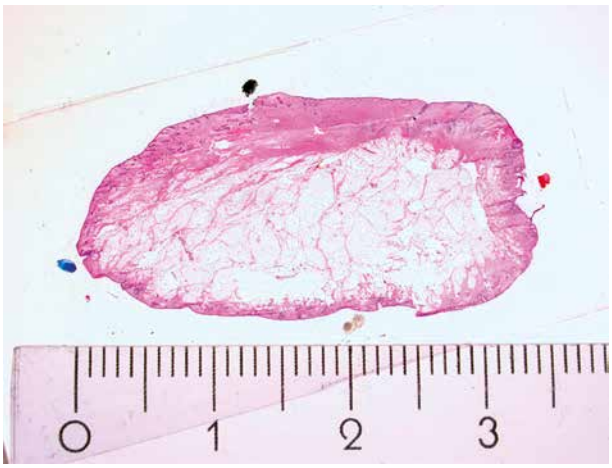

Fig. 4. The complete sections, even of the largest dimensions, are cut from the most superficial layers of the block. This tissue section of 35 mm in diameter was acquired at a depth of 80 micrometers from the block surface

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The authors declare no conflict of interest.

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References

1. Dogan MM, Snow SN, Lo J. Rapid skin edge elevation using the OCT compound droplet technique to obtain horizontal microsections in Mohs micrographic surgery. J Dermatol Surg Oncol 1991; 17: 857-860.
2. Selak LS. The Mohs Laboratory. In: Mikhail G (ed.). Mohs micrographic surgery. Saunders Phildelphia 1991; 299-308.
3. Yob EH. Cryostat and cryostat technique: Obtaining Quality Mohs Slides. In: Gross KG, Steinmann HK, Rapini RP (eds.). Mohs Surgery: Fundamentals and Techniques. Mosby, St Louis, Baltimore Boston 1998.
4. Miller LJ, Argenyi ZB, Whitaker DC. The preparation of frozen sections for Mohs micrographic surgery. A review of current metodology. J Dematol Surg Oncol 1993; 19: 1023-1029.

5. Silapunt S, Peterson R, Alcalay J, Goldberg LH. Mohs tissue mapping and processing: a survey study. Dermatol Surg 2003; 29: 1109-1112.
6. Hanke CW, Leonard AL, Reed AJ. Rapid preparation of high quality frozen sections using a membrane and vacuum system embedding machine. Dermatol Surg 2008; 34: 20-25.
7. Hanke CW, Lee MW. Cryostat use and tissue processing in Mohs micrographic surgery. J Dermatol Surg Oncol 1989; 15: 29-32.
8. Carter VH. A new method for preparing tissue blocks for cryostat sectioning. J Dermatol Surg Oncol 1985; 11: 687-689.
9. Gormley DE. Evaluation of a method for controlled tissue embedding for histologic evaluation of tumor margins. Am J Dermatopathol 1987; 9: 308-315.
10. Koehn GG. A new modification for preparing tissue blocks for cryostat sectioning. J Dermatol Surg Oncol 1992; 18: 485-486.
11. Motley RJ, Holt PJ. A simple device for optimal tissue preparation for Mohs micrographic surgery. Br J Dermatol 1992; 126: 57-59.
12. Marini L. Cryosystem: a new, complete device for tissue processing in micrographic surgery. J Eur Acad Dermat Venereol 1995; Supl. 1: 117.
13. Franks JW. A precision machine for mounting tissues for Mohs micrographic surgery. Dermatol Surg 1998; 24: 989-993.
14. Peters S. The Art of Embedding Tissue for Frozen Section, in: Stephen R. Peters (ed): A Practical Guide to Frozen Section Technique. Springer-Science+ Business Media, Pathology Innovations LLC 2010 [http://www.leicabiosystems.com/pathologyleaders/the-art-of-embedding-tissue-for-frozen-section/].
15. Tulli A. Mohs' micrographic surgery. In: Chu AC, Edelson RL (eds.). Malignant Tumors of the Skin. Oxford University Press, London, UK 1999; 381-395.
16. Rapini RP. Pitfalls of Mohs micrographic surgery. J Am Acad Dermatol 1990; 22: 681-686.
17. Soo Hoo W, Ruebner B, Vogt P, Wiese D. Orientation of small, flat, frozen-section specimens. Am J Surg Pathol 1988; 12: 573-574.
18. Randle HW, Zitelli J, Brodland DG, Roenigk RK. Histologic preparation for Mohs micrographic surgery. The single section method. J Dermatol Surg Oncol 1993; 19: 522-524.
19. Sukal SA, Tudisco M, Strippoli B, Nehal KS. Innovative laboratory techniques to facilitate processing of large Mohs cases. Dermatol Surg 2005; 31: 763-765.
20. Gloster HM. Surgical pearl: Large single sections in Mohs micrographic surgery. J Am Acad Dermatol 2003; 49: 506-508.
21. Ellis JI, Khrom T, Wong A, et al. Mohs math-where the error hides. BMC Dermatol 2006; 6: 10.

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