pathoDISCO-HE: Ultramicroscopy and fluorescent labelling of glioblastoma for 3D virtual HE imaging and improved histopathological evaluation

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Introduction

The gold standard for tumour histopathology is examination of H&E stained formalin fixed paraffin embedded sections; however, this technique has a number of limitations. Typically only a few sections from a 3-4mm thick tissue are produced and sections may be lost due to mechanical scoring or tearing. Overall, only a limited representation of the tumour specimen is available for examination by the histopathologist, resulting in the possibility of important pathological features being missed.

This situation could be improved by labelling and imaging entire specimens followed by 3D reconstruction and analysis. This approach would allow the histopathologist to digitally review the entire specimen.

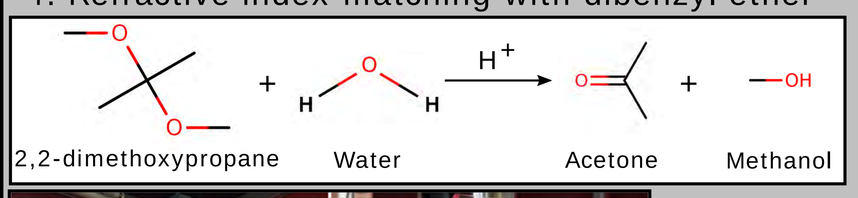
Histopathology is used to diagnose disease, make informed treatment decisions and also indicates patient prognosis. Improved histopathological analysis of cancer specimens is likely to improve the accuracy of diagnoses and prognoses, and may allow clinicians to make better decisions when considering treatment options; ultimately, these factors may improve patient outcomes.

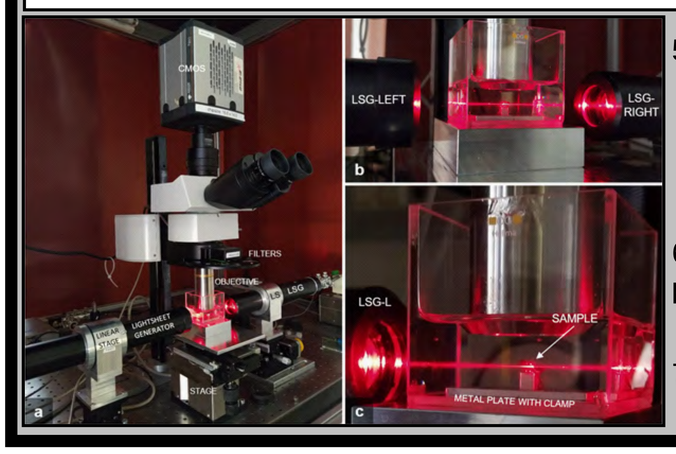
Glioblastoma (GB) is a highly aggressive malignancy beginning in the brain and with an incidence of around 7 in 100 000. In this study we applied tissue clearing and lightsheet microscopy to GB specimens. Subsequently, images were processed using a false colouring algorithm and 3D virtual HE reconstructions were produced. This is the first time that such a technique has been used in GB and represents an important step towards realising 3D histopathology in the clinical setting.

Methodology

GB specimens were prepared using a protocol we term "pathoDISCO-HE"

1. Bleaching of tissue with hydrogen peroxide
2. Permeabilisation of tissue with the zwitterionic detergent CHAPS and simultaneous labelling of nuclei with cresyl violet
3. Chemical dehydration of samples with 2,2-dimethoxypropane
4. Refractive index matching with dibenzyl ether



