High Throughput Bio-Printing with Individualized Piezoelectric Ejectors

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Commercial Impact at PARC

- **Inventions in nearly every Xerox product**
  - Laser Printing, Laser Diodes, Dual-beam Laser Diodes
  - DocuPrint NPS (1993-current)
  - Web XPress services infrastructure – delivered via cross-organizational collaboration
  - Color RIP for DocuColor 60
  - Scheduling software for IGen3

- **Seeded the Valley**
  - 8 companies based on early PARC research: 3Com, Adobe, VLSI, and KOMAG

- **19 Spin-offs since 1980**
  - 1983: Spectra Diode Laboratories, Inc. – acquired by JDS Uniphase in $41 Billion merger
  - 1985: Synoptics Communications, Inc. – became Bay Networks, acquired by Nortel in 1998
  - 2000: ContentGuard – digital property rights management, purchased by Microsoft.
  - 2001: Gyricon Media Inc. – Macy’s and Dow Jones as lead customers for retail signage

- **Licensing**
  - Dragon collaboration with Sun Microsystems on SPARC Station's multi-processor architecture
  - 3D Visualization techniques licensed to Microsoft Corporation
Biomedical Sciences at PARC

• Leverage PARC technical competencies
  – Ultra Small Drop Control
  – MEMS / Electronics / Applied Physics
  – Information Sciences

• Partner for life science competencies
  – Scripps Research Institute domain knowledge and industry contacts
  – Scripps-PARC Institute for Advanced Biomedical Science [www.scrippsparc.com](http://www.scrippsparc.com)
  – Collaborate in securing external funding
  – Attack big problems
Biomedical Science Efforts

- **Bio-Printing¹**: Individualized Piezoelectric Ejectors
  - Low cost, high volume DNA arrays
- **Universal Assay²**: Enthalpy Array
  - High throughput screening without assay development
- **Rare Cell³**: Fiber Array Scanning Technology
  - Early stage cancer detection and fetal analysis
- **Image Analysis⁴**
  - Protein crystal ID
  - CryoEM Sample ID
- **Mass Spec Data Analysis⁵**
- **Bio-Agent Concentration & Detection⁶**

6. ARO-JSAWM contracts: DAAD19-03-0116, W911NF-04-C-0034
Printing Technologies

**Acoustic Single Ejector**
- Micro-pipetting
- Printed organic electronics
- Drug inhaler

**Acoustic Multi-Ejector Printhead**
- Photofinishing
- LCD color filter deposition
- Printing on pills

**Piezo Single Ejector**
- Biological micro-arrays
- Printed organic electronics
- Micro-pipetting

**Piezo Multi-Ejector Printhead (OPB/Tektronix)**
- Office documents with solid ink
- Printed organic electronics
- LCD color filter deposition
- Decorative & packaging printing
- Printing on pills

**Ballistic Aerosol Marking**
- Powder deposition/processing
Piezoelectric Single Ejector

- Solve the plumbing problem of traditional inkjet bioprinter
- Eliminate cross-contamination
- Break the practical limit of number of print heads
- Provide low cost, high throughput solutions
Fluid Core of Single Ejector

Reservoir Volume=76µl

- Main Reservoir
- Aperture=76µm
- Fill Hole
- Compression Chamber
- Purge Reservoir
- Purge Hole
- Piezo Diaphragm
- Refill Ducts Behave Like Check Valves

Parc
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PARC’s Piezoelectric Ejector Technology

- Printing system with multiple fluids printed simultaneously
  - Individual piezo ejector for each fluid
  - Many piezo ejectors or fluids
  - All stainless steel construction
  - Non-contact
  - High fluid utilization
  - Reconfigurable
  - Versatile drop control (5pl – 300pl)
  - Prototype system demonstrated

Initial printing system:
  - X-Y scanning
  - Inspections
  - Building block for larger systems
Bioprinter Prototype

- PARC’s deep printing understanding maps well to bio-fluidics:
  - Manipulating drops & images
  - Manufacturing & modeling piezo ejectors
  - Precision motion & sensing systems
Reliable Droplet Ejection

- Counted ≈ 4M drops through 8 apertures
- Videos of drop ejection before and after a 4M drop run
Reliability – Idling in Ambient

