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Tissue expansion of lung bronchi due to tissue processing for histology – A comparative analysis of paraffin versus frozen sections in a pig model

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ABSTRACT

Aim: Tissue shrinking due to fixation and processing is well known. However, the degree of shrinking varies significantly with the tissue type as well as the processing method and is not well studied in various tissues. In daily pathological routine workflow, histological specimens from frozen and paraffin sections are performed from the same tissue. In the present study we compared the thickness of bronchus walls obtained from paraffin and frozen sections.

Methods: Pig lungs were frozen in ventilated condition in liquid nitrogen and 36 bronchi were isolated after dissection. Frozen sections of 5 µm thickness were performed and the remaining tissue was fixed and embedded in paraffin after fixation in 4% formalin. Frozen and paraffin sections from the same cutting edge were analysed after haematoxylin and eosin staining by measuring the wall thickness of the bronchi using high power fields of 400-fold magnification. In each bronchus 40 measurements were implemented at different wall positions distributed over the entire wall area. Summed up, in each group 1440 wall measurements were performed in total. Statistical analysis was conducted using the Wilcoxon test and *t*-test as well as Pearson's correlation coefficient with a significance level at $P < 0.05$.

Results: The bronchial wall thickness was significantly ($p < 0.001$) smaller in frozen sections (median: 0.50 mm; min: 0.37 mm; max: 0.97 mm) compared to paraffin sections (median: 0.58 mm; min: 0.35 mm; max: 1.06 mm). The median difference between paraffin and frozen sections was 0.05 mm (min: -0.11 mm; max: 0.22 mm). The wall thickness ratio of both groups was as follows: frozen/paraffin section = 0.8609, thus yielding a difference between paraffin and frozen of 13.91%. High correlation was found between wall thickness measurements on paraffin and frozen sections ($R = 0.87$, $p < 0.001$).

Conclusions: The bronchus wall thickness in the frozen section was 14% reduced compared to the paraffin section. In routine pathology as well as in scientific studies these results are of relevance, as airway wall thickness represents a relevant marker for pathological interpretation, especially using CT image techniques.

1. Introduction

Tissue shrinking due to fixation and tissue processing procedures is well documented in surgical pathology [1]. Multiple influences have been established, including fixatives such as alcohol, formalin [2] or formaldehyde [3,4], the embedding of tissue in paraffin as well as

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subsequent cutting and stretching [4]. Tissue shrinking also occurs when frozen sections are performed [5]. Besides histological preparation, shrinking artefacts are well known due to critical point drying and glutaraldehyde tissue processing for electron microscopy [6–9]. Even the exposition to air was documented as a cause of tissue retraction [1,10]. The extent of shrinking varies significantly depending on the used fixative, the type of tissue, the size of the fixed specimen and the method of tissue processing. Moreover, the age of the patient has been demonstrated to be a possible modulating factor [1,11].

Tissue shrinkage has been reported in various tissue types and preparation methods. Abramson et al. showed a fixation-associated difference in the length of optic nerves of about 30% between the surgeon's measurement immediately after enucleation and the measurement of the surgical pathologist prior to processing [1]. Other optic tissue types like uveal melanomas [12] cornea [13] and corneal epithelial cells also undergo shrinkage due to fixation and processing [6].

The investigation of human malignant melanomas revealed that the age of the patients influences tissue shrinkage [14] ranging from 15% to 25% [15]. An experimental study on human epithelial and melanocytic tumors revealed an initial tissue shrinkage due to loss of skin tension followed by increasing tumor thickness after histologic processing [16]. These findings suggested that the effect of initial shrinkage was reversed by the tissue processing procedure [16]. In this context, the skin location of the explant may play a role in tissue shrinkage, since in an animal study shrinkage after tissue removal occurred in samples from the thorax, abdomen and rear leg, whereas shrinkage due to formalin fixation was only found in samples from the thorax [17]. Tissue shrinkage and distortion were also reported in arteries following formalin fixation [18,19] and paraffin embedding [19]. Interestingly, a volume expansion of 4–13% was found when arteries were embedded in glycol methacrylate and of 8% when glutaraldehyde was used for fixation [19]. With a view to avoid shrinkage, tethering of bovine pericardium to original dimensions during fixation in glutaraldehyde led to a material extensibility nearly identical to that of fresh tissue [20]. In head and neck oncological surgery, the surgeon often finds significantly smaller tumor-free margins *in situ* than reported from the histopathologic measurement after excision, formalin fixation and slide preparation [21]. The measurement of human oral tongue carcinoma specimens prior to and after histopathological processing revealed an average tumor shrinkage of 20% [22]. Oral cavity mucosa and tongue muscle underlay shrinkage of 30% in lingual surface mucosa and of 35% in deep tongue tissue. The shrinkage of labio-buccal mucosa was 47%. Interestingly, in this study the greatest proportion of shrinkage occurred immediately after resection [21]. Also oral cavity tumors and squamous cell carcinomas were shown to shrink significantly after formalin fixation [23]. In contrast, fixation in formalin did not influence the tissue dimensions of palatal tonsils in comparison to direct *ex vivo* measurements even following extended fixation time periods [24].

