Visual Guide to FFPE materials preparation

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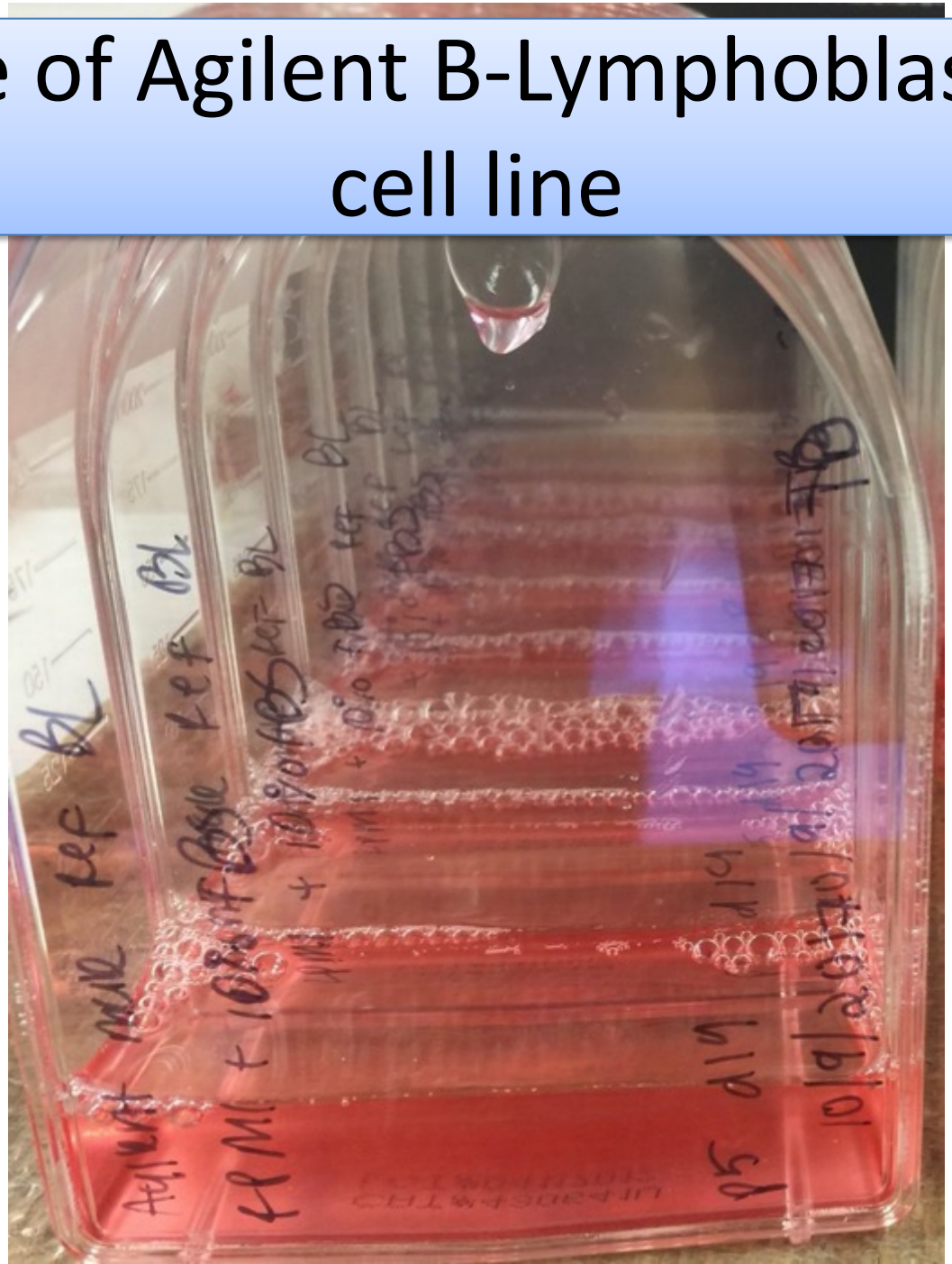
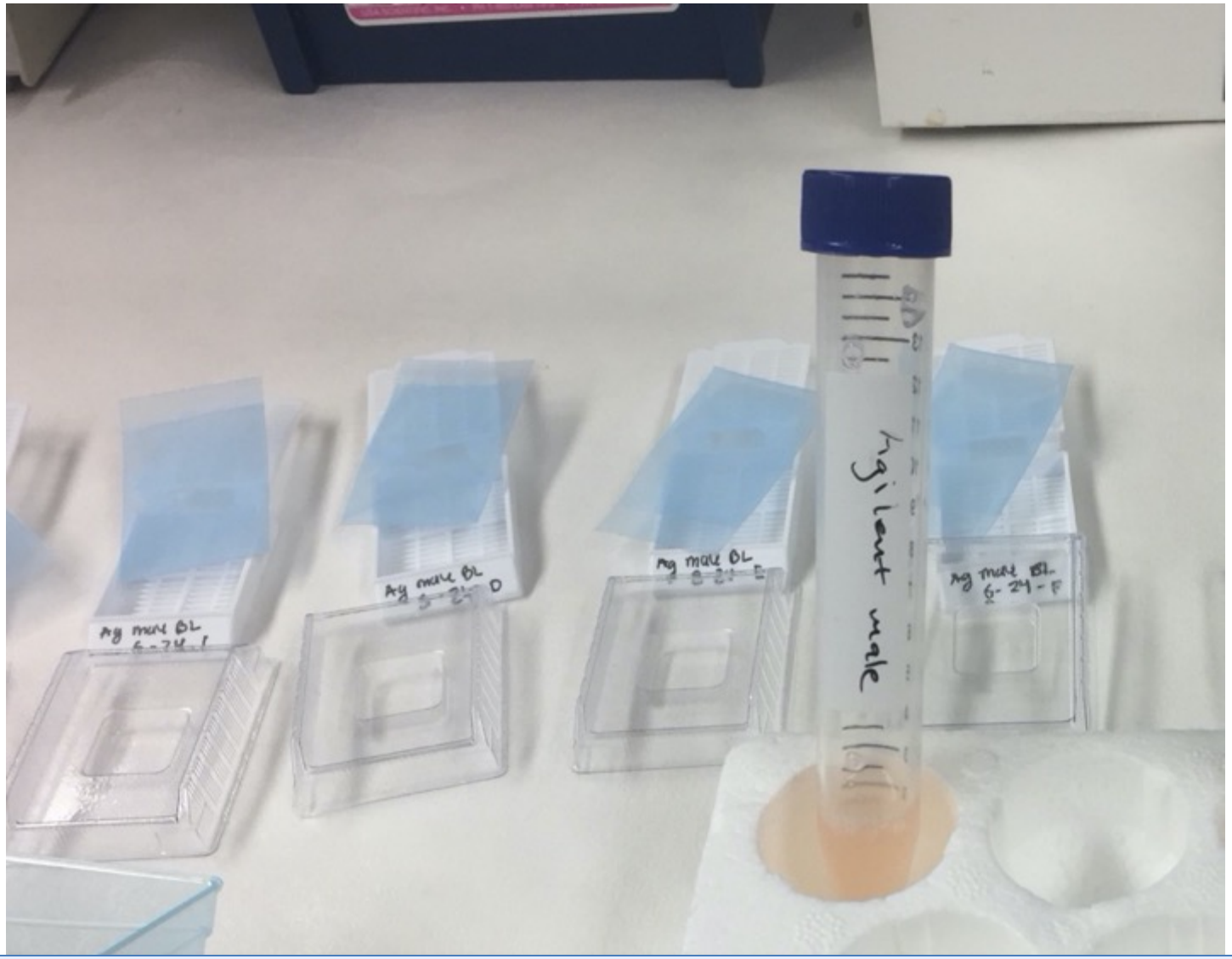
Culture of Agilent B-Lymphoblast Male cell line

