



Validation and optimization of the diatom L/D ratio as a diagnostic marker for drowning

Dominik Hagen¹ · Stefan Pittner² · Jian Zhao³ · Astrid Obermayer¹ · Walter Stoiber¹ · Peter Steinbacher¹ · Fabio C. Monticelli² · Walther Gotsmy²

Received: 2 December 2022 / Accepted: 8 February 2023 / Published online: 4 March 2023
© The Author(s) 2023

Abstract

If a dead body is discovered in water, it nearly always raises the question about the cause of death, often associated with the persistent problem to differentiate between a drowning incident and post-mortem immersion. In numerous cases, a reliable confirmation of death by drowning is often only possible by a combination of diagnoses obtained from autopsy and additional investigations. As to the latter, the use of diatoms has been suggested (and debated) since decades. Based on the consideration that diatoms are present in almost every natural waterbody and are unavoidably incorporated when water is inhaled, their presence in the lung and other tissues can provide evidence of drowning. However, the traditional diatom test methods are still subject of controversial discussion and suspected of erroneous outcome, predominantly through contamination. A promising alternative to minimize the risk of erroneous outcome seems to be disclosed by the recently suggested MD-VF-Auto SEM technique. Especially the establishment of a new diagnostic marker (L/D ratio), which represents the factorial proportion between the diatom concentration in lung tissue and the drowning medium, allows for clearer distinction of drowning and post-mortal immersion and is largely robust to contamination. However, this highly elaborated technique requires specific devices which are frequently unavailable. We therefore developed a modified method of SEM-based diatom testing to enable the use on more routinely available equipment. Process steps such as digestion, filtration, and image acquisition were thoroughly broken down, optimized, and ultimately validated in five confirmed drowning cases. Taking certain limitations into consideration, L/D ratio analysis provided promising results, even in cases of advanced decomposition. We conclude that our modified protocol indeed opens a way for a broader use of the method in forensic drowning investigation.

Keywords Diatoms · Drowning · L/D value · SEM

Introduction

The diagnosis of drowning is a very difficult, and yet crucial aspect in forensic routine. Although there are numerous known macroscopic and microscopic indicators of drowning, most of them are insufficiently specific, only transiently applicable, and/or suffer from significant limitations

depending upon injury, drowning medium, and/or post-mortem interval (PMI) [1, 2]. Especially in the case of advanced decomposition, conventional methods have limited scope. In search of a method that can provide reliable and unambiguous evidence, one repeatedly comes across the examination of organs for diatoms. These microscopic algae, present in almost every natural waterbody, are assumed to be incorporated by inhalation of the drowning medium during the drowning process and to (at least partially) pass the alveolo-capillary membrane, thus reaching distinct organs through distribution via the bloodstream [3]. If the examination of organs distant to the lungs, like the liver, kidney, or bone marrow, exposes a certain amount of diatoms, this can be regarded as supportive evidence for drowning. The previous methodology of the diatom test included the digestion of biological tissues with strong acids, followed by purification

✉ Walther Gotsmy
walther.gotsmy@plus.ac.at

¹ Department of Ecology and Evolution, Paris-Lodron University of Salzburg, Salzburg, Austria

² Department of Forensic Medicine and Forensic Psychiatry, Paris-Lodron University of Salzburg, Salzburg, Austria

³ Guangzhou Forensic Science Institute & Key Laboratory of Forensic Pathology, Ministry of Public Security, Guangzhou, China

and deacidification steps, and lastly the assessment of the acid-stable silica diatom frustules via light microscopy [4].

Over the years, various studies repeatedly confirmed or criticized the supportive evidence of the diatom test, not least due to its vulnerability for contamination effects and false positive results in non-drowning cases [5]. Especially the examination of diatoms in lung tissue leaves this subject in controversial discussion, as diatoms are presumed to infiltrate the lungs post-mortem during the submersion period [5, 6].

A recently suggested method, the microwave digestion–vacuum filtration–automated scanning electron microscopy technique (MD-VF-Auto SEM), appears to be a promising new development in the field of diatom examination to minimize erroneous results from contamination and diatom loss during centrifugation steps. Combining diatom-sensitive microwave digestion and membrane filtration with automated scanning electron microscopy, this method achieves a remarkable quality of diatom recovery and ensures qualitative and quantitative examination at high resolution [7]. The additional establishment of a diagnostic marker (L/D ratio), which represents the proportion between the diatom concentration in 1g of lung tissue and 1ml of drowning medium, allows clearer distinction between drowning and post-mortal immersion, as an active aspiration of fluid in the case of drowning results in a relatively higher concentration of diatoms in the lung tissue than in the drowning medium (L/D ratio >1), whereas in the case of post-mortal immersion, the diatom concentration in lung tissue can at most reach equality with the concentration of the drowning medium (L/D ratio ≤ 1) [8]. Despite these advantages, this highly elaborated technique impedes routine application by requiring expensive “high-tech” devices including automated SEM for reliable diatom identification and counting, which are frequently unavailable.

In order to enable routine application on existing equipment, process steps as digestion, filtration, and image acquisition were thoroughly broken down, optimized, and ultimately validated in confirmed drowning cases, without detracting the method’s reliability and precision.

Material and methods

Sample collection

All tissue samples analyzed in the present study were collected with thoroughly cleaned instruments during routine autopsies at the Department of Legal Medicine of the University of Salzburg. For each case, approximately 10 g of lung tissue (left superior lobe) was removed and preserved at –20°C until further investigations. Additionally, 10 g

of liver and kidney tissue was collected and stored under the same conditions to test diatom presence in peripheral tissues.

Water samples and putative drowning media (required for comparison, respectively L/D calculations) were collected in clean plastic bottles and sampled with caution, to avoid extraction close to the surface or close to benthic layers (minimum distance 20 cm, if possible).

