

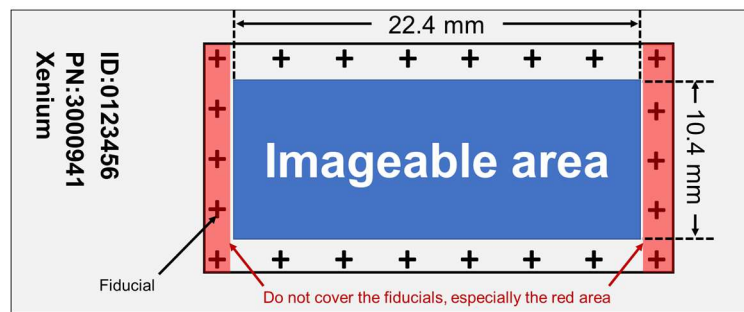
# Tissue Sectioning for Xenium Spatial Transcriptomics

## Guidelines:

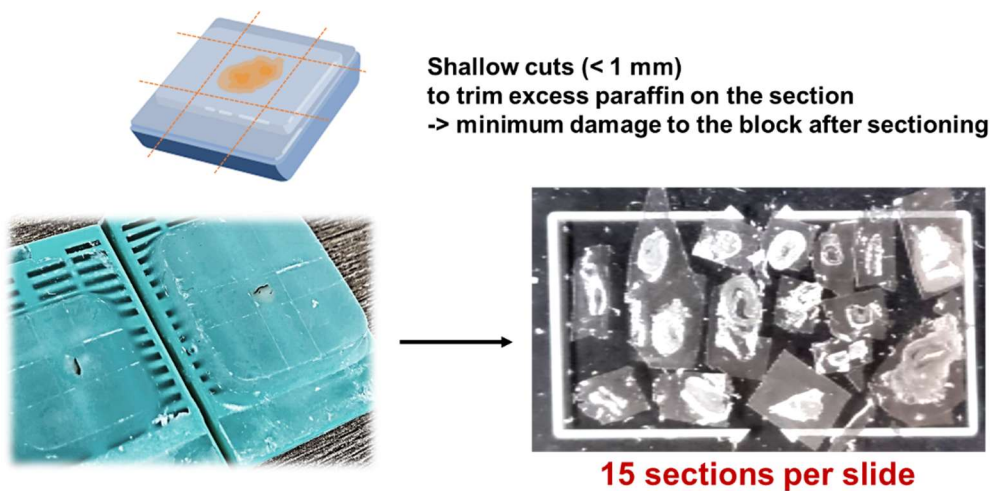
- The tissue section must be placed on specific Xenium Slide from 10x Genomics.
- Tissue slides should be freshly prepared, preferably within 4 weeks prior to Xenium Assay.
- The recommended section thickness is 5 µm for FFPE and 10 µm for fresh frozen samples.
- The tissue should have direct contact with the slide to prevent tissue detachment.
- The imageable area for Xenium is 10.4 mm × 22.4 mm per slide. Only the tissue placed within this area can be analyzed.

## Recommended procedure:

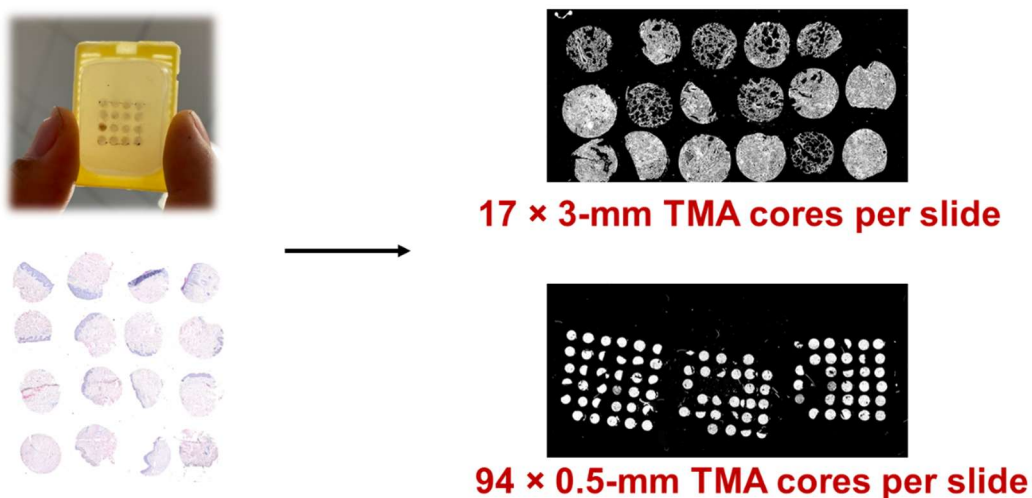
1. For FFPE samples, equilibrate the Xenium Slides (stored at -20°C) to room temperature for 30 minutes. Keep the Xenium Slide at -20°C for cryosection.
2. Place the tissue sections within the imageable area on the Xenium Slides. The fiducials should be avoided, especially the ones at the top and bottom of the area (**Figure 1**). For cryosections, keep the Xenium Slide at -20°C during sectioning.
3. It is recommended to trim or remove excess embedding material, e.g., paraffin or OCT, to achieve maximum utilization of the imageable area (**Figure 2**). Tissue microarray can also be used (**Figure 3**).
4. For FFPE sections, dry the section at room temperature for up to 30 min. Incubate the section at 42°C for 3 h.
5. Store the sections at room temperature (FFPE) or -80°C (cryosection) for up to 4 weeks. It is highly recommended to store the sections in a low moisture environment, e.g., with silicon desiccator.



**Figure 1 Xenium imageable area:** Only the tissue section placed within the imageable area can be analyzed. Avoid placing tissue on the fiducial, especially the area marked in red. Covering by paraffin or OCT should be fine, as it will be washed away during processing.



**Figure 2 Cost-efficient tissue arrangement:** Trimming tissue block is one of the methods to place multiple samples on one slide. The shallow cuts were made on the block so that the excess paraffin can easily be removed after sectioning.



**Figure 3 Compatibility with tissue microarray (TMA):** TMA should be the most cost-efficient method for Xenium. Depending on the size of tissue cores, 18 (3-mm TMA cores) or more than 100 samples (0.5-mm TMA cores) can be placed on one slide.