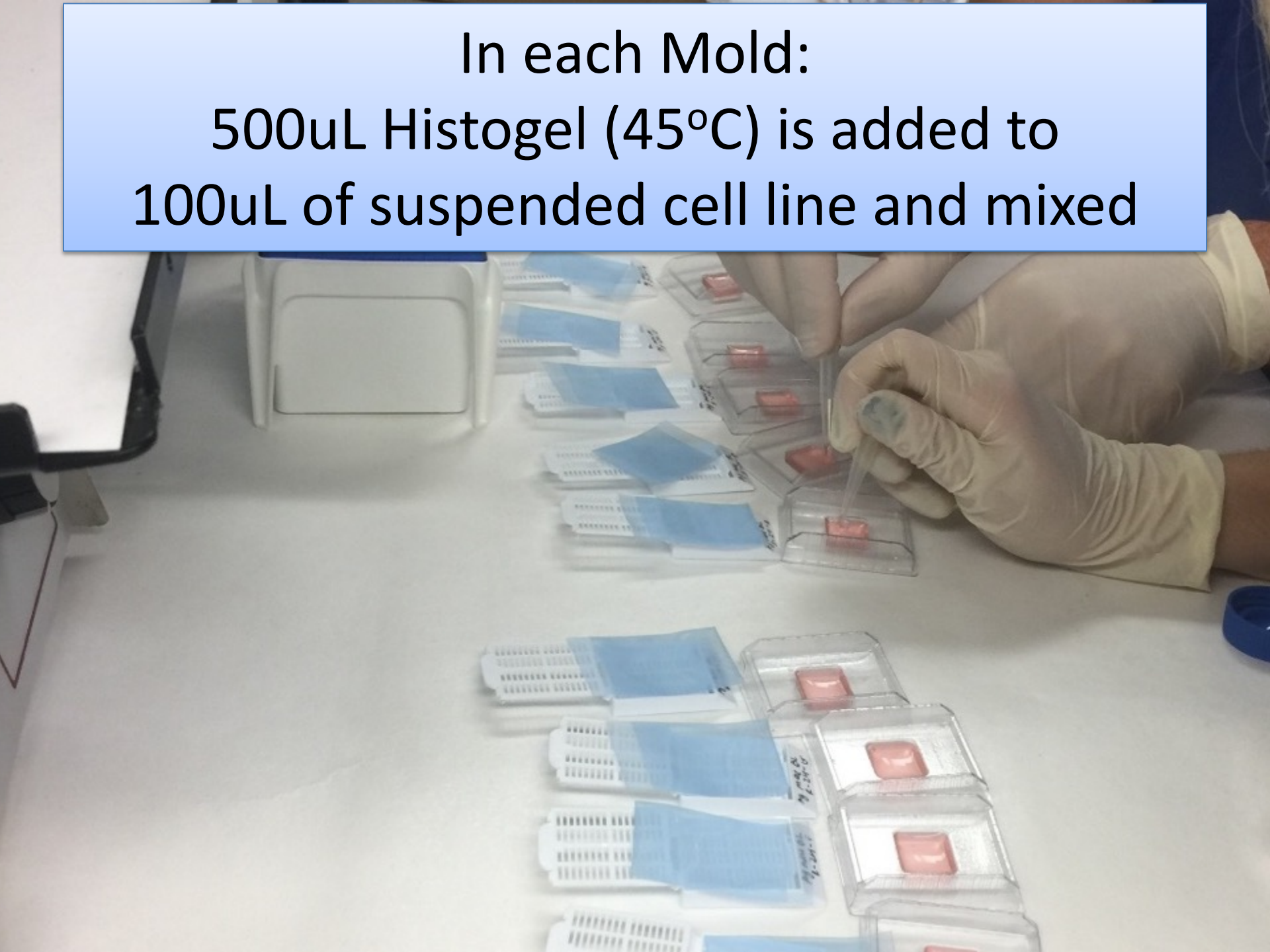


Harvesting

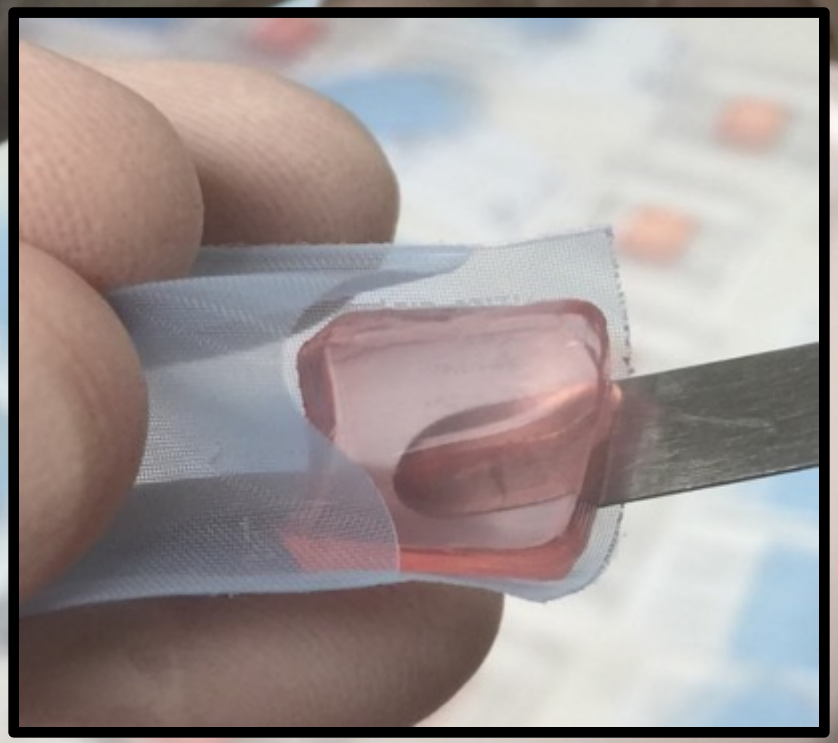
Set up for the Histogel embedding



Harvested cell pellets are suspended in 10% neutral buffered formalin


In each Mold:
500uL Histogel (45°C) is added to
100uL of suspended cell line and mixed