Control samples for protocol optimization

Lung tissue samples of three confirmed drowning cases served as controls (A, B, C) to evaluate, modify, and optimize the digestion procedure with nitric acid and hydrogen peroxide. In addition, these samples were tested for regular diatom dispersion and the correlation between diatom quantity and investigated tissue mass by assessing the number of diatoms from lung tissue samples of different weight. This was particularly important to enable adjustment of the tissue mass (dilution) in the case of membrane clogging or diatom-overload on the membrane, or to enhance digestive capability.

SEM image acquisition procedures were tested and validated at three dilutions (1:1, 1:2, 1:5) of diatom-rich water samples from a local pond.

Study cases

Five autopsy cases (three males, two females, all found during spring/summer) were selected to validate the adapted processing conditions and to perform L/D ratio calculations. Corresponding drowning media was collected by the police upon discovery of the bodies and stored in dark environment at 4°C until further analysis.

Four of the cases presented distinct classical drowning signs, such as emphysema aquosum, foamy liquid in the airways, splenic anemia, and/or liquid in the sphenoid sinuses (Svechnikov’s sign), and thus were diagnosed as drowning cases. By contrast, the autopsy of case 4, which was in a state of advanced decomposition, solely exhibited liquid in the sphenoid sinuses and drowning was only presumed. For each case, 0.5–1.0 g lung tissue and 10 ml putative drowning medium were processed and digested under previously established conditions (see the “[Acid digestion and filtration](#)” section) and analyzed via SEM (see the “[SEM analysis and image acquisition](#)” section) to enable calculation of respective L/D ratios. Notably, the putative drowning medium of case 5 contained a significant amount of debris (plant particles), but was also processed as described. Study case data are presented in Table 1.

Table 1 Demographic data and autopsy findings of five potential drowning cases. *EA* emphysema aquosum, *FL* foamy liquid in air passages, *LSC* liquid stomach content, *SA* splenic anemia, *SS* Svehnikov's sign

Case no.	Gender	Age	Season	Water body	Drowning signs	Additional findings
1	Male	70	Spring	River	EA, LSC, SS	-
2	Female	73	Summer	River	EA, SS, SA	-
3	Female	75	Spring	River	EA, FL	-
4	Male	53	Spring	River	SS	Advanced decomposition
5	Male	80	Summer	Pond	FL, SA, SS,	Debris in the drowning medium

Contamination tests

To rule out false positive tests due to diatom-containing chemicals and contamination effects during sample processing, digestion reagents (nitric acid, hydrogen peroxide) and all cleaning and rinsing components (ultrapure water, tap water, ethanol) were filtrated onto acid-stable membranes and separately investigated for diatom content via SEM. In addition, a diatom-rich water sample was subjected to an evaporation test to rule out possible diatom loss and/or cross-contamination of samples during the digestion process. For this purpose, the digestion tube was fitted with a membrane underneath its cap. All reagents and other liquids, just as the evaporation membrane, were found entirely free of diatoms (see Supplements 1).

Acid digestion and filtration

Overall preparation was conducted under sterile conditions and high safety precautions. All samples (i.e., study case tissues, control tissues, drowning media, and water controls) were transferred into 50-ml screw cap plastic tubes (Greiner) and as an essential safety measure, tube caps were provided with small perforation holes to enable gas emission. Samples were then treated with a digestive solution of nitric acid (HNO₃ 65%, CarlRoth) and hydrogen peroxide (H₂O₂ 30%, CarlRoth) while being heated in a water bath of 100°C until the solutions turned clear (at least for 2h) and afterwards left at room temperature for cooling. Instead of conventional deacidification by repetitive centrifugation and replacement of the supernatant, samples were then directly filtrated through acid-stable polyvinylidenefluoride (PVDF) membrane filters (Ø = 1.0 cm, pore size 0.45 µm) with a custom-built syringe pump system comprising NEMA 17 stepper motors linked to A4983 Big Easy driver chips and a PurrData-software-operated Teensy 3.2 microcontroller (Fig. 1). Membranes were subsequently deacidified with ultrapure water, desiccated with pure ethanol, and air dried at 40°C. As the digestive solution of lung tissue can contain relevant amounts of organic residues which potentially clog the membrane filters, provision was made to ascertain best conditions of organic matter digestion. Therefore, three different volumes of the nitric acid–hydrogen peroxide medium (5 ml, 10 ml, 15 ml) were each applied to 0.5 g, 1.0 g, and

1.5 g lung tissue control samples to test their digestive capability. In more detail, three samples of each weight were respectively digested with 4 ml HNO₃ + 1 ml H₂O₂, 8 ml HNO₃ + 2 ml H₂O₂, and 12 ml HNO₃ + 3 ml H₂O₂, and further processed as described above. Membranes were then qualitatively examined for the presence of diatom fragments or incompletely digested tissue remnants via SEM.

Based on the related results (see the “SEM imaging” section), study case tissue samples of 0.5–1.0 g weight and 10 ml of reference liquids (drowning media/water controls) were equally treated with a 10 ml batch of the HNO₃/H₂O₂ digestion medium.

SEM analysis and image acquisition

After being attached to aluminum pin stubs with double-sided adhesive carbon tabs, membranes were sputter-coated with gold and analyzed in a Philips/FEI XL30 ESEM scanning electron microscope at 15 kV.

Peripheral tissues (liver and kidney) were qualitatively examined for the presence of diatoms by classification into one of the following categories: – (0 diatoms), + (1–4 diatoms), ++ (5–9 diatoms), or +++ (10 or more diatoms).

To quantitatively assess lung and water samples, the membranes were manually scanned at a magnification of 1000× to cover areas of appropriate size ensuring representative quantification while also allowing for easy identification of small diatoms.

Due to the limitation of non-automated (manual) SEM imaging, full coverage of the membranes would hardly be feasible as requiring more than 1700 images. Therefore, two alternative strategies with reduced imaging demand—transectial acquisition and scatter acquisition—were applied on diatom-rich water samples in three dilutions (1:1, 1:2, 1:5) and tested for their time requirement and capability to assess diatom abundance of ≥ 95 % accuracy. Transectial image acquisition was performed in line of the filter's diameter as quarter, half, full, and double transect, whereas scatter image acquisition was performed as eighth, quarter, half, and full scatter at uniformly distributed coordinates (Fig. 2). Both strategies were evaluated concerning their efficiency (number of required images) to reach an extrapolation-accuracy of 95% to the total diatom count (i.e., double transects plus full scatter).

