APPLICATION NOTE

Comparing FISHArray low volume multi-welled slides to manually prepared FISH slides

Introduction

As demand for FISH testing continues to grow, pressures to reduce cost and increase output are forcing laboratories to find more efficient ways to generate a greater number of assays. Smart technologies, such as the BioDot CellWriter with FISHArray slides, have emerged and are reducing the cost per sample by utilizing smaller probe volumes, increasing the number of assays per slide and decreasing processing time with automation.

slide is created by the CellWriter™ workstation (Figure 1), a high-speed nanoliter printing system that both automates and miniaturizes the traditional FISH assay.

In order to maximize the benefits of the technology (consistency, data management, and miniaturization) and reduce risk (assay mix-up and cross contamination due to manual pipetting errors), the FISHArray technology is enabled only through the CellWriter workstation.

Humidity & Temperature Control

Process parameters set through system interface before starting.

24 Slide & 24 Tube Capacity

Designed to accept any slide and 15ml conical sample tubes.

BioJet™ Print Head with Barcode Scanner

High speed, non-contact nanoliter printing of cells and probe. Integrated barcode scanning allows loading of slides in any order.

Disposable Tips Loading & Unloading

Disposable tips decrease cycle times and eliminate cross contamination risk.

Figure 1: BioDot CellWriter S™ workstation

What is BioDot FISHArray?

Nanotechnology and multiplexing have enabled tremendous advancements in genomic sequencing, cell-based applications, and proteomics. As new technologies dramatically increase the accessibility of genetic information, cytogenetics labs are being challenged to find similar gains in speed, consistency, and cost. FISHArray was designed so that the cytogenetics laboratory can actively participate in the trends that are reshaping science.

FISHArray reduces the area on a slide that is needed to perform a FISH assay. Up to 8 unique assays can be hybridized simultaneously on a single slide. Each FISHArray

The user may choose to integrate the CellWriter platform and FISHArray technology with an automated scanning system, or use BioDot's software applications to guide manual scoring.

Materials

BioDot CellWriter materials

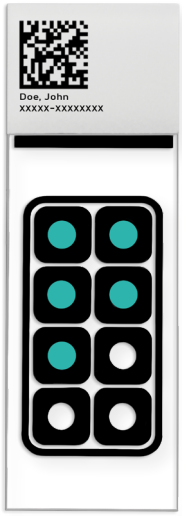
- CellWriter S2 Platform (Cat. #CellWriter S2)
- Phaselink Software (Cat. #Phaselink)
- Probe Inventory Management Software (Cat. #PIMS)
- 8-Well FISHArrayTM Microscope Slide (Cat. # 0004-0047)

Probes and reagents

- IGH Breakapart (Cytocell/OGT Cat. #LPS 032-A, 100µl)
- D13S319 Plus Deletion (Cytocell/OGT Cat. #LPH 068-A, 100µl)
- 12cen in Aqua Spectrum w/Hyb Sol B (Cytocell/OGT Cat. #LPE 012B-A, 30µl)
- PML/RARα(RARA) Translocation, Dual Fusion (Cytocell/OGT Cat. #LPH 023-A, 100µl)
- MYB Deletion (Cytocell/OGT Cat. #LPH 016-A,100µl)
- P53(TP53)/ATM Probe Combination (Cytocell/OGT Cat. #LPH 052-A,100µl)
- BCR/ABL(ABL1) Plus Translocation, Dual Fusion (Cytocell/OGT Cat. #LPH 038-A,100µl)
- DAPI 0.125µg/ml (Cytocell/OGT Cat. #DAPI DES1000L, 1000µL)

Additional equipment

- Oxford Gene Technology's Cytocell® FISH probes
- ThermoBrite®
- Olympus BX61 with ASI GenASIs software
- Thermo ScientificTM Ultra Frost Gold SealTM Microscope Slides (ThermoSci Cat # 3063-002)
- Normal Human Bone Marrow Specimens (in Carnoy's solution (3:1 methanol/acetic acid) fixative)



Adaptive Barcode Reading
Reduce lab IT disruption by using a lab's existing barcodes.

