ORIGINAL ARTICLE

Frozen section analysis of sentinel lymph nodes in patients with breast cancer does not impair the probability to detect lymph node metastases

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Abstract Intra-operative frozen section analysis (FS analysis) of sentinel lymph nodes (SLNs) in patients with breast cancer can prevent a second operation for axillary lymph node dissection. In contrast, loss of tissue during FS analysis may impair the probability to detect lymph node metastases. To determine the effect of tissue loss on the probability of detection of metastases, dimensions and tissue loss resulting from intra-operative frozen section analysis were measured for 21 SLNs. In a mathematical model, the influence of tissue loss on the probability to detect metastases was calculated in relation to SLN size for various pathology protocols: an American, a widely used European, the extensive 'Milan' and the Dutch protocol. For median-sized SLN $11 \times 8 \times 5$ mm (length×width× height), FS analysis led to a median loss of 680 μm (13.6%) of the height of the SLN. Irrespective of SLN size or used pathology protocol, the probability of detecting 2 mm metastases remained unchanged or even increased (0–12.8%). Moreover, the probability to detect 0.2 mm metastases increased for the majority of tested combinations of SLN size, tissue loss and used protocol. Only when combining maximum tissue loss and smallest SLN size in the Dutch protocol, or when applying the extensive Milan protocol on a median-sized SLN, the probability to detect 0.2 mm metastases decreased by 2.7% and 14.3%, respectively. Contrary to 'common knowledge', doing FS analysis of SLNs does not impair the probability to detect lymph node metastases.

Keywords Frozen section · Sentinel lymph node · Lymphatic metastasis · Breast cancer · Mathematical model

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Introduction

Sentinel lymph node (SLN) biopsy has proven to be an accurate staging procedure in breast cancer patients and has replaced axillary lymph node dissection (ALND) in clinically 'node negative' axillas [1]. Though the SLN procedure has led to a more thorough inspection of these lymph nodes in general, their specific examination varies substantially throughout Europe [2] and the USA [3].

Intra-operative frozen section (FS) analysis of an SLN enables ALND to be performed during the same operation if tumour metastases are detected. When an SLN is prepared for FS analysis, part of the lymph node is sacrificed to obtain a reliable cut from the SLN for pathological examination. This loss of tissue may influence the probability to detect small lymph node metastases. As such, it may be a reason to discourage the use of FS analysis [4–6].

In the present study, the loss of SLN tissue during FS analysis is measured and the effect of the tissue loss on the probability to detect metastases within an SLN for four different, commonly used protocols is calculated.

Materials and methods

Intra-operative FS analysis and definitive pathology examination of SLNs (applying the Dutch guideline for evaluating SLNs)

In our hospital since 2000, intra-operative FS analysis of SLNs is routinely performed in breast cancer patients. Following retrieval of axillary SLNs, lymph nodes are transported to the pathology department and processed by the pathologist. After clearing the node from surrounding fat, SLNs are bisected along their longest axis. The two emerging halves are then frozen in Tissue-tek (Klinipath, Duiven, The Netherlands) after which preparatory cuts are taken from the centre of the nodes at variable intervals until appropriately examinable cuts are obtained from both halves. Appropriate cuts are slices that comprise an (almost) complete cross section from the lymph node. These intra-operative cuts are stained with haematoxylin–eosin (HE) and are examined by the pathologist for the presence of metastases. The remaining tissue is subsequently formalin-fixed and examined according to the Dutch guideline for evaluating SLNs, i.e. taking three cuts from either half starting from the centre with 250 μm distance between two cuts. SLNs thicker than 10 mm are sectioned into 2-mm slices. According to the protocols, samples are stained both with HE and immunohistochemically with antibodies to cytokeratin [7].

The study was approved by the institutional ethics committee. The size of SLNs was measured and tissue loss during the FS procedure was assessed. For a range of SLN sizes, the influence of the varying amount of tissue loss on the probability to detect metastases was calculated in a mathematical model, representing the processing of SLNs according to the Dutch pathology protocol. Subsequently, the influence of applying existing, alternative examination protocols was determined.