In a study by Schned et al. prostate shrinkage caused by fixation was minimal (4%), but increased to 14.5% due to tissue processing. Rehydration and expansion on the flotation bath caused tissue swelling which adjusted shrinkage to some degree. Interestingly, formalin concentration, processing methods like whole-mount *versus* quadrant sections, tissue slice thickness, time period of alcohol dehydration and temperature of the flotation bath had no influence on prostate tissue shrinkage [25]. A neuropathological study on brains revealed a total shrinkage of 48%, more than half of which was caused by fixation. In contrast to the fixation time period, the formaldehyde concentration had no impact on the extent of shrinkage [26]. Tissue shrinkage complicates the evaluation of brain lesions, since sizes of hemispheres, infarction and oedema are influenced by processing induced shrinkage [27].

A study on oesophageal shrinkage due to processing after tumor resection revealed overall shrinkage after fixation of 50%, with the upper margins undergoing a greater degree of shrinkage than the lower margins and only little change of tumor length [28]. After low anterior resection of rectal cancer significant tissue shrinkage occurs, resulting in a poor correlation between the *in situ* measurement of length of the distal margin and that determined by the pathologist [29]. Approximately 70% of shrinkage of colorectal resection specimens occur during the first 10–20 min after removal and the remaining 30% occur after fixation [10]. The shrinkage of porcine small and large intestine after zinc salt fixation varied between 19% and 57% [30].

Lung tissue also underlies volume change due to fixation and histological processing [31,32]. The degree of shrinkage seems to vary considerably among different species and requires measurement of lung dimensions after fixation and histological processing in different species, particularly regarding interspecies physiologic studies [31]. Although shrinkage of lung tissue caused by fixation and histological processing has been reported, shrinkage of bronchial tissue and the comparison of the two most commonly used methods in surgical pathology, namely frozen and paraffin sectioning, have not yet been investigated. The present study was conducted to assess the size of bronchial tissue in histological paraffin and frozen sections and to investigate if there was a difference in size between these two methods in porcine bronchial tissue.

2. Material and methods

2.1. Animal experiment, tissue extraction and histological tissue preparation

The tissue samples used in this study derived from a previous radiological-pathological animal investigation performed by Achenbach et al. [33], which was further investigated histologically by one of the present authors (C. Schmitt) [34]. For the intention of the present study no additional animals were killed. A detailed description of the animal experiments and methods of retrieving the inspected bronchi are already given in the former publication [33,34]. In brief, the lungs of four pigs were removed from the body and frozen in a liquid nitrogen bath under ventilated conditions. The lungs were then cut under frozen conditions and from the obtained slices bronchi were isolated. Of these, frozen sections of 5 µm thickness were performed immediately [33]. The surface of the cutting edge was marked on the remaining tissue to guarantee the performance of a frozen and a paraffin section from exactly the same cutting edge. The tissue was then fixed in 4% formalin and embedded in paraffin. Afterwards, paraffin sections of 5 µm thickness were performed. Both, frozen and paraffin sections were stained with haematoxylin and eosin according to standardized methods. The slides were assessed for intactness of the anatomical structures. Only complete bronchi with an entire intact wall, including the epithelium, were taken for the histological investigation [34].

2.2. Histological evaluation

In total, 36 bronchi were investigated. For each bronchus one frozen and one paraffin section were analysed, so that a total of 72 slides were assessed. For histological evaluation a light microscope (BX45, Olympus, Hamburg, Germany) was used. Measurements were performed with help of the microscope software Cell sense entry (Olympus, Hamburg, Germany). Images were obtained using a microscope camera (Olympus SC30, Olympus, Hamburg, Germany). The wall thickness of the bronchi was investigated using high power fields (hpf) of 400-fold magnification. In each bronchus 40 measurements were implemented at different wall positions distributed over the entire wall area (Fig. 1) [34]. Hence, in each group 1440 wall measurements were performed in total.

2.3. Statistical analysis

Data collection and statistical analyses were conducted using Microsoft Excel (Microsoft Excel 2010, Microsoft Corporation,

