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Embedding automation methods: Perspective and prospects

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1. Introduction

Embedding automation is the laboratory management's lingering desire to eliminate the embedding procedure, the step between grossing and microtomy. The technical solution of the problem would be a sectionable processing cassette. The 1996–2007 Williamson's et al. patent for the Paraform insert laid ground for the practical implementation of embedding automation [1]. Almost two decades ago, Sakura Finetek USA Inc. (Torrance, CA), which owns the patent, pioneered embedding automation by offering the Tissue-Tek® AutoTec® Automated Embedding System with Tissue-Tek® Paraform® Sectionable Cassette System [2].

This author's article, published in 2010, underlined some issues with the sectionable cassette's insert design [3]. The main one was uncertainty of specimen orientation, which led to difficulties in alignment of the microtomy surface to provide a complete and representative section on the microscope slide. This would possibly be a substantial problem in biopsies.

The current article presents an overview of the embedding automation methods. Different options for the sectionable cassette design will be discussed. Examples of actual implementation are not mentioned to avoid marketing, but actually there have been only limited implementations in few laboratories in the US and Europe [4,5]. Although the issues of productivity and costs are concerns for everyone in pathology practice, they are also omitted because they require special reliable statistical study. The intention of the present article is to give readers, especially pathologists and decision-making administrators, more information about the methodological part of the embedding automation issue.

2. Methodological approaches to embedding automation

2.1. Tissue-Tek® AutoTEC® a120 Automated Embedding System (Sakura Finetek)

The core of the method is the Tissue-Tek® Paraform® Sectionable Cassette System, actually an insert, which is placed into a specially designed plastic cassette frame (Fig. 1). In recent years, the company has concentrated its efforts predominantly in designing the embedding automation tissue processor instrument (AutoTEC® a120), while making some changes in the design of the original variants of the sectionable inserts.

Six types of the inserts are used for different types of specimens. They are made from a proprietary resin material (Paraform). They can be easily bent and twisted. Different is size and perforation at the bottom, they have a lid which seals the tissue sample to prevent any movement outside the insert while in the tissue processor. The inserts were described in some detail in the previous article [3]. The current article is concentrating on their application experience and changes in design.

Large and middle size standard inserts are used or samples which do not require any orientation (Fig. 2). Gradually the laboratory becomes used to them.

A round space insert with a lid, which includes a spongy polyester foam pad, is used for very small biopsies without orientation (Fig. 3a). A skin punch in the insert (Fig. 3b) and after tissue processing (Fig. 3c).

An insert with multiple projectails, “pegs” or “posts”, as they are called in the original patent, surves the need to place a sample on the edge for a vertical immobilization. It is called Shaved Biopsy cassette Fig. 4a). They are placed indiscriminately or in some row order. The insert's lid is used for additional immobillization. A shave biopsy is placed with vertical immobilization/orientation (Fig. 4b and c).

An insert for core needle biopsies has rows of wells with conelike bristles extending from the surface down almost to the bottom of the well to immobilize of the core sample at the microtome cutting surface (Fig. 5). The insert has a lid with pins. (Some comments on this insert will be presented later.)

The company suggested a Tissue-Tek Paraform Orientation Biopsy Gel pad for immobilization of an oriented in the insert biopsy specimen (Fig. 6 a-c).

On the one hand, it makes sense to have the gel pad in the grooved core biopsy insert to prevent damage to fragile tissue from the insert lid's sharp edge, on the other hand, the pad is useless when placed above the insert's projections for immobilization of small (less 3 mm in greater dimension (core biopsies) biopsies (Fig. 7). Cores are able to move under conditions of vacoom and pressure in the tissue processor.

The gel pad has a tendency of shrinking during tissue processing if it is pressed on the projections (Fig. 8).

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Fig. 1. The Tissue-Tek® Paraform® Sectionable Cassette System insert in the plastic cassette frame.

