# Custom Tissue Microarray (TMA) Development for Maximized Capture Area in Spatial Transcriptomic Profiling by Xenium

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## Introduction / Background

Tissue microarrays (TMAs) are widely used in cancer research due to their ability to analyze multiple tissue samples in a single paraffin block. This format makes histological and molecular studies more efficient while conserving the samples and reagents. In lung cancer, where tumor heterogeneity requires broad sample representation, TMAs provide a scalable and cost-effective way to compare multiple lung cancer tissue samples. However, conventional TMA molds often have limited capacity, wide spacing, and inconsistent stability during sectioning. These issues can lead to tissue loss or distortion, reducing the reliability of results. Recent studies have described silicone molds created from 3D-printed designs, but most use larger spacing and standard paraffin wax, which restricts block density and durability (Pazaitis et al., 2023). To overcome these limitations, an existing protocol was modified to produce custom TMA molds with higher capacity, improved stability, and smoother sectioning.

## Purpose and Objectives

The purpose of the project was to design, fabricate, and validate custom tissue microarray (TMA) molds optimized for lung cancer tissue studies. The objectives focused on improving various aspects of TMA production and usability. First, the project aimed to increase the capture area within each paraffin block by reducing the inter-core spacing and adjusting the well dimensions for greater sample density (Remotti, 2013). Second, we aimed to improve sectioning quality using streamlined embedding procedures and harder paraffin wax, to enhance the integrity of tissue sections during microtomy. Finally, the project aimed to refine the reproducibility of tissue core placement by improving mold release and demolding techniques, ensuring consistent alignment and reducing potential errors.

## Methods and Procedures

Mold Design and Fabrication**:** Polylactic acid (PLA) molds were custom designed using CAD software and fabricated via 3D printing. To maximize block density, inter-core spacing was reduced to 0.5 mm. Core wells were adjusted to improve fit: +0.05 mm for 1 mm, +.07mm for 1.5 mm wells, and +0.1 mm for 1-2 mm wells. These modifications ensured smoother insertion and reduced mechanical stress on tissue cores (Fowler, 2011).

Silicone Casting: PLA molds served as masters for silicone casting. 30 mL of silicone was poured per mold to keep block thickness manageable. The silicone was degassed under vacuum to eliminate air bubbles, improving mold fidelity. Mineral oil was applied as release agents to prevent adhesion to PLA. After curing, the silicone mold was separated using a soap-assisted demolding step, which preserved fine structural features.

Paraffin Embedding: Tissue cores were embedded using Paraplast X-TRA, a harder paraffin formulation that provides improved block integrity and facilitates thin sectioning. Compared to conventional paraffin, Paraplast X-TRA yielded blocks with higher resistance to tearing and compression during microtomy. Sectioning workflows were iteratively refined in collaboration with lab members to minimize tissue displacement.

## Results

The redesigned TMA molds showed improvements in capacity and sectioning performance compared to conventional wax molds. Five prototypes with core diameters from 0.5 mm to 3.0 mm were created to evaluate performance across sampling scales. Reducing inter-core spacing and optimizing well dimensions increased capture capacity per paraffin block by 33%, based on the number of usable cores and surface area utilization compared to standard molds. Pilot hematoxylin and eosin (H&E) sections showed tissue alignment within fiducial markers, with minimal displacement or overlapping across rows. Sectioning quality improved with Paraplast X-TRA, which reduced tissue shearing and core dislocation, producing uniform ribbons and preserving cellular morphology during microtomy. Reproducibility testing confirmed the reliability of the fabrication process; vacuum-degassed silicone allowed multiple duplicate casts of each PLA mold without structural degradation or dimensional loss. Overall, the redesigned molds improved experimental throughput and the consistency of histological preparation in lung cancer tissue studies.

## Conclusions and Recommendations

This project demonstrated the feasibility and benefits of fabricating high-capacity custom TMAs by modifying existing silicone mold protocols (Battifora, 1986). The redesigned molds increased capture area by 33%, reduced tissue damage during sectioning, and improved core alignment reproducibility. These outcomes are especially valuable for lung cancer research, where maximizing sample representation and preserving tissue integrity are essential for reliable histological and molecular analysis. Future work should include printing a precise bottle neck, optimizing hole refinement /expansion using drill bits, and adapting the mold design for integration with spatial transcriptomic workflows. Overall, our study shows that targeted engineering refinements can substantially improve TMA performance, providing a scalable and cost-effective approach for cancer research laboratories.

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