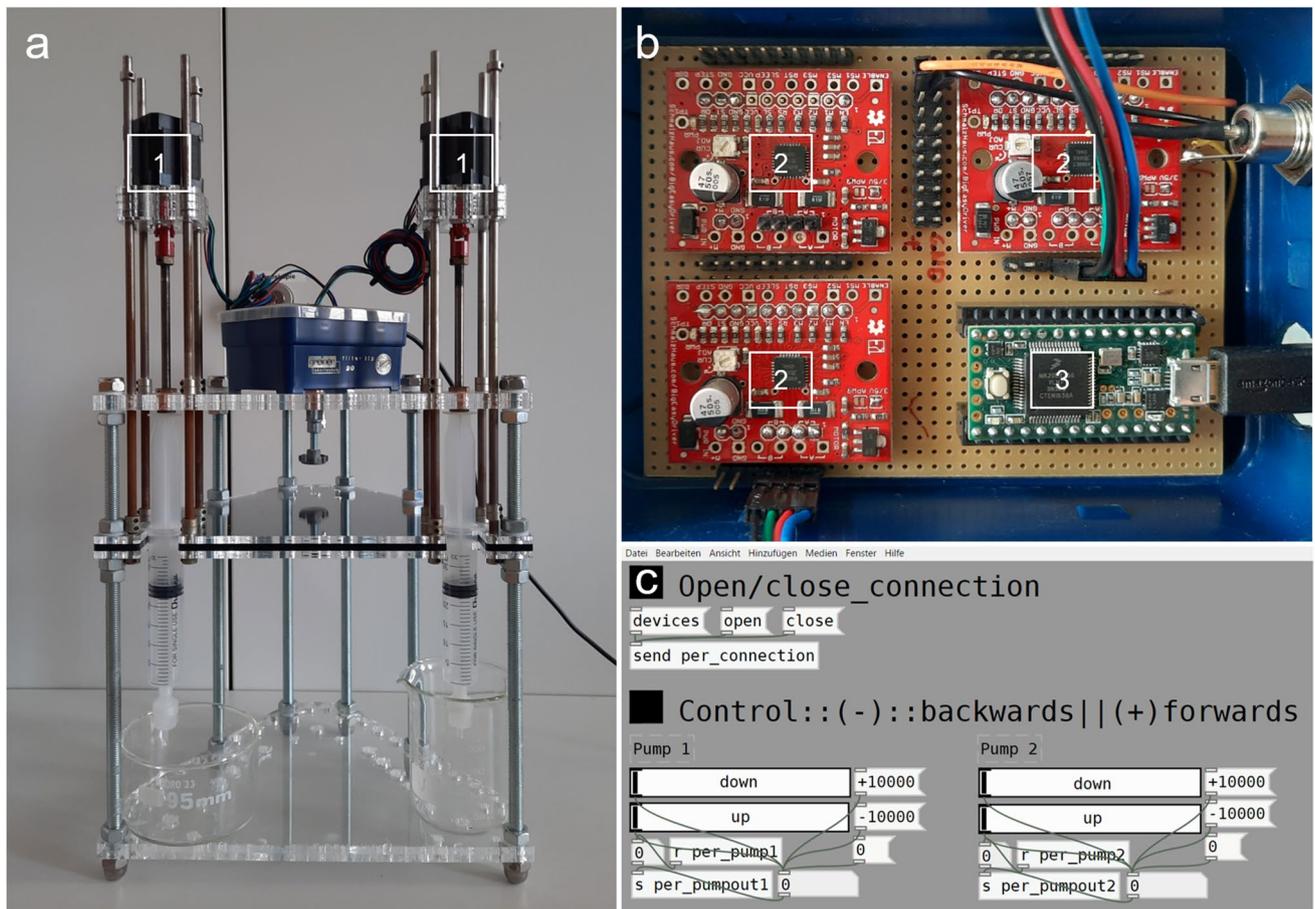
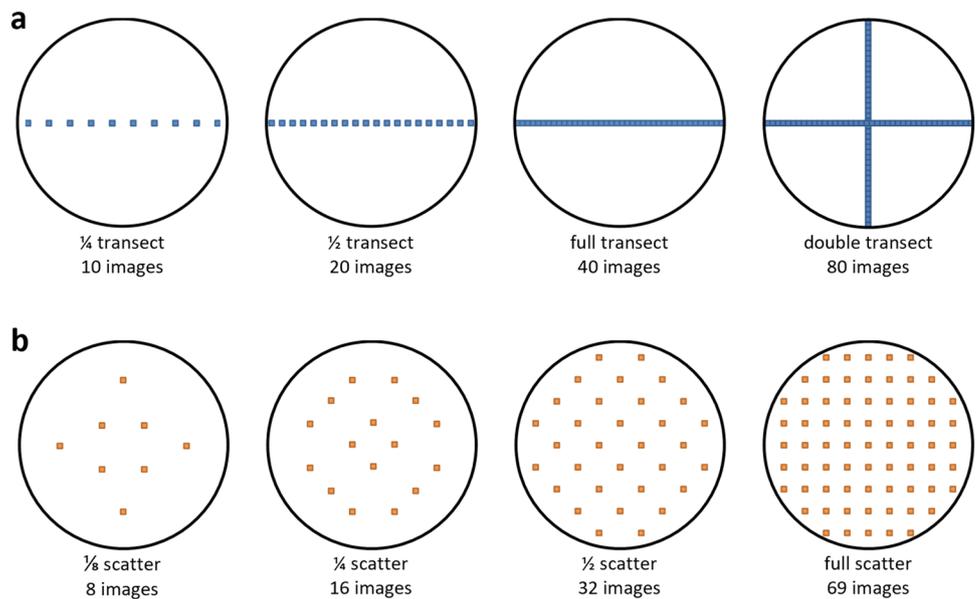