2.2. SYNERGY™ auto-embedding (milestone medical)

Milestone/Klinipath V.P. SYNERGY (Kalamazoo, MI) presented an all-in-one system of automatic embedding (Fig. 9). The method is based on Klinipath's patent [6]. The specimen, for example prostate biopsy core is placed at the base of the plastic mold [7]. A ubiquitous polyfoam blue pad goes on top to maintain the position. The cassette is clipped with a perforated lid to seal the mold. The cassette assembly is inserted in the rack for processing in Milestone's Logos or Pathos Delta tissue processor. However, other processors can be also used.

After the rack removal from the processor by the operator, one by one the cassettes are placed on a standard cold plate. After 10 min, the cassettes are ready to be opened. The mold is broken to release the block for microtomy. With the SYNERGY method, trimming is reduced.

2.3. Other approaches to embedding automation

2.3.1. Biopsy Chip™ (BxChip®)

The Biopsy Chip™ (now BxChip® Leavitt Medical, Inc. d.b.a. LUMEA, Inc.) is an insert in the processing cassette (Fig. 10a and b). The chip is a grooved, sectionable 2 mm matrix made from a proprietary biomimetic protein polymer. It can be used for aligning the specimens during the biopsy procedure, as well as during grossing. Up to 12 core biopsies can be placed in one individual sectionable matrix for simultaneous processing and sectioning. The chip, loaded with cores, is sandwiched between two foam pads in a tissue cassette to prevent any movement during processing. The biomimetic polymer shrinks during processing to the same extent as the cores themselves that provides immobilization. Upon tissue processing, the chip is embedded and the paraffin block is sectioned. The design of the insert has been changed (Fig. 10b). BxChip® was used for the collection and analysis as a multiplex method [8,9].

2.3.2. QuickMBed™

In 2009, QuickMBed, LLC filed an international patent application “Scaffold for tissue sample orientation” for automatic embedding. QuickMBed™ first suggested a silicone pad and later a hydrogel pad that has a flexible base and rows of stems with flared ends on the top of the stems (Fig. 11a and b). The Fig. 11b shows a prostate needle biopsy core

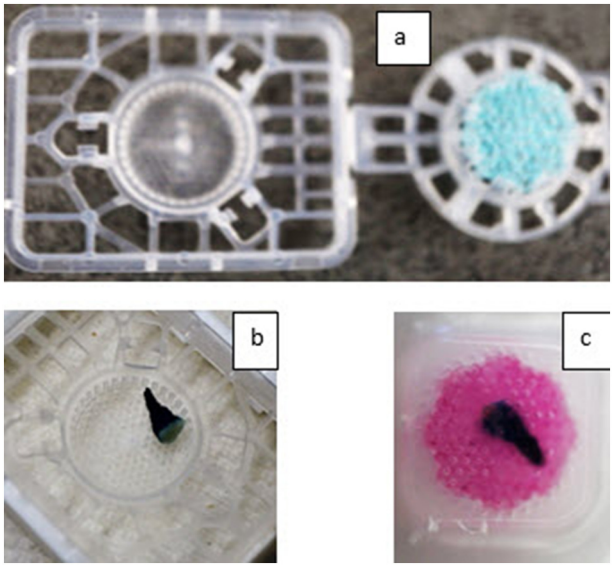

Fig. 3. (a-c). A skin punch biopsy in a small biopsy insert (a), a skin punch biopsy in the insert (b), and after tissue processing (c).

between stems of a flexible pad. The grasp of the stems maintains a tissue sample in a particular pre-determined orientation. Even if the pad falls on the floor, the specimen remains unmoved, in the same place. The pad can be placed in a standard cassette. The sectionable pad is intended for needle core biopsies and shave skin biopsies. However, this proposal was abandoned due to a threat of patent infringement litigation from a large automated instrument manufacturer [10]. Even the domain no longer exists.

2.3.3. SmartBx™

UC-CARE Medical Systems, Ltd. (Israel) proposed a different approach, which introduced the semi-automatic prostate specimens downloading system SmartBx™ technology, a device that allows clinicians to collect a prostate biopsy maintaining its in-gland orientation and direction throughout the grossing and embedding process [11]. The SmartBx™ System is a complementary tool to the Navigo™, a Fusion Navigation System for Prostate Biopsies, also manufactured by the same company. The SmartBx™ method abandoned the idea of a sectionable cassette but solved the main problem of core immobilization during processing through adhesiveness to the matrix (proprietary paper membrane), which is embedded in the paraffin block.