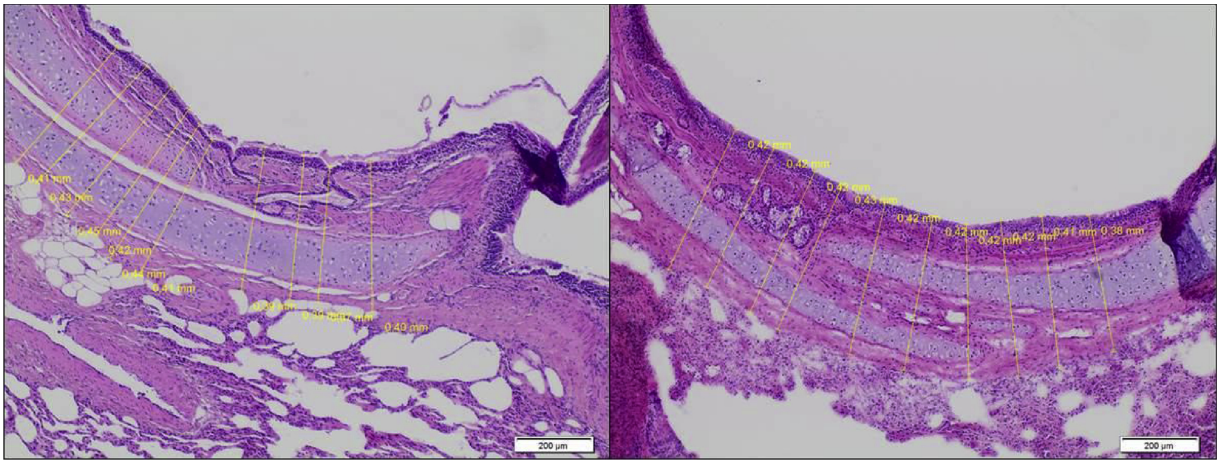

Fig. 1. Bronchus wall measurement.
In paraffin (left) and frozen sections (right) the wall thickness of bronchi was measured using high power fields (hpf) of 400-fold magnification with 40 measurements at different wall positions in each bronchus.

Redmond, WA, USA) and SPSS Statistics 20 (IBM Deutschland GmbH, Ehningen, Germany). Wilcoxon test and *t*-test were used as well as linear regression analyses to calculate Pearson's correlation coefficient. The significance level was set at $P < 0.05$.

3. Results

The bronchial wall thickness of frozen sections was smaller compared to paraffin sections (Fig. 2) [34]. The difference in size between paraffin and frozen sections was statistically highly significant ($p < 0.001$).

In detail, the measurement of bronchial wall thickness of paraffin sections gave the following values: median 0.58 mm, and mean value 0.60 mm, with a minimum of 0.35 mm and a maximum of 1.06 mm. The first quartile (Q1, percentile 25) amounted 0.46 mm and the third quartile (Q3, percentile 75) 0.71 mm. In frozen sections, the bronchi

Table 1
Results of the bronchial wall thickness measurements of paraffin and frozen sections.

		Paraffin sections	Frozen sections
n		36	36
Median		0.5750	0.4950
Mean value		0.5992	0.5444
Standard deviation		0.16674	0.16792
Minimum		0.35	0.37
Maximum		1.06	0.97
Percentiles	25	0.4600	0.4200
	50	0.5750	0.4950
	75	0.7100	0.6225

All values except the count of samples (n) are represented in the measuring unit mm.

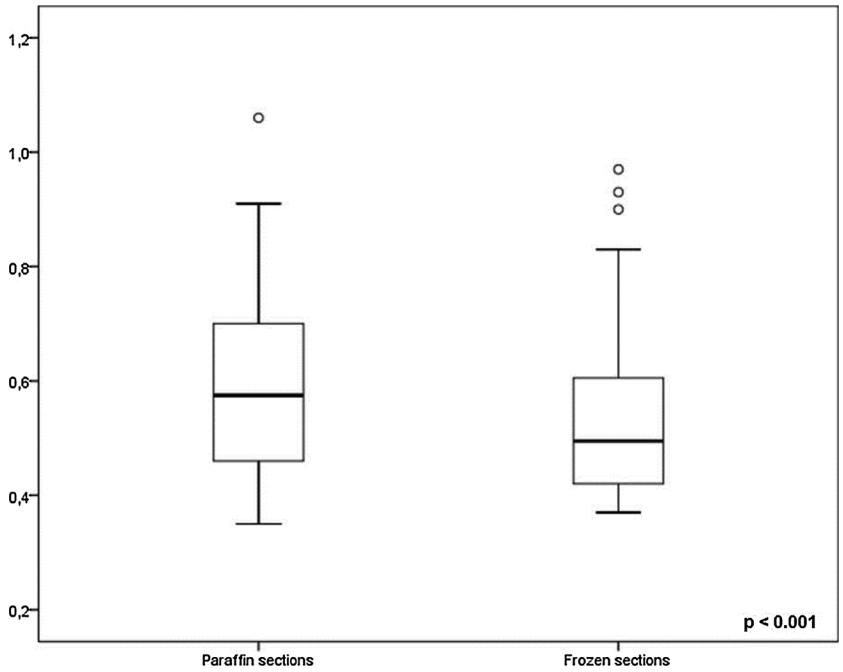

Fig. 2. Bronchial wall thickness in paraffin and frozen sections.
Boxplots showing the bronchial wall thickness measurements of paraffin and frozen sections with a significantly smaller size of the bronchial wall in the frozen specimens. The scale bar is scored in the measurement unit mm.

Table 2
Results of the difference between paraffin and frozen section measurements.

