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Digital microfluidic (DMF) hub Integration

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LDRD
LABORATORY DIRECTED RESEARCH & DEVELOPMENT

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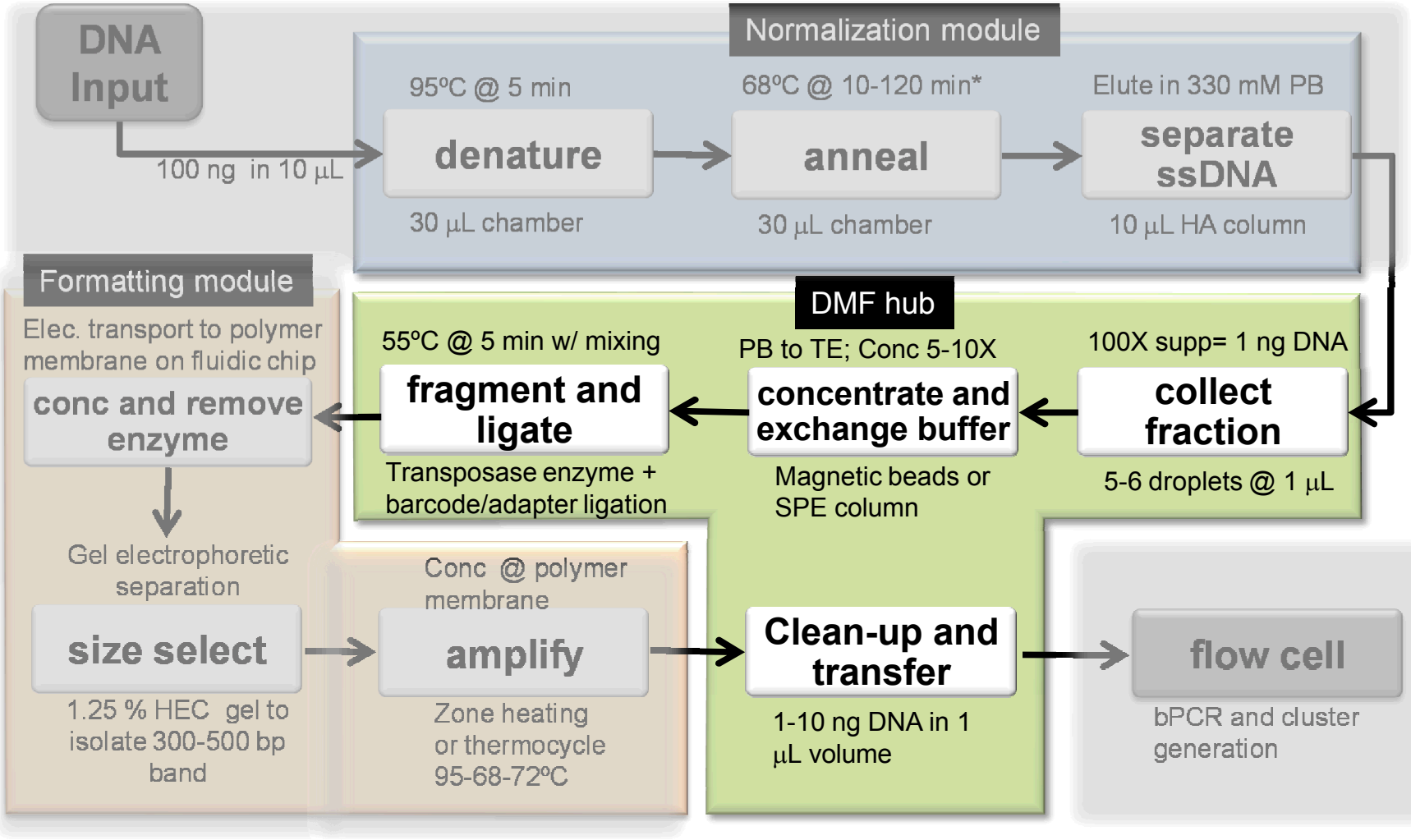
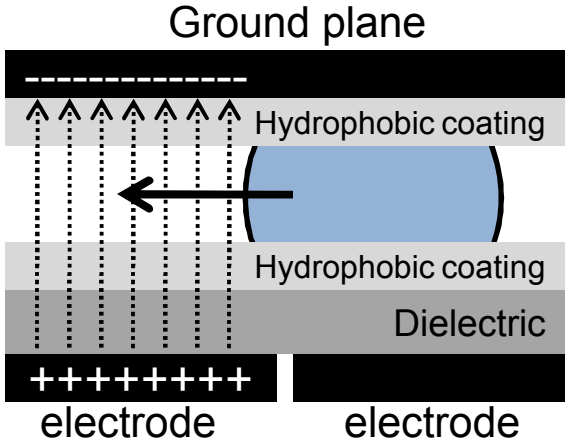
Gen 1 prototype process flow diagram for normalizing + formatting the reference sample

