7 Applications for Formalin-Fixed Paraffin-Embedded (FFPE) Tissue Specimens

As technologies and methodologies advance, so do the potential applications for biospecimens. In this article, we explore 7 uses of FFPE, ranging from the common to the cutting-edge.

1. **Immunohistochemistry.** FFPE tissues are frequently used in immunohistochemistry (IHC) for determining the distribution of an antigen or biomarker of interest in tissues. IHC is used widely in oncology for making diagnoses, evaluating prognosis, and predicting response to therapy. Additionally, it plays a critical role in biological research and drug development for immunology, hematology, infectious diseases, and neurodegenerative disorders.

   IHC is also utilized in the development of therapeutic antibodies or antibody-like molecules, particularly for preclinical tissue cross reactivity (TCR) studies, where a series of IHC screening assays are used to not only identify off-target binding, but also to detect previously unknown sites of on-target binding for novel biotherapeutics. The FDA generally recommends fresh frozen tissues for TCR studies; however, certain cytoplasmic or nuclear antigens may be better preserved in FFPE specimens.[1] Tissue microarrays containing FFPE tissue core samples may also be useful for preliminary evaluations of cross-reactivity focused on lead identification or optimization.

   Advances in digital pathology platforms and artificial intelligence (AI) tools are improving the efficiency and accuracy of IHC. For example, there are approved digital pathology platforms that enable pathologists to score or diagnose from a computer, allowing for quicker turnaround times and remote collaboration. Precision for Medicine supported the development and approval of several of these platforms, including first digital pathology solution for primary diagnostic use in the US, the Philips PIPS (Philips IntelliSite Pathology Solution).

   Additionally, given its ability to retain the spatial coordinates of every cell and the pathologist annotations, digital pathology can be used to quantify distances between cells and other objects. These cell-to-cell or cell-to-structure distances give us an idea of how different subpopulations of cells are interacting with each other, or how different markers are expressed on similar cell types, depending on their localization. For example, immune cells far away from the tumor might have different levels of expression of some markers compared to immune cells that are very close to the tumor, or even infiltrating the tumor. These distances also allow scientists to use the tissue architecture to better assess the tumor microenvironment, much more so than the traditional percentage of positive cells or area.

AI algorithms are also making it easier for pathologists to classify tissues or regions, perform more accurate annotations more efficiently, quantify stained areas, and evaluate single-cell resolution data. Beyond their research applications, some of these algorithms are being developed and evaluated with the intention of validating them as companion diagnostics (CDx) at some point.

2. **Transcriptome and gene expression analysis.** Historically, fresh frozen tissue has been the sample of choice for RNA sequencing as it contains relatively large amounts of RNA. However, collection and storage of fresh frozen tissue can be costly and involve complex logistics. FFPE samples can be challenging for molecular analysis due to high variability, low yield, and higher degradation of RNA compared to other tissue sources. Newer transcriptomic technologies have made it possible to reliably perform gene expression analysis studies on FFPE specimens.

   The NanoString nCounter® Analysis System, for example, does not require any amplification steps and can detect and quantify hundreds of unique transcripts in a single reaction.[2] Studies have shown that data generated by nCounter from FFPE specimens correlate with data generated from matched frozen tissue and is superior to qPCR for gene expression analysis.[3]

   RNA-sequencing with next-generation sequencing (NGS) is becoming the method of choice for studying the transcriptome. Compared to gene expression arrays, RNA-sequencing offers a broader dynamic range and is not limited by prior knowledge, so it captures both known and novel features—including transcript isoforms, gene fusions, and single nucleotide variants—in a single assay.[4]

3. **Multiplex immunofluorescence for spatial signatures.** Multiplex immunofluorescence (mIF) is a technique that preserves the architectural features of a sample and reveals the spatial relationships between cells.[5] Multiplexing allows simultaneous analysis of multiple markers for exploration of the spatial relationships and physical interactions among them. FFPE samples of solid tumors can be used for mIF, providing insights into the tumor microenvironment and the extent and spatial distribution of immune cell infiltration, such as PD-L1 expression and the average distance to CD8 cells.

   For example, in one study, multiplex IHC/immunofluorescence showed diagnostic accuracy comparable to multimodality cross-platform composite approaches, including tumor mutational burden (TMB), gene expression profiling, and PD-L1 IHC, in predicting response to anti–PD-1/PD-L1.[6] While the optimal signatures are yet to be discovered, leveraging spatial context has the potential to significantly increase the ability to predict response to treatment.

   In addition, multiplexing is developing rapidly and highly multiplexed technologies are emerging. Some of these are IF-based, while others use barcodes or mass cytometry. These technologies further increase the number of markers that can be analyzed on the same slide. While this may increase the complexity of data, researchers are developing computational solutions that leverage AI to discover new signatures or relevant cell populations involved in patient response.

