

Abstract

Preparing whole mounted Histology preparations of diagnostic quality provides a significantly superior Histopathological registration for surgical specimens that have undergone *in vivo* MRI. The whole mounted slides offer excellent Pathology correlation with MRI imaging derived, 3D printed patient specific whole mount molds for prostate and kidney surgical specimens. An important requirement for MRI Pathology correlation is adequate image registration. Histopathologic analysis using standard tissue based processes where the prostate and kidney are sectioned in multiple routine tissue blocks results in challenges of correlation between the *in vivo* MRI findings and the reference Histopathological standard.

Patient specific 3D printed mold was created utilizing the *in vivo* MRI data and utilized to prepare slabs of tissue for processing. Specimen orientations were marked with tissue color and grossed into mega cassettes with adequate slot perforations for good reagent exchange. Processing schedule was developed on the Milestone LOGOS microwave processor for specimens of a standardized tissue thickness of 5mm. The protocol was optimized to produce diagnostic quality histologic preparations in addition to good tissue preservation options including special stains, IHC and molecular that would assist technologist prepare sections or tissue on regular slides.

In the current design radiologist and pathologist work independently for diagnostic medicine. Whole mounted histological preparations on 3D printed patient specific MRI offers a unique opportunity for the two disciplines to collaborate. In this setting pre-operative multi-parametric MRI data when correlated with whole mounted Histo-Pathological findings can provide valuable insights into MRI detected suspicious lesions by assisting in characterizing the tumor and assigning a Gleason score before surgical intervention.

Introduction

A tissue diagnosis of adenocarcinoma is often essential for establishing a diagnosis of prostate cancer, and the foundation for a tissue diagnosis is currently light microscopic examination of hematoxylin and eosin stained section. Cancer is often not visible at gross examination, and the tumor extent is always underestimated by the naked eye. Correlations between MRI and Histological whole mount slides provides valuable diagnostic insights. The panoramic view of the large tissue sections provide significant advantage for kidney tumors as well.

Steps followed in a prostate cancer patient include:

- **Magnetic Resonance Imaging done *in vivo***
- ***Ex-vivo* MRI is done within 12 hours after radical prostatectomy.**
- **The specimen is inked for margin and lateral side identification.**

Specimen is submitted for histopathology

- The gross examination is done which includes measurement in three dimensions, weighing the prostate after removal of the seminal vesicles.
- Tissue is grossed using 3D printed Mold and horizontally cut in approximately 3-5 mm
 - If tissue is too big, slab is divided to fit in the cassette.
- The tissue is submitted in formalin, post fixed and processed.
- The tissue sections were processed in a microwave histoprocessor
 - **Embedding performed in mega cassette**
 - **Sectioning done using special chuck adapter**
 - **Section obtained at 4 micron on mega slide**
 - **H&E stain performed using staining rack adapter**

Materials and Methods

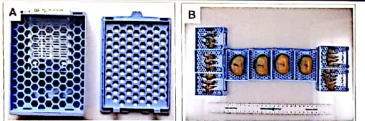


Fig 1A: Whole mount cassette with regular cassette insert. Fig 1B: Whole mount sections of Prostate (5mm Thick)

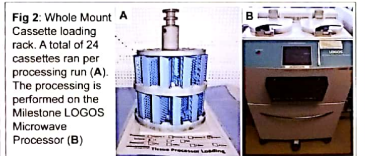


Fig 2: Whole Mount Cassette loading rack. A total of 24 cassettes can run per processing run (A). The processing is performed on the Milestone LOGOS Microwave Processor (B)

Tissue Processing Protocol

Step	Name	Reagent	Time (mins)	Temperature	Max power	Vacuum
1	Fixation	10%formalin	01:10:00	50° C	750 Watts	No
2	Flushing	70% Alcohol	2 00:01:00	35° C	N/A	No
3	Rinsing 1	100% Alcohol	3 00:02:00	35° C	N/A	No
4	Rinsing 2	100% Alcohol	4 00:02:00	35° C	N/A	No
5	Ethanol	100% Alcohol	5 02:05:00	70° C	1050 Watts	No
6	Isopropanol	Isopropanol	6 02:05:00	70° C	750 Watts	No
7	Vaporization	N/A	N/A 00:1:5:00	70° C	750 Watts	Yes
8	Wax	Paraffin	Wax 02:31:00	70° C	750 Watts	Yes

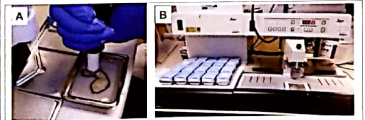


Fig 3: Embedding of whole mount tissue section using tamper to lay flat (A). Whole mounts are embedded and left on cooling plate for a minimum of 1hr (B).

