Precision Staining of Histological Samples Using Thermal Inkjet Technology

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Statement of Purpose: In this abstract, we present a reagent deposition system capable of using both traditional and fluorescent stains to produce patterns on tissue at a small scale using thermal inkjet technology.

Staining techniques can be separated into three categories: bulk, low volume, and ultra-low volume or drop-on-demand. The bulk category incorporates traditional immunohistochemical methods in which the sample is fully immersed in the reagent. The low volume approach involves exposing the tissue or part of the tissue to the stain using a mask. The drop-on-demand approach uses deposition systems, such as thermal inkjet technology, that control the amount and location of stain application down to the individual drop. The potential advantages to drop-on-demand staining include the minimization of expensive reagents and the ability to apply multiple stains on one sample.

The ability to stain may itself be beneficial. Some structures, like absorbable sutures can be harmed by traditional staining processes. A drop-on demand system could stain around a sensitive structure. In an educational setting, histology classes are best taught by hands-on study of many samples. General areas of interest as well as specific structures could be highlighted for ease of demonstration and study. An emerging application that will require precision staining is the 3D re-creation of structures using destructive sectioning and surface imaging [2].

Methods: The drop-on-demand system used in this paper is built around thermal inkjet technology, which uses a heater in the printhead to eject a drop from a nozzle. The cartridge used in these experiments was an HP26 inkjet cartridge traditionally used in the HP500 series of inkjet printers. The native resolution of the HP26 cartridge and for this system is 300 dots per inch [1]. To prepare for staining, an inkjet cartridge must be drained of ink and loaded with different staining agents. The HP26 cartridge is mounted above a two-axis motorized stage and controlled with a real-time computer system. The computer processes binary images and controls the firing of each nozzle as the stage moves the sample underneath.

To test the capabilities of our system, several types of samples were stained. Bovine dermal tissue and bovine spleen tissue were stained using Aniline Blue. A confluent monolayer of D1 mesenchymal stem cells was labeled with Invitrogen CellTracker green (Excitation 450 nm, Emission 517 nm) and red (Excitation 550 nm, Emission 602 nm) fluorescent probes. Circle and arrow patterns were printed on the dermal and spleen tissue to determine how different tissue textures would respond to the individual drop staining technique. Using similar patterns, the last experiment involved staining a monolayer of D1 cells that was seeded onto a polystyrene slide.

Results: As Figure 1 shows, the patterns printed on the dermal and spleen tissues were very recognizable as an arrow and a circle. The smoother texture of the spleen sample allowed a better picture of how the individual drops of stain were being deposited. The monolayer samples also produced well formed patterns.

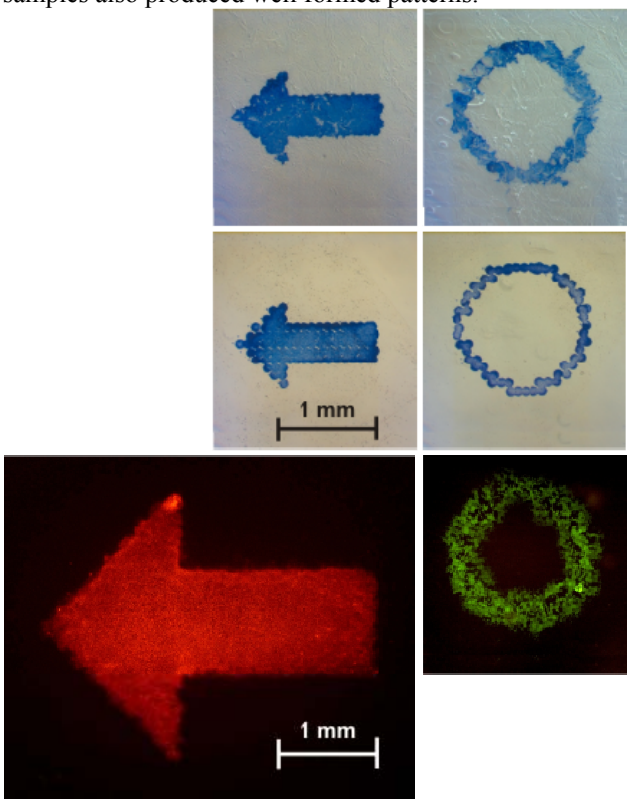

Figure 1. The top pair of images are arrow and circle Analine Blue patterns on bovine dermal tissue. The middle pair are Analine Blue on bovine spleen tissue. The bottom pair of images depicts fluorescent staining of a monolayer of D1 mesenchymal stem cells. All pictures are shown at the same scale.

Conclusions: The results demonstrate the accurate placement and retention of scale of the patterns over a range of different reagents with images and patterns less than 40 pixels square (2.4 mm). This ability to precisely dispense an exact amount of reagent could minimize reagent use, incur less cost, and facilitate enhanced labeling.

References:

[1] May et al., "Data to Dots in the HP DeskJet Printer", *Hewlett-Packard J.* 1988;39(5):76-80.

[2] Sands et al., "Automated Extended Volume Imaging of Tissue Using Confocal and Optical Microscopy", *Proc. 28th IEEE EMBS Conf.,* New York, NY, 8-2006.

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