Fig. 1 Filtration apparatus; **A** syringe-pump-system with stepper motors (1), **B** chip-setup with Big Easy drivers (2) and teensy microcontroller (3), and **C** operating software (PurrData); **filtration time: approx. 17 ml/min (7500 rpm)**

Fig. 2 SEM imaging methods; **a** transectal image acquisition (quarter, half, full and double transects) and **b** scatter image acquisition (eight, quarter, half and full scatter)



Data processing and statistical analysis

Images of transectal and scatter image acquisition were quantitatively analyzed for diatoms by manual counting, using the cell counter tool of the ImageJ 1.53p software to keep track of the process and document the results. Individual counts were compiled to a total number of diatoms per filter, allowing to calculate diatoms per g values for tissue samples and diatoms per ml values for drowning media, which were then used to determine L/D ratios. Statistical analyses, including correlation analysis between diatom number and tissue weight, diatom count projection, and L/D calculations, were performed using Microsoft

Excel and SPSS 27.0 software, regarding $p < 0.05$ as statistically significant.

Results

Digestive capability

Digestive capability tests, performed with different volumes of the $\text{HNO}_3/\text{H}_2\text{O}_2$ digestion reactant on lung tissue samples of variable weight, showed the following results: The 5-ml batch (4 ml $\text{HNO}_3 + 1$ ml H_2O_2) reached optimal digestion results with samples of 0.5 g (Fig. 3a) but resulted in partial (incomplete) digestion with samples of ≥ 1.0 g (Fig. 3b–c).

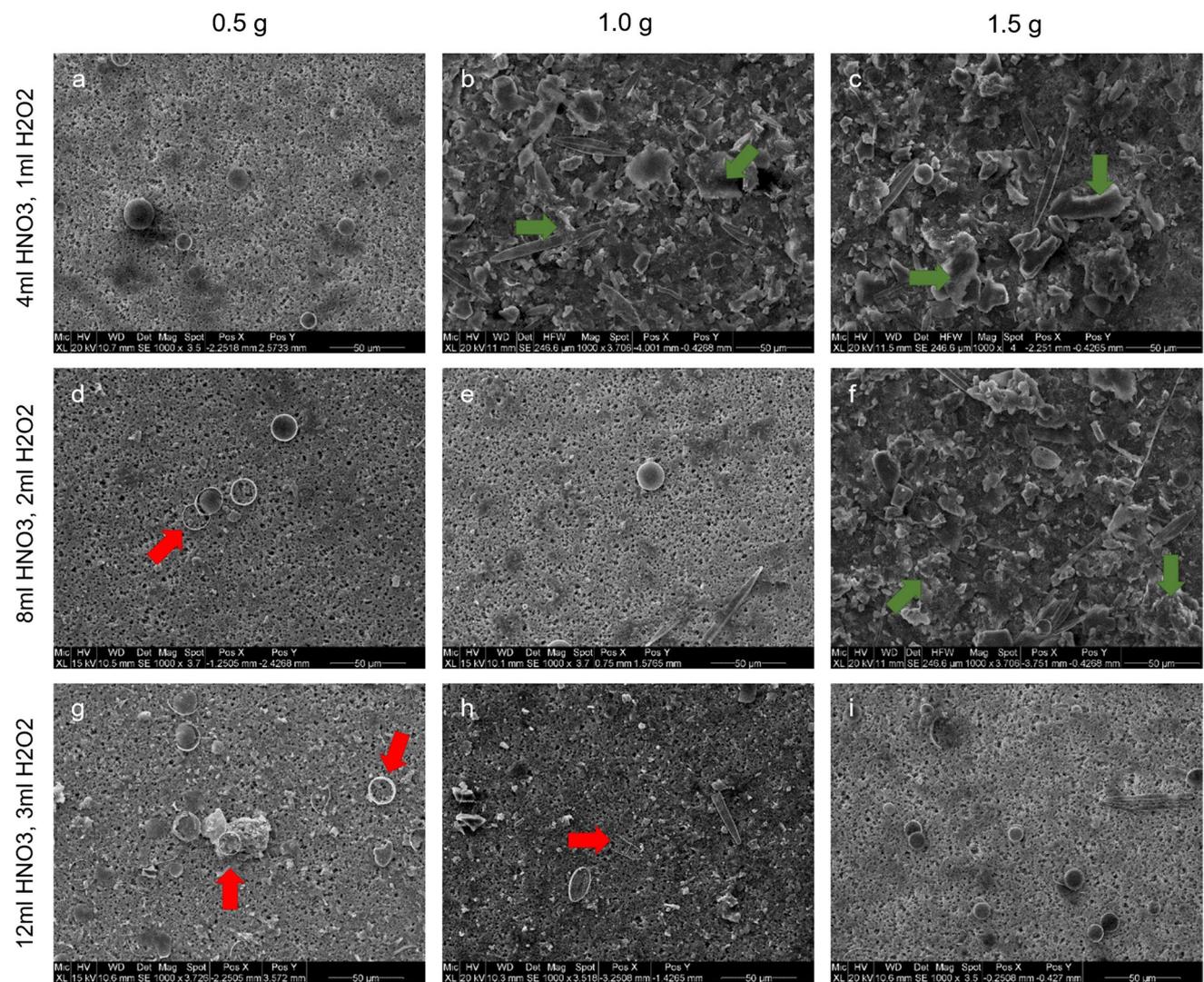


Fig. 3 Digestive capability of different volumes of reagent: 0.5 g (**a**, **d**, **g**), 1.0 g (**b**, **e**, **h**), and 1.5 g (**c**, **f**, **i**) of lung tissue digested with 4 ml nitric acid and 1 ml hydrogen peroxide (**a**, **b**, **c**); 8 ml nitric acid and 2 ml hydrogen peroxide (**d**, **e**, **f**); and 12 ml nitric acid and 3 ml

hydrogen peroxide (**g**, **h**, **i**); green arrows indicate organic remains (tissue residue); red arrows indicate partially disintegrated diatom frustules

By contrast, 10 ml of the reactant (8 ml HNO₃ + 2 ml H₂O₂) was capable to completely dissolve samples of 0.5 g and 1.0 g (Fig. 3d–e) with slight diatom-dissolution at lower tissue weight (Fig. 3d), while tissue residues persisted with 1.5 g (Fig. 3f). Fifteen milliliters of the reagent (12 ml HNO₃ + 3 ml H₂O₂) had a clearly higher digestive potential with 1.5 g samples (Fig. 3i) but promoted diatom disintegration in samples of lower weight (Fig. 3g–h). In addition, higher total volumes required longer filtration times, also potentially affecting diatom integrity.