Difference paraffin and frozen sections		
n		36
Mean value		0.0547
Median		0.0450
Standard deviation		0.08389
Minimum		−0.11
Maximum		0.22
Percentiles	25	0.0025
	50	0.0450
	75	0.1375

All values except the count of samples (n) are represented in the measuring unit mm.

wall thickness had a median of 0.50 mm and a mean value of 0.54 mm, with a minimum of 0.37 mm and a maximum of 0.97 mm. The first quartile (Q1, percentile 25) was 0.42 mm and the third quartile (Q3, percentile 75) amounted 0.62 mm (Table 1) [34].

The difference between paraffin and frozen sections amounted to 0.05 mm for both the median and mean values, with a minimum of -0.11 mm and a maximum of 0.22 mm. The first quartile was 0.0025 mm and the third quartile was 0.14 mm (Table 2) [34]. Comparing the wall thicknesses using the ratios of the medians (paraffin sections: 0.575 mm; frozen sections: 0.495 mm) and mean values (paraffin sections: 0.5992 mm; frozen sections: 0.5444 mm) of both groups gave values of frozen/paraffin section = 0.8609 (median) and frozen/paraffin section = 0.9085 (mean value). Thus, in relative terms the difference between paraffin and frozen sections amounted to 13.91% (median) or 9.15% (mean value), respectively.

Pearson's correlation coefficient revealed a highly significant correlation between paraffin and frozen sections with R = 0.87 for both median and mean value with $p < 0.001$ (Table 3 and Fig. 3).

4. Discussion

Rapid intraoperative frozen sectioning with subsequent confirmation of the result using paraffin sections is an important method in contemporary patient care. Tissue shrinkage caused by tissue fixation and processing has been reported for several tissue types, including optic tissue [1,6,12,13], skin [14–17], blood vessels [18,19], pericardium [20], head and neck tissue [21–24,35], prostate [25], gynecological specimens [36], neural tissue [26,27,37], tissue from the gastrointestinal tract [10,28–30] and lung tissue [31,32,38,39]. Furthermore, it is well known that discrepancies may occur in the evaluation of the most commonly used histological processing methods, namely paraffin and frozen sections [40–49]. This has a great impact on the daily routine work of surgical pathologists and scientific laboratories which use these methods. The present study represents the first investigation to compare the thickness of bronchial tissue of histological paraffin and frozen sections. In the present analyses, the bronchial wall thickness of paraffin and frozen sections gave a difference of nearly 14%, with the larger values being obtained for the paraffin sections. Achenbach et al. [33] have shown that the wall thickness was overestimated by 11% on the CT images, but the correlation between the wall thickness measurements on the frozen sections and on the CT images was very high (R = 0.951). In the present study, a high correlation between the wall thickness estimation on the frozen sections and on the paraffin sections was found (Pearson's R = 0.87, p-value < 0.001). This suggests that there is also a high correlation between the wall thickness determination on the paraffin sections and on the CT images. However, it has always to be kept in mind that the absolute wall thickness values differ significantly between frozen and paraffin sections and frozen section and CT images.

The present results are highly relevant for intraoperative tumor or resection margin evaluation using frozen sections and their subsequent verification by paraffin sections. Fixation- and processing-induced tissue shrinkage are crucial when samples are compared after different processing procedures [30] and must be considered in the evaluation of oncological specimens with respect to tumor size, resection margins or correctness of the size of the anatomical site [21]. Tissue shrinkage may occur even before the specimen reaches the pathologist. Thus, in giant cell arteritis it was suggested to resect routinely longer temporal artery biopsy specimens in order to reduce the risk of diagnostic inaccuracy due to shrinkage caused by chemical fixation [18]. A significant discrepancy in size between *in situ* reported tumor-free margins and the subsequent histopathological measurement is often found in head and neck oncological surgery. In one study specimens of oral cavity mucosal and tongue muscle shrank significantly after resection and it could be shown that an *in situ* margin of resection of at least 8–10 mm was necessary to obtain a pathologically clear margin of 5 mm [21]. Due to significant shrinkage of human head and neck tissue caused by formalin fixation immediate measurement of the tumor after explantation is required to avoid underestimation of the tumor size [23].

After low anterior resection of rectal cancer pinning of the specimens was suggested as a feasible method to maintain the length of the distal margin between the lower border of the tumor and the level of division, since no significant difference before and after fixation was found if the specimen had been pinned, but significant shrinkage did occur with fixation when the specimen had not been pinned [29]. Considerable shrinkage has also been observed in esophagus specimens, causing discrepancies when comparing measurements by surgeons and pathologists regarding the length of the margins after tumor resection [28]. Furthermore, in the evaluation of brain tissue shrinkage due to processing complicates the assessment of lesions. It was therefore suggested to express the size of brain lesions like infarction and edema in relative terms to the hemisphere rather than by absolute measures, since this represents a robust method to state brain lesions independent of the investigated fixation procedures [27]. Regarding prostate cancer, correction factors were developed to calculate tumor volumes from microscopic slides with the aim of compensating for shrinkage of prostate tissue due to fixation and processing. However, also this method was shown to be vulnerable, since the available tissue-shrinkage correction factors may not be applicable for all laboratories on account of interlaboratory variations in tissue-processing procedures or differences in measuring shrinkage [25]. Moreover, some calculated tumor volumes that have been used for prognostic thresholds may be too high because of inflated tissue-shrinkage correction factors [25].