The specimen harvested by the clinician biopsy is placed onto the specially engineered disposable cassette that is built out of plastic, polyester, and the paper membrane during the manufacturing stage of the cassette. The polyester part is the technology for downloading the specimen from the biopsy gun's needle onto the paper membrane. The cassette with biopsy material adhered (“stuck”) to the paper membrane is transported in fixative to pathology. In the gross room, the grossing

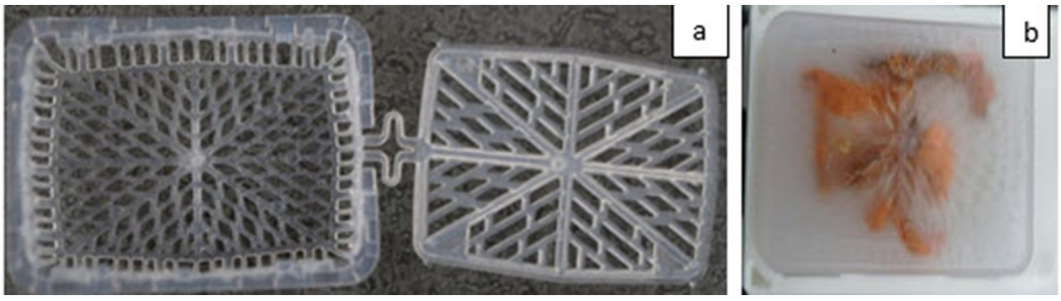

Fig. 2. Large insert (a) with endometrial curettage specimen after tissue processing (b).

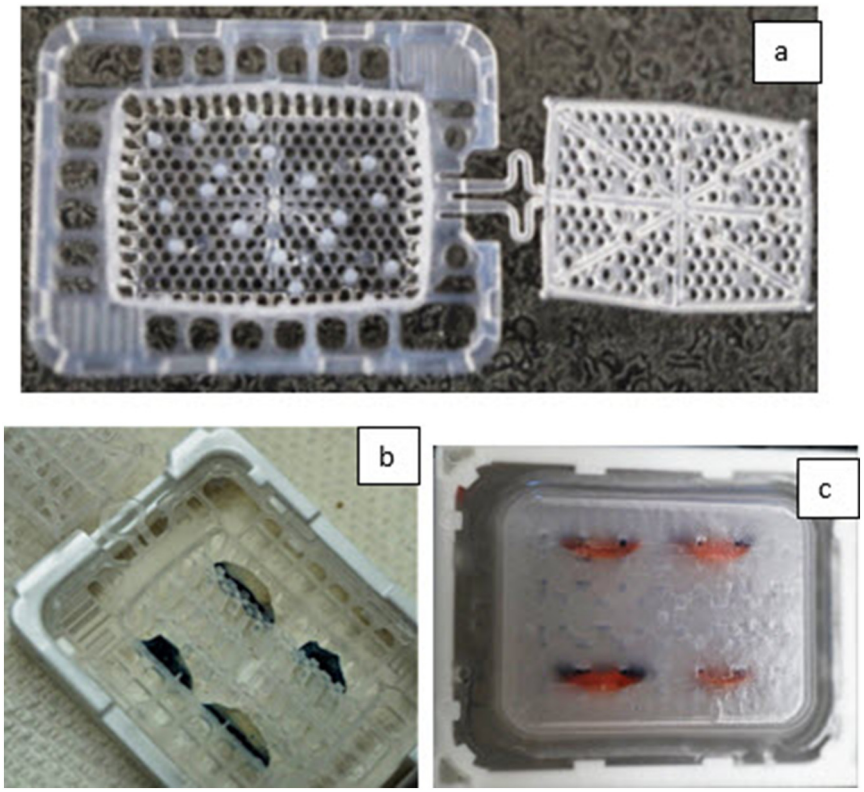

Fig. 4. (a-c). An insert (a) with skin biopsy on the edge orientation (b) and after tissue processing (c).