4. **Epigenetic profiling.** The study of epigenetic markers is an emerging approach to not only understand the molecular basis of carcinogenesis, but also to gain insight into cancer diagnosis and potential targeted

therapies. As with gene expression analysis, the first methods for epigenetic profiling required high-quality DNA from fresh frozen tissues. Now, technologies such as Precision for Medicine's proprietary EpiontisID can detect differential methylation in DNA extracted from FFPE samples. With over 69,000 samples analyzed across more than 100 clinical trials, EpiontisID is a qPCR-based immune cell phenotyping platform based on epigenetic cell counting. Studies have shown that EpiontisID results correlate strongly with flow cytometry, making it an effective solution for immune monitoring in both early- and late-stage clinical trials.[7]

EpiontisID can be performed on FFPE samples to monitor more than 30 immune cell types, for which fully validated, automated epigenetic assays have been developed. This includes B and T cells or subsets of T cells, such as Treg cells, all expressed as percentage of total cells. For instance, FFPE samples of atopic dermatitis or psoriasis can be characterized for a wide range of infiltrating immune cells including Th17 cells. Bisulfite-specific NGS, which characterizes the methylation status of a genomic region, can complement investigations with additional markers beyond the cell type-specific epigenetic biomarkers that are already validated and available.

5. **Spatial transcriptomics.** This term describes the ability to locate and localize transcripts down to the subcellular level, providing a three-dimensional map of RNA targets throughout tissue sections.[8] NanoString's GeoMX® Digital Spatial Profiling platform provides spatial resolution of both RNA and protein detection on all sample types, including FFPE. NanoString has also developed protein assays for over 300 validated antibodies that enable multi-analyte analysis for both nCounter and NGS readouts.

   10x Genomics has developed Visium Spatial Gene Expression for FFPE, which combines histological spatial information with whole transcriptome analysis. Visium can provide spatial profiling of RNA expression for over 18,000 genes in human and mouse FFPE samples. It can be combined with IF for simultaneous visualization of protein and gene expression or with H&E staining for morphological context.

   The ability to understand transcriptomic profiles in a highly multiplexed manner, while still retaining tissue architecture, can provide a more comprehensive understanding of a tumor and its surrounding environment. Researchers are also developing clustering algorithms to better generate meaningful insights from these highly multiplexed data, and the potential to correlate spatial transcriptomics with spatial protein information would increase the data obtained from each sample.

6. **Tumor profiling.** Cancer profiling using FFPE is commonly used for determining eligibility for treatment and for discovering new biomarkers. There are a number of commercially available assays, including the Oncomine Precision Assay and the TruSight Oncology 500 (TSO500) assay, which analyze variants across known cancer-related genes using NGS. The Oncomine Precision Assay analyzes known variants across 50 key genes. The TSO500 includes pan-cancer biomarker content that supports identification of all relevant DNA and RNA variants implicated in a variety of solid tumor types. The assay also measures microsatellite instability (MSI) and TMB and is available in a high throughput version.

7. **RNAscope®.** This is a novel in situ hybridization (ISH) assay for detection of target RNA within intact cells. With its proprietary probe design that amplifies target-specific signals, but not

background noise, the assay represents a major advance in RNA ISH approaches. RNAscope can be multiplexed and even combined with protein assays.

Key Considerations

With technological and methodological advances, FFPE tissues are becoming an increasingly valuable source of DNA, RNA, and proteins for research and therapeutic applications. The quality of the sample is critical, and researchers should seek tissues that have been prepared using the most stringent standards and quality control measures. Precision for Medicine offers an extensive inventory of pathologist-reviewed FFPE tissue blocks, slides, curls, cores, and tissue microarrays from both normal and diseased subjects, all collected under IRB-approved protocols. Each sample comes with pathology reports and, optionally, medical history, disease characterization, and outcomes data. To learn more about our FFPE tissue library, click here.

Precision for Medicine also has 7 full-service, accredited labs providing custom assay development, testing, and clinical trial support. You can learn more about these services at: www.PrecisionforMedicine.com.

References:

1. Leach MW, et al. Use of tissue cross-reactivity studies in the development of antibody-based biopharmaceuticals: history, experience, methodology, and future directions. *Toxicol Pathol*. 2010;38(7):1138-1166.
2. NanoString. FFPE Simplified. Available at https://www.nanostring.com/products/ncounter-analysis-system/ncounter-systems-overview/ffpe-simplified/
3. Reis PP, et al. mRNA transcript quantification in archival samples using multiplexed, color-coded probes. *BMC Biotechnol*. 2011;11:46.
4. Illumina. Study gene expression using RNA sequencing. Available at https://www.illumina.com/techniques/sequencing/rna-sequencing.html
5. Lee CW, et al. Multiplex immunofluorescence staining and image analysis assay for diffuse large B cell lymphoma. *J Immunol Methods*. 2020;478:112714.
6. Lu S, et al. Comparison of biomarker modalities for predicting response to PD-1/PD-L1 checkpoint blockade: A systematic review and meta-analysis. *JAMA Oncol*. 2019;5(8):1195-1204.
7. Baron U, et al. Epigenetic immune cell counting in human blood samples for immunodiagnostics. *Sci Transl Med*. 2018;10(452):eaan3508.
8. Nanostring. Spatial Transcriptomics: Birth of GeoMx® Digital Spatial Profiler, May 24, 2021. Available at https://www.nanostring.com/blog/pioneers-in-spatial-transcriptomics-the-birth-of-geomx-dsp/