450nl Assays

Smart Cell Dispensing
Conserve sample by recognizing which wells have been assigned probes and only dispenses cells to these regions.

On-the-fly Sample Concentration
Sample concentration is measured as part of the dropping process. If the initial concentration is low, CellWriter adjusts by printing more sample to the wells. The fix evaporates and you are left with more cells.

Figure 2: nanoFISH FISHArray™ multi-welled slide

Real-time Volume Tracking
Checks fill volumes before each run and updates the inventory system as probes are used.

Unique Vial Barcodes
PIMS tracks ongoing probe inventory by probe, lot, and tube.

Tube Data:
Probe
Lot Number Expiration Date Open Date
Storage Conditions
Samples Hybridized

Incoming & Ready For Use Tracking
PIMS catalogues both incoming probe vials and working probe vials. Real-time inventory levels for these groups are presented separately.

300µl Fill Volume
Approximately 600 assays, designed for long-term storage of probes.

Figure 3: BioDot PIMS tubes

Methods

Sample and Slide Preparation

1. FISH assays were processed using 3-month-old normal human bone marrow specimens fixed in 3:1 methanol:acetic acid. The pellets were spun down, the supernatant was removed and they were supplied with fresh fixative prior to use.
2. Manual FISH slides were prepared by dropping 20µl of sample onto the slides (Thermo Scientific) and allowing them to air dry on the benchtop. A total of 10µl FISH probe* was used for each manual FISH assay.
3. FISHArray slides were prepared on the CellWriter S2 platform with integrated pellet normalization; a total of 2µl specimen was deposited onto each target well. On the CellWriter platform, the total amount of specimen used per slide varied depending on the concentration of the sample. The CellWriter has an integrated reader that allows the system to dynamically determine the total drop volume per well; the baseline is 1µl total per target well. All seven FISH probes were processed on a single FISHArray slide; a total of 0.45µl probe was applied to each target well.

*Prior to probe-spotting the slides underwent the BioDot automated on deck FISH Pre-treatment protocol; this process ensured consistently clean slides with low amounts of debris.

FISH Assay Conditions

1. Both manual slides and FISHArray slides were denatured and hybridized using the BioDot protocol on Thermobrites. (Note that the latest version of the CellWriter S2 platform has a heated nest allowing on-board denature/hybridization).
2. Two sets of slides were prepared: overnight hybridization and 2hr hybridization (BCR/ABL Plus and PML/RARA only).
3. Overnight Hybridization: the slides and probes were codenatured at 78°C for 3 minutes and hybridized at 37°C for 12 hrs.
4. 2hr Hybridization: the slides and probes were codenatured at 78°C for 3 minutes and hybridized at 37°C for 2hrs.

BioDot Recommended Post Hybridization Wash Protocol

1. 0.4x SSC with 0.3% IGEPAL, pH 7 at 73°C for 2 minutes
2. DIH2O at Room Temperature for 5 seconds
3. 70% EtOH at Room Temperature for 5 seconds
4. 85% EtOH at Room Temperature for 5 seconds
5. 100% EtOH at Room Temperature for 5 seconds

FISH imaging methods

The images were captured using the ASI GenASIsTM SpotCount platform. The images included in this application note were not enhanced.