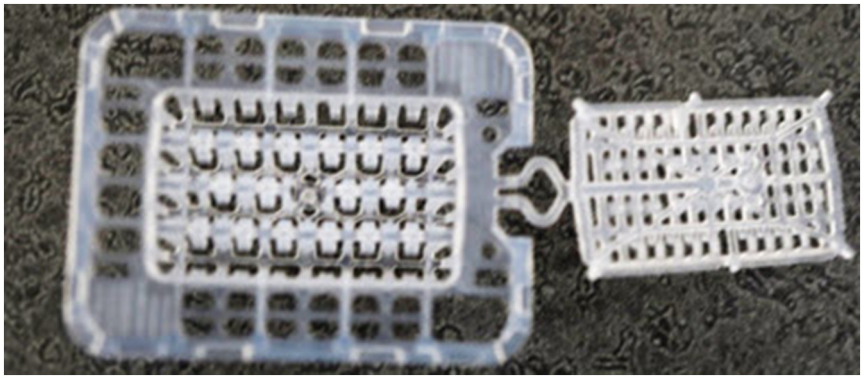

Fig. 5. An insert for cone needle biopsies.

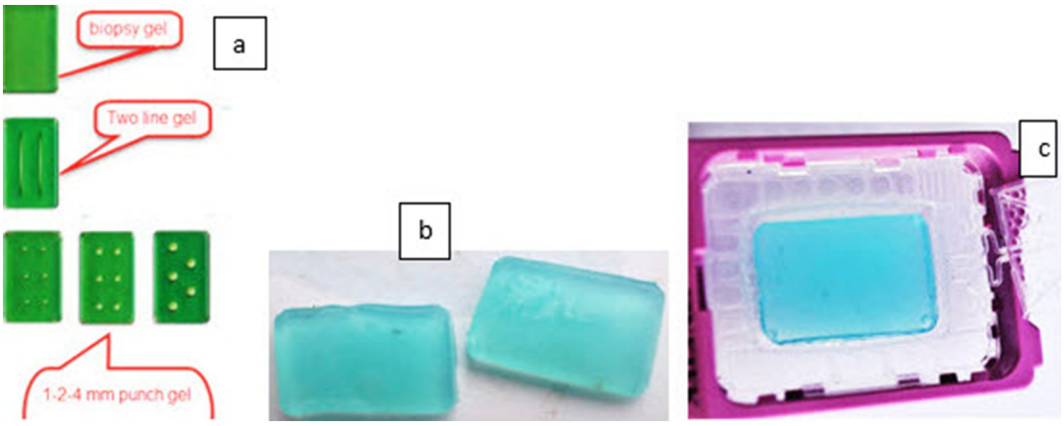

Fig. 6. Tissue-Tek® Paraform® Orientation Biopsy Gel pad (a) variants of the gel pad, b) unperforated gel pad, c) a gel pad in the insert.

Fig. 7. The Tissue-Tek® Paraform® Orientation Biopsy Gel pad (a) above the inserts projections (b).

Fig. 8. The Tissue-Tek® Paraform® Orientation Biopsy Gel pad pressed on the insert's projections.

person opens the cassette, makes the necessary measurements and descriptions, and disposes the propriety cassette. The paper with adhered biopsy material is placed into a regular tissue processing cassette onto a regular "blue" polyester pad. Then, the paper with the biopsy material is removed from the cassette and placed biopsy-side down onto a special SmartBx™ embedding mold that is paired with a special tamper (Figs. 12 and 13).