Initial State
(surface is clean except for 2 stains from filling)

Ejection after sitting idle at ambient RH for 4 hours:

- RH = 19.2% at 23°C
- BSA/CAPS deposits formed on ejector surface but do not appear to interfere with drop ejection
- No appearance of drop abnormalities

Features Printed after sitting idle at ambient RH for 4 hours:

(Drops per feature varies from 1000 to 10 drops)
Reliability-Idling in Humidity

Initial State  
Ejection after sitting idle with RH control for 3 hours  
Ejection after sitting idle with RH control for an additional 14 hours

- BSA/CAPS nominal solution

Results:
- Surface around aperture remains clean
- Drop trajectory remains the same
- No appearance of drop abnormalities
Feature Size With Varying Droplet Qty.

Single Ejector #1610
Three strips measured.
BSA with 0.01 % dye.
No Motion
Very circular features.

Drop Size = 73 um
\[ y = 76.076 \ln(x) - 13.297 \]

Drop Size = 59 um
\[ y = 51.644 \ln(x) + 9.832 \]
Feature Size Comparison Before and After Hybridization

Each drop is 74 um diameter = 200 pLiter = 65 fg of probe per drop

Probes are 20 mers long @ 0.325 ug/ml
Bio Materials Successfully Printed

- **Genomic DNA solution (100ng/uL)**
  - viscous, long DNA fiber
  - PCR verified printed DNA
- **Aqueous DMSO (5- 50%)**
  - low surface tension
  - at 4°C, 22°C, 37°C
- **Oligonucleotide solutions**
  - arrays hybridized successfully
- **BSA and Human IgG protein (1mg/ml)**
- **Human cell line TIB180 (10µm)**
  - at 4°C, 22°C, 37°C
  - cell settlement occurred over time
  - agitation (ultrasound) could reduce settlement
Bio-Array Printing Applications

• Clinical
  – high volume, low cost: $10
  – medium density: 100 to 1000 features
  – diagnostic, metabolic, reproductive, forensic

• Research
  – low volume, high cost: $100 to $500
  – high density: 1000 to 10,000 features
  – population arrays, drug discovery, customized
Strip or Array Production

Web Fed, Dual Speed, 500 um Feature Pitch, 27x46mm Strip

Arrays per 8 Hr Shift vs. Droplets per Feature

- 96 features per strip
- 384
- 1536

XY Platen, Dual Speed, 500 um Feature Pitch, 27x46mm Strip

Arrays per 8 Hr Shift

- 96 features per strip
- 384
- 1536
Companies with Micro Droplet Technologies for **Array** Generation
(from 2002 literature)

Legend
Solid: contact, Open: non-contact
red: quill pin
cyan: piezoelectric
blue: micro dosage head
green: solid pin
orange: micro solenoid valve
Symbol Size ~ minimum drop size

Affy: density: >250k
Agilent: density: up to 22575/slide

Density (Features per Strip)

Production (Arrays per Shift)

red lines indicate dead volume in nano liters
Future Work

• “Image” (feature) quality & reliability
  – In many ways, conventional images demand more accurate printing than bio applications.
  – Orderly, consistent bioarrays are within reach, but for large arrays and lots of ejectors, concerns are:
    • mechanical issues like alignment
    • set up time
    • droplet trajectory changes due to meniscus changes
• Automatic filling station for precious fluids
• Inexpensive ejectors: injection molding
• Extend ejector performance
• Materials studies
• System architecture studies
Injection Molded Plastic Ejector
96 Ejector Array
System Architectures

- XY Platen, Auto Fill
- XY Platen, Manual Fill
- Web, Auto Fill, Auto Tune, Auto Tune, PreRacked
- Web, Manual Fill
Potential Applications

• Bio-defense
  – printing small arrays in large quantity
  – example arrays include bio-agents such as microbial genomes, toxin Abs, viruses, or chemical arrays
  – inexpensive

• On-demand printing and dispensing of biomaterials
  – library of bio-molecules stored in ejectors
  – computerized, automated pick-and-print

• Handheld nano-liter dispenser
• Protein arrays for research and diagnostics
• Tissue, organ, or live cell printing
...Continuous Innovation

Thank you! Questions?

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