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Development of an Automated Microfluidic FISH Platform for Point-of-Care Risk Stratification of Multiple Myeloma

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INTRODUCTION

Chromosomal analysis is a critically important diagnostic tool for disease stratification and treatment decisions, but fluorescence in situ hybridization (FISH) is not available in many centers due to cost and complexity. With these limitations in mind, we have developed a novel robotic FISH platform that incorporates lab-on-a-chip technology and machine intelligence to "call" the results for FISH staining of bone marrow cells localized in a microfluidic channel.

In this work we present examples with unfractionated bone marrow mononuclear cells because they provide internal normal cell controls to validate the staining process (as in the results in Figure 1); however, the described technology is equally suitable for staining of purified plasma cells.

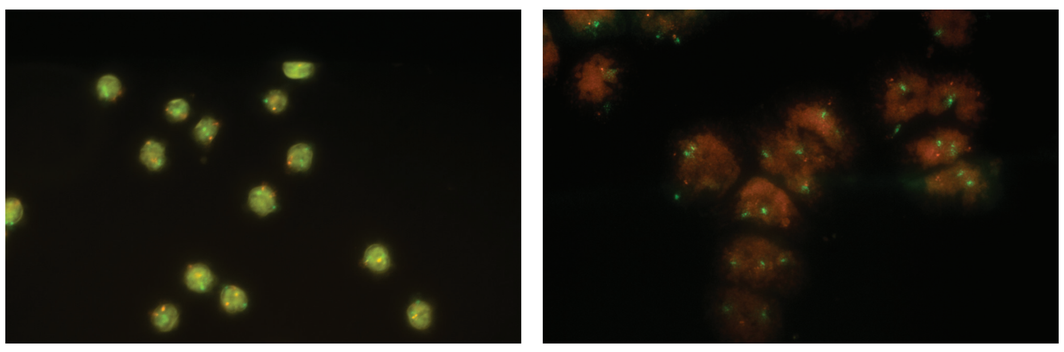

Figure 1: FISH images from microfluidic chip and slide.

ROBOTIC AUTOMATION

We have developed a novel robotic FISH platform that incorporates lab-on-a-chip technology. An alpha prototype has been developed for processing FISH (staining immobilized cells within a microfluidic channel) integrating a custom dispenser with a bank of positive displacement syringe pumps. The prototype includes heating/cooling steps, and is ready to accept an integrated reagent delivery system and image collection capabilities, run by a menu-driven user interface. It incorporates robotic back & forth movement of a stage holding three FISH chips, side to side movement for the reagent holder, and up/down movement for reagent pick-up & dispensing by syringes with steel needles for piercing reagent reservoir packs. Needles are designed to pierce each reagent reservoir for each of the steps in the FISH staining protocol. The syringes have been engineered to accurately deliver 10-100nl droplets to wells of each channel on the microfluidic FISH chip, with robotic vacuum systems to "pull" reagents into the chip. The robotic stage has a humidity box and a sealed lid to maintain the level of humidity needed to prevent evaporation of reagents. Once fully optimized, when a chip is placed in the dispenser the reagent placement, rinsing, and thermal cycling steps will run "hands free" as far as the operator is concerned. The robotic stage for the alpha prototype accepts multiple chips, enabling high throughput for clinical use. Each chip can test a different patient or different panels of probe sets, allowing versatility in diagnostics and monitoring.

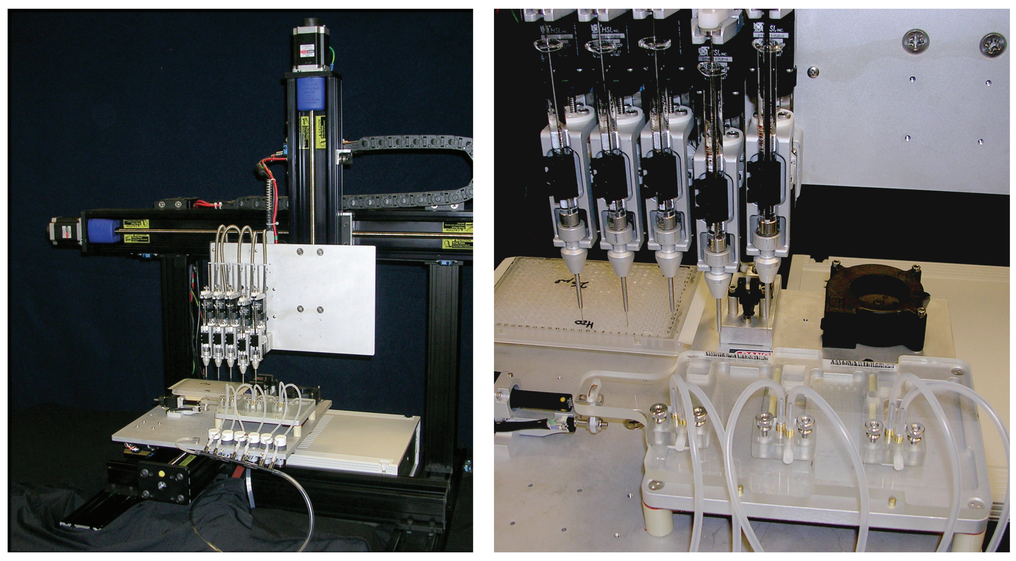

Figure 2: Robotic platform for rapid FISH analysis.