Establishing the size of an SLN

The dimensions of 21 SLNs obtained during breast surgery of ten consecutive female patients with cT1-2N0 breast cancer were measured by a pathologist after clearance of surrounding fat and before fixation. Length, height and width of the SLN were measured in millimetres (mm). In order to estimate the volume of an SLN, a triaxial, ellipsoid shape is assumed with parameters (half-lengths) a_x (width), a_y (depth) and b (height) (Appendix A; Fig. 4)

Tissue loss associated with frozen section analysis

For intra-operative FS analysis, an SLN is first bisected along its longest axis. FS are prepared from the cut surface (centre part) and preparatory FS are cut until a first section can be obtained that contains a complete cross section from the SLN (Fig. 1). Tissue loss was estimated by counting the number of preparatory sections cut on a cryostat (Leica CM1850) multiplied by the section thickness.

Calculating the influence of tissue loss on the probability of detecting metastases in the SLN

The Dutch guideline for evaluating SLNs advocates to bisect SLNs and to take three cuts from both halves starting from the centre with 250 μm distance between two cuts (or cuts at 2-mm intervals when an SLN is thicker than 10 mm). In a previous study [8], a model was presented to calculate the probability of detecting metastases in an SLN. This probability can be interpreted as the complementary risk of missing metastases that remain unnoticed due to their location in the outer areas of the SLNs or in the tissue between the cuts.

In the present study, we adopt and adapt this model to assess the probability of detecting the smallest (0.2 mm) and the largest (2 mm) single micrometastasis (TNM-N-class pN1mi) when performing FS analysis. The metastasis is represented as a ball shape with diameter m. A circular shape of a micrometastasis and a random location of the micrometastasis within the SLN are assumed (Appendix A; Fig. 4). The probability of detecting a metastasis in a particular tissue is roughly determined as the ratio of the volume covered by the histological examination and the total volume of the tissue (V). The pathology protocols, described below, all divide the unfixed tissue into two halves, after which a number of cuts n with height c and a distance d are taken from either half. The number of cuts depends on the protocol and the size of the SLN. If a metastasis with size m is present in the tissue and intersects with the examined section, it is assumed to be discovered with certainty. For preparing intra-operative FS, the two halves are levelled, cut
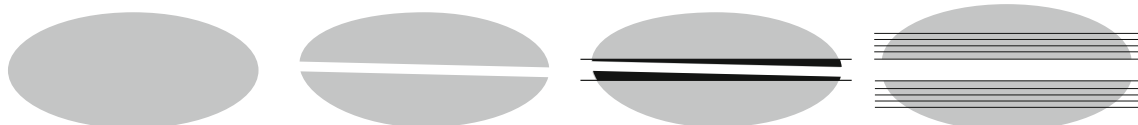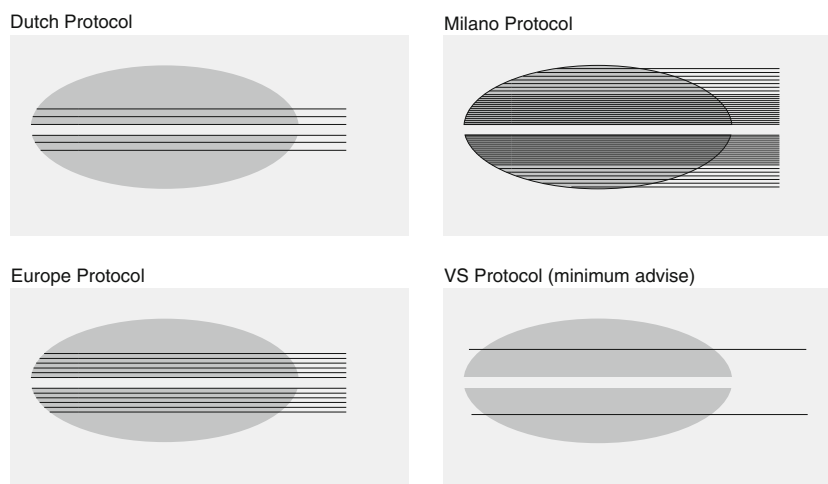