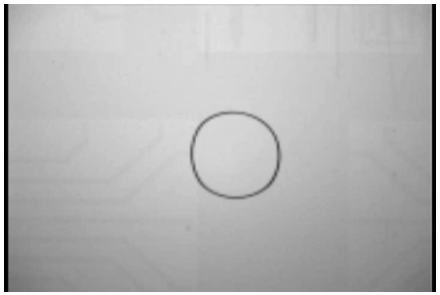


DMF technology is an excellent tool to manipulate microliter droplets

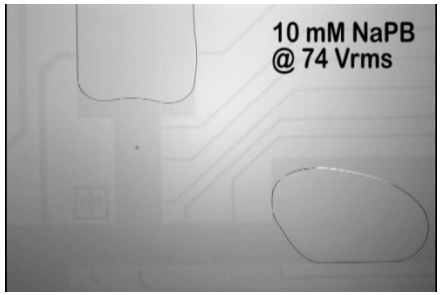
- **Droplets are ideal micro-cargo containers**
  - Operated “digital” fashion (Virtual tubes)
  - Nanoliter to microliter in volume
  - Merge, mix, split, etc (Virtual Pipetting)
- Based on principle of electrowetting-on-dielectric
  - Applying voltage to target pad
- Micromanipulation of droplets using electrowetting is known as **digital microfluidics (DMF)** (Wheeler et al, 2009)



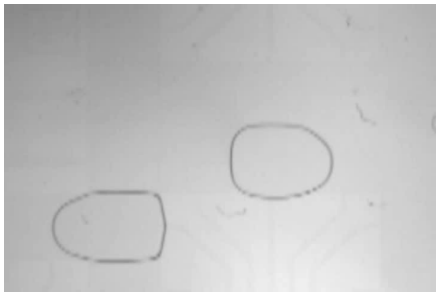
Actuation



Splitting

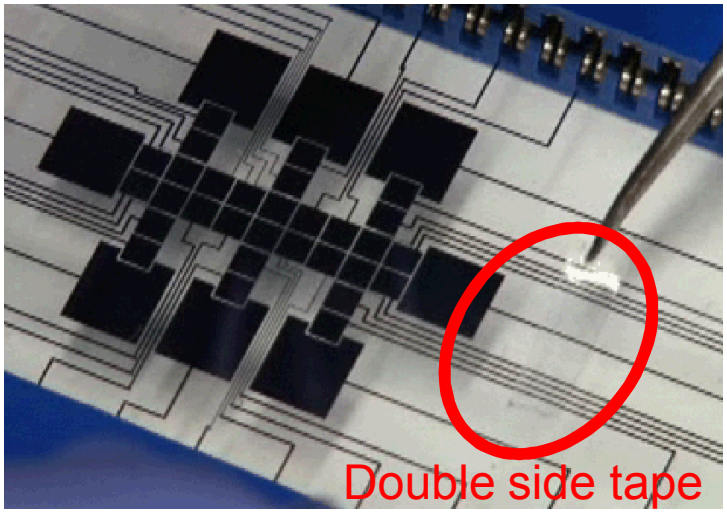


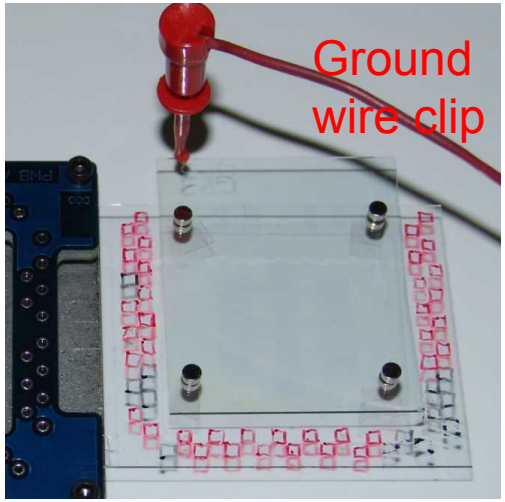
Merge and mix



DMF technology requires a solid engineered platform

- Gap height created by double-side tapes with hand-press
  - Inconsistency between DMF integration
  - Coating layers damaged
- No fluidic interface
  - Sample reservoirs with limited volume are required
  - Limited waste disposal