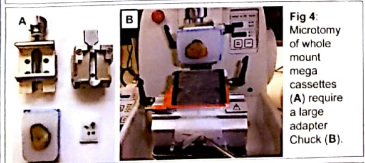


Fig 4: Microtomy of whole mount mega cassettes (A) require a large adapter Chuck (B).

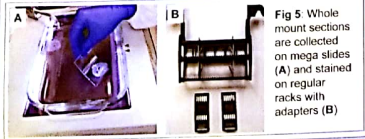


Fig 5: Whole mount sections are collected on mega slides (A) and stained on regular racks with adapters (B).

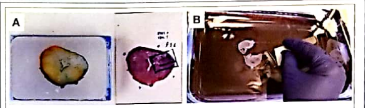
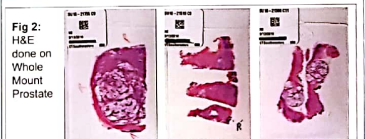
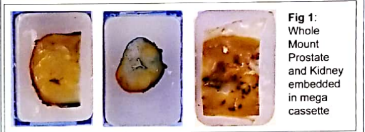


Fig 6: IHC and special stain testing for whole mounts are performed by obtaining partial sections on regular sized slides (B). The area of interest is marked by a Pathologist (A) and the appropriate area of the tissue is obtained by splitting section in flotation bath (B)

Whole Mount Prostate and Kidney



Quality issues and challenges



Fig 1: Example of tissue that is inadequately fixed resulting in sub-optimal quality H&E stained section with holes in tissue

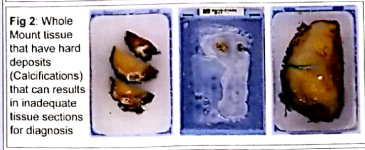


Fig 2: Whole Mount tissue that have hard deposits (Calcifications) that can result in inadequate tissue sections for diagnosis

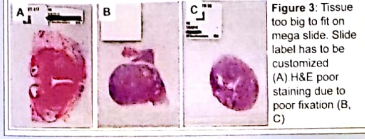


Fig 3: Tissue too big to fit on mega slide. Slide label has to be customized (A) H&E poor staining due to poor fixation (B, C)

Results

- Magnetic resonance Imaging (MRI) of the prostate has become an essential modality for staging and characterizing prostate cancer.
- Correlations with whole mount histology diagnostic slides and MRI has facilitated the detection of tumors *in vivo*.
- Pathologist have liked whole mount sections since it gives them a better overview and the identification of multiple separate tumor foci in not only prostate but other tumors including Kidney.
- Whole Mount slide provides pathologist panoramic and comprehensive view of the microscopic section enabling them identifying significant previously unsuspected findings.
- Compared to conventional histopathology processing, whole mount processing has advantages in terms of cost and efficiency.

Staining Quality Samples

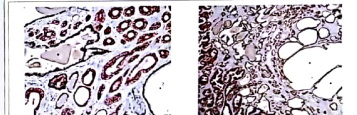
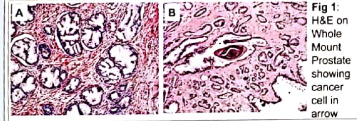


Fig 2: PIN 4 Immunohistochemical staining on Whole Mount Prostate showing benign prostatic glands and invasive adenocarcinoma

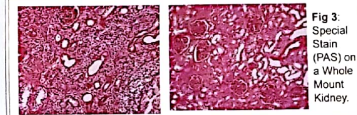


Fig 3: Special Stain (PAS) on a Whole Mount Kidney.

References

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