Histogel is set (~5 minutes)
Mold has about 0.5-1.0 million cells each
Cell molds are placed in Nylon Bags

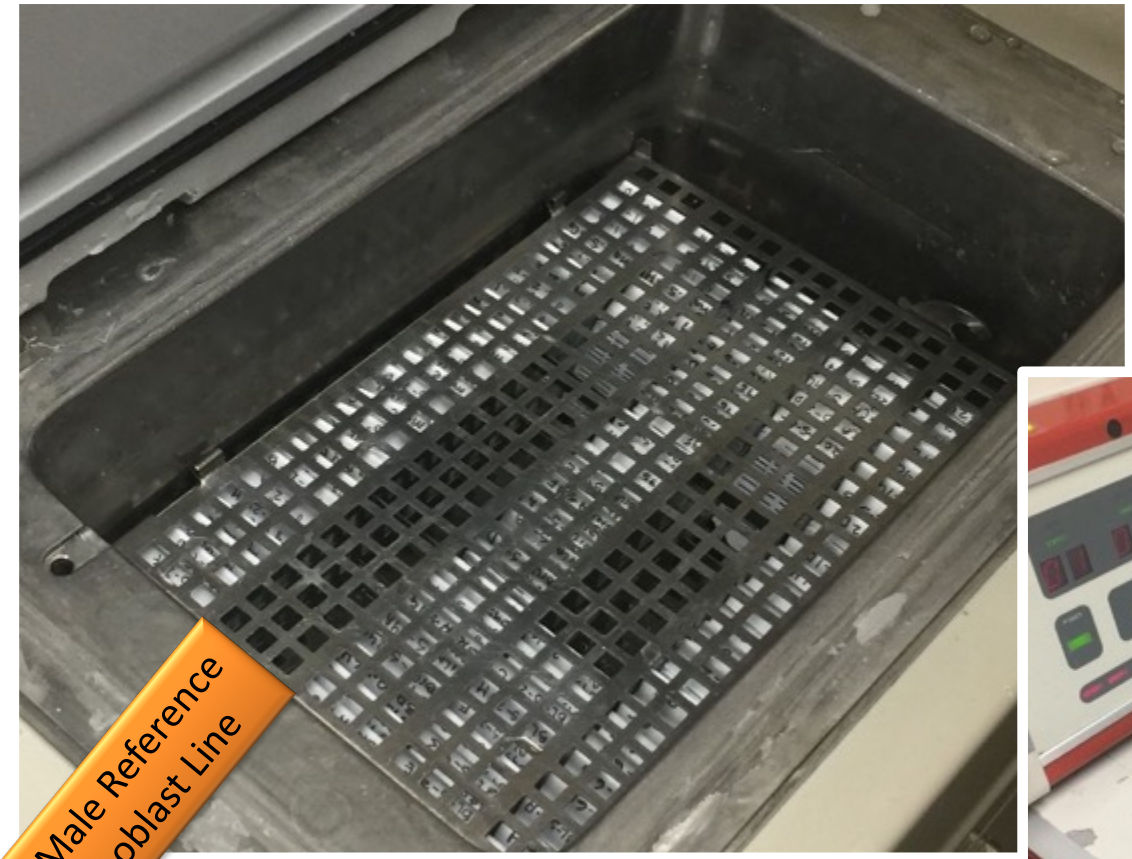


Separate spatulas were used for each time point

Nylon bags with histogel cell molds are placed in cassettes and then in 10% neutral buffered formalin for 24, 6, 2, or 1 hours.

Agilent Male B-Lymphoblast Line

After 24, 6, 2 or 1 hour pre-processing formalin fixation: Cassettes are placed in tissue processor for “Routine” Run



Agilent Male Reference B-Lymphoblast Line

UTMC-Pathology “Routine” Run on Sakura Tissue Tek VIP 5 Tissue Processor

STATION 1	10% Neutral Buffered Formalin	1 Hour
STATION 2	10% Neutral Buffered Formalin	1 Hour
STATION 3	70% Ethanol	1 Hour
STATION 4	80% Ethanol	1 Hour
STATION 5	95% Ethanol	45 minutes
STATION 6	95% Ethanol	45 minutes
STATION 7	100% Ethanol	45 minutes
STATION 8	100% Ethanol	45 minutes
STATION 9	Xylene	45 minutes
STATION 10	Xylene	45 minutes
STATION 11	Paraffin @ 60° Celsius	30 minutes
STATION 12	Paraffin @ 60° Celsius	30 minutes
STATION 13	Paraffin @ 60° Celsius	30 minutes
STATION 14	Paraffin @ 60° Celsius	0 minutes