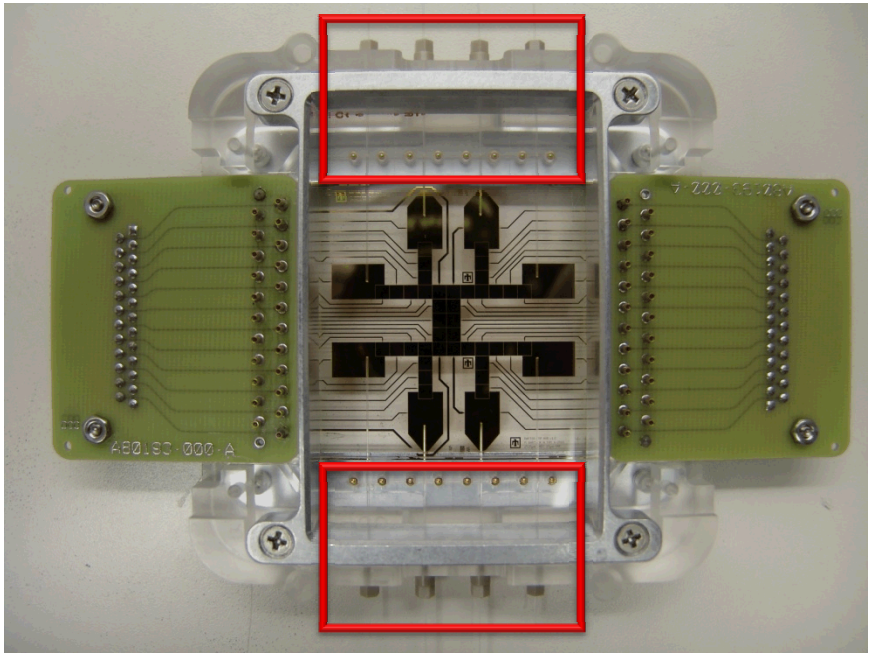


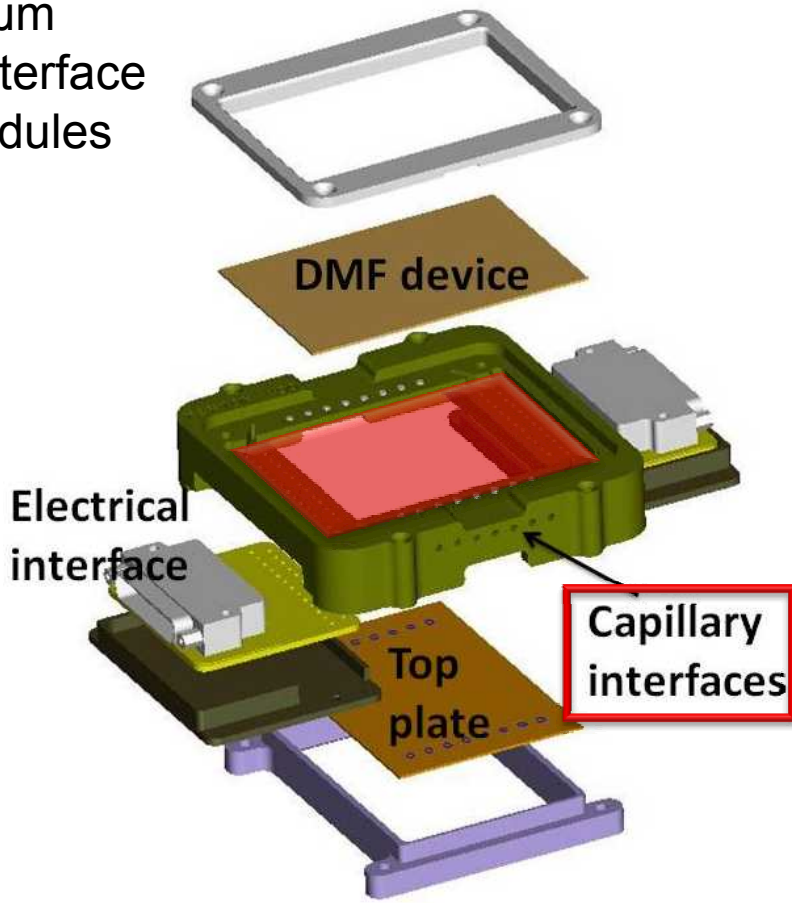


Captured from a Video published by Wheeler's group
Jebrail et al., 2009, Journal of Visualized Experiments

DMF hub platform developed for reliable and flexible sample processing

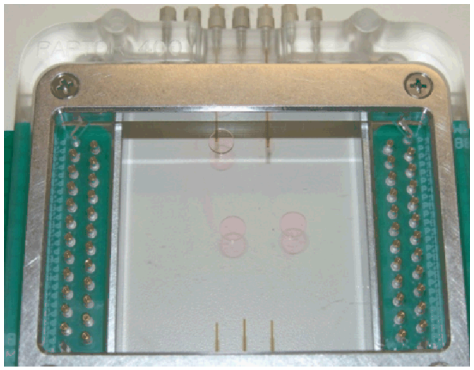
- Reliable and Reproducible Self-registering pocket
  - Built-in fixed gap height of 185, 400 μm
  - Dedicated circuit board for electric interface
- Flexible fluidic interface to peripheral modules
  - Capillary interface
  - Via-hole interface manifold

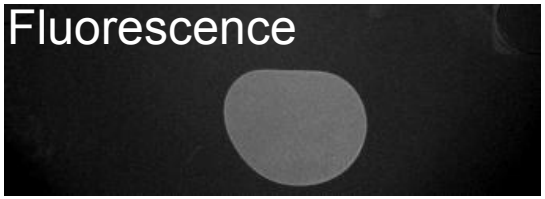


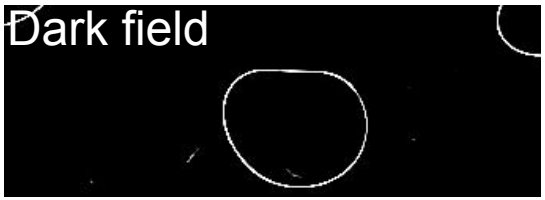


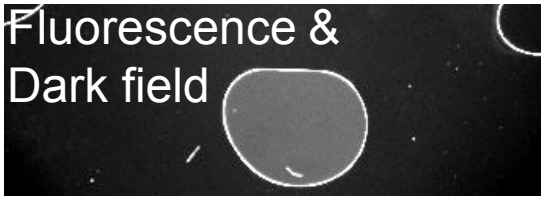
DMF hub platform developed for reliable and flexible sample processing

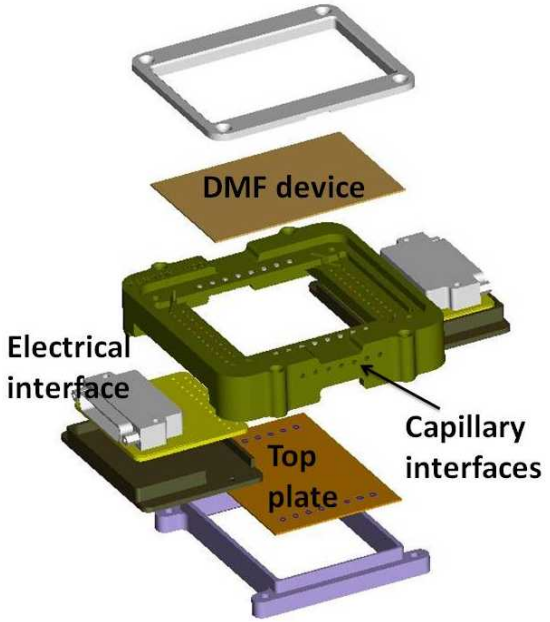
- Transparent ITO DMF device for flexible detection schemes
  - Optical access from top and bottom
  - Critical for feedback control for a fluorescence assay
  - Flexible combinations of optical detection schemes