Shrinkage is not just a phenomenon in fixation and processing of paraffin sections, but is also well-known in processing frozen sections. In an interesting study by Gardner et al. the degree of tissue shrinkage was assessed in specimens from Mohs micrographic surgery [5]. These were processed using routine frozen sections and subsequent hematoxylin and eosin staining. The group revealed significant alterations in

Table 3
Correlation between paraffin and frozen sections.

	R	95% confidence interval	Standard deviation	P-value
paraffin_frozen (median)	0.874	0.700; 1,036	0.083	< 0.001
paraffin_frozen (mean value)	0.869	0.696; 1.041	0.085	< 0.001

Linear regression analyses calculating Pearson's correlation coefficient with a significance level at P < 0.05. N = 36. R: Pearson's correlation.

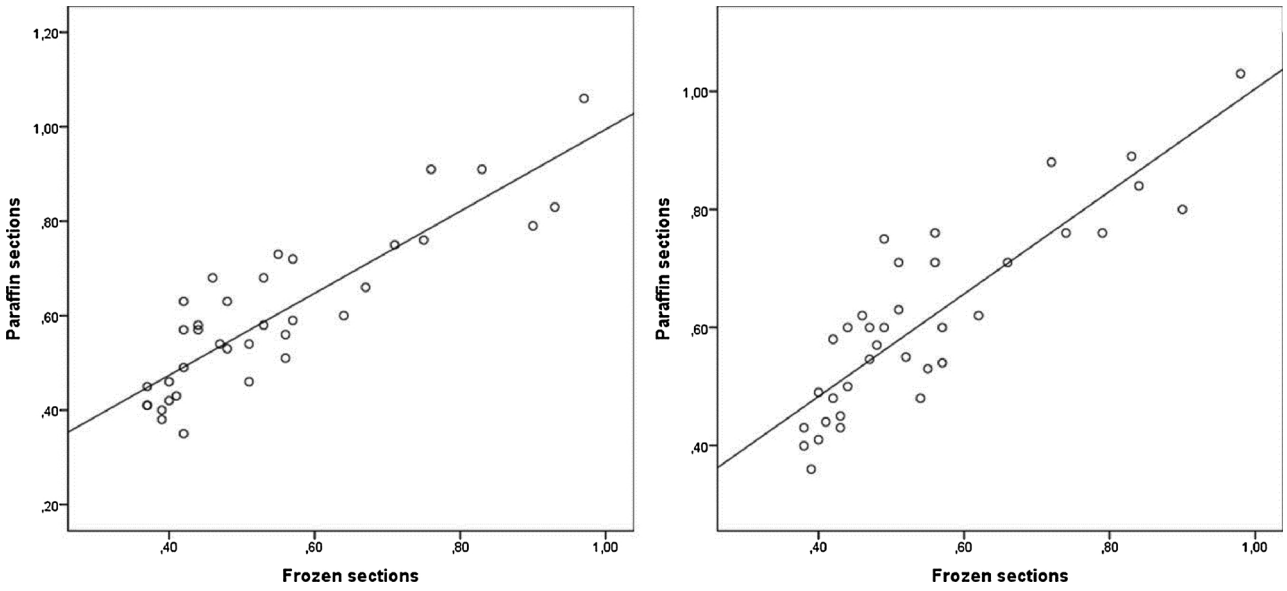

Fig. 3. Correlation between paraffin and frozen sections.
Scatterplots showing the correlation between paraffin and frozen section of median (left) and mean value (right). The scale bar is scored in the measurement unit mm.

length, with specimens being 12% shorter after histological processing compared to the measurements of the same specimens obtained immediately after surgical excision. Tissue specimens obtained from the trunk or extremities showed a greater degree of tissue shrinkage (16%) than specimens obtained from the head and neck (10%) [5]. In the present study, for the first time the bronchial wall thickness in paraffin and frozen sections was compared. A significant difference could be found between both groups, and high correlation between the wall thickness estimation on the frozen sections and paraffin sections was found. These are important results for current clinical routine practice as well as for future studies. For example, by using airway analysis in CT images it could be shown that bronchial dimensions depend on the smoking status. Smoking-induced airway remodeling can be partially reversible after smoking cessation even in long-term heavy smokers [50]. In another study, CT based airway analysis was used to show that bronchial thermoplasty reduced airway wall thickness in patients with severe asthma [51]. For the evaluation and improvement of radiological methods, the results of the present study concerning differences in airway dimension between paraffin and frozen sections are of great importance.

