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Original Research

A 3-dimensional histology computer model of malignant melanoma and its implications for digital pathology

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Abstract ***Background:*** Historically, cancer diagnoses have been made by pathologists using two-dimensional histological slides. However, with the advent of digital pathology and artificial intelligence, slides are being digitised, providing new opportunities to integrate their information. Since nature is 3-dimensional (3D), it seems intuitive to digitally reassemble the 3D structure for diagnosis.
Objective: To develop the first human-3D-melanoma-histology-model with full data and code availability. Further, to evaluate the 3D-simulation together with experienced pathologists in the field and discuss the implications of digital 3D-models for the future of digital pathology.
Methods: A malignant melanoma of the skin was digitised via 3 µm cuts by a slide scanner; an open-source software was then leveraged to construct the 3D model. A total of nine pathologists from four different countries with at least 10 years of experience in the histologic diagnosis of melanoma tested the model and discussed their experiences as well as implications for future pathology.
Results: We successfully constructed and tested the first 3D-model of human melanoma. Based on testing, 88.9% of pathologists believe that the technology is likely to enter routine pathology within the next 10 years; advantages include a better reflectance of anatomy, 3D assessment of symmetry and the opportunity to simultaneously evaluate different tissue levels at the same time; limitations include the high consumption of tissue and a yet inferior resolution due to computational limitations.
Conclusions: 3D-histology-models are promising for digital pathology of cancer and melanoma specifically, however, there are yet limitations which need to be carefully addressed.

1. Introduction

Digital pathology is rapidly evolving, providing novel opportunities for expert support, such as with the help of artificial intelligence (AI). [1] Given the lack of experienced pathologists, the promise of AI and digital tools for pathology in general lies in their ability to enhance the accessibility, efficiency, and precision of tissue analysis.

In the field of skin cancer, considerable promise for computer-assisted digital tissue analysis has been demonstrated in various studies focusing on melanoma [2–4] as well as non-melanoma skin cancers [5].

All of these approaches build on 2-dimensional tissue analysis, which is a conventional approach that dates back to a time when the microscope was the standard vehicle to diagnose for pathologists. The emergence of digital pathology has presented a unique opportunity to reconnect tissue slides back to the original tumour, enabling pathologists to "fly through tumours" and challenging the slide-by-slide approach. Further opportunities include the digital predictions based on whole tumours instead of single slides; i.e. of metastasis or survival in cancer patients. [6–8].

The history of generating 3D models from histology dates back to the early 2000s and these early approaches have been covered in the survey paper by Pichat et al. [9]. Recent works focus on building 3D models from histology for organs like the human brain [10–12] or the mouse prostate [13–15]; but do not include human experts in the evaluation of the generated model. Additionally, there is currently no published 3-dimensional model for the primary diagnosis of human skin cancer.

Consequently, we developed the first fully 3-dimensional model of a human melanoma and tested it with nine experienced pathologists from four countries who specialise in the diagnosis of melanocytic lesions. We share full code and data to ensure reproducibility and further development.

2. Methods

2.1. Study design

After ethics consent was obtained, one half of an archived, fully anonymized human melanoma was collected from a local laboratory (DK), cut into 3 µm thin sections, which were then digitised and reassembled to a 3D-melanoma-model, that can be viewed from any angle. All slides were digitised with a slide scanner and retained by DK. Then, 17 expert pathologists were invited to test the model via a link and fill out a related survey via email within the next 14 days. A total of nine expert pathologists completed the task within time. Details on each of the steps are found below.

2.2. Ethics approval

Ethics approval was obtained from the ethics committee of the Medical Faculty of Mannheim of the University

of Heidelberg, 68131 Mannheim, Germany, before the study was conducted.

2.3. Preparation of the tissue sample

A fully anonymized, human melanoma (superficial spreading melanoma, Breslow thickness 0.7 mm, no ulceration or regression, no nevus-association, pT1a,), was obtained from a local dermatopathology lab (DK). The tissue originated from a formalin-fixed, paraffin-embedded block. The tissue block was vertically cut into half, the slides used originally for diagnosis were retained by DK; the remaining tissue was cut into sections of 3 µm thickness. The sections were put on glass slides in the order from proximal to distal. Both halves were stained with Hematoxylin and Eosin. The originating Hematoxylin and Eosin slides were digitised with a Leica Aperio AT2 slide scanner and all tissue slides were then archived by DK.