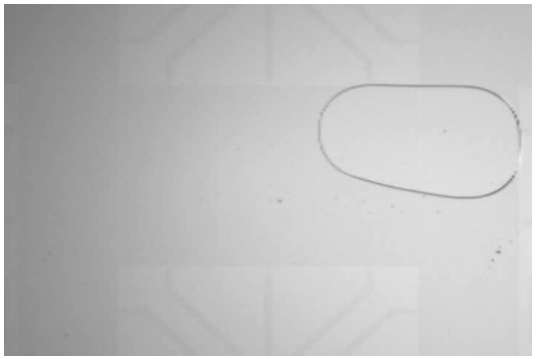


In-house ITO DMF devices fabrication process established

DMF hub platform developed for reliable and flexible sample processing

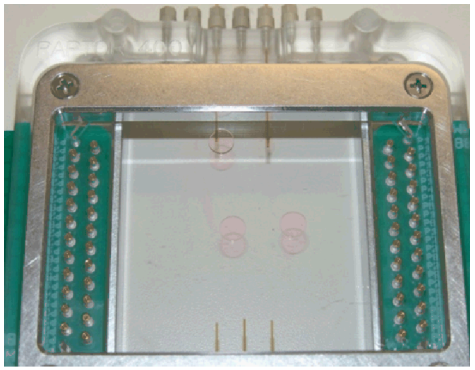
- Sample size flexibility
  - Ranging from 1.2 to 10's of µl (current design)

