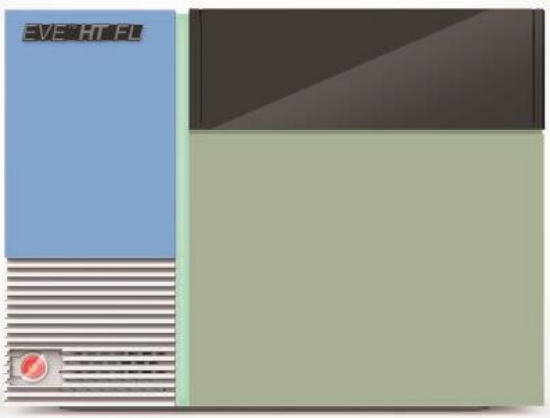
EVE HT FL

Application Note



Sharing innovation

NanoEntek

Robust FFPE Nuclei Quantification Using EVE™ HT FL Automated Cell Counters

Introduction

The global demand for formalin-fixed paraffin-embedded (FFPE) tissue analysis continues to expand. Driven by advancements in precision medicine, these specimens have become increasingly important for biomarker discovery and targeted therapy development. Recently, FFPE tissues have been successfully integrated into advanced genomic workflows, such as single-nucleus RNA sequencing (snRNA-seq). Prior to snRNA-seq, precise nuclear counting is required to maximize target discovery rates while minimizing doublets.

However, FFPE samples undergo extensive molecular cross-linking and structural alterations during the fixation and embedding processes. Furthermore, isolating nuclei from these specimens requires aggressive mechanical and enzymatic tissue dissociation. These processing steps frequently generate excessive extracellular matrix debris and fragmented nuclei. Under these conditions, AO exhibits non-specific binding, which overestimates the number of isolated nuclei. In this study, we demonstrate accurate quantification of FFPE derived nuclei using DAPI or PI with NanoEntek's fluorescence cell counters.

Material & Method

Materials

- Mouse FFPE tissue sample
- Human FFPE tissue sample
- Xylene
- Ethanol (100%)
- Nuclease Free Water
- PBS
- Collagenase I & II (Roche, 5401135001)
- Proteinase K (Roche, 311586001)
- Mortar and pestle
- Cell strainer (porosity 30µm)
- Quenching buffer (10xGenomics, 1000414)

Deparaffinization and Rehydration of FFPE tissues

1. Wash twice in xylene for 10 min each.
2. Wash twice in 100% ethanol for 5 min each.
3. Wash once in 70% ethanol for 3 min.
4. Wash once in 50% ethanol for 3 min.
5. Wash once in nuclease-free water for 3 min
6. Wash once in cold PBS for 5 min.

Tissue Dissociation and Nuclei isolation *

1. Suspend deparaffinized and rehydrated tissues in an enzyme solution like collagenase. Optionally, one can use proteinase K for hard-to-dissociate tissues.
2. Mechanically dissociate tissues in enzyme solution using a pestle
3. Pass everything through a 30 µm filter
4. Centrifuge at 700 RCF for 5 min
5. Resuspend pellet in 1x quenching buffer
6. Centrifuge at 700 RCF for 5 min
7. Resuspend pellet in PBS
8. Run cell counting