2.4. Construction of the 3D-model-architecture

In Fig. 1, the model generation process is visualised. From the digitised slides, each tissue section was cropped out separately, the background was removed using Otsu's thresholding method [16] and the files were stored in sequential order. Through this procedure, 66 images were obtained for one half of the melanoma, with each image representing one section in z-direction. While the original scan resolution was 0.25 µm / pixel (40x magnification), the images were downscaled to a resolution of 1 µm / pixel, due to computational limitations in the alignment method, and the images were further adjusted such that each image had the same size of 8700 × 3900 pixels. For spatial alignment of the image sequence, the TrakEM2 plugin of the open-source software Fiji [17] was used. For the alignment of the image sequence, the Elastic Stack Alignment [18] algorithm was chosen and the hyperparameters were iterated for multiple rounds to obtain a visually satisfying overlap between the images.

From the TrakEM2 plugin, the aligned images were exported in the Tagged Image File Format file format and had to be re-adjusted to the input size of 8700×3900 pixels. To store the aligned sections in single file format, the Neuroimaging Informatics Technology Initiative file format, established in Neuroimaging, was chosen. Since MRI images have a significantly lower resolution than macroscopic images, the aligned images were once again downsampled to 870×390 pixels. The resulting 3D Model therefore has dimensions of $870 \times 390 \times 66$ pixels with three colour values (Red, Green, Blue) for each pixel. The generated file was provided in a Web Viewer for the conducted survey among pathologists.

2.5. Survey Design

An online survey with 14 questions (starting with name & experience, continuing with specific questions about the tested model, and finalising with questions on general implications for digital pathology). The full survey, including all questions and all answers can be found in the Supplementary Material 1.

3. Results

3.1. Model

The full 3D-model can be tested, downloaded and reproduced as described in the code & data availability statement below.

3.2. Survey

3.2.1. Participants

Nine pathologists from four European countries (France, Germany, Italy, Spain), not personally known to the investigators but experienced in the diagnosis of melanocytic lesions, were invited via E-Mail to test the model and filled out the survey. Seven possessed more

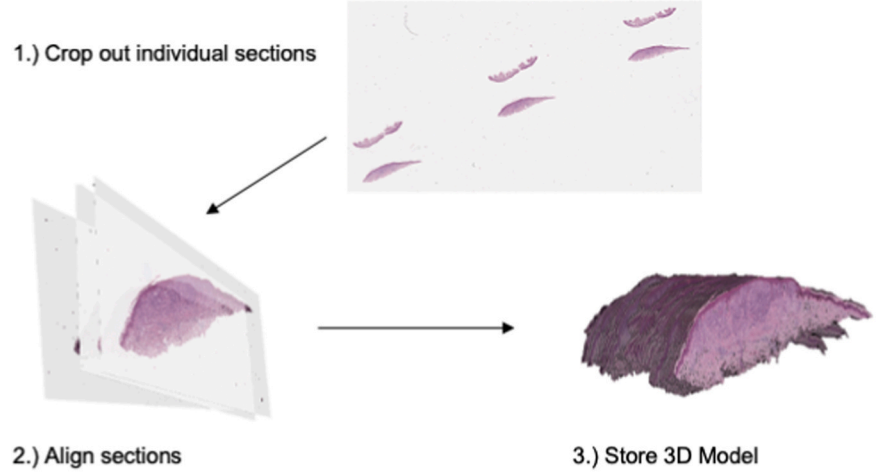

Fig. 1. Schematic of the procedure for generating the 3D melanoma model from digitised histological slides.

than 15 years of diagnostic experience as a pathologist, two between 11 and 15 years. All participants claimed to have diagnosed digitised tissue sections in the past, only five use digital pathology in their daily routine.

3.2.2. Transfer of 3D-models to routine care

Eight out of nine pathologists think it is likely (n = 5) or very likely (n = 3) that digitised 3D-models of tumours will enter the diagnostic routine within the next 10 years (rather unlikely n = 1). Within the next 30 years, all participants think it is rather likely or very likely (n = 5). If asked for melanoma specifically ("In how many years do you expect 3D-tumour-models to enter routine care in digital pathology for the diagnosis of melanoma?"), seven pathologists expect 3D-models to enter routine care within the next 5–10 years, one in 11–20 years and one in 1–5 years.