The SmartBx™ method has a predecessor in James B. McCormick's

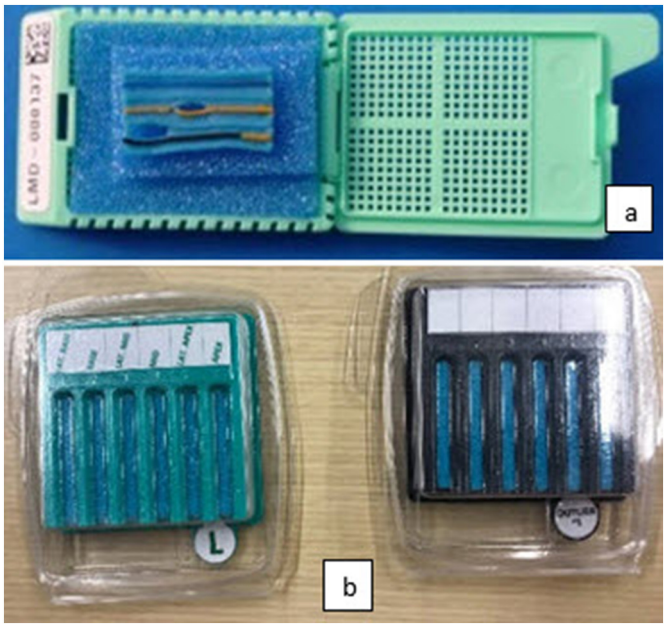

Fig. 10. Biopsy Chip™ insert: a) initial variant; b) current variant.

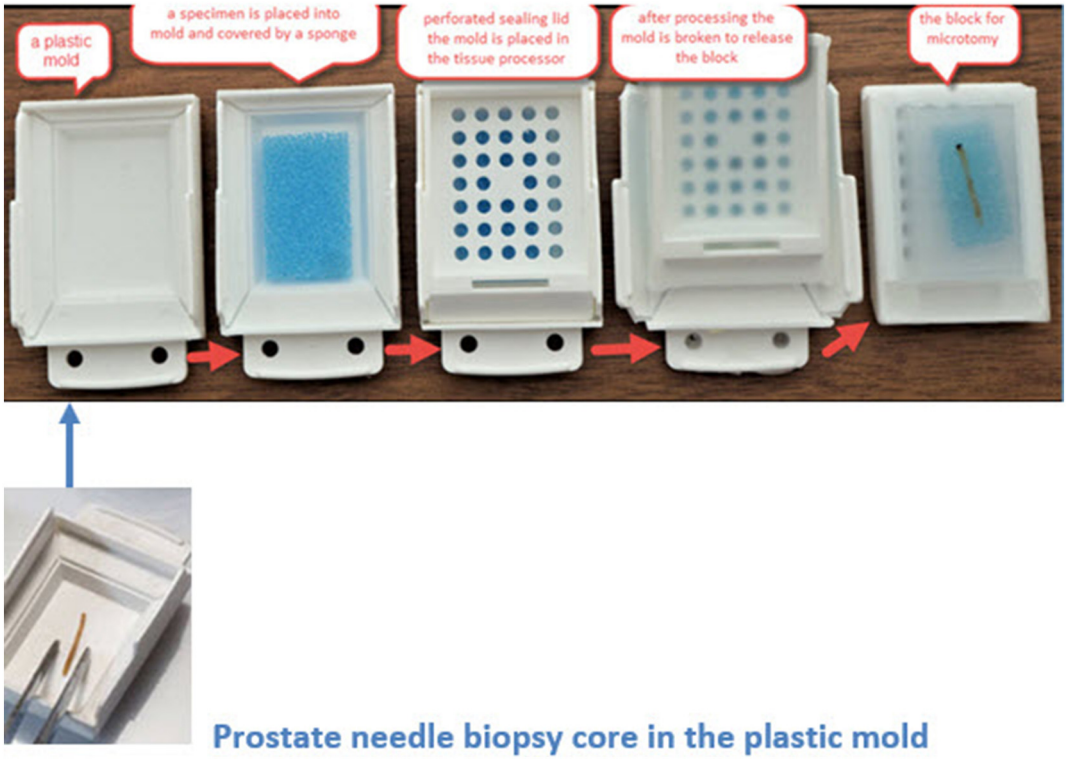

Fig. 9. The sequence of Milestone/Klinipath V.P. SYNERGY embedding automatic procedure.

