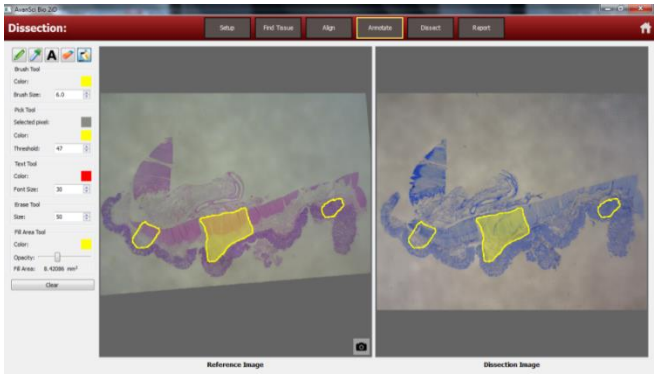
AvanSci Bio Technical Application:
FISH Analysis on Mesodissected Fragments of Tissue

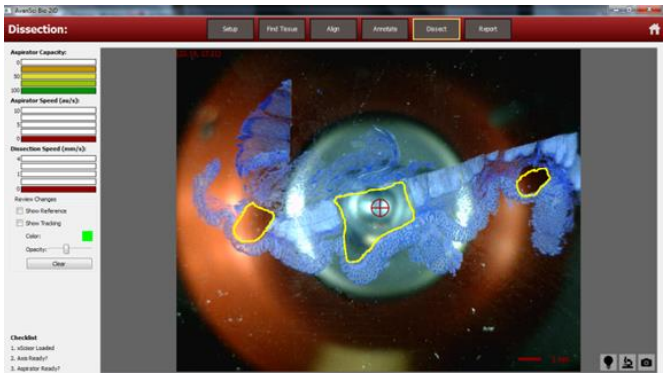
This technical application presents a protocol and example results for re-adhering mesodissected tissue fragments from slide-mounted tissue sections onto new slides and performing FISH analysis on these tissue fragments. This application has the potential to automate and increase the throughput of FISH on tissue sections while greatly reducing sample consumption.

The AvanSci Bio mesodissection system is essentially a micro tissue mill that employs a specialized disposable mill bit termed the xScisor® that simultaneously dispenses liquid, cuts tissue from the slide surface, and aspirates the liquid along with the displaced tissue fragments. The accompanying 2iD software package is capable of transferring digitally annotated AOI(s) (Areas Of Interest) between images of serially cut tissue sections to guide dissection and generate an electronic record of the process. In this manner, the AOI(s) can be identified on a cover slipped H&E slide, dissected from the analogous regions of a single non-stained non-cover slipped serial section, and spotted onto multiple locations on one or more new slides for FISH analysis with multiple probes.

Identification of AOI(s) to Dissect on Serial Tissue Sections

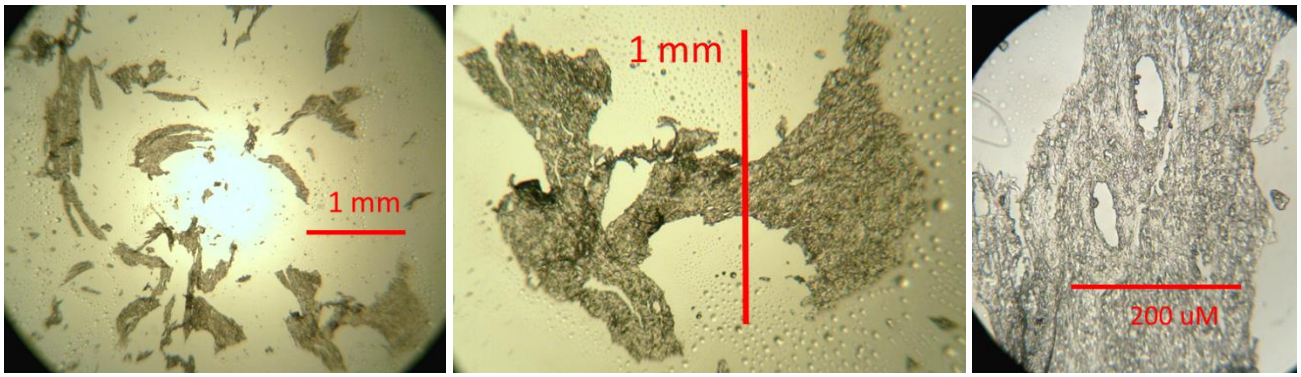
In the example below, three AOIs were indicated on a digital image of an H&E stained slide. This digital image was aligned to a digital image of a deparaffinized and Analine Blue stained serial section acquired from the digital microscope situated below the stage of the mesodissection instrument. The AOIs were transferred to overlay the live view of the Analine Blue stained section. These digital AOIs move as the instrument stage moves providing guidance of mesodissection, either by hand using the MeSectr or automated dissection using the MilliSect.