3.2.3. Core advantages of 3D-models compared to 2D-models

Pathologists could express what they reflect as core advantages in a free text field; the key points are summarised here, repetitions were deleted (see Suppl. 1 for full, unedited answers):

- Diagnostic procedures become more complete and biologically analogous.
- Pathologists can easily fuse diagnostic and macro aspects of lesions with microscopy images.
- Enhanced safety for diagnosis and margin evaluation.
- Three-dimensional symmetry serves as the primary golden standard for distinguishing benignancy from malignancy.
- "Stacking" multiple levels of sections allows for advanced 2-dimensional diagnostic procedures.
- Simultaneous evaluation of different tissue levels enhances accuracy and research applications.
- Greater tissue sampling leads to more accurate diagnosis and prognosis.
- Better tumour staging aids in prognosis and adjuvant therapy decisions.
- Improved detection of lymph and blood vessel invasion.
- Provides a better basis for AI-assisted diagnosis.
- Good image quality/magnification can reduce tissue consumption.

3.2.4. Core disadvantages of 3D-models compared to 2D-tissue-slides in digital pathology

Core points to this question included:

- High tissue consumption depending on the technique used.
- Manual "stacking" of up to 100 sections may be time-consuming.
- More technical work and sections needed in laboratories.
- Combining 2D-histology with ex-vivo-confocal microscopy for 3D-models without irreversible tissue consumption.
- The need for a proper cross-platform, intuitive, and fast navigation system.
- Adequate download speed for large files.
- Powerful client system required for proper interaction with big files.
- Current lack of training in diagnosis with these systems, which will improve over time.

3.2.5. What improvements on the tested 3D-model specifically should be made from a clinician standpoint?

Improvement suggestions include enclosing both halves of the tumour specimen, using magnification 40x for all sections, and ensuring precise alignment in the stacking mode. The current navigation app lacks resolution, proper interaction controls, and a magnifying feature, making it difficult to evaluate images from a histopathological standpoint.

3.2.6. What do you think is already working quite well in the current software?

Summary of the feedback (Details in Supplementary 1): Initially, adjusting to the moving image lines can be complicated, but it becomes intuitive. The "multi" program in Display mode is fascinating, allowing selection of suspicious planes, zooming, and comparison with other sections. Ability to navigate among different levels and rapidly examine multiple tissue planes. Appreciation for the visualisation of the 3D architecture.

3.2.7. Do you think digital pathology for the diagnosis of melanoma should be 2D or 3D?

The question was asked under the condition that "2D and 3D models were both equally technically advanced and the education of pathologists would teach both equally well". Seven experienced pathologists answered with "3D", and two answered with "2D".

4. Discussion

We successfully created and evaluated the first 3D-Model of human melanoma with an overall positive reception by expert pathologists. Due to the full availability of code, survey and specimen, our work is fully reproducible.

4.1. Discussion of methods

4.1.1. Preparation of specimen & technical considerations

One insight from the performed reconstruction is that the sections should all be cut in sequential order, which is the reason why only one half of the provided melanoma could be reconstructed straightforwardly. Also we recommend using a 3D scanning modality such as micro computertomography to generate a 3D reference volume before cutting the tissue block into sections.

When performing the alignment of the image stack with the Elastic Stack Alignment algorithm included in the Fiji software, we speculate that optimising the algorithm for the reconstructed tissue entity may yield improved results. For the example of aligning sections

of a melanoma, greater emphasis should be placed on correctly aligning the epidermis compared to other parts of the tissue.

A central feedback we received on the current 3D model is that the resolution is too low to perform pathological analysis on a cellular level. This leads to the conclusion that the used NIfTI file format is not well-suited for pathological use cases, as it is not designed to store sections with such a high resolution as required in pathological routines. On the other hand, the possibility to view each section in the 3D model separately is of high relevance for pathologists, which makes data formats such as a regular point cloud less applicable. We therefore see the need to potentially extend the properties of the NIfTI file format, or establish a new file format for pathological use cases in order to make constructing and analysing a 3D tissue model an actual use case in pathological workflows.

The method does not come without its limitations. Since only 3 μm thick sections were used, which is already thinner than the cuts done in clinical routine, the 3D-model is only capable of imaging and connecting those sections. Even thinner cuts may be necessary in order to image every detail of the tissue with maximum resolution. Furthermore, it should be noted that the current resolution of the present model is lower than possible (40x magnification) due to computational limitations. However, this can be resolved in the near future.

Despite the fact that none of the participating pathologists is personally known to the principal investigator and the personal opinion of the Principal Investigator was not disclosed to the authors when inviting them to the survey, ascertainment bias can not be fully excluded in any survey study.

