# MicroFISH High-Speed Microvolume Liquid Handling Automation Reduces Reagent Consumption and Technologist Time

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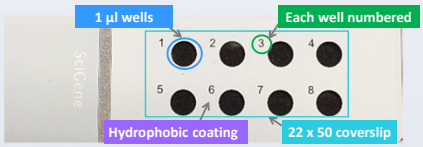
## ABSTRACT

Fluorescent in situ hybridization (FISH) is an effective method for detecting recurrent sizable copy number changes and translocations in human cancers. The combination of high cost DNA probes, labor intensive process, complex workflow, and shrinking reimbursements is causing mounting economic strain on cytogenetics laboratories.

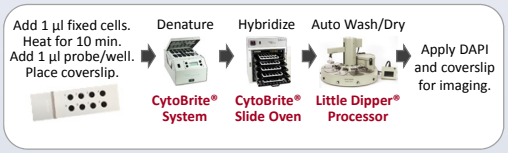
We present clinical validation data of 8-well slides with a special hydrophobic coating that eliminates coverslip sealant and only requires 1 µl cell samples and 1 µl probe during the hybridization process (MicroFISH Assay slides; SciGene, Sunnyvale, CA).

### MicroFISH Assay Slide



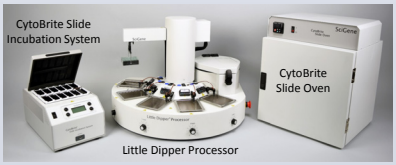

Coating retains samples in wells. Coverslip seals without rubber cement.

### MicroFISH Workflow




In a clinical validation of the MicroFISH system, 100 patient samples were run in parallel using the MicroFISH assay and standard methodology. Subjective grading on a 1 to 5 scale by technologists reading both sets indicated comparable hybridization quality. The amount of hybridization spots requiring a reset and clinical cut-off value were similar for both methods.

### Slide Processing Equipment for MicroFISH Assay




More efficiency is gained using a high speed microvolume liquid handling work station to drop 1 µl of fixed cells onto assay slides and apply 1 µl probe (Scorpion; Art Robbins Instruments, Sunnyvale, CA) readying them for hybridization.

The liquid handler reduces hands-on time for slide dropping and probe application, provides comprehensive batch report data, including lot and tube numbers of the probe with expiration date, and produces patient-specific scoresheets for technologists to use during slide reading.

## CONTACT INFORMATION

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## MATERIALS AND METHODS

**Probes and Reagents**: Probes (Cytocell, Cambridge UK) were prepared following the manufacturer's recommendations. Slides were washed in 0.4X SSC; 0.3% NP40 (VWR) at the prescribed temperature and counterstained with DAPI (Vector).

**Equipment**: A CytoBrite Slide Incubation System and CytoBrite Slide Oven (SciGene) were used for performing denaturation and incubation steps respectively followed by a Little Dipper Processor (SciGene) for automated coverslip removal and post-hybridization washing and drying. Slides were imaged using standard methods (Cytovision) after manual examination.

**Manual Slide Preparation**: 1 µl of fixed blood or bone marrow cells in fresh Carnoy's was dropped in each well to be used on MicroFISH slides held in the CytoBrite slide tray. Slide trays were then transferred to a 90oC oven for 10 minutes. 1 µl of probe was then placed in the center of each well containing cells and a single 22 x 50 mm coverslip placed over all eight wells. No coverslip sealant was used.

**Hybridization**: CytoBrite slide trays with coverslipped MicroFISH slides were transferred to a CytoBrite Slide Incubation System and heated at 76°C for 2 minutes. Trays were then transferred to a sealed chamber with absorbent pad moistened with 100 ml of water and placed in a CytoBrite Slide Oven set at 37°C overnight.

**Post-Hybridization Processing**: After incubation, the sealed humidity chamber was taken from the oven and CytoBrite slide trays removed. MicroFISH slides were immediately placed in a slide rack for the Little Dipper Processor (SciGene) to process:

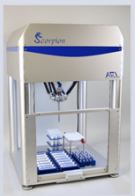
| Step | Location | Time (sec) | Conditions |
|---|---|---|---|
| 1 | Bath # 1 | 5 | Wash Buffer @ RT with agitation* |
| 2 | Bath # 2 | 60 | Wash Buffer @ 72°C without agitation |
| 3 | Bath # 3 | 30 | Wash Buffer @ RT without agitation |
| 4 | Centrifuge | 30 | Drying step |

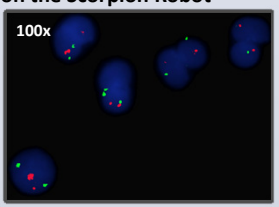
* Coverslips shake off automatically with 600 RPM agitation during step 1.