5. Conclusions

For the first time, tissue shrinkage was compared in paraffin and frozen sections of the bronchus wall. In the present animal study bronchus wall thickness significantly differed between the two methods. These results are of interest in both daily surgical pathology routine, e. g. the postoperative evaluation of tumor-free margins, and in scientific issues, since bronchus wall thickness represents a relevant marker for pathological interpretation especially by CT image analysis.

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Declaration of interest

None.

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References

[1] D.H. Abramson, A.C. Schefler, D. Almeida, R. Folberg, Optic nerve tissue shrinkage during pathologic processing after enucleation for retinoblastoma, Arch. Ophthalmol. 121 (1) (2003) 73–75.
[2] M. Noguchi, S. Furuya, T. Takeuchi, S. Hirohashi, Modified formalin and methanol fixation methods for molecular biological and morphological analyses, Pathol. Int. 47 (10) (1997) 685–691.
[3] C.B. Moelans, N. ter Hoeve, J.-W. van Ginkel, F.J. ten Kate, P.J. van Diest, Formaldehyde substitute fixatives. Analysis of macroscopy, morphologic analysis, and immunohistochemical analysis, Am. J. Clin. Pathol. 136 (4) (2011) 548–556, https://doi.org/10.1309/ajcphh1b0cocbgom.
[4] C.H. Fox, F.B. Johnson, J. Whiting, P.P. Roller, Formaldehyde fixation, J. Histochem. Cytochem. 33 (8) (1985) 845–853.
[5] E.S. Gardner, W.T. Sumner, J.L. Cook, Predictable tissue shrinkage during frozen section histopathologic processing for Mohs micrographic surgery, Dermatol. Surg. 27 (9) (2001) 813–818.
[6] M.J. Doughty, J.P. Bergmanson, Y. Blocker, Shrinkage and distortion of the rabbit corneal endothelial cell mosaic caused by a high osmolality glutaraldehyde-formaldehyde fixative compared to glutaraldehyde, Tissue Cell 29 (5) (1997) 533–547.
[7] R. Hanschke, F. Schauer, Improved ultrastructural preservation of yeast cells for scanning electron microscopy, J. Microsc. 184 (Pt 2) (1996) 81–87.
[8] D. Gusnard, R.H. Kirschner, Cell and organelle shrinkage during preparation for scanning electron microscopy: effects of fixation, dehydration and critical point drying, J. Microsc. 110 (1) (1977) 51–57.
[9] L. Wollweber, R. Stracke, U. Gothe, The use of a simple method to avoid cell shrinkage during SEM preparation, J. Microsc. 121 (Pt 2) (1981) 185–189.
[10] N.S. Goldstein, A. Soman, J. Sacksner, Disparate surgical margin lengths of colorectal resection specimens between in vivo and in vitro measurements. The effects of surgical resection and formalin fixation on organ shrinkage, Am. J. Clin. Pathol. 111 (3) (1999) 349–351.
[11] M.J.J. Kerns, M.A. Darst, T.G. Olsen, M. Fenster, P. Hall, S. Grevey, Shrinkage of cutaneous specimens: formalin or other factors involved? J. Cutan. Pathol. 35 (12) (2008) 1093–1096, https://doi.org/10.1111/j.1600-0560.2007.00943.x.
[12] D.H. Nicholson, S. Frazier-Byrne, M.T. Chiu, J. Schiffman, J.R. Hughes, E.K. Novinski, Echographic and histologic tumor height measurements in uveal melanoma, Am. J. Ophthalmol. 100 (3) (1985) 454–457.
[13] D.A. Hoeltzel, P. Altman, K. Buzard, K. Choe, Strip extensiometry for comparison of the mechanical response of bovine, rabbit, and human corneas, J. Biomech. Eng. 114 (2) (1992) 202–215.
[14] F.M. Golomb, J.P. Doyle, C.M. Grin, A.W. Kopf, M.K. Silverman, M.J. Levenstein, Determination of preexcision surgical margins of melanomas from fixed-tissue specimens, Plast. Reconstr. Surg. 88 (5) (1991) 804–809.
[15] M.K. Silverman, F.M. Golomb, A.W. Kopf, C.M. Grin-Jorgensen, K.A. Vossaert, J.P. Doyle, M.J. Levenstein, Verification of a formula for determination of