Fig. 1 Graphical presentation of bisecting an SLN, wasting tissue (*black*) by cutting preparative sections for FS analysis and further examination of the SLN. *SLN* sentinel lymph node

Fig. 2 Graphical presentation of the various pathology protocols

surface up, to ensure that the FS covers the full surface of both halves (Fig. 1). Thus, the potential waste is taken from the middle segment of the lymph node. The wasted tissue is assumed to have a total height w, of which w_L concerns the lower half, and w_U the upper half of the tissue. The choice of which half of the tissue is the lower or upper part is immaterial; the distinction between different amounts of waste from both halves merely adds flexibility to the model. Conceptually, wasting tissue has two opposite effects on the probability of detecting metastases during FS examination. On the one hand, the wasting tissue might cause the pathologist to miss metastases which reduces the probability of detection (the pure waste effect). On the other hand, levelling of the tissue halves has the effect of covering more of the periphery of the lymph node, which may actually increase the probability of detection (the advancement effect). The precise consequences of wasted tissue for the detection probability will depend on the pathology protocol, the size of the metastasis, the wasted volume and the size of the SLN. The specifics of the mathematical model are summarised in Appendix A.

The effect of FS analysis when alternative pathology protocols for the examination of SLNs followed

The effect of FS analysis has been studied for three commonly used protocols in addition to the Dutch protocol: the American pathology guideline [4], the 'Milan' protocol as described by Veronesi [1] and the protocol described by Cserni [2] as the most commonly used practice in Europe. While the Dutch protocol takes three cuts from either half of the tissue at a distance of $d=250$ μm, American guidelines suggest sectioning SLNs at 2-mm intervals after bisecting the SLN. The protocol described by Cserni as the most commonly used in Europe takes six cuts from both halves at a distance of $d=150$ μm. The most extensive Milan protocol takes 15 cuts from both halves at a distance of $d=50$ μm, after which the remaining tissue is cut at 100 μm. The vertical size of the cuts is $c=5$ μm for all protocols except the Milan protocol in which $c=4$ μm. The specifics of these protocols are graphically represented in Fig. 2. The effect of FS analysis on the probability of detection has been assessed for various combinations of the

Table 1 Probability to detect 0.2-mm metastases in SLNs without and with frozen section analysis

	Small SLN		Median SLN		Large SLN	
	7×6×4 mm		11×8×5 mm		20×10×10 mm	
	Without FS	With FS	Without FS	With FS	Without FS	With FS
Minimum amount of lost tissue during FS (72 μm)	44.0%	46.7%	35.8%	38.1%	18.3%	19.5%
Median amount of lost tissue during FS (680 μm)	44.0%	47.4%	35.8%	39.8%	18.3%	21.2%
Maximum amount of lost tissue during FS (1,250 μm)	44.0%	41.3%	35.8%	36.7%	18.3%	20.8%

Probability values were calculated for the various combinations of minimum, median and maximum tissue loss and minimum, median and maximum SLN size

SLN sentinel lymph node, *FS* frozen section

Table 2 Probability to detect 2-mm metastases in SLNs without and with frozen section analysis

	Small SLN		Median SLN		Large SLN	
	7×6×4 mm		11×8×5 mm		20×10×10 mm	
	Without FS	With FS	Without FS	With FS	Without FS	With FS
Minimum amount of lost tissue during FS (72 μm)	100%	100%	100%	100%	69.1%	70.0%
Median amount of lost tissue during FS (680 μm)	100%	100%	100%	100%	69.1%	76.4%
Maximum amount of lost tissue during FS (1,250 μm)	100%	100%	100%	100%	69.1%	81.9%

Probability values were calculated for the various combinations of minimum, median and maximum tissue loss and minimum, median and maximum SLN size

SLN sentinel lymph node, *FS* frozen section

different sized SLNs and the different amounts of wasted tissue corresponding with the different pathology protocols.