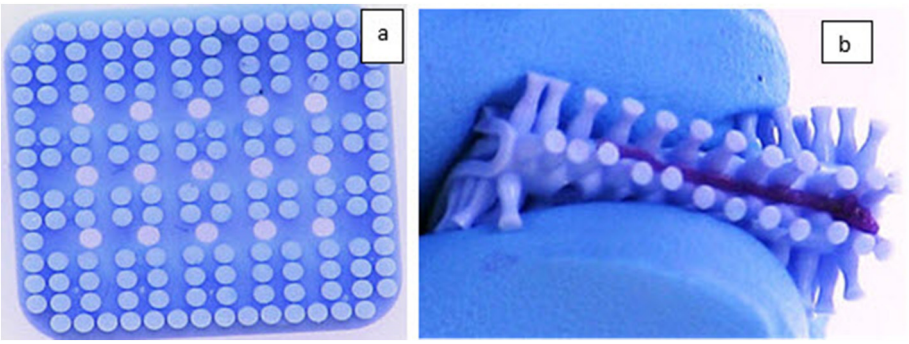

Fig. 11. QuickMBed™ silicone pad (a), b) a prostate needle biopsy core between the pad's stems.

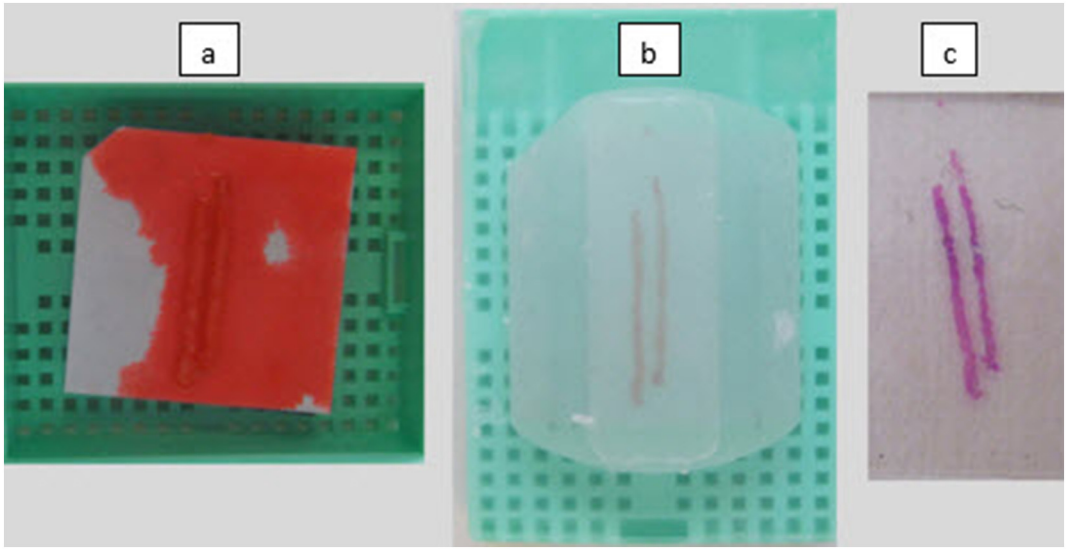

Fig. 12. SmartBx™ a) disposable proprietary cassette; b) after tissue processor; c) microscope slide.

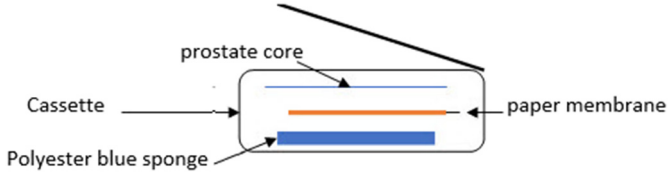

Fig. 13. Diagram for SmartBx™. The paper with adhered biopsy material is placed into regular tissue processing cassette.

2006 patent, which suggested using a perforated card for receiving a tissue specimen to be processed for histological examination [12].