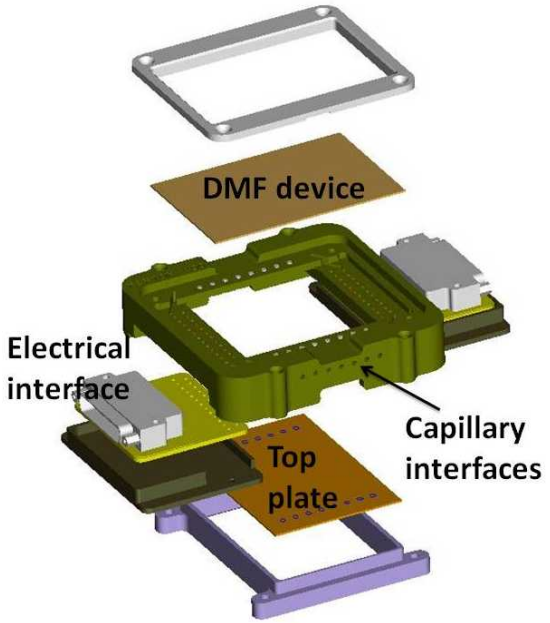
1.2 µl droplet



2.4 µl droplet

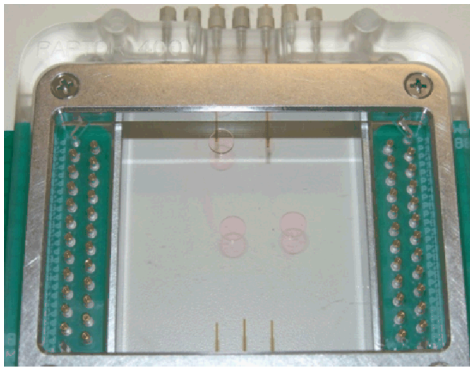
3.6 µl droplet

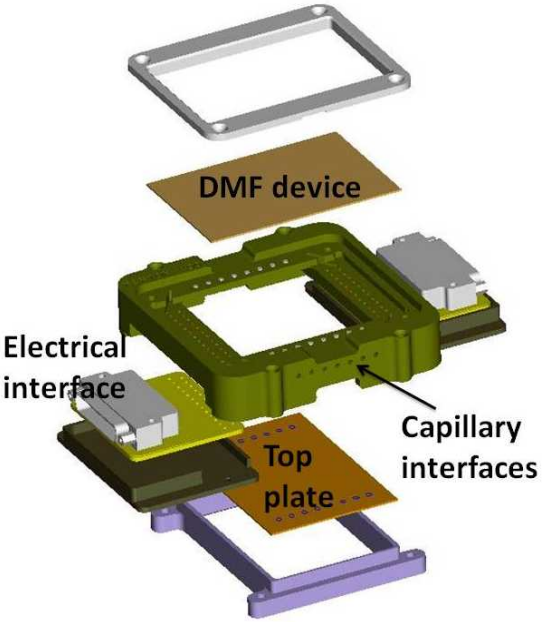


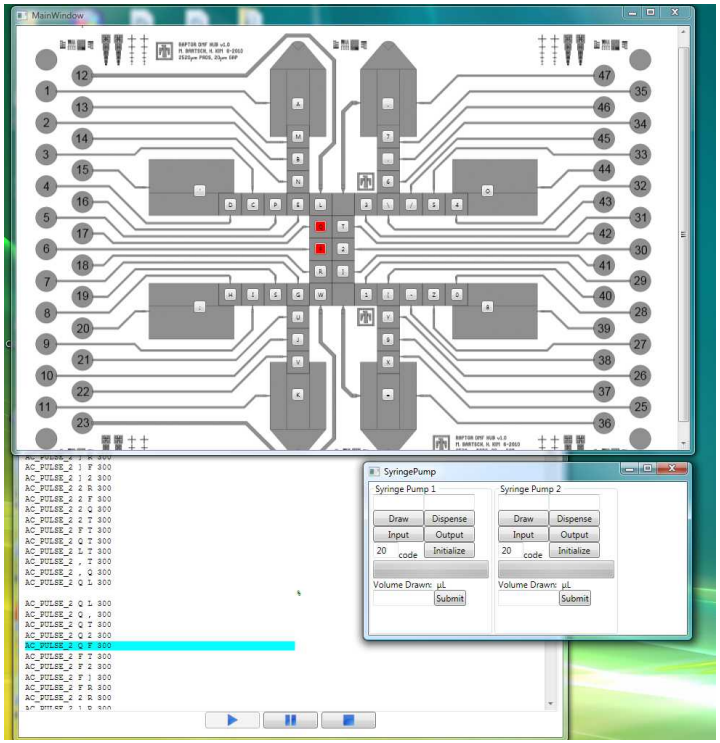


DMF hub platform developed for reliable and flexible sample processing

- Graphic User Interface developed
  - Intuitive designs
  - Manual Key strokes or scripting
  - Expandable to simultaneous control of peripheral modules (ex: normalization module)







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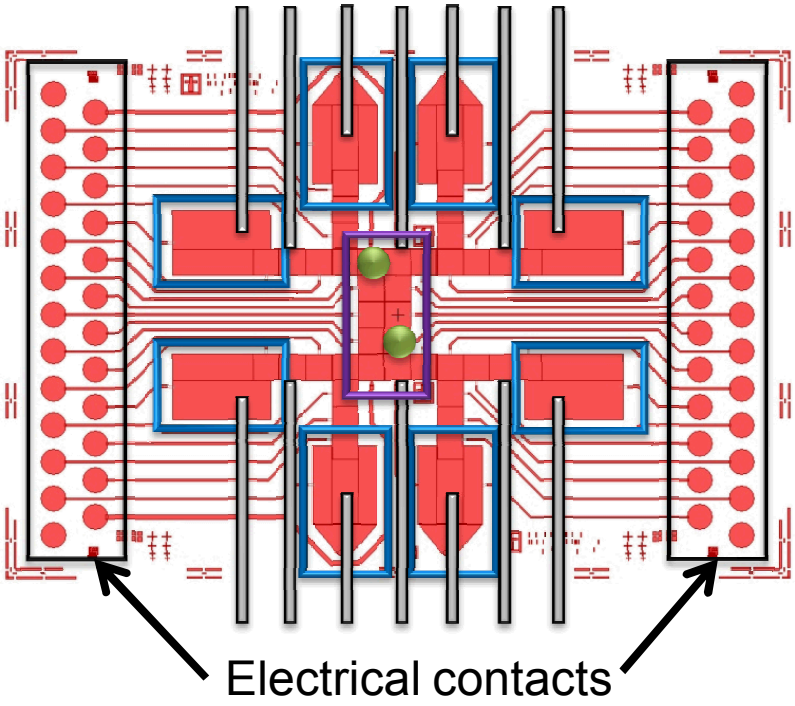
DMF hub operation optimized and tested compatible with DNA processing buffers

- Frequency: 15kHz (0 to 20kHz Tested)
- Dielectric: Parylene C (Su-8, PMMA, Glass tested)
- Actuation voltage

		Actuation voltage	Surface fouling
DI water		>100 Vrms	No
TE buffer		77 Vrms	No
Elution buffer (10 mM Tris-HCl, pH 8.5)		~70 Vrms	No
Buffers from normalization module	NaPB (10mM)	56 Vrms	No
	NaPB (160mM)	66 Vrms	No
	NaPB (330mM)	<70 Vrms	No
Ethanol		< 70 Vrms	No
DNA zap (DNA cleaning agent)		<70 Vrms	No
5% bleaching solution		<70 Vrms	No
Buffers for magnetic beads DNA capturing kit	Purification buffer	<70 Vrms	Controllable
	Beads solution	<70 Vrms	No
	Wash buffer	<70 Vrms	No
PBS		<70 Vrms	No