Results

The median size of the 21 examined SLNs was 11×8×5 mm (range 7×6×4–20×10×10 mm; length×width×height). After bisecting the SLN, cutting preparatory sections from the centre of the SLN led to a median loss in the height of tissue of 680 μm (range 72–1,250 μm). This tissue loss comprised 13.6% (range 1.4–25%) of the height of a median SLN.

In Table 1, the probability to detect metastatic involvement in SLNs of different sizes as well as the influence of FS related tissue loss is shown for 0.2-mm metastases when SLNs are processed according to the Dutch pathology protocol. The probability to detect 0.2-mm metastases only decreased when the maximum amount of tissue was wasted when examining the smallest SLN. In that case, the probability to detect a 0.2-mm metastasis decreased from 44.0% to 41.3%. For all other combinations, the probability of detection increased when doing FS analysis, the increase of this chance to detect a 0.2-mm metastasis ranging between 0.9% and 4.0%.

The probabilities of detecting 2.0-mm metastases are shown in Table 2. For small- and median-sized SLNs, the probability of detection remained 100%, irrespective of doing FS analysis. For large SLNs, in which the probability of detection of 2.0-mm metastasis without FS analysis is 69.1%,
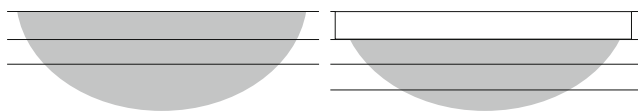
the use of FS analysis increased the probability of detection, and the increase of the detection probability varied between 0.9% and 12.8%.

The effect of FS analysis is graphically represented in Fig. 3. When applying the Dutch guideline for the pathology examination of SLNs, the loss of tissue from the centre of the SLN resulted in an advancement of the examined area towards the outer areas of the SLN, on average leading to a higher probability to detect metastases.

In Table 3, the effect of tissue loss resulting from FS analysis is summarised for the alternative pathology protocols, i.e. the Milan, the European and the American protocol. For 0.2-mm metastases, the probability of detection increased for the European and American protocol, from 55.8% to 57.7% and from 12.3 to 18.1% respectively. Only in the extensive Milan protocol, a 14.3% decrease to detect 0.2-mm metastases was observed when doing FS analysis. The probability of detecting 2.0-mm metastases remained the same for the European and the Milan protocol, i.e. 100%, and it increased when applying the American protocol from 94.5% to 99.5%.

Table 3 Effect of frozen section on detection probabilities when applying alternative pathology protocols for SLN examination

	Metastasis size	Without FS	With FS
European protocol	0.2 mm	55.8%	57.7%
	2 mm	100%	100%
Milan protocol	0.2 mm	100%	85.7%
	2 mm	100%	100%
Dutch protocol	0.2 mm	35.8%	39.8%
	2 mm	100%	100%
American protocol	0.2 mm	12.3%	18.1%
	2 mm	94.5%	99.5%

SLN sentinel lymph node, *FS* frozen section

Fig. 3 Graphical presentation of the effect of tissue loss from the centre of the SLN: advancement of examined tissue towards the outer area. *SLN* sentinel lymph node

Discussion

In the present study, a beneficial effect of FS analysis on the probability to detect micrometastases in SLNs is observed. Despite losing approximately 14% of tissue from the centre of the SLN, the probability to detect the smallest micrometastasis in an SLN of median size with median tissue loss increased by 4%, while the probability to detect the smallest macrometastasis remained unchanged (100%) for the Dutch pathology protocol. Variations in the probability of detection depend on the size of the SLN and the amount of wasted tissue. These paradoxical findings were confirmed for most of the other pathology protocols.

According to our knowledge, no studies have been performed that actually measure the potentially adverse effect of tissue loss resulting from FS analysis. However, various authors have hypothesised a substantial effect of lost tissue due to FS analysis [5, 6]. Based on this assumption, 'conventional wisdom' has been ground for dissuasion of this intra-operative technique. In addition, this presumed effect is the main reason to advocate other intra-operative techniques such as touch imprint cytology or intra-operatively assessing greater volumes of SLNs or even whole lymph nodes without losing tissue by one-step nucleic acid amplification [9, 10].