MicroFISH slides were removed from the rack, a bead of DAPI applied down the center of each slide and a 22x50 mm coverslip placed over the wells.

**Imaging**: MicroFISH slides were imaged under 100X oil objective and scored following standard methods.

### Cells Probed with EGR1 Using MicroFISH Slides Prepared on the Scorpion Robot






**Automated Slide Preparation**: The Scorpion liquid handler was configured with 12 MicroFISH slides in two CytoBrite slide trays, 12 patient tubes of fixed blood or bone marrow cells in fresh Carnoy's and a rack of probe solution (CytoCell) tubes. Patient information and probe panel data were entered and the run started. The instrument deposited 1 µl of fixed cells into each well, paused for trays to be removed and heated for 10 minutes at 90°C in an oven and returned, and then added 1 µl of probe to assigned wells. Trays were removed and one 22x50 mm coverslip was placed per slide, without sealant. Slides were then processed as described above.

## RESULTS

We have previously shown (Crawford et al., ACMG 2015 poster #182) that multi-well slides with a hydrophobic coating (MicroFISH; SciGene) used in a simplified workflow allowed for more rapid processing of FISH samples using 1 µl probe volumes. We report here the completion of the clinical validation of the platform and automation of the slide preparation steps in the workflow.

For the clinical validation study, blood cell samples from 100 patients were analyzed in parallel utilizing the MicroFISH slides and workflow vs. the laboratory's standard method. Each slide set was scored by two readers who graded overall cell quality on a 1 (failure) to 5 (very good) scale. The average quality grade for MicroFISH slides was 3.77 compared to 3.73 for our standard method. Both methods had identical probe spot failure rates of 1.4% and 100% concordance on successfully analyzed pairs of spots interpreted as abnormal. Significant variation was noted in one run which was attributed to oversaturation of the humidity chambers during hybridization. In serial hybridizations, 100 ml total volume of water provided optimal signal quality in a MicroFISH humidity chamber.

### 100 Patient Clinical Validation Study

| GAI Standard Method | | | | | | | |
|---|---|---|---|---|---|---|---|
| Probe | Normal | 1r2g | 1r1g | 2r1g | 3r3g | 1r1g12f | 1r1g1f |
| 5q | 95% | 2% | 1% | | | | |
| 7q | 95% | 1% | 2% | 1% | | | |
| 8 | 95% | | | | 1% | | |
| 20q | 93% | 1% | 2% | 1% | | | |
| BCR;ABL1 | 92% | | | | | 1% | 3% |
| **MicroFISH Method** | | | | | | | |
| Probe | Normal | 1r2g | 1r1g | 2r1g | 3r3g | 1r1g12f | 1r1g1f |
| 5q | 94% | 2% | 1% | | | | |
| 7q | 95% | 1% | 2% | 1% | | | |
| 8 | 94% | | | | 1% | | |
| 20q | 95% | 1% | 3% | 1% | | | |
| BCR;ABL1 | 93% | | | | | 1% | 3% |

Incorporating automation of cell dropping and probe dispensing onto MicroFISH slides using the Scorpion robot (Art Robbins Instruments) provides high speed setup of MicroFISH slides ready for processing. We found instrument performance to be superior to hand pipetting. For example, it delivers 1 µl of fixed cells from 12 patients to all eight wells of 12 MicroFISH slides in 6.5 minutes. Similarly, it precisely dispenses 1 µl of probe to the center of the wells of 12 slides in 4.5 minutes. Just as importantly, we have found the quality of results from MicroFISH slides setup using the instrument equivalent to manually prepared slides. The processing capacity of one Scorpion robot meets our laboratory requirements.

### Probe Economics of the MicroFISH System

| Probe Supplier A - $8/µl | | | |
|---|---|---|---|
| # Cases per Year | Annual Probe Cost | | Annual Savings |
| | * Standard Method | MicroFISH | |
| 5000 | $960,000 | $240,000 | $720,000 |
| 10000 | $1,920,000 | $480,000 | $1,440,000 |
| * 6 probe panel / 4 µl probe per sample | | | |

## CONCLUSIONS

In our laboratory, we continue to show that the MicroFISH slide system yields comparable results to standard methods in terms of slide quality, clinical cut-off values, and result concordance with reduction of hands-on technologist time, repetitive motion, and probe consumption. Further gains in technologist time, repetitive motion, and gain of reproducibility and traceability can be achieved by incorporating automation of slide dropping and probe dispensing.