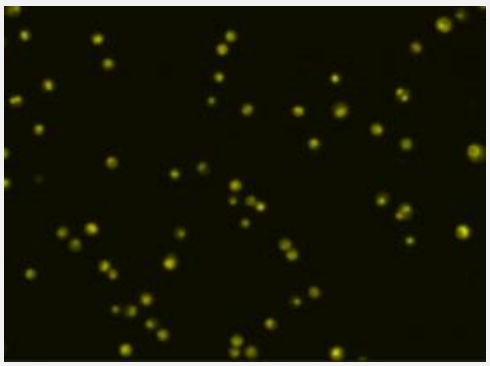
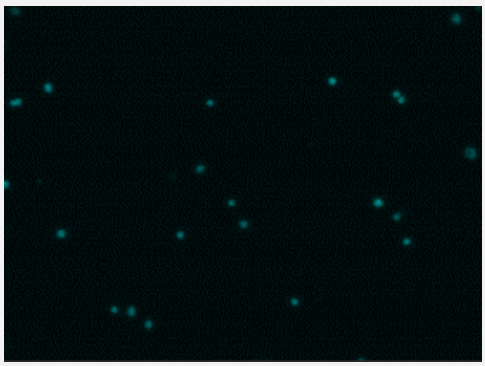
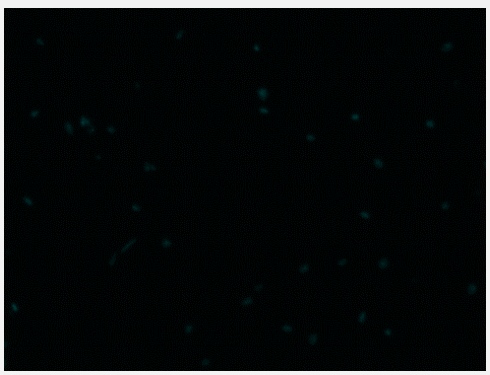
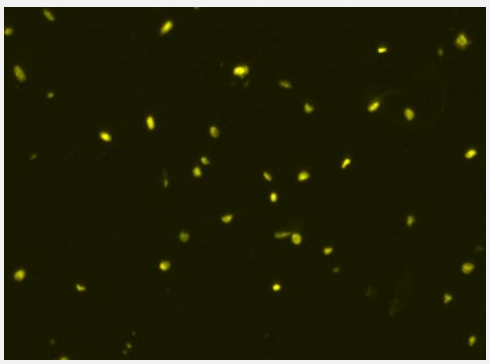
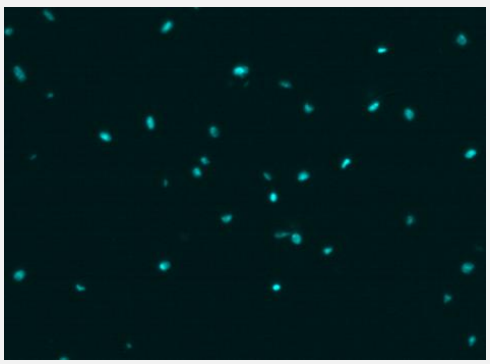
**** Nuclei isolation protocol can vary depending on tissue types and institutions. Users are expected to use a protocol that produces nuclei with little clumps or debris.***

Staining dye & Instrument

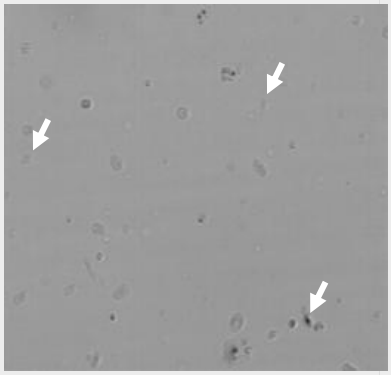
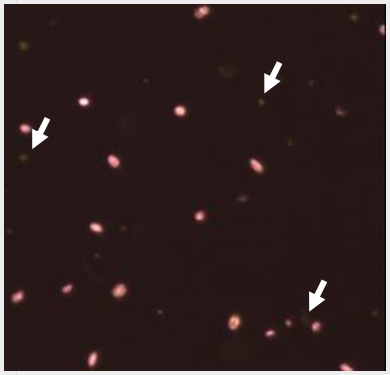
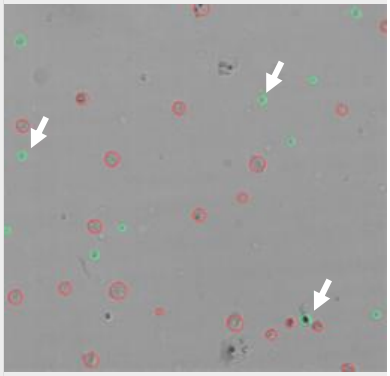
- AO/DAPI with EVE™ HT FL
- YOYO-1 with EVE™ HT FL
- PI (N solution) with ADAM™ MC2