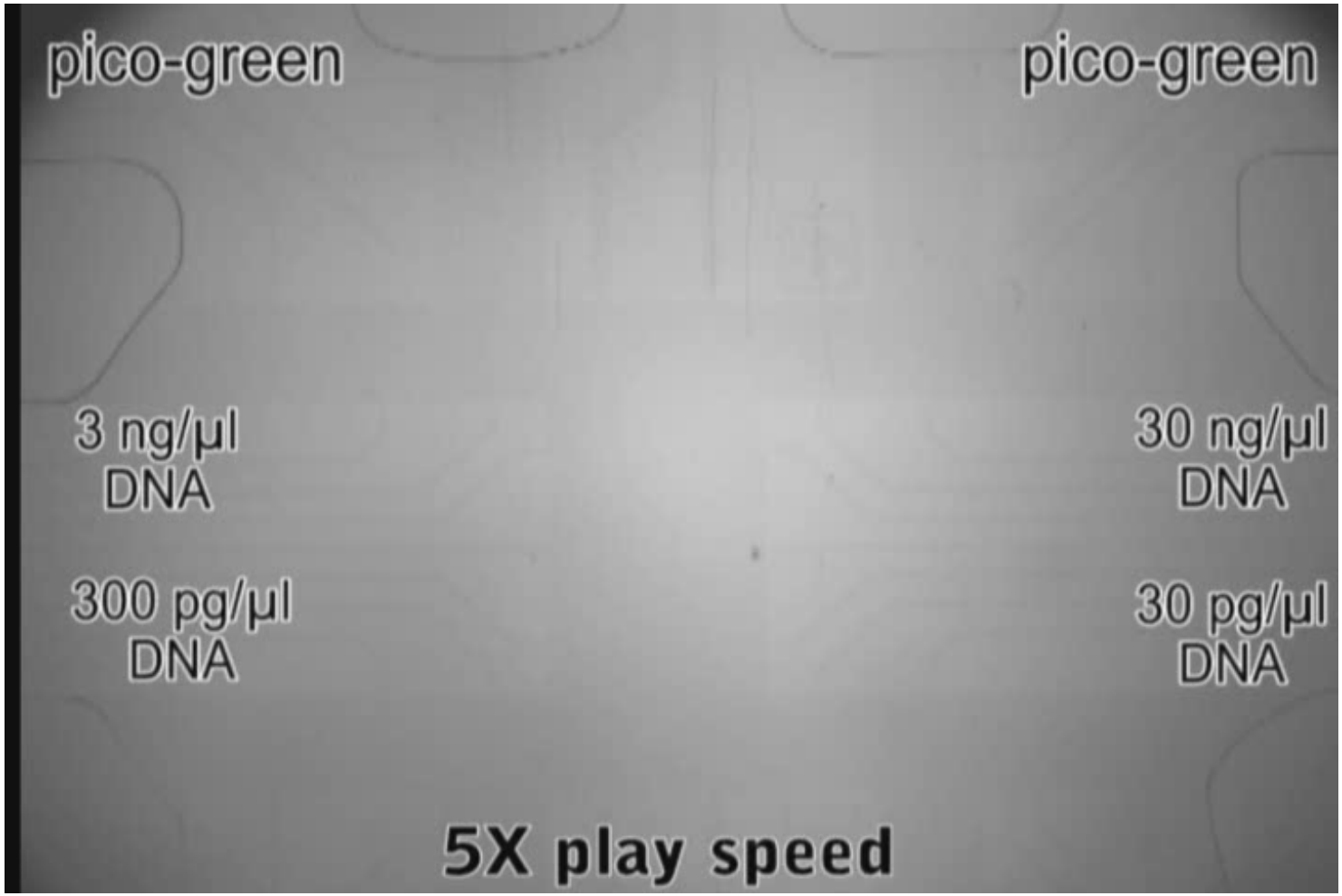
DMF design for sample processing



Hub 1

- 48 addressable pads (2.5 x 2.5 mm actuation pad)
- Single droplet volume of 1.2 or 2.5 µl (185 or 400 µm gap height)
- 8 sample reservoirs(12 to 25 µl)
- Pads in line with 14 capillary tubes
- Central mixing zone

Single assay using picogreen: 10^3 fold DNA concentration quantification



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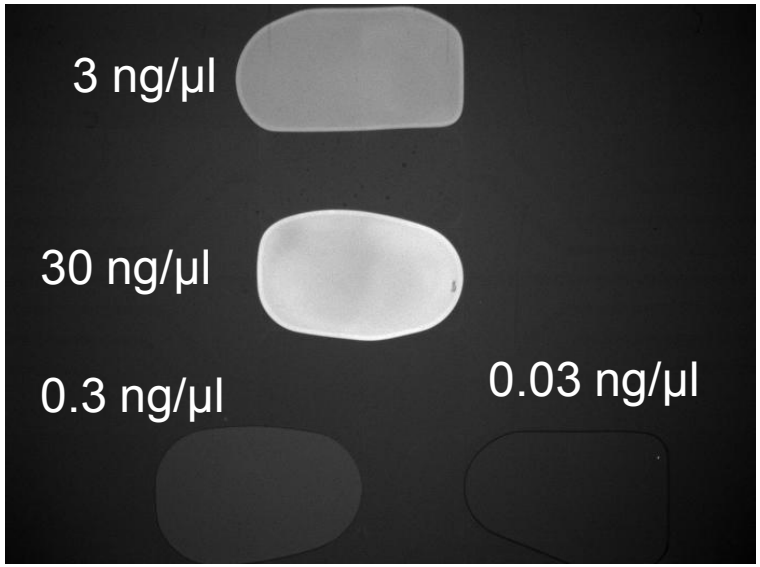
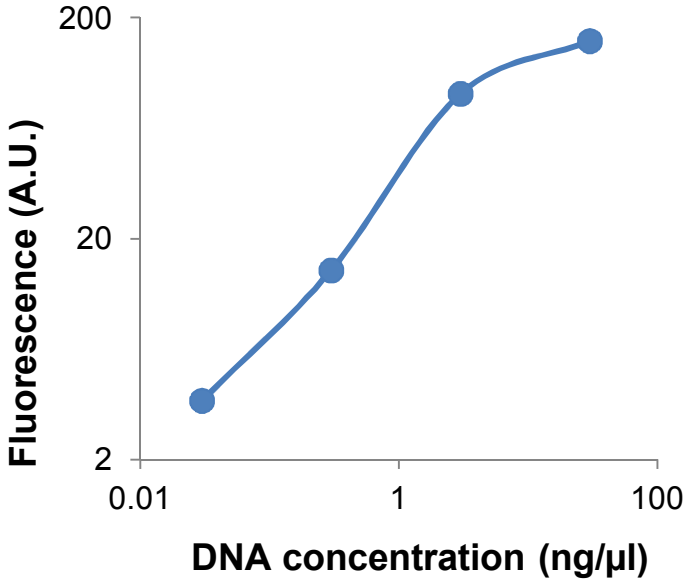
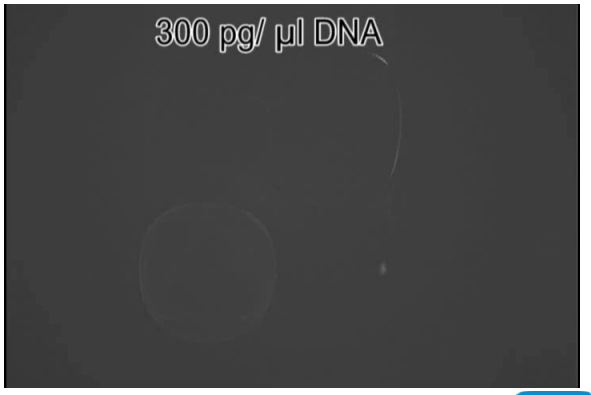