Cell blocks (Post-tissue processing) are embedded in paraffin

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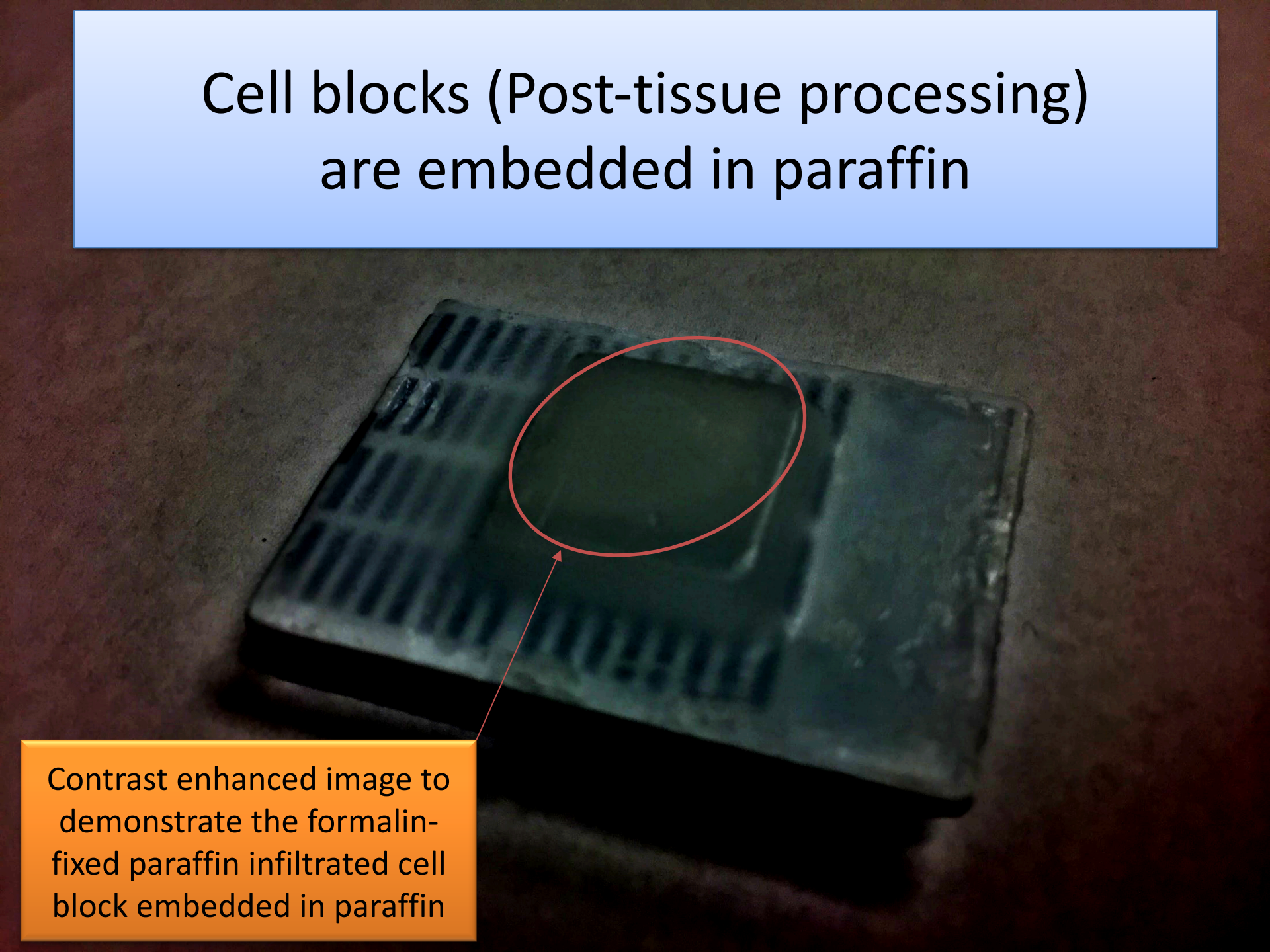
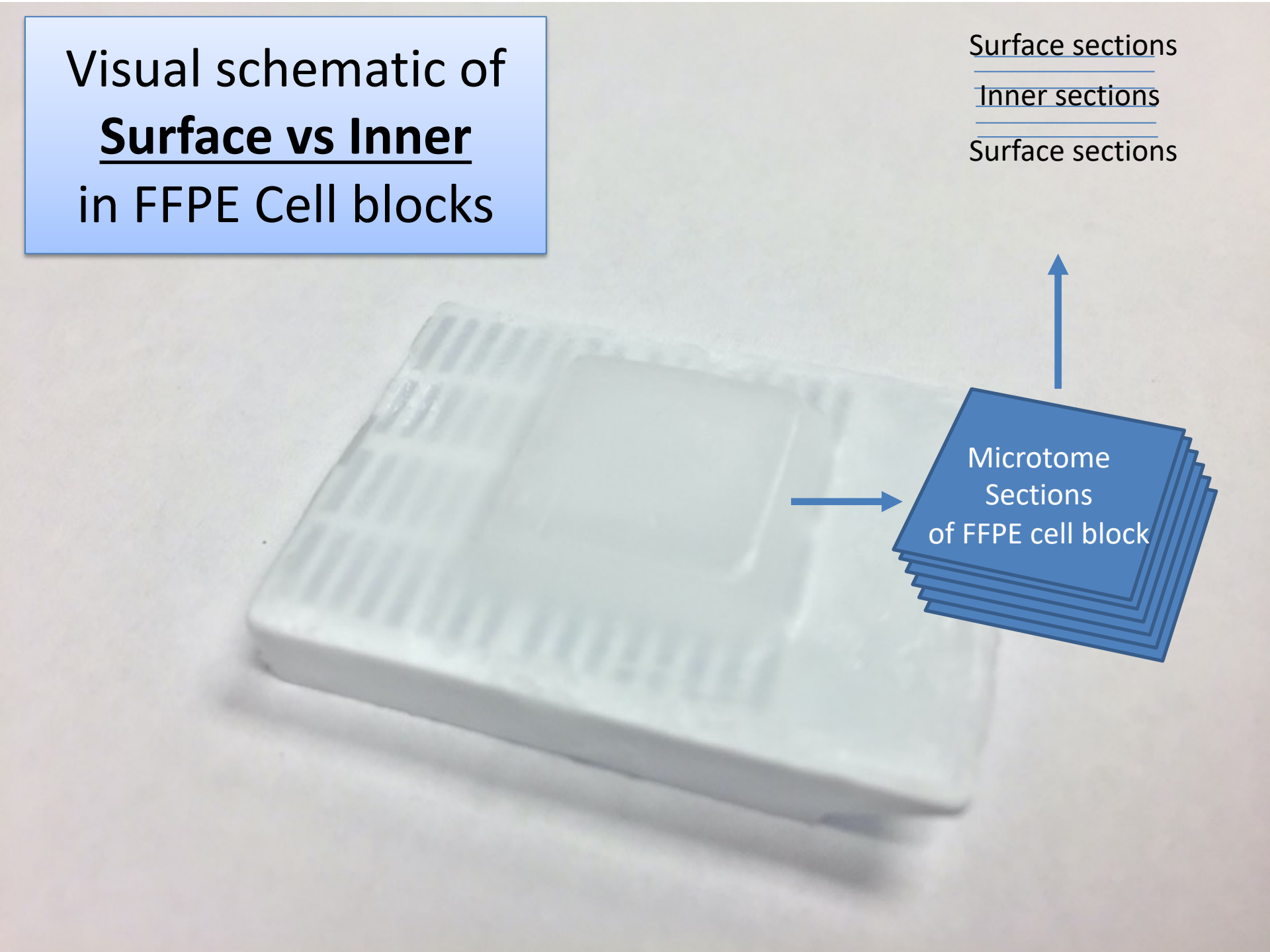


