MUSE (Microscopy with UV Surface Excitation):

A Novel Ex-Vivo Microscopy in Surgical Pathology

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ABSTRACT

AIM: MUSE is a novel ex-vivo microscopy method that employs 280-nm UV excitation and oblique cis-illumination to generate high-quality images from cut surfaces of fresh or fixed tissue in approximately 2 minutes without requiring thin-sectioning via cryomicrotomy or standard histological processing. MUSE staining and imaging does not impair the tissue for future use in traditional H&E preparation or molecular testing. The aim of this study was to evaluate the MUSE in assessing a wide range of surgical pathology cases.

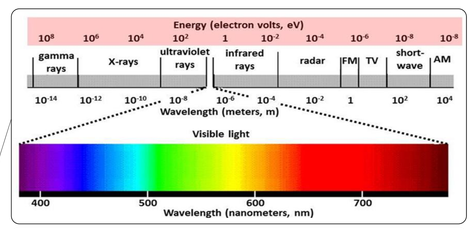
Methods: 25 samples of 10 different tissues (kidney, thyroid, colon, rectum, small intestine, breast, prostate, lung, liver and skin) were examined by two surgical pathologists and two dermatopathologists with a few minutes of training as well as by two pathology residents with at least two months of experience on MUSE. The MUSE diagnostic score was calculated by the percentage of correct diagnosis of MUSE images. MUSE comparison score was assessed by the concordance between paired images captured by MUSE and correlated H&E images generated by a whole slide scanner (Score 0: MUSE was not useful/ Score 1: useful but not diagnostic/ Score 2: diagnostic but weaker than H&E / Score 3: Equal to H&E / Score 4: Stronger than H&E). Preliminary results indicate that the MUSE method shows promise as a diagnostic approach to surgical specimens by achieving a total diagnostic score of 82.5%. It was most effective on thyroid, skin, GI, and breast samples, with diagnostic scores of 100%, 89%, 79% and 75% respectively. MUSE also received an average comparison score of 2.22 which shows that it is a useful diagnostic tool, but slightly weaker than H&E on providing all the histologic details.

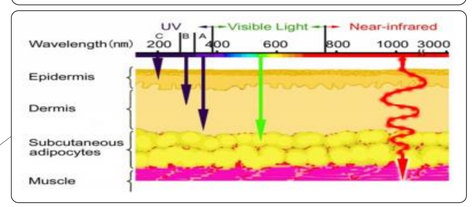
Conclusion: MUSE can be a fast, reliable and inexpensive approach for evaluating surgical specimens. The utility of MUSE in intraoperative consultation, especially in Mohs surgery, will be a focus of future work.

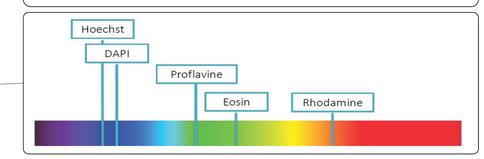
➢ 3 types of microscopy:
- ❑ Conventional microscopy
  - ❖ Require traditional fixation, thin-sectioning and staining
- ❑ Ex-vivo microscopy (Slide-free)
  - ❖ Rapid imaging of biopsy material
- ❑ In-Vivo microscopy (Biopsy-free)
  - ❖ Evaluation of human tissue microstructure in real time

➢ What is MUSE?
- ❑ A novel Ex-Vivo microscopy
  - ❖ Slide-free method developed at UC Davis
  - ❖ First in evaluating on human tissue
- ❑ Microscopy with UV Surface Excitation (MUSE)
  - ❖ Using UV-emitting LED with wavelength of 275 to 285 nm
  - ❖ Digital camera captures the emission light

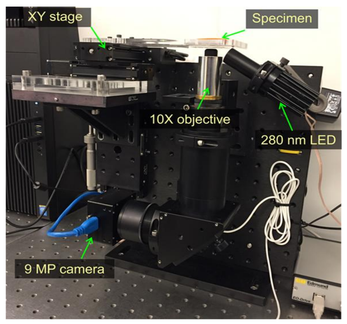
➢ How does MUSE work?
- ❑ Ultraviolet (UV) is an electromagnetic radiation
  - ❖ Wavelength: 10 nm to 400 nm (Shorter than visible light)
- ❑ Light penetration in tissue depends on the wavelength
- ❑ 275 to 285 nm UV light penetrates about 10 microns
  - ❖ Close to the thickness of a conventional tissue section
- ❑ UV light can excite dyes or endogenous autofluorescence
  - ❖ The emission light varies from blue to red
- ❑ A digital camera can capture the emitted lights
  - ❖ 3 microns thickness from the surface of the specimen
  - ❖ Morphology is similar to H&E



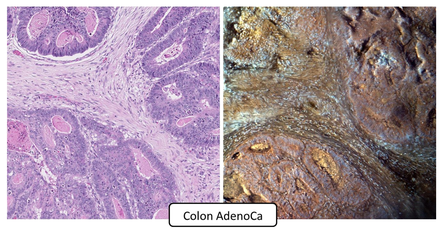


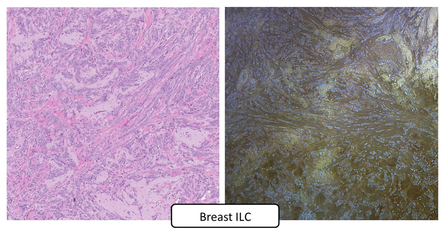


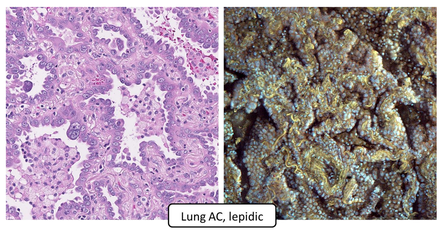
➢ MUSE setup:
- ❑ Prepare flat tissue surface
- ❑ Staining (50 sec total)
  - Dyes used include:
  - ❖ Rhodamine B
  - ❖ Hoechst 33342
  - ❖ Eosin
  - ❖ Propidium iodide
- ❑ Capture images