3. Summary of main embedding automation methodology problems

Besides specific technical concerns, such as silicone or Paraform wearing blades out faster than paraffin alone, embedding automation methodology has two main problems, which require considerable attention: openness of the processing cassette with the insert for fluid perfusion in the tissue processor and immobilization of the sample oriented during grossing.

The adequate perfusion of fluids during tissue processing is the essence of James McCormick's worldwide and ubiquitous histology processing cassette. In contrast to Tissue-Tek® Paraform® Sectionable Cassette System, the Synergy® plastic mold and Biopsy Chip™ both open to the fluid perfusion from one side. If the perfusion still matters and the openness of McCormick's cassette is not a superfluous design feature of the old pre-microwave vacuum pressure tissue processors, every deviation from the fluids moving through the cassette should be considered a methodology deficiency. Standardization of the tissue processing was always important; however, under conditions of emerging digital pathology Whole Slide Imaging (WSI), it is especially necessary.

The sample's immobilization after orientation during grossing is the sticking point in the wider implementation of biopsies and small specimens embedding automation. The variety of presented options is evidence that the search is ongoing for this problem's optimal solution.

The ubiquitous polyester sponge is used for securing orientation and fluid perfusion through the net of relative sharp threads. It is prone to pressure artifact and carrying over fluids in a vacuum/pressure tissue processor. The pressure artifact is irrelevant for homogenous tissues, but it is significant for fragile specimens, especially core needle biopsies, when they are used most frequently. The perforated gel pad might be a move in the right direction to eliminate the sponge. Perhaps as the first step, it would be reasonable to use high permeability filtration paper, like Kimberly-Clark FluidShield® mask's fourth layer, to separate the fragile specimen and the polyester sponge [13].

One option for the secure orientation of small biopsies in the sectionable cassette might be to use an old method of agar pre-embedding by employing HistoGel®- like media. Currently, HistoGel® is used for cellblocks, while the old method of agar pre-embedding (double embedding) is rarely employed for small biopsies. Practical application of this method in embedding automation requires an array of organizational efforts and some additional technical contrivances, while keeping in mind the assembly line pattern of grossing room processing in a regular histology laboratory. These issues are discussed in detail in the

book *Grossing Technology: A Guide for Biopsies and Small Specimens*, where a prototype of the mini dissection board for orientation immobilization of the specimen by agar or HistoGel® is presented [14]. The portable mini board takes minimal space on the grossing table and provides the optimal condition for solidifying media during specimen orientation while maintaining the high speed of processing that is crucial for implementing the method in everyday practice.

4. Discussion

Advancements in tissue processing equipment and acceleration in pathology diagnosis reports turnaround time dramatically changed the specimen processing in histology laboratories. Times are gone when embedding started in mornings before sunrise. Embedding, especially biopsies, is done now during the day, sometimes repeatedly. Embedding procedure is standing in the line for automation in the histology laboratory environment when automatic stainer, cover slipper, even microtomes are not a novelty anymore.

Traditionally, embedding was the histotechnologists responsibility. Embedding automation makes the grossing person the last participant in specimen processing before microtomy. There are technical benefits to this switch in roles; for example, the specimen is not covered by a gray paraffin film, which makes different parts of the specimen less distinguishable or there is no race against the paraffin solidifying, which makes the sticky forceps less manageable. The grossing person has more information than the histotech about the specimen (type of tissue, clinical diagnosis, areas of diagnostic significance, etc.). Embedding automation thus becomes a part of grossing technology through the final pre-microtomy orientation by the grossing person.

The final orientation of the specimen before microtomy should be the main criteria for the evaluation embedding automation methodology. Neither productivity increase nor financial gains, which are particularly questionable at this stage of implementation, should dominate the approach to embedding automation. The current tide of an enormous number of biopsies will gradually be replaced with a more discretional approach to diagnostically significant areas of the biopsy.

There are two directions in maintaining final biopsy specimen orientation before microtomy: while the clinician is harvesting the biopsy material for traditional embedding and at the grossing table with following embedding automation. It seems that the latter is achievable in practice first.