Contrast enhanced image to demonstrate the formalin-fixed paraffin infiltrated cell block embedded in paraffin

These cell blocks were then serially sectioned using a microtome at 5 micrometer thickness and this shaved materials was placed into individual Lobind Eppendorf tubes and sent to vendors for extraction, library preparation, and NGS analysis

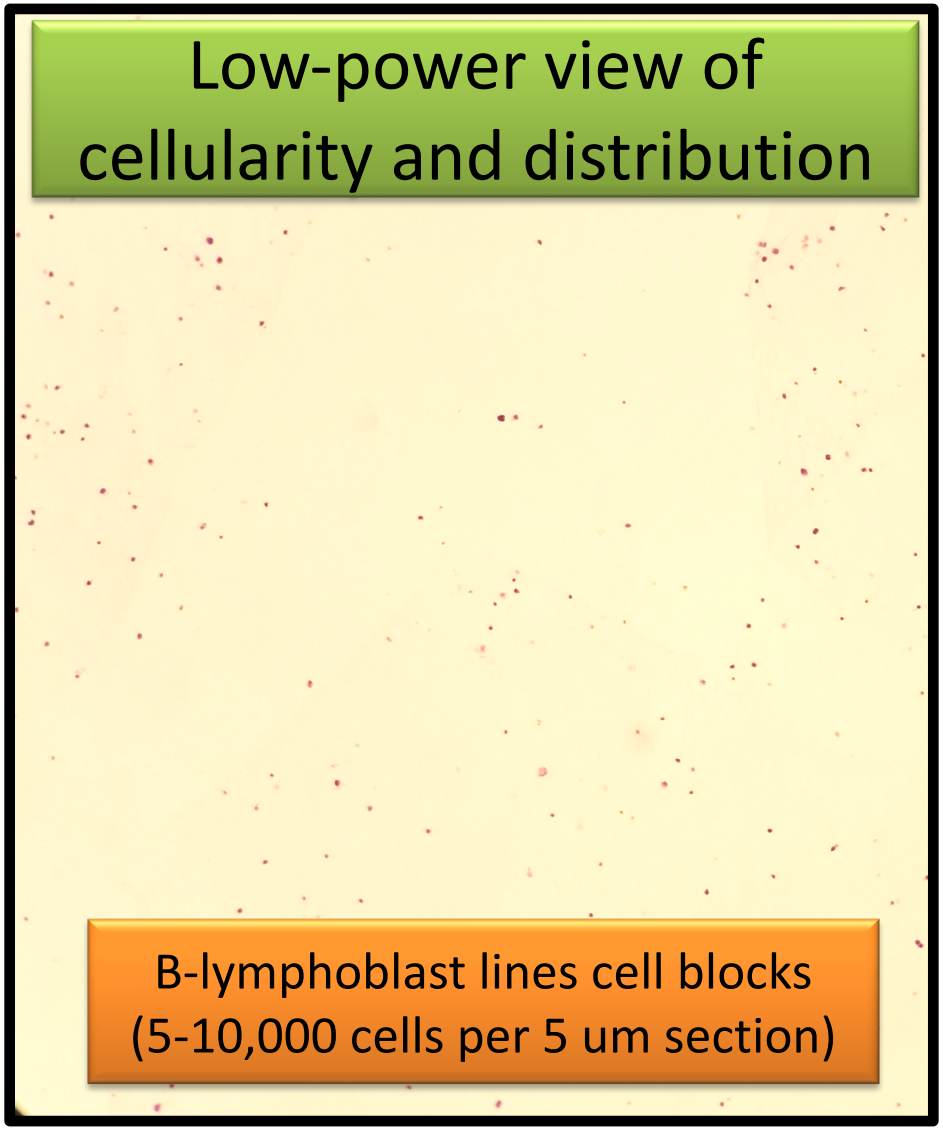
Every 8 sections, a representative section was taken for routine microscopic analysis with H&E staining for cell-counting

Visual schematic of **Surface vs Inner** in FFPE Cell blocks



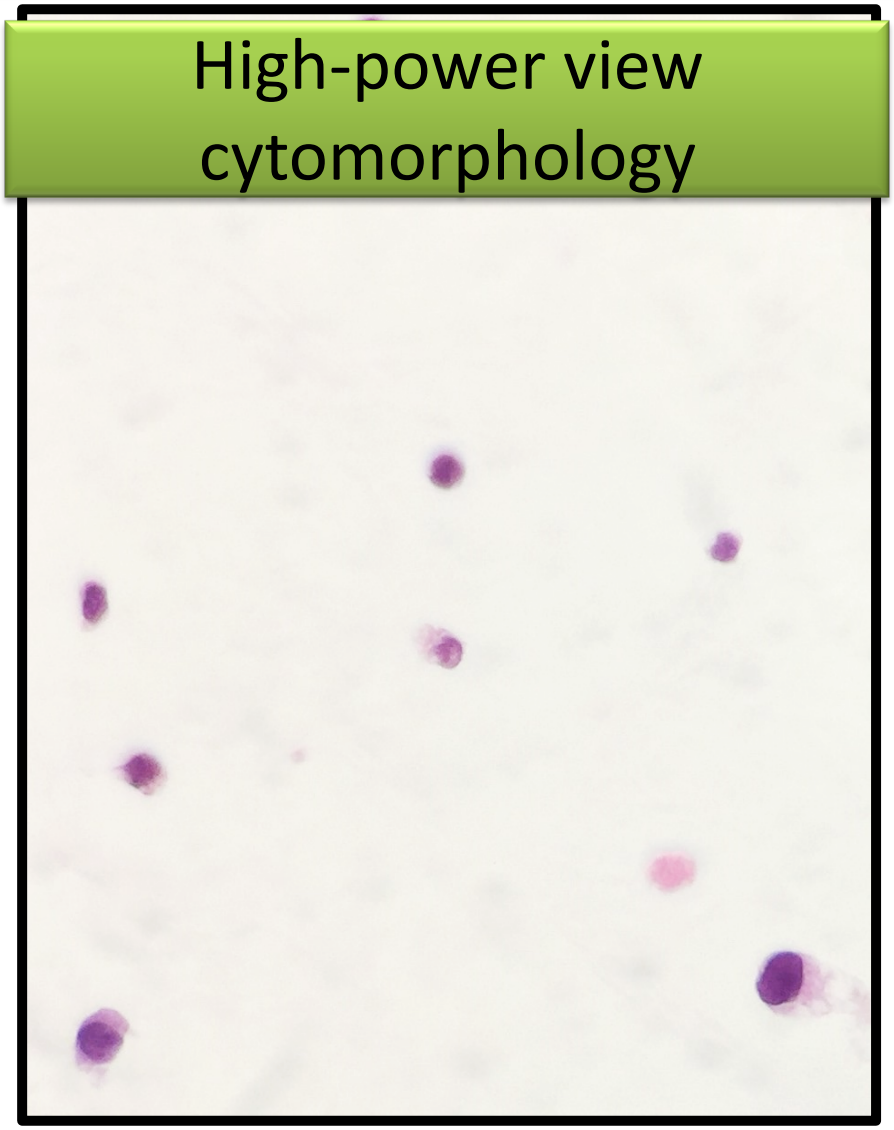
Hematoxylin and Eosin stained sections from FFPE cell blocks