MICROFLUIDIC CHIP-BASED DIAGNOTICS

The use of a multi-channel microfluidic chip (Fig. 3, top) allows simultaneous independent testing with 10 different probe sets or samples at a cost lower than that for a single conventional FISH test. Plastic chips fabricated from COC or PMMA were also made and tested. BMC adhere to the plastic channels, staining is excellent for MM BMC, and the chips are optically transparent as indicated in Fig. 3, bottom (63x magnification). This offers significant advantages for automated FISH-on-a-Chip since plastic chips can be readily fabricated by hot embossing or injection molding to produce an inexpensive chip that appears to perform as well as glass. Furthermore, the low cost ensures that chips can be disposable as is essential for a clinically useful test platform.

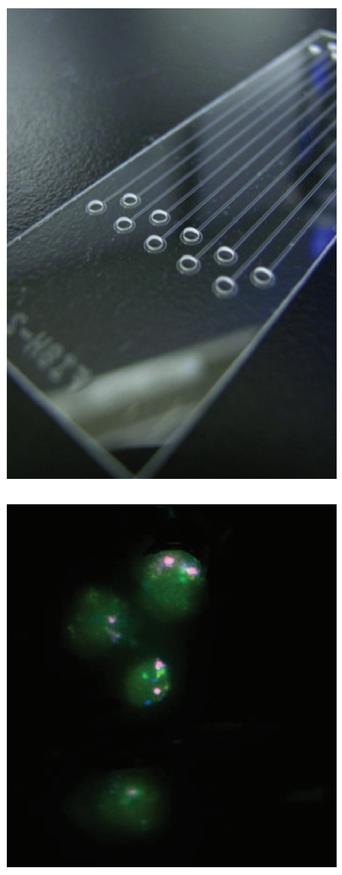

Figure 3: 10-channel microfluidic chip.

AUTOMATED SAMPLE ANALYSIS

Machine learning and pattern analysis software will enhance the automation of this FISH-on-a-Chip platform by making it possible to intelligently set thresholds and evaluate each test in the context of the probe approach being used, the presence of controls, regional population differences, and end-user preferences. Our approach also enables the calling of FISH results even with low-resolution images, minimizing computational requirements and greatly reducing the overall cost and size of the imaging system. Software currently provides "one-click" integration of individual cell detection, FISH probe detection, probe relationship analysis for different assay types, and end-stage processing that summarizes pattern-related statistics for all probe sets within a diagnostic context. Obtained cell detection and probe analysis agrees with expert-labeled images (Fig. 4).

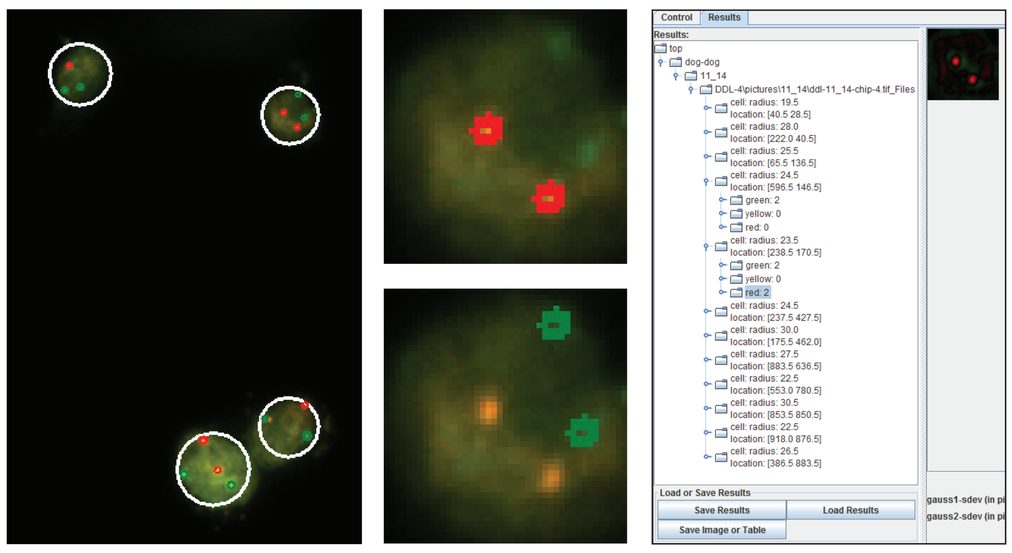

Figure 4: Pattern analysis system for on-chip FISH.

SUMMARY & FUTURE IMPACT

Collectively, these physical and computational improvements will increase the level of automation during screening while reducing the cost-per-test. The plastic chips used by the system can also be readily mass produced for significant cost savings. The result is a viable approach for near point-of-care diagnostics. This platform will make FISH more widely accessible as a standardized screening strategy for multiple myeloma, performed at near point of care, with results in less than 24 hours.

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