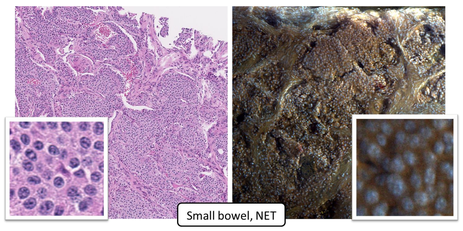


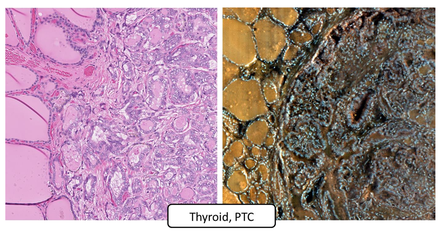
- ❑Pros:
  - ❖Robust method
    - Simple physical & chemical principles
    - Fast (2 minutes)
    - Fresh, formalin- or alcohol-fixed
  - ❖MUSE images:
    - Multi-color and 3-dimensional (more informative)
    - Similar to H&E (orientation/thickness)
    - High diagnostic value (even for fresh eyes)
  - ❖Ex-vivo microscopy:
    - Inexpensive (No histology)
    - Preserves tissue (for downstream molecular testing)
    - Potential use in intraoperative consultation
    - Can potentially be used as POC
  - ❖Digital pathology:
    - Provide service to low-resource areas
- ❑Cons:
  - ❖Prior to imaging:
    - Hard to orient very small specimens
  - ❖Image:
    - Can be hard to capture nuclear features (melanocytic, inflammatory)
    - Unfamiliar colors
  - ❖Post-image:
    - Large data sets (as with whole-slide imaging)

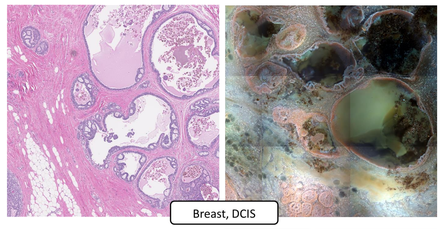


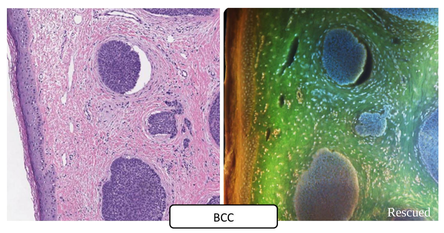


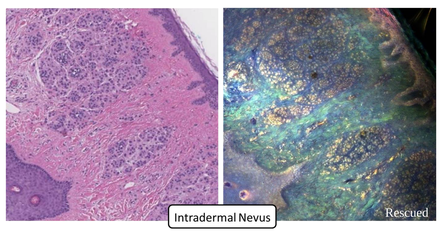


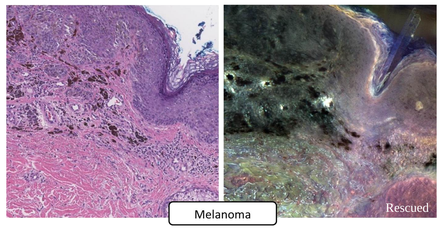


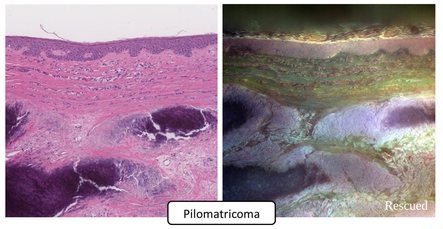


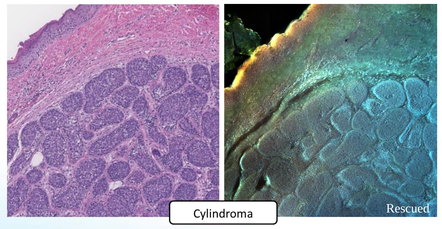


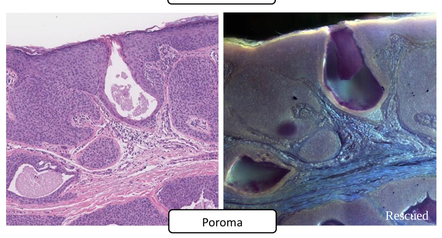


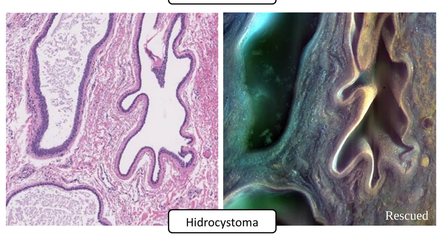


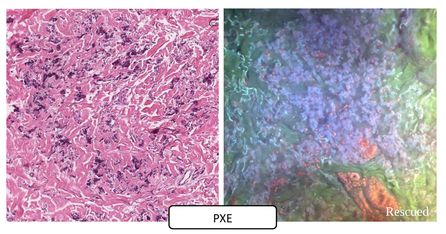












Disclosure of interest :
Dr. Levenson is co-founder and CEO of MUSE Microscopy, Inc., a start-up that intends to commercialize this technology . The remaining authors declare no conflicts of interest.

Reference: Farzad Fereidouni et al., “Microscopy with ultraviolet surface excitation for rapid slide-free histology” *Nature Biomedical Engineering* **volume 1**, pages957–966(2017). doi:10.1038/s41551-017-0165-y