Microscopy with Ultraviolet Surface Excitation (MUSE): Innovations in Diagnostics of Neuropathological Tumors

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Introduction: In the era of molecular diagnostics and personalized medicine, it is becoming increasingly important to save tissue for downstream testing for optimal pathologic diagnosis. Unfortunately, conventional histology processing and its expenditure of tissue for H&E imaging often results in inadequate material for essential molecular tests downstream. Microscopy Using Ultraviolet Excitation (MUSE) has emerged as a promising potential answer in providing a novel tissue-sparing method of generating morphologic imaging without the need to fix or cut fresh tissue. We aim to standardize protocols for imaging an array of CNS tumor samples and demonstrate equivalency to traditional FFPE H&E in terms of generating images for tumor diagnostics.

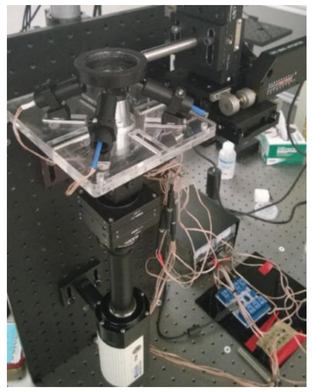
Materials and Methods: 24 CNS tumor biopsy specimens were imaged using the MUSE interface, then subsequently fixed and paraffin-embedded for traditional H&E staining. Each pair of slides (MUSE and H&E) were then read by a panel of 4 neuropathologists, and the diagnosis by each reader was recorded as correct or wrong. Combined accuracy was calculated within each diagnosis category and for each pathologist.

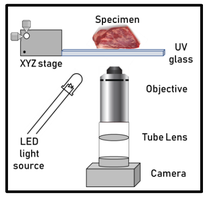
Results: In surgical resections of 24 adult patients (mean age 54 years) with newly diagnosed brain and spinal cord tumors, 7/24 were diagnosed by conventional methodology with diffuse astrocytic/oligodendroglial tumors, 8/24 with meningiomas, 3/24 with ependymal/choroid plexus tumors, 3/24 with tumors of cranial/paraspinal nerves, and 3/24 with metastatic tumors. 97% concordance was observed among MUSE versus light microscopy diagnostics, with 94% within the pathologist panel.

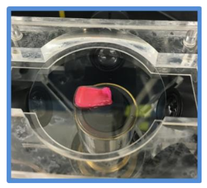
Conclusions: MUSE imaging appears to have been successful in reliably generating diagnostic-quality histological images of CNS tumors. This is supported by inter-pathologist concordance on diagnoses made through both MUSE and traditional H&E images. Ongoing studies are expected to expand to assessments of grading MUSE images of more diagnostically difficult brain and spinal cord tumors.

MUSE OPTICAL SCHEMATIC

The MUSE prototype is designed to feature an inverted interface, in which irregularly shaped specimens lie flush against UV-permeable quartz/sapphire. No coverslip is needed in this configuration. Excitation light is provided by 275 nanometer wavelength UV-emitting LEDs.







IMAGING RESULTS

H&E RECONSTRUCTION

Once MUSE images are captured (left), inversion and color conversion of RGB channels can be configured through Adobe Photoshop to achieve pseudo-H&E appearance (middle). Compared to the traditional FFPE H&E image (right), results closely resemble original.

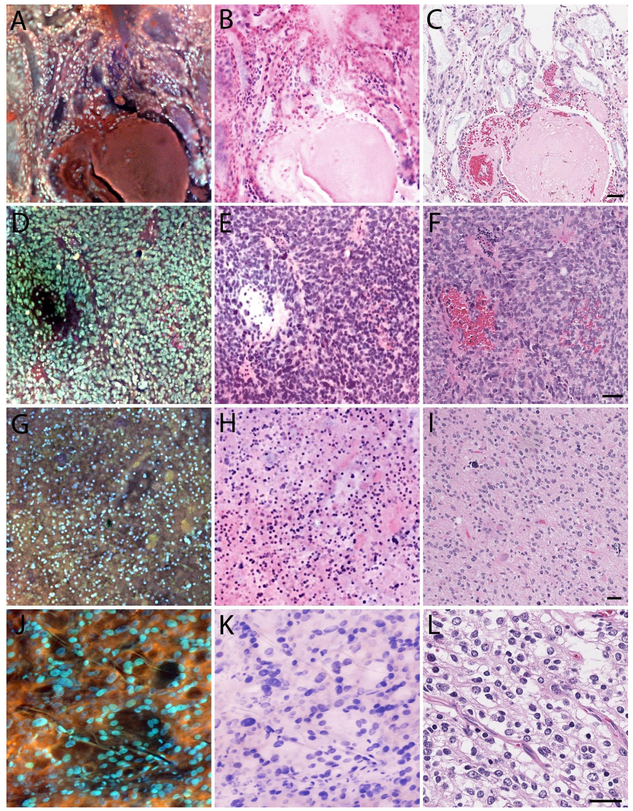


Figure 1: Selected CNS tumors via MUSE and H&E reconstruction. A-C) Myxopapillary Ependymoma. D-F) Anaplastic Ependymoma. G-I) Glioblastoma multiforme. J-L) Oligodendroglioma. Scale bar 50 um.