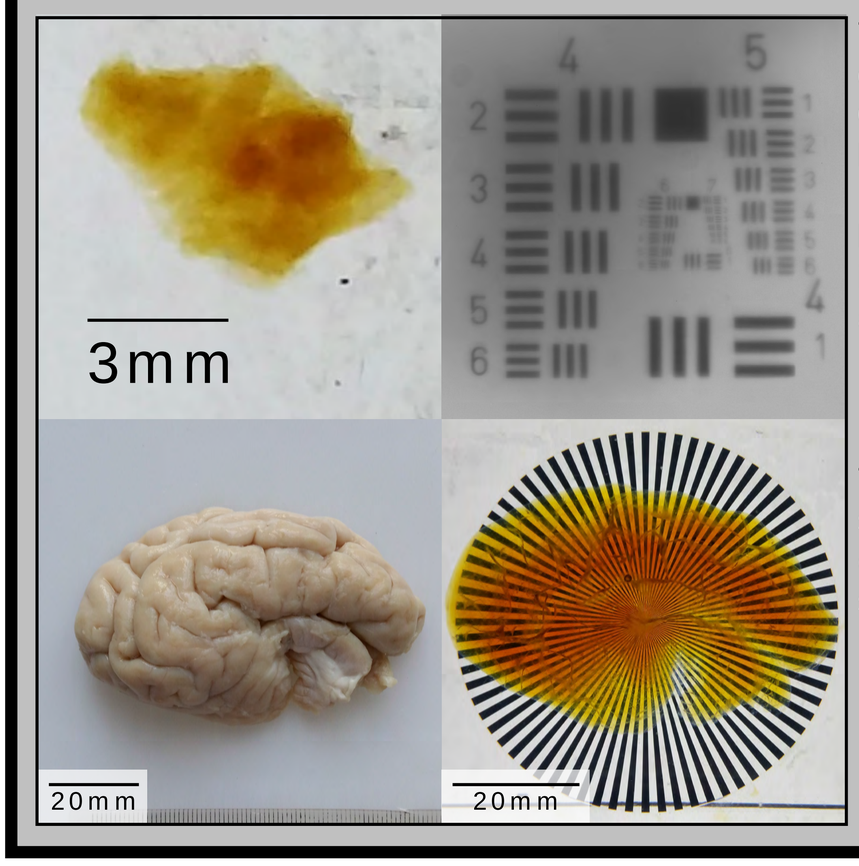


5. Imaging by lightsheet microscopy
   - two stacks recorded:
     - Nuclei (cresyl violet)
     - Cytoplasm (autofluorescence)

6. Post-processing of data and virtual HE labelling

7. 3D reconstruction

Results - Tissue clearing



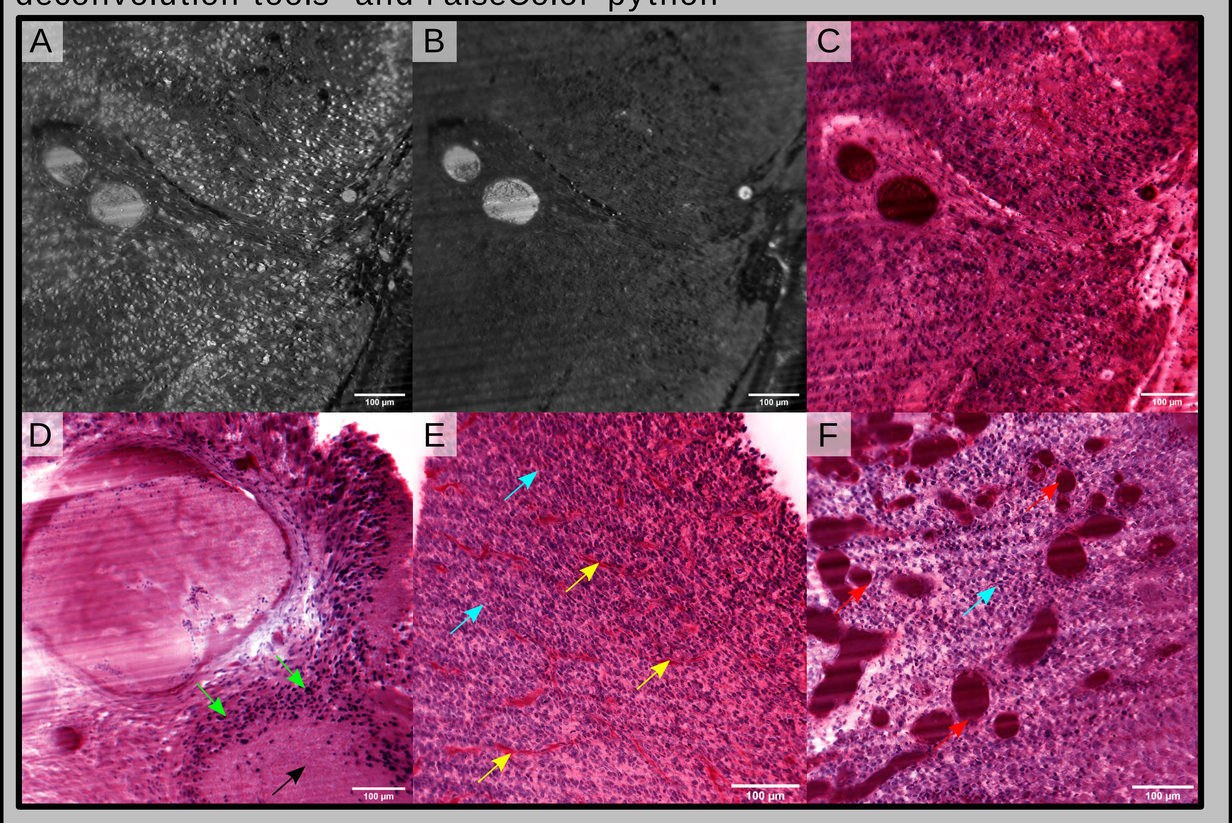
The above protocol can adequately clear GB specimens for light sheet microscopy (top left). USAF chart imaging indicates an acheievable resolution of <3.1µm (top right).