preexcision surgical margins from fixed-tissue melanoma specimens, J. Am. Acad. Dermatol. 27 (2 Pt 1) (1992) 214–219.
[16] W. Salmhofer, E. Rieger, H.P. Soyer, J. Smolle, H. Kerl, Influence of skin tension and formalin fixation on sonographic measurement of tumor thickness, J. Am. Acad. Dermatol. 34 (1) (1996) 34–39.
[17] J.L. Miller, M.J. Dark, Evaluation of the effect of formalin fixation on skin specimens in dogs and cats, PeerJ 2 (2014) e307, https://doi.org/10.7717/peerj.307.
[18] H.V. Danesh-Meyer, P.J. Savino, J.R. Bilyk, R.C. Eagle, R.C. Sergott, Shrinkage: fact or fiction? Arch. Ophthalmol. 119 (8) (2001) 1217.
[19] P.B. Dobrin, Effect of histologic preparation on the cross-sectional area of arterial rings, J. Surg. Res. 61 (2) (1996) 413–415, https://doi.org/10.1006/jsre.1996.0138.
[20] J.M. Lee, R. Corrente, S.A. Haberer, The bovine pericardial xenograft: II. Effect of tethering or pressurization during fixation on the tensile viscoelastic properties of bovine pericardium, J. Biomed. Mater. Res. 23 (5) (1989) 477–489, https://doi.org/10.1002/jbm.820230503.
[21] R.E. Johnson, J.D. Sigman, G.F. Funk, R.A. Robinson, H.T. Hoffman, Quantification of surgical margin shrinkage in the oral cavity, Head Neck 19 (4) (1997) 281–286.
[22] D. Brotherston, I. Poon, R. Peerani, S. Raphael, K. Higgins, D. Enepekides, J. Lee, J. Davidson, M. Yaffe, Tumor shrinkage associated with whole-mount histopathologic techniques in oral tongue carcinoma, Pathol. Res. Pract. (2015), https://doi.org/10.1016/j.prp.2015.01.009.
[23] C.-H. Chen, M.-Y. Hsu, R.-S. Jiang, S.-H. Wu, F.-J. Chen, S.-A. Liu, Shrinkage of head and neck cancer specimens after formalin fixation, J. Chin. Med. Assoc. 75 (3) (2012) 109–113, https://doi.org/10.1016/j.jcma.2012.02.006.
[24] J. Vent, C. Zimmermann, U. Drebber, I. Wedemeyer, H.E. Eckel, K.B. Huettenbrink, S.F. Preuss, Influence of formalin fixation on tissue dimensions in palatal tonsils, Pathol. Res. Pract. 210 (1) (2014) 59–61, https://doi.org/10.1016/j.prp.2013.10.002.
[25] A.R. Schned, K.J. Wheeler, C.A. Hodorowski, J.A. Heaney, M.S. Ernstoff, R.J. Amdur, R.D. Harris, Tissue-shrinkage correction factor in the calculation of prostate cancer volume, Am. J. Surg. Pathol. 20 (12) (1996) 1501–1506.
[26] A. Mouritzen Dam, Shrinkage of the brain during histological procedures with fixation in formaldehyde solutions of different concentrations, J. Hirnforsch. 20 (2) (1979) 115–119.
[27] K. Overgaard, P. Meden, Influence of different fixation procedures on the quantification of infarction and oedema in a rat model of stroke, Neuropathol. Appl. Neurobiol. 26 (3) (2000) 243–250.
[28] K.F. Siu, H.C. Cheung, J. Wong, Shrinkage of the esophagus after resection for carcinoma, Ann. Surg. 203 (2) (1986) 173–176.
[29] K. Søndenaa, K.H. Kjellevold, A prospective study of the length of the distal margin after low anterior resection for rectal cancer, Int. J. Colorectal Dis. 5 (2) (1990) 103–105.
[30] J. Rieger, S. Twardziok, H. Huenigen, R.M. Hirschberg, J. Plendl, Porcine intestinal mast cells. Evaluation of different fixatives for histochemical staining techniques considering tissue shrinkage, Eur. J. Histochem. 57 (3) (2013) e21, https://doi.org/10.4081/ejh.2013.e21.
[31] H. Lum, W. Mitzner, Effects of 10% formalin fixation on fixed lung volume and lung tissue shrinkage. A comparison of eleven laboratory species, Am. Rev. Respir. Dis. 132 (5) (1985) 1078–1083.
[32] S. Sutinen, P. Pääkko, R. Lahti, Post-mortem inflation, radiography, and fixation of human lungs. A method for radiological and pathological correlations and morphometric studies, Scand. J. Respir. Dis. 60 (1) (1979) 29–35.
[33] T. Achenbach, O. Weinheimer, C. Brochhausen, D. Hollemann, B. Baumbach, A. Scholz, C. Düber, Accuracy of automatic airway morphometry in computed tomography-correlation of radiological-pathological findings, Eur. J. Radiol. 81 (1) (2012) 183–188, https://doi.org/10.1016/j.ejrad.2010.09.012.