Fluorescence image

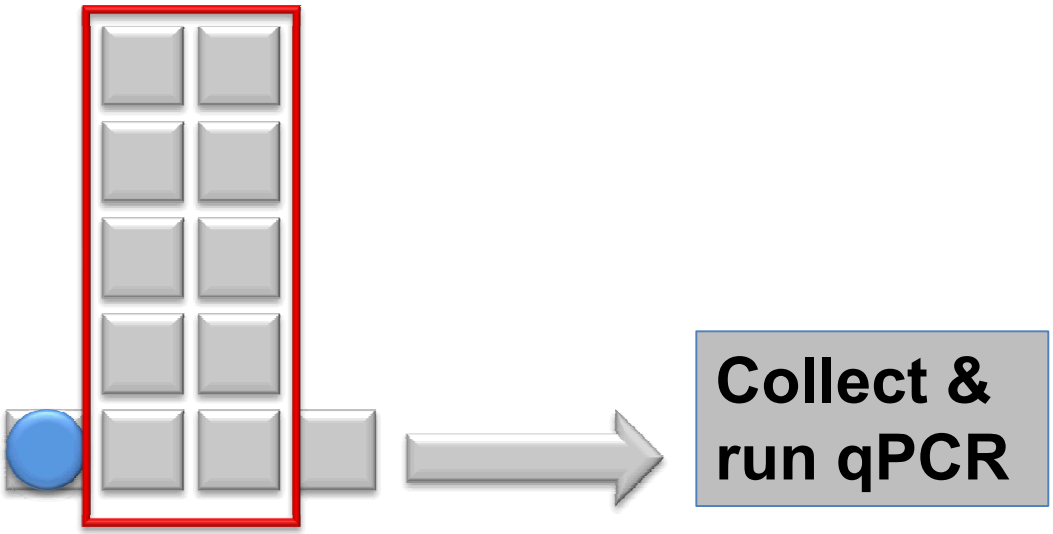




Carry over well controlled (10^6-fold dilution)

Sequence of Droplet introduced into 10-pads carryover test zone

1. **Elution buffer on a clean DMF for baseline**
2. **cDNA for contamination**
3. **Elution buffer for carry over**
4. Cleaning agent (bleaching solution, DNA zap…)
5. Rinse
6. **Elution buffer for carry over**
7. **Recover droplet and run qPCR (beta actin)**



Carry over well controlled (10^6-fold dilution)

Only 1 out of ~1,000,000 DNA molecules left behind and picked up by subsequent buffer droplet on DMF