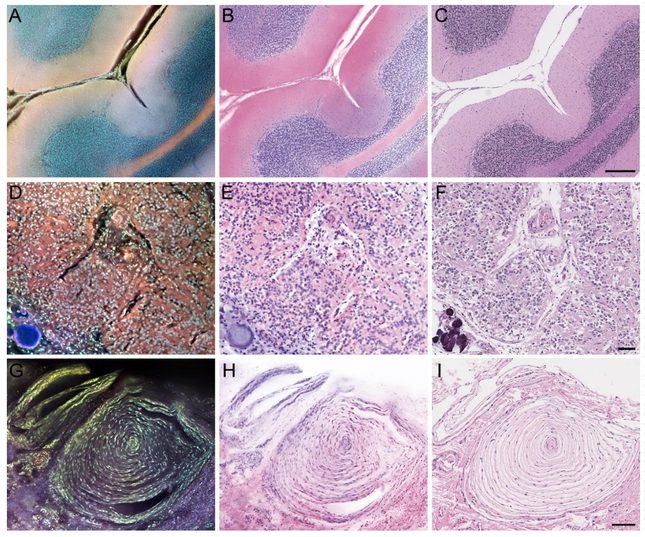


Figure 2: Additional Samples of MUSE and H&E reconstruction on Normal Anatomical Structures. A-C) Cerebellum. D-F) Pineal gland (note visible calcification). G-I) Pacinian corpuscle. Scale bars 300, 100, 100 um.

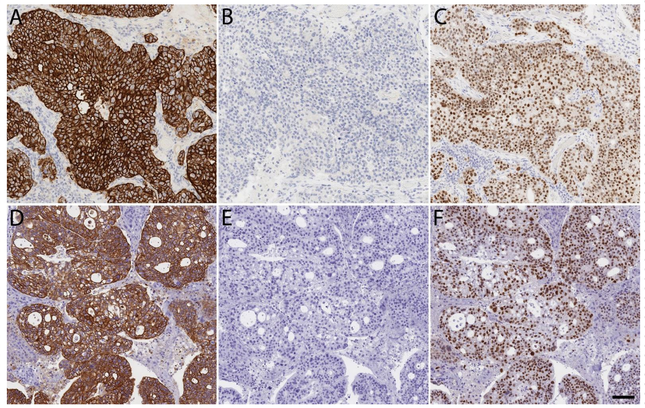


Figure 3: Immunohistochemical staining integrity preserved following MUSE. Comparable quality of routine FFPE immunohistochemical staining of cerebellar metastatic Mullerian adenocarcinoma following standard 10% formalin fixation and without exposure to UV light (A-C) compared to identical immunohistochemical staining procedure after a short exposure to UV light during MUSE microscopy (D-F). A and D: Cytokeratin 7; B and E: Cytokeratin 20; C and F: PAX-8. 200x magnification. Scale bar 150 um.

PRELIMINARY CONCORDANCE DATA

Table 1: MUSE vs Conventional Histology.

Tumor Type	Image	NP 1 Correct	NP 1 Wrong	NP 2 Correct	NP 2 Wrong	NP 3 Correct	NP 3 Wrong	NP 4 Correct	NP 4 Wrong	Combined Accuracy (%)
Diffuse Astrocytic Oligodendroglial	MUSE	7	0	6	1	7	0	6	1	93
	H&E	7	0	7	0	7	0	6	1	96
Meningioma	MUSE	8	0	8	0	8	0	8	0	100
	H&E	8	0	8	0	8	0	8	0	100
Ependymal & Choroid Plexus	MUSE	3	0	3	0	3	0	2	1	92
	H&E	3	0	3	0	2	1	3	0	92
Cranial or Paraspinal Nerves	MUSE	3	0	3	0	3	0	3	0	100
	H&E	3	0	3	0	3	0	3	0	100
Metastatic	MUSE	3	0	3	0	3	0	3	0	100
	H&E	3	0	3	0	3	0	3	0	100
	Combined Accuracy (%)	100		98		98		94		97

- Across all tumor types and pathologists, an overall 97% concordance rate was achieved.
- Within the pathologist panel, there was an overall 94% concordance rate.
- Within the pathologist panel, there was an 87% concordance rate based on tumor grade.

CONCLUSIONS

MUSE images are comparable or enhanced in resolution compared to traditional FFPE H&E images, as evidenced by retained morphological characteristics on H&E reconstruction, preserved immunohistochemical staining patterns, and high concordance rate among pathologists in the diagnosis of multiple CNS tumor types. This modality represents a promising opportunity to generate diagnostic-quality histological images of brain and spinal cord tumors on a rapid, sample-sparing basis.

Future directions include assessment of concordance data in the setting of diagnostically difficult neuropathology cases, as well as in assessments of tumor grading.

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