The observed paradoxically increased probability to detect small lymph node metastases when doing FS analysis can be explained in a number of ways. Firstly, the median loss of tissue due to FS analysis was 680 μm. Serial sectioning is in itself accompanied by a risk of 'missing' metastases that are smaller than the interval between the cuts (250 μm according to the Dutch guidelines). Conceptually, the observed loss of 680 μm may be viewed as an extension of the cut interval by 430 μm (680 instead of 250 μm). Furthermore, if a metastasis is larger than the height of wasted tissue, the probability to detect such a metastasis remains unaffected. Finally, losing tissue from the centre of the node leads to an advancement of the sections to be analysed microscopically towards the outer areas of the SLN. So, the loss of 430 μm from the centre of the node is accompanied by examining 680 μm deeper into the periphery of an SLN. This 'advancement' effect increases the probability to detect metastases in case of pathology protocols that advocate examining the central part of the lymph node. This effect is absent in case of protocols advocating extensive serial sectioning of the entire SLN, e.g. the Milan protocol.

Thus, preparing SLNs during FS analysis and thereby sacrificing tissue will initially have a favourable effect on the probability to detect metastases but, with on-going loss, will eventually result in a decreased probability. Differences in applied protocols and the absence of consensus regarding a minimum metastasis size that should be detected under all circumstances preclude formulating a rule of thumb that can be used to suggest what proportion of an SLN can be sacrificed during FS analysis without compromising the detection probability. Then again, the results of the present study demonstrate that the wasted tissue never compromised the chance to detect lymph node metastases when commonly used protocols are applied.

Only when using the protocol as described by Veronese et al. [1], which implies cutting the entire SLN at 50-μm intervals, the probability to detect 0.2 mm was decreased by 14.3% (from 100% to 85.7%) while the chance to detect the smallest macrometastasis was unimpaired. Then again, few other institutions advocate such an extensive examination of SLNs. In this respect, it is noteworthy that the probability to detect a 0.2-mm metastasis was very limited in all protocols except for the Milan protocol, as demonstrated in a previous study [8]. Given the ongoing discussion regarding the prognostic effect of micrometastases and isolated tumour cells [11, 12], this may be a reason to consider a more extensive protocol.

The present study has a number of limitations. In designing the model, a number of assumptions were adopted such as the ellipsoid shape of an SLN, the circular-shaped metastasis, as well as the random position of the metastasis within an SLN. Furthermore, we assumed that subsequent definitive pathology examination was not associated with tissue loss irrespective of the use of FS analysis, whereas in reality, tissue loss accompanies the final pathological examination too. Furthermore, in this model, a lymph node metastasis is assumed to be single, whereas the probability of detecting metastases increases with the number of metastases in the SLN. As a rule of thumb, the probability to detect at least one of multiple metastases will increase for all examination protocols, and the effect of FS analysis will never lead to an increased risk of missing metastases.

In summary, contrary to our expectation as well as 'common knowledge', FS analysis of sentinel lymph nodes by bisecting lymph nodes, wasting of preparative sections from the centre does not impair the probability to detect lymph node metastases.
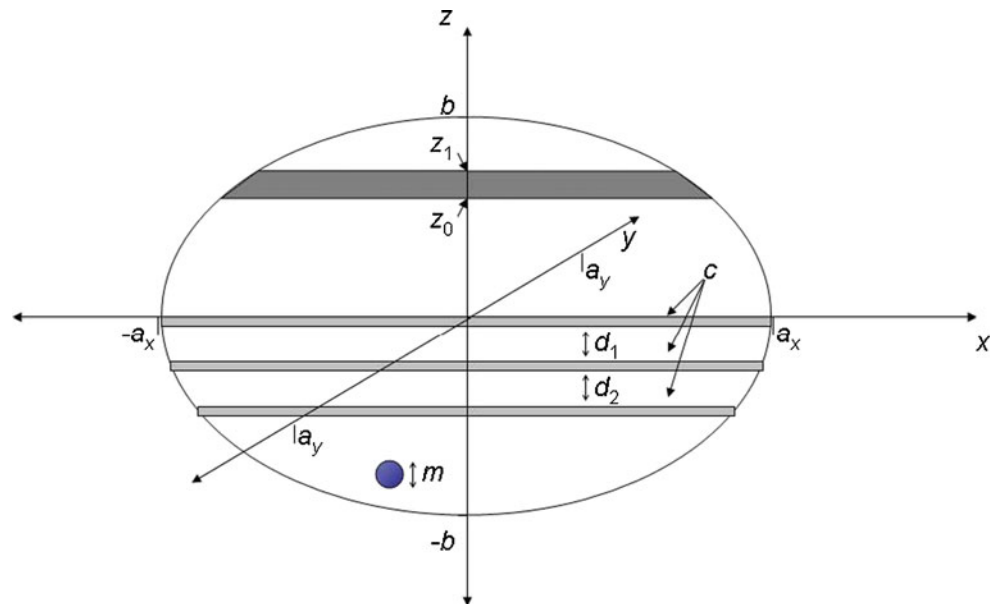
Appendix A