[34] C. Schmitt, Bronchialwandvermessung in der modernen Diagnostik - Vergleich histologischer und bildgebender Verfahren im Tierversuch, Springer Research, Wiesbaden, Germany, 2017.
[35] M. Kimura, N. Tayama, R.W. Chan, Geometrical deformation of vocal fold tissues induced by formalin fixation, Laryngoscope 113 (4) (2003) 607–613, https://doi.org/10.1097/00005537-200304000-00005.
[36] H. Boonstra, J.W. Oosterhuis, A.M. Oosterhuis, G.J. Fleuren, Cervical tissue shrinkage by formaldehyde fixation, paraffin wax embedding, section cutting and mounting, Virchows Arch. A Pathol. Anat. Histopathol. 402 (2) (1983) 195–201.
[37] D. Bucher, M. Scholz, M. Stetter, K. Obermayer, H.J. Pflüger, Correction methods for three-dimensional reconstructions from confocal images: I. Tissue shrinking and axial scaling, J. Neurosci. Methods 100 (1-2) (2000) 135–143.
[38] P.-K. Hsu, H.-C. Huang, C.-C. Hsieh, H.-S. Hsu, Y.-C. Wu, M.-H. Huang, W.-H. Hsu, Effect of formalin fixation on tumor size determination in stage I non-small cell lung cancer, Ann. Thorac. Surg. 84 (6) (2007) 1825–1829, https://doi.org/10.1016/j.athoracsur.2007.07.016.
[39] R.W. Mazzone, S. Kornblau, C.M. Durand, Shrinkage of lung after chemical fixation for analysis of pulmonary structure-function relations, J. Appl. Physiol. Respir. Environ. Exerc. Physiol. 48 (2) (1980) 382–385.
[40] N.R. Abu-Rustum, D.S. Chi, B.A. Wiatrowska, G. Guiter, P.E. Saigo, R.R. Barakat, The accuracy of frozen-section diagnosis in metastatic breast and colorectal carcinoma to the adnexa, Gynecol. Oncol. 73 (1) (1999) 102–105, https://doi.org/10.1006/gyno.1998.5312.
[41] Z. Ahmad, M.A. Barakzai, R. Idrees, Y. Bhurgri, Correlation of intra-operative frozen section consultation with the final diagnosis at a referral center in Karachi, Pakistan, Indian J. Pathol. Microbiol. 51 (4) (2008) 469–473.
[42] P. Baker, E. Oliva, A practical approach to intraoperative consultation in gynecological pathology, Int. J. Gynecol. Pathol. 27 (3) (2008) 353–365, https://doi.org/10.1097/PGP.0b013e31815c24fe.
[43] N. Behtash, M. Karimi Zarchi, B. Hamedi, F. Azmoode Ardalan, A. Tehranian, The value of frozen sectioning for the evaluation of resection margins in cases of conization, Arch. Gynecol. Obstet. 276 (5) (2007) 529–532, https://doi.org/10.1007/s00404-007-0384-7.
[44] D. Boriboonhirunsarn, A. Sermboon, Accuracy of frozen section in the diagnosis of malignant ovarian tumor, J. Obstet. Gynaecol. Res. 30 (5) (2004) 394–399, https://doi.org/10.1111/j.1447-0756.2004.00218.x.
[45] S.S. Connolly, F.T. D'Arcy, H.C. Bredin, J. Callaghan, M.O. Corcoran, Value of frozen section analysis with suspected testicular malignancy, Urology 67 (1) (2006) 162–165, https://doi.org/10.1016/j.urology.2005.07.041.
[46] E.K. Dankwa, J.D. Davies, Frozen section diagnosis: an audit, J. Clin. Pathol. 38 (11) (1985) 1235–1240.
[47] P. Geomini, G. Bremer, R. Kruitwagen, B.W.J. Mol, Diagnostic accuracy of frozen section diagnosis of the adnexal mass: a metaanalysis, Gynecol. Oncol. 96 (1) (2005) 1–9, https://doi.org/10.1016/j.ygyno.2004.09.042.
[48] R. Golouh, M. Bracko, Accuracy of frozen section diagnosis in soft tissue tumors, Mod. Pathol. 3 (6) (1990) 729–733.
[49] A. Sienko, T.C. Allen, D.S. Zander, P.T. Cagle, Frozen section of lung specimens, Arch. Pathol. Lab. Med. 129 (12) (2005) 1602–1609, https://doi.org/10.1043/1543-2165(2005)129[1602:fsols]2.0.co;2.
[50] B.J. Jobst, O. Weinheimer, T. Buschulte, M. Trauth, J. Tremper, S. Delorme, N. Becker, E. Motsch, M.L. Gross, A. Trotter, M. Eichinger, H.U. Kauczor, M.O. Wielputz, Longitudinal airway remodeling in active and past smokers in a lung cancer screening population, Eur. Radiol. (2018), https://doi.org/10.1007/s00330-018-5890-4.
[51] P. Konietzke, O. Weinheimer, M.O. Wielputz, W.L. Wagner, P. Kaukel, R. Eberhardt, C.P. Heussel, H.U. Kauczor, F.J. Herth, M. Schuhmann, Quantitative CT detects changes in airway dimensions and air-trapping after bronchial thermoplasty for severe asthma, Eur. J. Radiol. 107 (2018) 33–38, https://doi.org/10.1016/j.ejrad.2018.08.007.