SEM imaging

Diatom abundances of each imaging strategy (transectial acquisition and scatter acquisition), initially evaluated in three dilutions (1:1, 1:2, 1:5) of diatom-rich control water samples and eventually combined for coherent accuracy assessment, yielded the following results: diatom count extrapolations from quarter transects (10 images), half transects (20 images), and full transects (40 images) achieved accuracy levels of 79.8%, 87.1%, and 92.2%, thus remaining below the required confidence limit of 95%. By contrast, the double transect method reached a value above the limit (95.6%) but required 80 images. Results from scatter acquisition proved distinctly different from those of transect acquisition. While the eighth scatter (8 images), quarter scatter (16 images) and half scatter (32 images) strategies (86.1%, 91.0%, and 93.8%) remained below the confidence limit as their counterparts in transect acquisition, the full scatter method achieved a value of 97% based on the analysis of only 69 images. Calculations to reach the 95% accuracy limit using the obtained logarithmic regression formulae

resulted in theoretical thresholds of 78 images for transectial acquisition and 50 images for scatter acquisition (Fig. 4).

Correlation between diatom quantity and tissue weight

Correlation analyses results between diatom quantity and tissue mass (weight) of three control drowning cases (A, B, C) are summarized in Table 2. The Kruskal-Wallis comparison did not indicate significant differences between corresponding lung tissue samples of each case. Spearman’s correlation analysis showed a positive correlation between diatom number and tissue weight at high significance levels for all samples. Diatoms per gram calculations showed almost identical values for lung tissue samples within each respective case at a maximal deviation of 2.5% (Fig. 5).

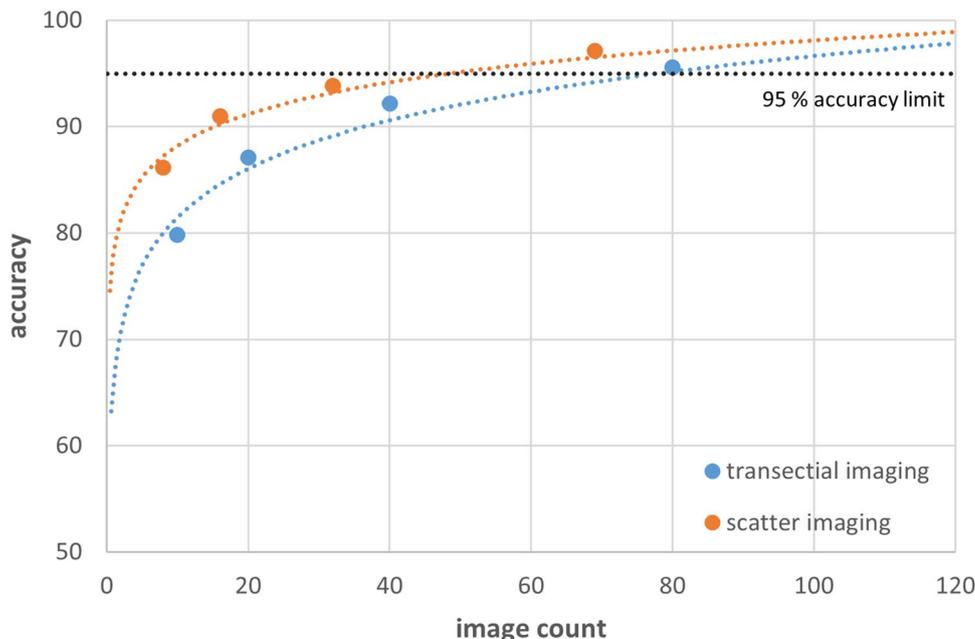
Case analysis applying L/D ratios

All investigated drowning media and lung tissue samples contained diatoms in quantities sufficient for analytical application. Peripheral tissue analysis (qualitative SEM

Table 2 Statistical analysis and correlation of three lung tissue samples of different weight with their respective diatom counts in three different drowning cases

Control cases	A	B	C
Tissue weights	0.5/1.1/1.5	1.0/2.0/3.0	0.4/0.9/1.4
Number of diatoms	57/108/155	207/444/640	55/108/181
The Kruskal-Wallis test	<i>p</i> = 0.149	<i>p</i> = 0.229	<i>p</i> = 0.841
Spearman’s correlation	<i>r</i> = 0.406 <i>p</i> = 0.000	<i>r</i> = 0.681 <i>p</i> = 0.000	<i>r</i> = 0.573 <i>p</i> = 0.000

Fig. 4 Accuracy of transectial image acquisition and scatter imaging compared with the number of images required to reach a 95% accuracy limit; formula of transectial imaging $y = 6.5832\ln(x) + 66.32$; formula of scatter imaging $y = 4.3587\ln(x) + 77.99$