The protocol can be scaled up for clearing of larger specimens - such as this intact pig cerebral hemisphere (bottom).

[1]Becker et al, J. Biophotonics 2022, https://doi.org/10.1002/jbio.202100290
[2]Serafin et al, PLOS ONE 2022,https://doi.org/10.1371/journal.pone.0233198

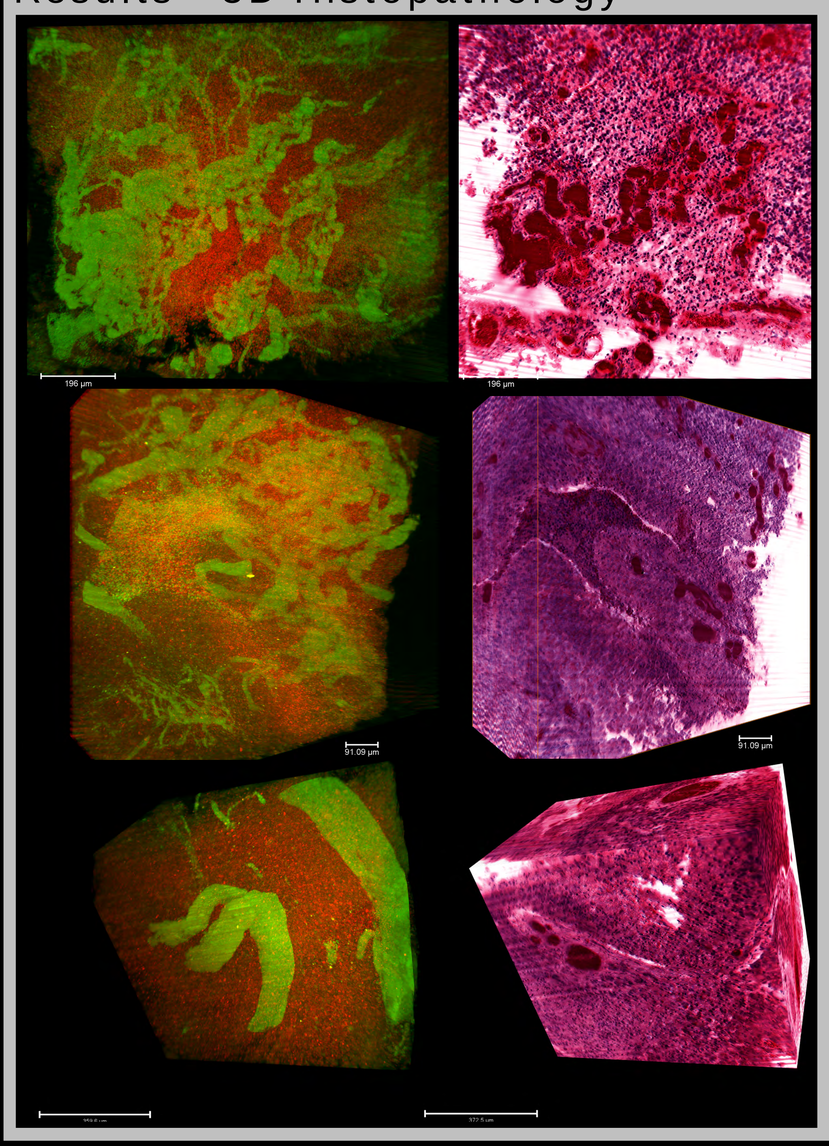
Results - Virtual HE labelling

Post-processing and virtual HE labelling were performed using Wiener deconvolution tools[1] and FalseColor-python[2]



Optical glioblastoma sections imaged at 16x magnification. A+B: greyscale image of cresyl violet labelled nuclei and autofluorescence. C: The same optical section after combining and false coloring of A+B. D-F: Identification of common pathological features in GBM. Arrows: Green - pseudopallisading, black - necrosis, blue - hypercellularity, yellow - vascular proliferation, red - vascular garlands.

Results - 3D Histopathology



In addition to 2D virtual HE sections, 3D reconstructions could also be produced. On the left are three different GBM samples displayed using maximum intensity projection - allowing visualisation of the internal structure including nuclei (red) and vasculature and cytoplasm (green).

On the right are corresponding "solid" volume renderings using traditional HE colours. Specimens can be viewed from any angle and at any depth with good resolution even in the z-axis.

Summary

1. 3D histopathology is desirable to improve analysis of patient specimens

2. We have developed a novel protocol for clearing and virtual HE visualisation of glioblastoma specimens

3. Characteristic pathological features of glioblastoma can be observed in our virtual HE preparations

4. Virtual HE can be viewed in 3D, from any angle and at any depth in the tissue

5. Specimens can also be visualisied using maximum intensity projection to characterise tumour vasculature - an important feature of tumour pathology