Results

FISHArray assay results were comparable to 10µl control slides

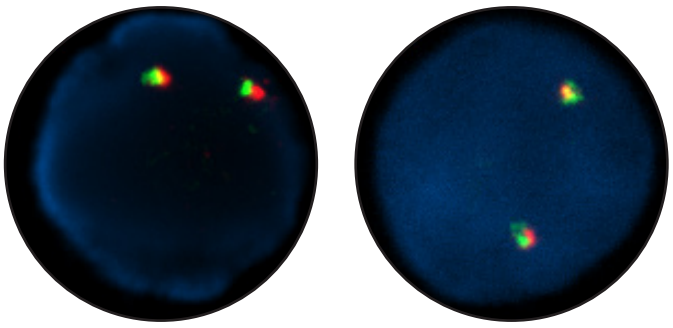
The results obtained on the FISHArray multi-welled slides (0.45µl of FISH probe) were comparable to those observed on the manual control slides (10µl of FISH probe). Strong, distinct signals were observed on FISHArray slides after an overnight hybridization for IGH Breakapart, D13S319 Deletion, 12 Centromere, BCR/ABL(ABL1) Plus Translocation, Dual Fusion, and PML/RARα(RARA). We also conducted a 2 hour hybridization using BCR/ABL and PML/RARA; both probes showed strong, distinct signals on the 0.45µl FISHArray assays and the 10µl controls.

The FISH signals were also able to be read manually using individual filters. All samples used in this experiment were normal thus the images seen below represent the normal signal pattern expected for each probe. The captured images are shown below; the 10µl control is on the left and the FISHArray image is on the right side.

Minimal background and low debris were observed on both the FISHArray slides and the manual control slides.

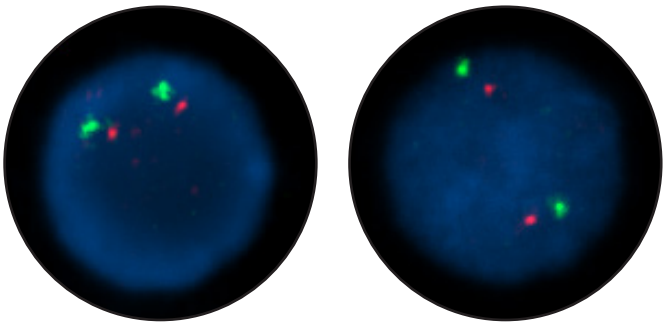
Overnight Hybridization

IGH Breakapart



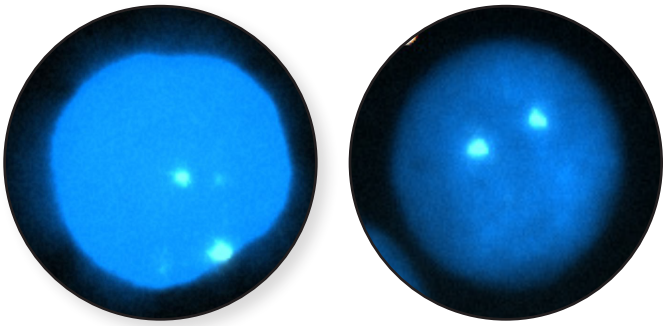
10µl control | 0.45µl FISHArray

D13S319 Plus Deletion



10µl control | 0.45µl FISHArray

12Cen in Aqua Spectrum (with Hyb Solution B)



10µl control | 0.45µl FISHArray

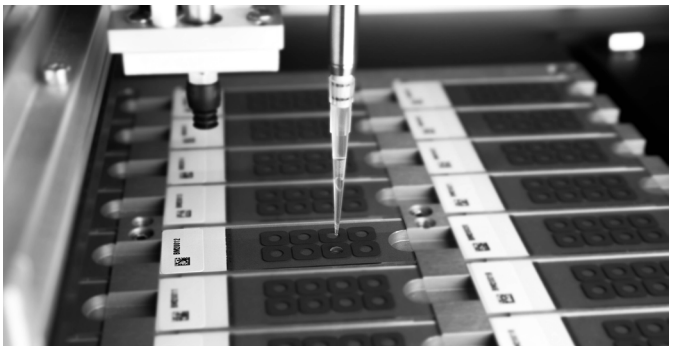
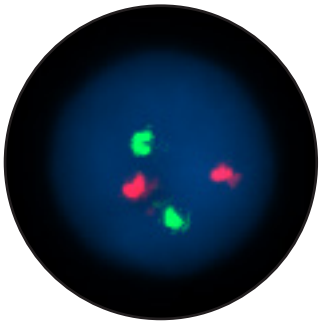