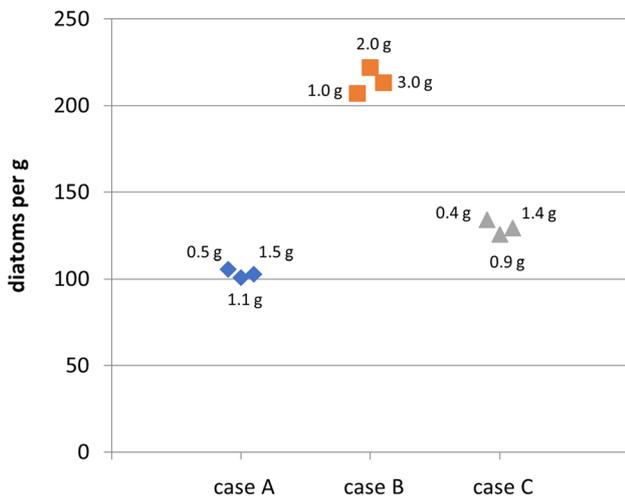


Fig. 5 Correlation between lung tissue samples of different weight and their calculated diatom counts per gram in three control drowning cases

analysis) revealed diatom presence in all kidney samples, as well as in liver tissue samples from cases 1, 4, and 5 (Supplements 2). All cases showed species conformance between tissues and drowning media. Except for case 5, all lung tissue samples displayed higher diatom numbers than the corresponding drowning media, consequently resulting in L/D ratios above 1, even exceeding a value of 2 (Fig. 6), thus showing that the diatom concentration in lung tissue was at least twice as high than in the corresponding drowning

medium. By contrast, case 5—although displaying a relatively high diatom number per gram of lung tissue—reached an L/D ratio of 0.1, as the concentration of diatoms per ml drowning medium reached a value almost ten times higher than in the lung (Table 3).

Discussion

The diatom test has long been a controversial technique in forensic drowning diagnosis, most prominently due to its sensitivity to contamination and risk of incorrect results even in qualitative analysis [5, 9–12]. In recent years, an increasing interest of quantitative diatom testing has promoted re-evaluation of the approach [4, 13–15]. Especially the comparison of diatom content in lung tissue and drowning medium (L/D value) has changed the significance of the method [8]. Rather than utilizing timesaving and less expensive light microscopy, the preference of SEM application empowered diatom-based forensic drowning diagnosis providing much higher resolution and the options for automated scanning and diatom counting [16, 17], as well as species identification by artificial intelligence [18–20]. However, to date the method is only applied in a limited number of institutes, not least because of its technical requirements. Results of the present work enabled the establishment of a protocol for quantitative diatom analysis using/requiring comparably limited resources.

Fig. 6 L/D ratios of cases 1–5 on logarithmic scales of diatom count in lung tissue and drowning medium. L/D values >1 assume drowning, L/D values <1 assume post-mortal immersion

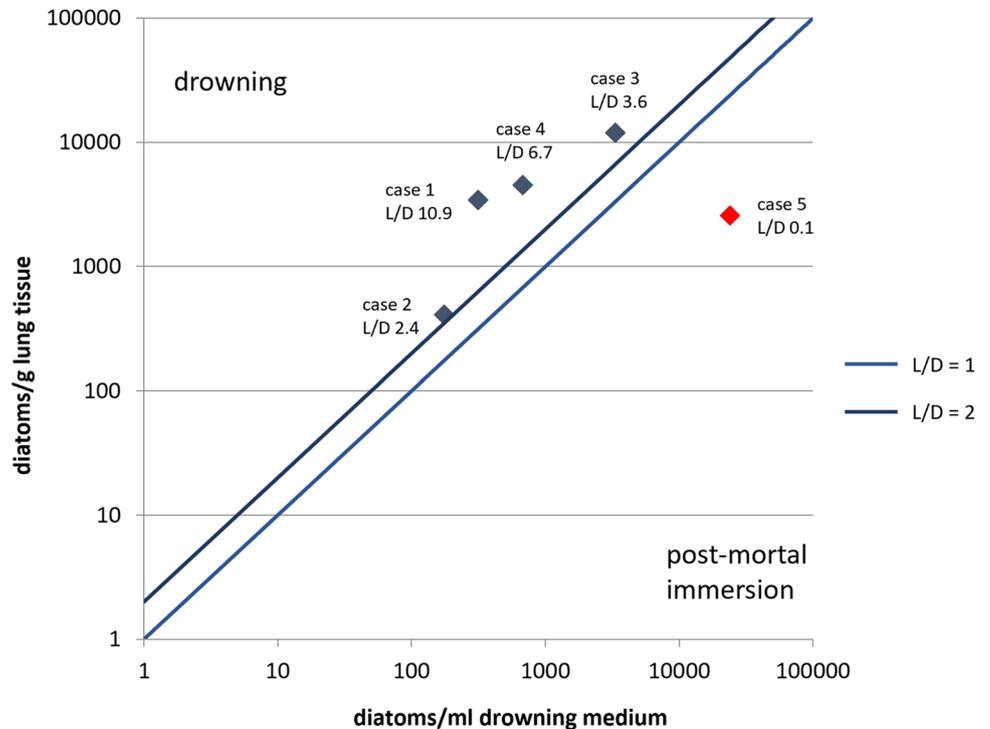


Table 3 Diatoms per gram lung tissue and per ml drowning medium and calculated L/D ratios of cases 1–5

Case no.	Diatoms per g lung tissue	Diatoms per ml drowning medium	L/D ratio
1	3426	315	10.9
2	410	175	2.3
3	11928	3336	3.6
4	4521	675	6.7
5	2567	24148	0.1

Notes on methodology

To warrant the accuracy of the modified method for SEM-based forensic diatom testing, some critical aspects have to be considered:

- (i) All reagents and supportive materials need to be validated for diatom absence. This is of particular relevance as diatomaceous earth is a commonly used filtration aid, traces of which may remain in chemicals [21]. Compared to qualitative investigation, these traces may be deemed marginal in quantitative approaches [15, 22], but are of special importance for bodies of water with low diatom concentrations.
- (ii) Diatom treatment (protocols) should have the capability to assure complete dissolution of all organic matter while leaving diatom frustule integrity intact. It appears optimal to digest tissue samples at around 1.0 g with 10 ml of a digestion medium composed of 8 ml HNO₃ and 2 ml H₂O₂, whereas samples of 0.5 g or less may also be sufficiently digested at smaller volume of the digestion reagent. Analogously, tissue samples over 1.0 g in weight should be treated with a digestive volume larger than 10 ml to reach satisfactory results, which clearly confirmed that the volume of digestion media applied to a certain amount of tissue can substantially influence the quality of the performance, regardless of whether quantification is carried out automated or manually.
- (iii) In this context, the present work specified the necessity to assess the replicability of the methods outcome. We could show that, regardless of the applied tissue mass, calculated diatom concentrations per gram are highly stable. This seems especially important when membranes clog during filtration and/or when the initial analysis results in very high (diatoms in clusters or layers) or very low diatom numbers on the filter. In such cases, it may be necessary to modify (enlarge or reduce) tissue sample volumes in order to ensure a reliable analysis of the diatom number.
- (iv) Another crucial aspect for a wider practical application is to optimize the method's cost and effort without negatively influencing the diagnostic quality. In this respect, a major task was to determine the minimum number of required images to obtain representative data for the entire membrane, irrespective of whether dissolved tissue or diatom-containing water (drowning media) are examined. Experimental conditions revealed that regular diatom distribution on the membrane is not granted. An evaluation of 10 equidistantly scattered images over the filter resulted in a mean error of 12%. This error accumulated to 19% when the same number of images were taken equidistantly along a transect, clearly suggesting that scatter imaging should be preferred over transectal acquisition. Our data indicate that a total of 50 equidistant scatter images distributed over the entire filter should be sufficient to produce reliable results beyond the 95% probability limit.

Significance in practical application

Despite the small sample size, the present trial conducted on five autopsy cases indicates that the modified method of SEM-based diatom testing has indeed true application value in forensic drowning diagnosis.

Previous studies reported drowning probabilities of 96% for L/D values >1 and even 100% for L/D values >2, while yet evincing limited conclusiveness between drowning and post-mortal immersion at values ≤1 [8]. Investigation of the cases 1–3, which all presented distinct classical drowning signs [23, 24], achieved L/D values between 2.4 and 10.9, which therefore can be considered strong evidence for drowning [25–27].

Case 4 showed insufficient evidence to be diagnosed as drowning case based on classical drowning signs, most likely due to advanced decomposition. However, the determined L/D value of 6.7 confirmed drowning as the cause of death, as a 6.7-fold diatom concentration in the lung compared to the drowning medium is very difficult to explain by other mechanisms than active aspiration and pressure filtration of the drowning medium in the lung [22, 28, 29]. This case underlines the diagnostic potential of the modified SEM-based diatom test in cases of advanced decomposition which frequently lack other drowning signs [30].

Case 5, although exhibiting several classical drowning signs, only reached an L/D value of 0.1, rather indicating post-mortal immersion than drowning. However, specific circumstances of this case leave some caveats. The collected drowning medium contained plenty of debris from aquatic plants, which is most likely the cause for the very high number of diatoms detected (>24.000 diatoms per ml), as diatoms are known to colonize on aquatic plants epiphytically [31]. It

must be questioned whether the secured materials represent the actual diatom concentration of the drowning medium at the time the body got into the water. Perhaps, actively aspired water contained less diatoms as suggested by the present analysis, consequently the debris would have had a major effect on the L/D value and the interpretation of the result. This depicts the limitation, that methods of SEM-based diatom testing are inherently dependent on the reliability of the secured drowning medium. Consequently, sampling requires special caution to not distract the water body's diatom homogeneity. Other factors potentially affecting this reliability include sampling from wrong depth or location [32] and delayed sampling, allowing for diatom concentration changes in the waterbody [26, 33], e.g., after heavy weather conditions [34]. In this relation, additional research providing reference data on species and abundances from spatial, temporal, and seasonal diatom mapping of local natural waters, as already implemented in some countries, could greatly improve the validation of drowning media [35, 36].

Conclusion

With some adaptations in sample processing and SEM imaging, we were able to apply a new setup of quantitative diatom assessment at our institute. Our data show that the modified method of SEM-based diatom testing has high potential to become a standard technique in forensic drowning investigation, particularly in cases of advanced decomposition, despite the necessity to critically consider the limitations of the application and outcome interpretation.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s00414-023-02970-x>.

Funding Open access funding provided by Paris Lodron University of Salzburg.

Data availability All data generated or analyzed in course of this study are included in the article.

Declarations

Ethical approval Not applicable. Tissue collection and analysis were case specifically indicated; no additional tissue was specifically collected for research purposes.

Consent to participate Not applicable (see above).

Research involving human participants and/or animals All applicable international, national, and/or institutional guidelines and standards were followed.

Conflict of interest The authors declare no competing interests.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source,

provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

- Piette MHA, De Letter EA (2006) Drowning: still a difficult autopsy diagnosis. *Forensic Sci Int* 163:1–9
- Armstrong EJ, Erskine KL (2018) Investigation of drowning deaths: a practical review. *Acad Forensic Pathol* 8:8–43
- Farrugia A, Ludes B (2010) Diatomeennachweis und -identifizierung: Bedeutung für die Diagnose des Ertrinkungstodes. *Rechtsmedizin* 20:49–58
- Pachar JV, Cameron JM (1993) The diagnosis of drowning by quantitative and qualitative diatom analysis. *Med Sci Law* 33:291–299
- Foged N (1983) Diatoms and drowning: once more. *Forensic Sci Int* 21:153–159
- Neidhart DA, Greendyke RM (1967) The significance of diatom demonstration in the diagnosis of death by drowning. *Am J Clin Pathol* 48:377–382
- Zhao J, Liu C, Hu S, He S, Lu S (2013) Microwave digestion—vacuum filtration-automated scanning electron microscopy as a sensitive method for forensic diatom test. *Int J Legal Med* 127:459–463
- Zhao J, Ma Y, Liu C, Wen J, Hu S, Shi H, Zhu L (2016) A quantitative comparison analysis of diatoms in the lung tissues and the drowning medium as an indicator of drowning. *J Forensic Legal Med* 42:75–78
- Kakizaki E, Shinkawa N, Sonoda A, Yukawa N (2022) Conventional diatom testing using strong acid: notable false-positive results caused by an underestimated contamination source (blind spot). *Forensic Sci Int* 330:111131
- Peabody AJ (1980) Diatoms and drowning - a review. *Med Sci Law* 20:254–261
- Reh H (1968) Zur Diatomeenfrage. *Dtsch Z Für Gerichtl Med* 3
- Schellmann B, Sperl W (1979) Diatomeen-Nachweis im Knochenmark (Femur) Nichtertrunkener. *Z Für Rechtsmed* 83:319–324
- Horton BP, Boreham S, Hillier C (2006) The development and application of a diatom-based quantitative reconstruction technique in forensic science. *J Forensic Sci* 51:643–650
- Hürlimann J, Feer P, Elber F, Niederberger K, Dirnhofer R, Wyler D (2000) Diatom detection in the diagnosis of death by drowning. *Int J Legal Med* 114:6–14
- Lunetta P, Miettinen A, Spilling K, Sajantila A (2013) False-positive diatom test: a real challenge? A post-mortem study using standardized protocols. *Legal Med* 15:229–234
- Wen J, Hu S, Liu C, Dai W, Wang S, Su HF, Zhao J (2012) Automatic scanning by scanning electron microscopy: the first step towards automation of forensic diatom test. In: 2012 Int. Conf. Biomed. Eng. Biotechnol. IEEE, Macau, Macao, pp 754–757
- Yu W, Xue Y, Knoops R et al (2021) Automated diatom searching in the digital scanning electron microscopy images of drowning cases using the deep neural networks. *Int J Legal Med* 135:497–508
- Yu W, Xiang Q, Hu Y et al (2022) An improved automated diatom detection method based on YOLOv5 framework and its preliminary study for taxonomy recognition in the forensic diatom test. *Front Microbiol* 13:963059

19. Zhang J, Zhou Y, Vieira DN et al (2021) An efficient method for building a database of diatom populations for drowning site inference using a deep learning algorithm. *Int J Legal Med* 135:817–827
20. Zhou Y, Zhang J, Huang J et al (2019) Digital whole-slide image analysis for automated diatom test in forensic cases of drowning using a convolutional neural network algorithm. *Forensic Sci Int* 302:109922
21. Shen X, Liu Y, Xiao C, Zheng C, Huang J, Shi H, Xu Q, Cheng J, Liu C, Zhao J (2019) Analysis of false-positive results of diatom test in the diagnosis of drowning—would not be an impediment. *Int J Legal Med* 133:1819–1824
22. Bortolotti F, Del Balzo G, Calza R, Valerio F, Tagliaro F (2011) Testing the specificity of the diatom test: search for false-positives. *Med Sci Law* 51:7–10
23. Madea B (ed) (2015) *Rechtsmedizin*. <https://doi.org/10.1007/978-3-662-43500-7>
24. Schneppe S, Dokter M, Bockholdt B (2021) Macromorphological findings in cases of death in water: a critical view on “drowning signs”. *Int J Legal Med* 135:281–291
25. Kihara Y, Makino Y, Nakajima M, Tsuneya S, Tanaka A, Yamaguchi R, Torimitsu S, Hayama S, Iwase H (2021) Experimental water injection into lungs using an animal model: verification of the diatom concentration test to diagnose drowning. *Forensic Sci Int* 327:110983
26. Li Z, Wu B, Cheng X, Wu Y, Zhang P, Shi H, Zheng D, Cheng J, Liu C, Zhao J (2019) Evaluation of L/D ratio in a water-related case for the differentiation between drowning and postmortem immersion. *Forensic Sci Int Synergy* 1:68–70
27. Zhao J, Liu C, Bardeesi ASA, Wu Y, Ma Y, Hu S, Shi H, Cheng J (2017) The diagnostic value of quantitative assessment of diatom test for drowning: an analysis of 128 water-related death cases using microwave digestion-vacuum filtration-automated scanning electron microscopy. *J Forensic Sci* 62:1638–1642
28. Lunetta P, Penttilä A, Hällfors G (1998) Scanning and transmission electron microscopical evidence of the capacity of diatoms to penetrate the alveolo-capillary barrier in drowning. *Int J Legal Med* 111:229–237
29. Zhang P, Kang X, Zhang S, Xiao C, Ma Y, Shi H, Xu Q, Zhao J, Chen L, Liu C (2020) The length and width of diatoms in drowning cases as the evidence of diatoms penetrating the alveoli-capillary barrier. *Int J Legal Med* 134:1037–1042
30. Farrugia A, Ludes B (2011) Diagnostic of drowning in forensic medicine. *Forensic Med - Old Probl New Chall*. <https://doi.org/10.5772/19234>
31. Letáková M, Fránková M, Poulíčková A (2018) Ecology and applications of freshwater epiphytic diatoms — review. *Cryptogam Algol* 39:3–22
32. Besse-Lototskaya A, Verdonschot PFM, Sinkeldam JA (2006) Uncertainty in diatom assessment: sampling, identification and counting variation. *Hydrobiologia* 566:247–260
33. Du Y, Liu J, Li Q et al (2022) Concordance analysis of diatom types and patterns in lung tissue and drowning medium in laboratory animal model. *Int J Legal Med* 136(3):911–917. <https://doi.org/10.1007/s00414-021-02768-9>
34. Li H, Kang X, Zheng D et al (2022) Are diatom types or patterns in the organs and water samples of drowning cases always consistent? *Aust J Forensic Sci* 54:376–385
35. Thakar MK, Singh R (2010) Diatomological mapping of water bodies for the diagnosis of drowning cases. *J Forensic Legal Med* 17:18–25
36. Zhao J, Wang Y, Zhang Y, Hu S, Liu C (2015) Types of diatoms in China’s three major rivers and the possible application for an automatic forensic diatom test. *Aust J Forensic Sci* 47:268–274

Publisher’s note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.