Results

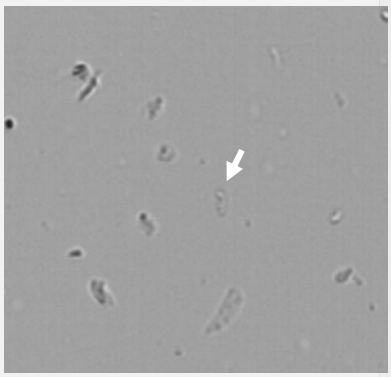
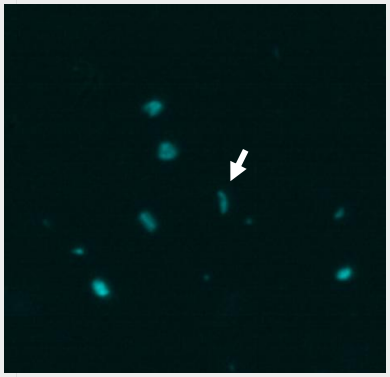
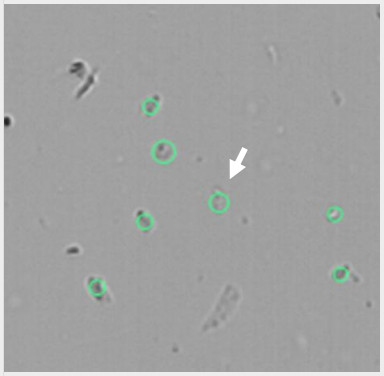
We first tried to stain nuclei isolated from FFPE tissues (Mouse) with EVE HT FL reagent (AO/DAPI). While mammalian cells stained with EVE HT FL reagents were bright in both AO and DAPI channels, FFPE nuclei stained with EVE HT FL reagents were very dim in both fluorescence channels. After increasing exposure time significantly, we were able to make them bright, and we found that nuclei were bright enough to be counted with EVE HT FL.

	AO channel	DAPI channel
Example images of cells		
Example images of nuclei isolated from FFPE with a standard setting		
Example images of nuclei isolated from FFPE with an increased exposure time		

When we looked at images closely, we found that AO stained not only nuclei but also debris that did not look cell nuclei. However, DAPI seemed to stain almost all nuclei and EVE HT FL software could count nuclei with various shapes including elongated one as shown in the example below.

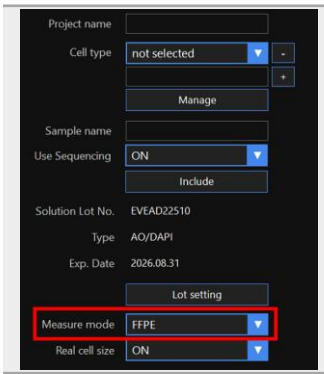
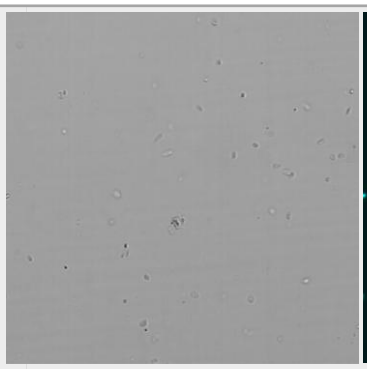
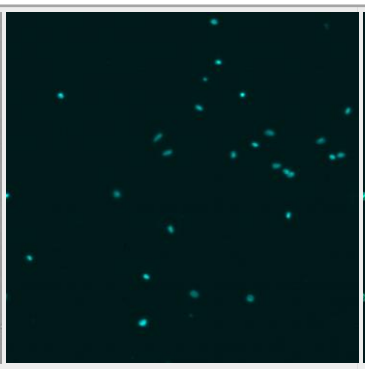
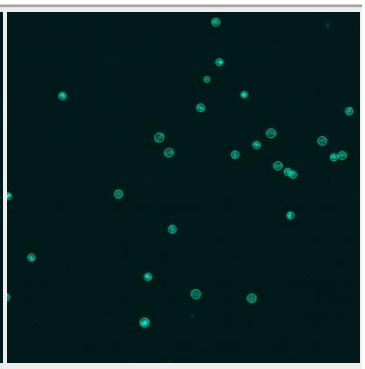
Bright field	Fluorescence channel	FL image with counting circles
	AO/DAPI merged 	Red (AO&DAPI) Green (AO only) 

※ Green circles indicate AO-detected debris (AO/DAPI merged) or detected nuclei (DAPI-only). Red circles indicate DAPI-detected nuclei in AO/DAPI mode.

	DAPI 	Circles based on DAPI 

Adding a new FFPE mode

Because AO has high probability to stain not only nuclei but also some of cell debris, counting based on AO images could be misleading. On the other end, DAPI was found to have little tendency to stain non-nucleous debris. Therefore, we developed a new FFPE mode in which only bright field and DAPI channels will be taken, and nuclei counts will be measured solely from DAPI.

	Bright field	DAPI	With counting circles
			

Comparison with other staining methods

NanoEntek's single slide fluorescence cell counter, ADAM MC2 uses PI (propidium iodide) to count nucleated cells. Because PI is known to be nuclei-specific, we compared PI and DAPI with nuclei isolated from FFPE tissues. In addition to them, we have also tried YOYO-1. We have tested these 3 dyes with various FFPE tissues. All the results suggest that DAPI on EVE HT FL and PI on ADAM MC2 gave very similar nuclei counts.