4.2. Discussion of survey results

In general, the overall perception of the 3D-histology model by the pathologists was positive; a core advantage being the inherent-3D-structure by the stacking mode, which allows for direct checking of symmetry/asymmetry in three angles instead of two.

The stacking mode has become very popular in other fields such as macro and astrophotography, where a sequence of photographs is captured either in different optical planes (micro/macro) or at staggered time intervals (astro). Afterwards, computer algorithms are used to process the images, allowing the ultra-sharp imaging of subjects, such as a tiny ant, from head to tail, which would otherwise be impossible due to the depth of field. Here, stacking is based on "merging". In the method used for our 3D-model ("inverse stacking"), it is the other way around: it provides a merged stack which allows to 1) see the whole structure immediately, and 2) unfold individual pages.

The core disadvantages discussed were that the current workup is complicated, costs more time, and potential difficulties in integrating it in the current workflow. However, this will improve over the years with automation of the processes needed; which may include automation of destaining/restaining of tissue (for instance for immunohistochemistry or for molecular analyses). At the moment, however, there are still no practical tools available to cut sections automatically and whether multistain-3D-models are currently achievable should be addressed in future research. The process of constructing the model out of digitised slides is partly automatable already now which led to a quite fast construction of the model by the used software (~40 min for converting and alignment). Along these lines, a comparison to *in vivo* methods was drawn, such as optical coherence tomography, micro computertomography and other 3D-*in vivo* technologies which may also improve and may provide a more efficient, non-invasive workup than a histologic-3D-model. The authors assume that several techniques can be combined: confocal microscopy has its limitations in the depth of tissue penetration, but it displays the whole tumour. If these images are combined with vertical sections of conventional histology, this might be an interesting new image to evaluate.

Nonetheless, looking directly at the digitised tissue instead of using *in vivo*-procedures may provide a higher resolution of biology. In addition, the third dimension is inherent in our stacking procedure - whereas in all other situations the 3rd dimension from other procedures such as reflected light microscopy, coherence tomography must be integrated into the 2-dimensional histological section. This makes a decisive difference since the 3D-histologic model enables the 3rd dimension at a glance.

Furthermore, it is worth considering whether all of the cuts are indeed necessary for the 3D model. If one would simply omit/discard every second cut, as done with the so-called "step cuts" in the current clinical routine, it would result in a time-saving measure with minimal loss of information. In the opinion of the authors, these kinds of trade-offs could be omitted with improved destaining/restaining methods in the future.

4.3. Extension of 3D-models

3D-histology-models could be extended to multiple stainings also by immunohistochemistry, but also by other layers of molecular analyses such as spatial transcriptomics, adding genetical / RNA-layers to morphology which would enable the enhancement of image-based genetic testing. In general, full 3D-tumours with heterogeneous spatially resolved profiles (morphology, genomics, proteomics, etc.) would be able to predict with a much higher precision overall tumour behaviour (metastasis, growth, immune system interaction) or targetable characteristics for therapy.

4.4. Conclusion

3D models are promising for the digital pathology of cancer and melanoma specifically, however, there are yet limitations which need to be carefully addressed in future research.

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CRediT authorship contribution statement

Alexander Kurz: Software, Investigation, Conceptualization, Methodology, Writing of initial draft, Visualization. **Dieter Krahl:** Conceptualization, Data collection, Resources, Software testing, Survey participation, Writing – review & editing, Preparation of the specimen. **Heinz Kutzner, Raymond Barnhill, Antonio Perasole, Maria Teresa Fernandez Figueras, Gerardo Ferrara, Stephan A. Braun, Hans Starz, Mar Llamas-Velasco:** Software testing, Survey participation, Writing – review & editing. **Jochen Sven Utikal, Stefan Fröhling, Christof von Kalle, Jakob Nikolas Kather, Lucas Schneider:** Writing – review & editing, Conceptualization. **Titus J. Brinker:** Initiation, Conceptualization, Investigation, Methodology, Supervision, Funding, Writing of initial draft, Writing – review & editing, Resources.

Data availability

The sequence of sections used for the alignment and the generated 3D model are available at https://doi.org/10.5281/zenodo.8155124. The code for processing the image sequence and building the 3D file is available at https://github.com/DBO-DKFZ/3d-histo.

Declaration of Competing Interest

TJB would like to disclose that he is the owner of Smart Health Heidelberg GmbH (Handschuhsheimer Landstr. 9/1, 69120 Heidelberg, Germany), outside of the scope of the submitted work. The other authors declare that they have no conflicts of interest.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ejca.2023.113294.

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