Fig. 4 The model to determine the probability of detecting micrometastases in SLN with and without frozen section analysis

This appendix presents the mathematical model to determine the probability of detecting micrometastases in an SLN with and without frozen section analysis. The pathological protocols considered in the present paper all divide the raw tissue into two halves. From each half, one frozen section with height fc and n cuts with height c are taken at inter-cut distance d; if no frozen section analysis is applied, then the height of the frozen section is simply equal to zero, $fc=0$. The number of cuts depends on the protocol and the size of the tissue. If a metastasis with size m is present in the tissue and intersects with the inspected cuts, it is assumed to be detected with certainty. During the preparation of the cutting process, the two halves are levelled to ensure that the first cut covers the full surface of the remaining half. The implied waste is taken from the midst of the original tissue. It is assumed to have a total height w, of which w_L concerns the lower half, and w_U the upper half of the tissue. The choice of which half of the tissue is the lower or upper part is immaterial; the distinction between different amounts of waste from both halves merely adds flexibility to the model. The occurrence of pathological waste has two opposite effects on the probability of detecting metastases. On the one hand, waste may lead the pathologists to definitively miss a metastasis, which reduces the probability of detection (the pure waste effect). On the other hand, levelling of the tissue halves causes the volume covered by the pathological inspection to include more of the outer parts of the tissue, which may actually increase the probability of detection (the advance-

ment effect). The precise consequences on the detection probability of wasted tissue will depend on the size of the metastasis, the wasted volume and the total volume of the tissue.

General assumptions

We model a given tissue by means of a triaxial ellipsoid with parameters (half-lengths): a_x (width), a_y (depth) and b (height); $a_x \geq a_y \geq b > 0$. The volume of the entire ellipsoid is obtained as $V = 4\pi\, a_x\, a_y\, b/3$, while the volume of an arbitrary (horizontal) slice from this ellipsoid between points z_0 and z_1, $-b \leq z_0 \leq z_1 \leq b$, can be derived as:

$$I(z_0, z_1) = \pi a_x a_y \left((z_1 - z_0) - \frac{1}{3b^2}\left(z_1^3 - z_0^3\right) \right) \qquad (1)$$

Obviously, $V = I(-b, b)$. The probability p of detecting a metastasis, if it is present, is defined as the total tissue volume covered by the pathological inspection (V_{PI}) corrected for waste (U_W) and unobserved volume due to metastases smaller than the inter-cut distance ($U_{m<d}$) divided by the tissue volume (V):

$$p = \frac{V_{PI} - U_W - U_{m<d}}{V} \qquad (2)$$

Further formalisation will distinguish between situations without and with pathological waste.