Low-power view of cellularity and distribution



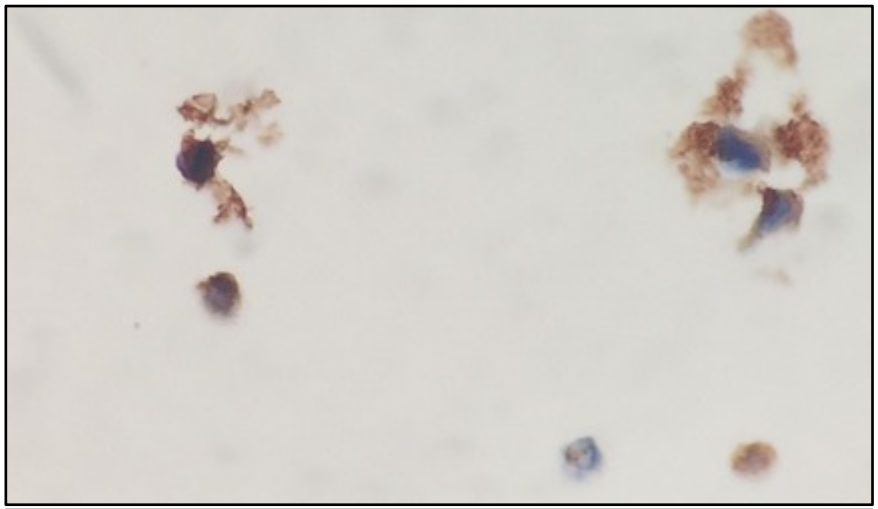
B-lymphoblast lines cell blocks (5-10,000 cells per 5 um section)

High-power view cytomorphology

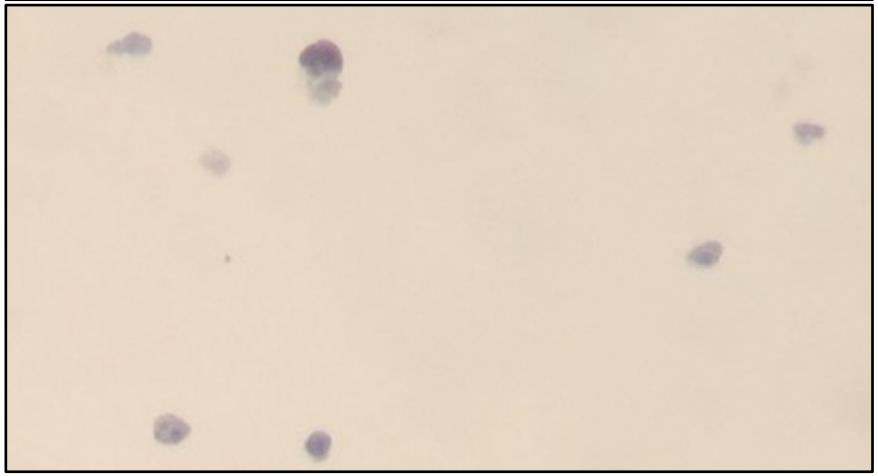


Immunohistochemical staining of sections from FFPE cell blocks

Leukocyte Common Antigen (CD45)



PanKeratin



FFPE Agilent B-lymphoblast cell-line cell-block