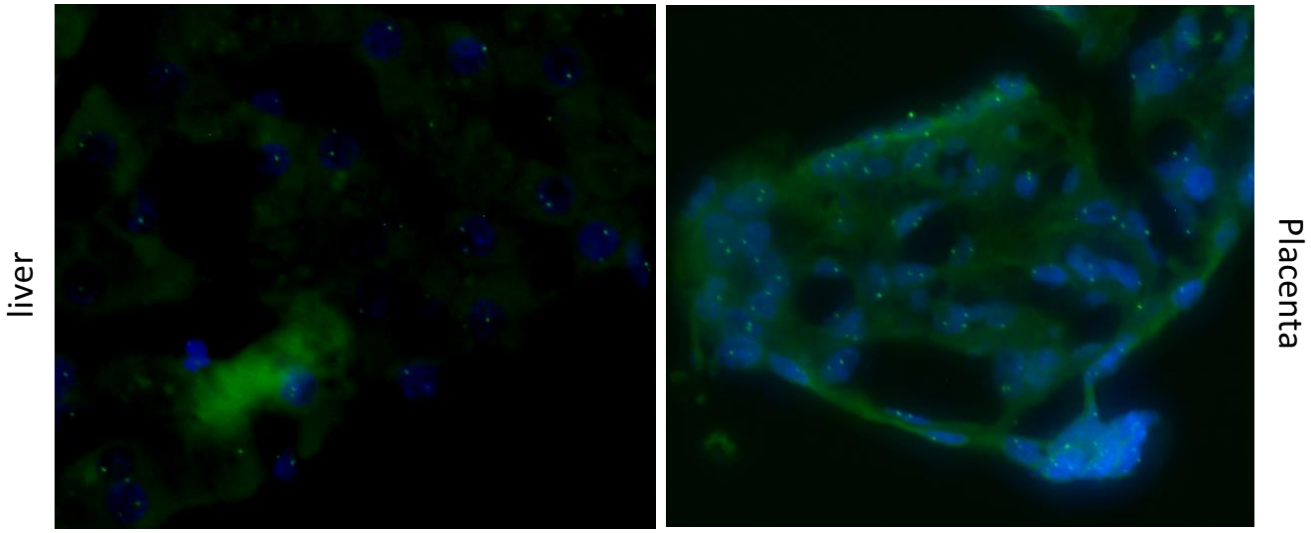
Mesodissection of Tissue Fragments

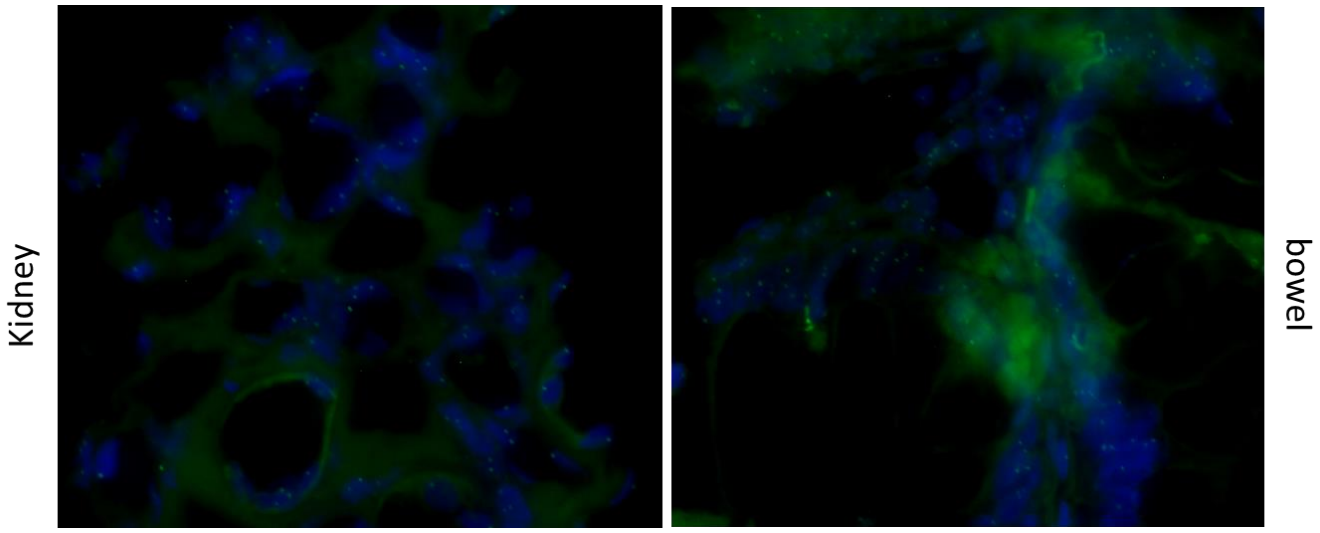
To dissect the slide-mounted tissue, the xScisor was loaded with TE plus 0.1% Tween-20 to be used as the tissue fragment aspiration (milling) solution. The recovered tissue fragments were ejected into a microfuge tube, centrifuged for 2 minutes, and the majority of the supernatant discarded. The tissue fragments were gently resuspended using a large bore pipet (a P-200 tip that had been cut short with a razor blade), and 2 µl aliquots were spotted in a grid pattern onto Fisher Scientific Capillary Gap plus slides and baked at 65^{o}C for 2 hours. The majority of the fragments were present in a single thickness (not folded nor stacked) with minimal obvious damage.