The case of no waste

In the absence of waste, assuming frozen section and symmetric halves, the total tissue volume covered by the pathological inspection is equal to:

$$V_{PI} = I[-(fc + c + (n-1)(c+d) + m); \\ fc + c + (n-1)(c+d) + m] \tag{3}$$

with n the number of cuts. The volume unseen due to waste is obviously void, $U_W = 0$. Metastases with size equal or larger than the inter-cut space ($m \geq d$) will always be detected, if they are within this inspected volume ($U_{m<d}=0$). Smaller metastases ($m<d$) within the inspected volume may be missed when they lie between two cuts without intersecting them. Considering cuts j and $j+1$, $j=1, \ldots, n-1$, the inter-cut volume unobserved by the pathologist can be approximated as the average of $I[fc + c + (j-1)(c+d); fc + j(c+d) - m]$ and $I[fc + c + (j-1)(c+d) + m; fc + j(c+d)]$. Alternatively, one could approximate this unseen volume by $I[fc + c + (j-1)(c+d) + m/2; fc + j(c+d) - m/2)$, but the difference between the two approaches is marginal. Assuming symmetry, the total volume unseen by the pathological inspection in the case of small metastases ($m<d$) is equal to:

$$U_{m<d} = \sum_{j=1}^{n-1} [I[fc + c + (j-1)(c+d); \quad fc + j(c+d) - m] \\ + I[fc + c + (j-1)(c+d) + m; \quad fc + j(c+d)]] \tag{4}$$

The probability of detection can now be calculated using Eq. 2.

The case of waste

In the case that the pathological process involves waste, the probability of detection is affected through a larger total volume covered by the inspection (the advancement effect) and a possibly unseen part of wasted tissue (the pure waste effect). Relaxing the assumption of symmetric tissue halves

and recalling that w_L and w_U refer to the wasted heights from the lower and upper tissue halves, respectively, the total inspected volume before correction becomes:

$$V_{PI} = I[-(w_L + fc + c + (n_L - 1)(c+d) + m); \\ 0] + I[0; \quad w_U + fc + c + (n_U - 1)(c+d) + m] \tag{5}$$

with n_L and n_U the possibly different numbers of cuts from the lower and upper tissue halves, respectively. The total tissue volume covered by the inspection is larger than that in the case of no waste, which positively affects the probability of detection. However, the occurrence of pathological waste implies that metastases in the wasted tissue might go undetected. Again, two situations may be discerned: large metastases with a size greater than the total height of the wasted material ($m \geq w = w_L + w_U$) and small metastases ($m<w$). In the former case, $m \geq w$, the metastasis will always intersect with the first cut on either half. Consequently, the wasting has no adverse effects on the detection of the metastases, and no waste correction is needed ($U_W = 0$). In the latter case, $m<w$, some metastases may be missed when they fall in between the first cuts on both tissue halves without intersecting them. In line with the unobserved parts of the inter-cut spaces, we approximate the unobserved part of the wasted tissue by:

$$U_W = (I[-w_L + m; w_U] + I[-w_L; w_U - m])/2 \tag{6}$$

Again, this could be approximated by $I(-w_L + m/2; w_U - m/2)$, leading to a marginally different outcome. Furthermore, the unobserved parts of the inter-cut spaces due to small metastases ($m<d$) simply generalise from Eq. 4. Taking into account the waste (w_L and w_U), possibly different numbers of cuts on the upper and lower halves (n_L and n_U), and not imposing symmetry, the unseen volume of tissue during pathological inspection ($U_{m<d}$) is obtained as:

$$U_{m<d,L} = \sum_{j=1}^{n_L - 1} [I[-(w_L + fc + c + (j-1)(c+d); -(w_L + fc + j(c+d) - m)] + \\ + I[-(w_L + fc + c + (j-1)(c+d) + m); -(w_L + fc + c + j(c+d))]]/2 \\ U_{m<d,U} = \sum_{j=1}^{n_U - 1} [I[w_U + fc + c + (j-1)(c+d); w_U + fc + j(c+d) - m] + \\ + I[w_U + fc + c + (j-1)(c+d) + m; w_U + fc + c + j(c+d)]]/2 \\ U_{m<d} = U_{m<d,L} + U_{m<d,U} \tag{7}$$

Having the corrections for waste and unseen inter-cut spaces, the probability of detection is determined using Eq. 2.

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