Different solutions might be available for maintaining diagnostically informative orientation during embedding automation. Perhaps some biopsies, selected by local protocols, can be excluded from the main workload of specimens by triage and can be processed using a different designated method, such as the already mentioned modified agar or HistoGel® pre-embedding method. 3D printer technology also opens opportunities for developing sectionable inserts that are customized for the specific needs of a particular histology laboratory.

The embedding automation technique requires testing with large quantities of specimens. Perhaps pharmaceutical research, with its significant resources and less diagnostic responsibility for concrete patients, can take on such an endeavor. While understanding that this suggestion has a problematic opportunity in the real world, the surgical pathology laboratory should continue to work on stabilizing the orientation to make embedding automation a standard procedure in practice. Again, this article does not discuss productivity or cost effectiveness, and it concentrates only on strictly methodological issues to provide decision-making professionals with information on the implementation of embedding automation, which is, in this author view, the future of histology laboratory practice.

5. Conclusion

The presented material shows that there are diverse embedding automation options. Securing diagnostically informative orientation remains an unsolved problem in the embedding automation implementation in practice.

References

[1] Williamson, IV, Warren P. et al. Apparatus and method for harvesting and handling tissue samples for biopsy analysis. United States Patent Full-Text Image database: 7156814. http://www.patentstorm.us/ patents/7156814/description.html [accessed 31 May 2019].
[2] Diederichsen Chris, Whitlatch Stephen. Description and preliminary results of a novel cassette system (Tissue-Tek Paraform Cassette System). HistoLogic 1999;31(2):28–30.
[3] Dimenstein IB. Sectionable cassette for embedding automation in surgical pathology. Ann Diagn Pathol 2010;14:100–6.
[4] Goldberg MS, Wetherington SJ, Susa JS, Wickless SC, Cockerell CJ. Characterization of tissue and slide artifacts from automated embedding systems. Am J Dermatopathol 2015;37(11):846–9.
[5] Phelan Sine M. Impact of the introduction of a novel automated embedding system on quality in a university hospital histopathology department. Journal of Histology & Histopathology 2014. https://doi.org/10.7243/2055-091X-1-3.
[6] Method and device for preparing tissue and mould for pretreating tissue material. http://www.google.com/patents/EP2439510A1?cl=en, Accessed date: 31 May 2019.
[7] Pegolo Enrico, Pandolfi Maura, Di Loreto Carla. Appl Immunohistochem Mol Morphol 2013;21(4):362–70.
[8] Farcas CP, Spinu AD, Petrescu A, Mucat S, et al. High throughput processing and analysis of prostate biopsies for research applications. Revista Romana de Urologie 2014;13:9–16.
[9] Wojno K, Al-Jundi R, Mazurco A, Hamzawy H. MP16-19 BxChip™ clinical tissue array increases cancer detection rate & amount of tissue available for pathologist review. J Urol 2016;195(4S):e168. https://doi.org/10.1016/j.juro.2016.02.2584.
[10] www.plainsite.org/dockets/2y6jvs5mu/california-central-district-court/sakura-fine, Accessed date: 31 May 2019.
[11] https://www.uc-care.com/smartbx [accessed 25 January 27, 2019]. https://www.accessdata.fda.gov/SCRIPTS/cdrh/cfdocs/cfRL/rl.cfm?lid=333559&lpcd=KXG [accessed 31 May 2019]. www.focaltherapy.org/2013/Poster_Abstracts/Focal_2013_PP-22a.pdf [Poster].
[12] Apparatus and method for histological processing of a tissue specimen with means for maintaining the orientation of the specimen throughout the processing tissue Nov 29, 2005 Jun 8 McCormick Scient L L Chttp://www.google.com/patents/WO2006060317A1?cl=en; 2006, Accessed date: 25 January 2019.
[13] Dimenstein IB. The complete prostate needle biopsy submission during grossing and embedding. Journal of Histotechnology 2016;39(3):76–80.
[14] Dimenstein IB. Grossing technology: A guide for biopsies and small specimens. CreateSpace. Amazon.com; 2018. p. 246–53.