Testing using a variety of human tissue samples (5 microns thick) and dissection conditions found that fragment size was variable ranging from just a few cells to over a millimeter in diameter. However, for a given dissection condition, over 90% of the total tissue area was within a 2-3 fold size range. Dissection of well adhered tissue (for example paraffinized tissue dissected using mineral oil) produced small fragments (2-10 cells in diameter) whereas dissection of less well adhered tissue (for example deparaffinized pre moistened tissue) produced larger fragments (half to one millimeter in diameter). No obvious tissue type specific effects on tissue fragment size were noted, but we did observe that the tissue tended to tear along natural boundaries. We also noted some physical dissection and handling effects on fragment size. For example, larger slower rotating xScisor blades and gentle use of pipet tips with larger openings in the subsequent handling steps tended to produce larger tissue fragments.

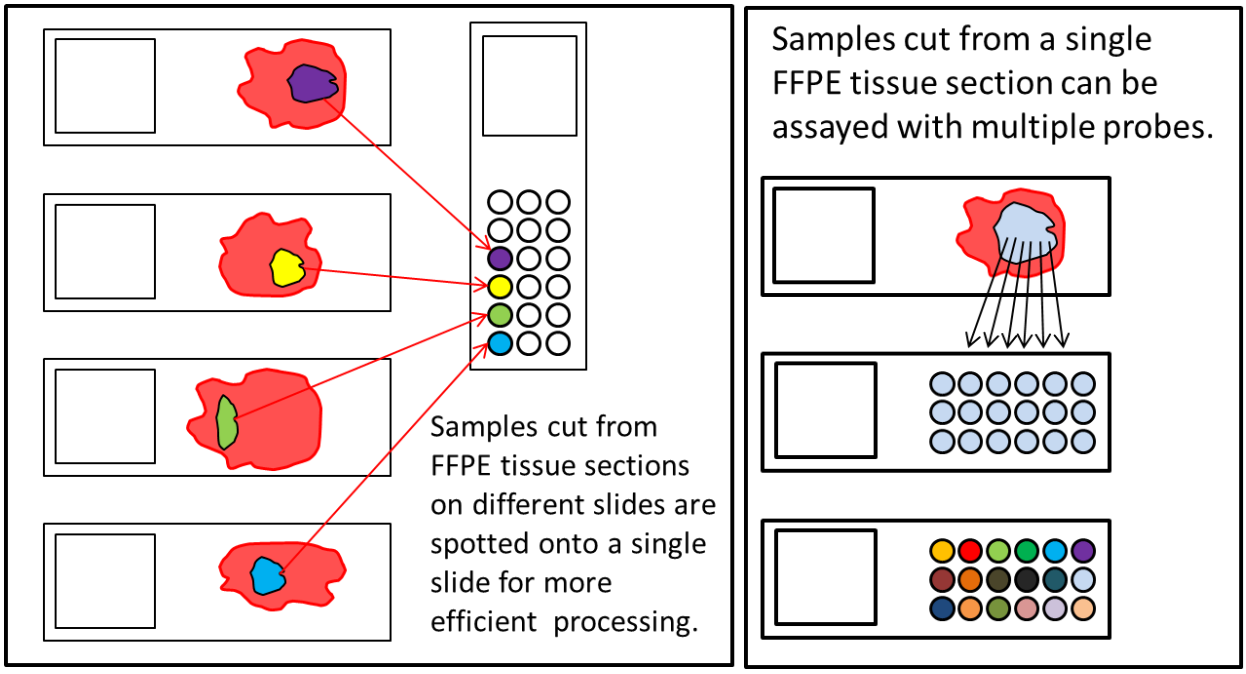
The immobilized tissue fragments were then subject to tissue FISH processing using standard conditions (In this case, the Kreatech recommended FFPE Tissue FISH protocol). The tissue fragments remained bound to the slides throughout the subsequent FISH processing steps and produced good quality FISH chromosomal signals upon hybridization with a chromosome 17 centromere probe.





Summary

This application of the mesodissection technology has the potential to increase FISH throughput. For example, multiple samples from different slides can be mesodissected and placed on a single slide minimizing the number of slides that need to be processed. In addition, a single tissue section can be mesodissected and placed in multiple locations on a slide allowing it to be hybridized with a panel of probes thereby minimizing the use of tissue sections. The digital record of the dissection process captured by the mesodissection system allows review of the histology context the collected fragments.



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