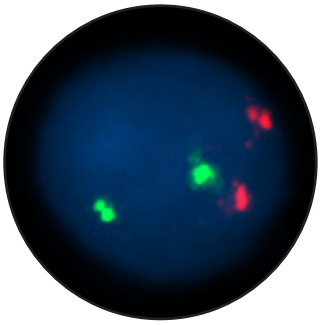
Figure 4: BioDot CellWriter™ workstation applies FISH probe to nanoFISH FISHArray™ multi-welled slides

Overnight Hybridization vs 2 Hour Hybridization

PML/RARa(RARA) Translocation, Dual Fusion - overnight hybridization

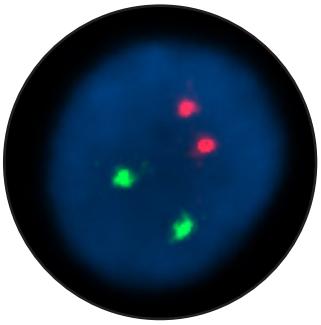


10µl control

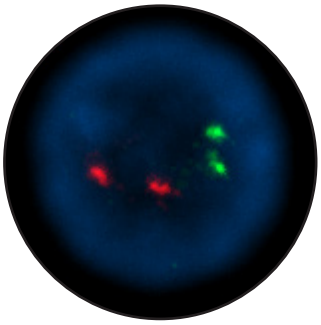


0.45µl FISHArray

PML/RARa (RARA) Translocation, Dual Fusion - 2hr hybridization



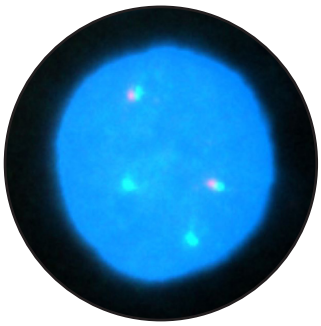
10µl control



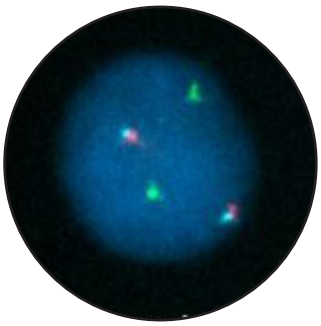
0.45µl FISHArray

BCR/ABL(ABL1) Plus Translocation, Dual Fusion - overnight hybridization

- ABL1, 9q34.11-q34.12, Red
- BCR, 22q11.22-q11.23, Green
- ASS1, 9q34.11-q34.12, Blue



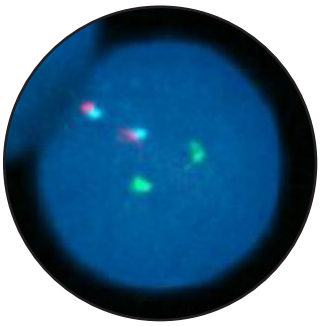
10µl control



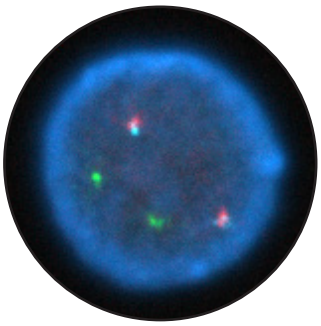
0.45µl FISHArray

BCR/ABL(ABL1) Plus Translocation, Dual Fusion - 2hr hybridization

- ABL1, 9q34.11-q34.12, Red
- BCR, 22q11.22-q11.23, Green
- ASS1, 9q34.11-q34.12, Blue



10µl control



0.45µl FISHArray

Conclusion

As the FISH market expands and laboratories are faced with the demands of their growing sample volumes whilst reducing costs, synergies between new technologies can create improved efficiencies for the end user. The comparable results produced on the BioDot CellWriter system, utilizing minimal volumes of FISH probe, will allow laboratories with increased assay demands to gain speed, maintain consistency, and reduce costs, without sacrificing quality.

References
1. ASI/BioDot Time Study – 03/07/2016

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