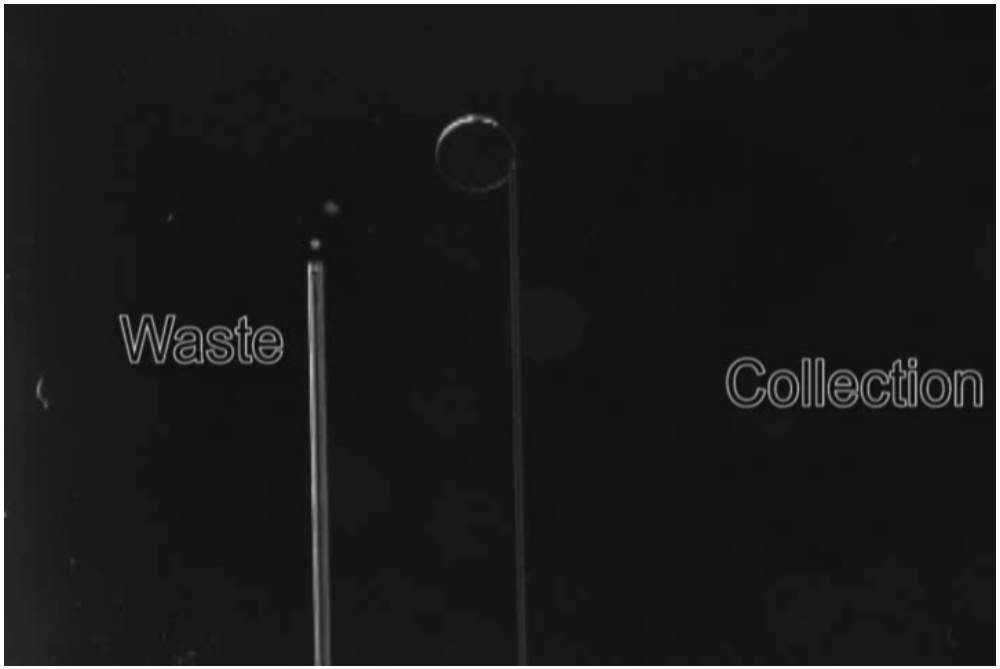
- No Cleaning agent

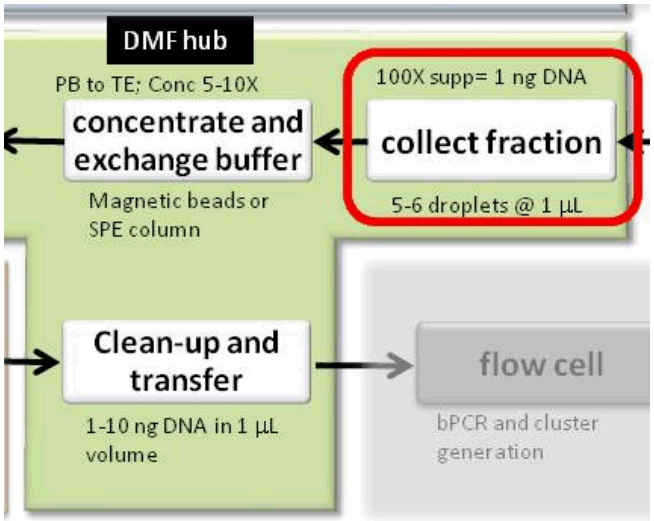
	Buffer drop (Baseline)	cDNA Drop	Buffer Drop	Buffer Drop	Buffer Drop
Ratio to cDNA	$<10^{-8}$		$\sim 10^{-6}$	$\sim 10^{-7}$	$\sim 10^{-8}$

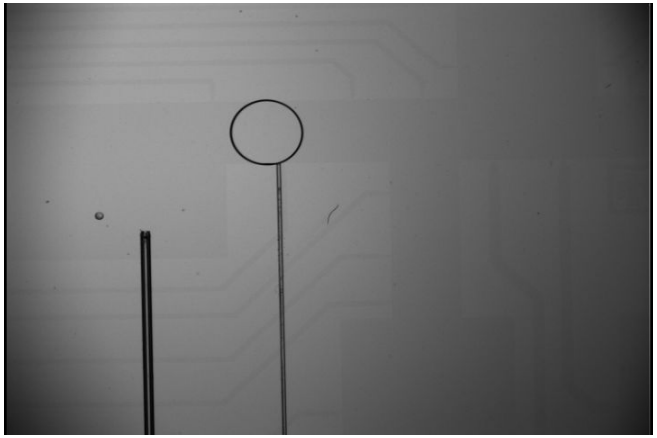
- Cleaning agents
  - DNAzap: ~ $<10^{-8}$
  - 5% bleaching solution: ~$<10^{-8}$

DMF hub collects fraction from continuous flow through capillary interface

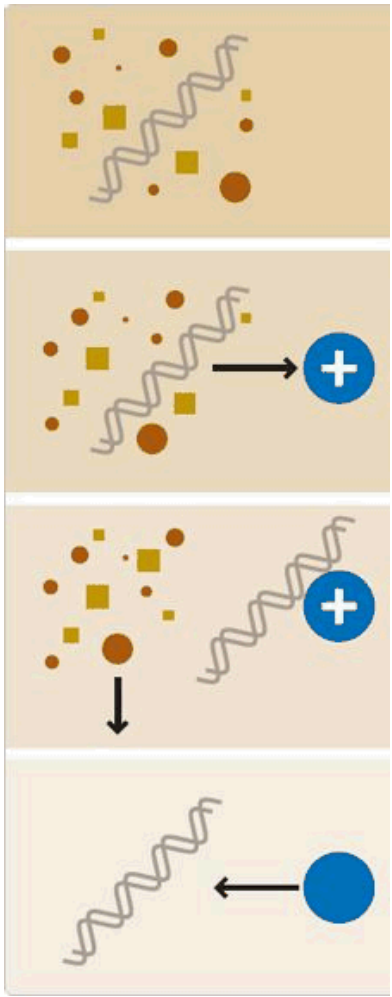
Collection of 5 µl fraction from continuous buffer flow @1.5mL/hr







Magnetic beads assay performed on DMF captures DNA effectively



DNA + Contaminants

DNA with contaminants
Ex) Excessive salts, enzymes

DNA binding

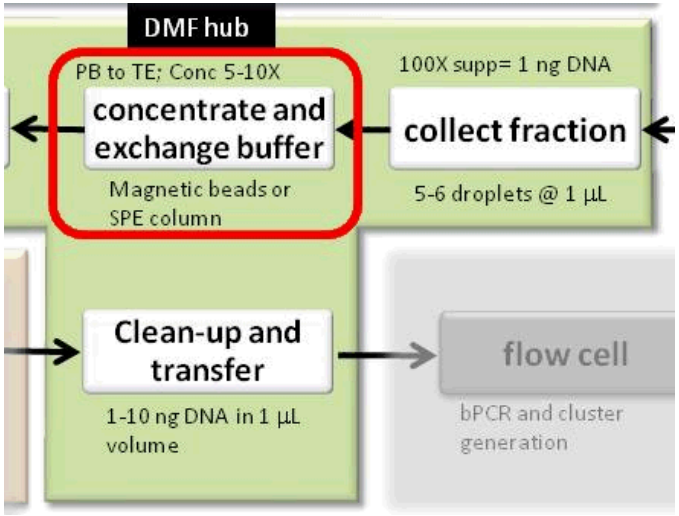
Purification buffer lowers pH
Beads added take positive charges & bind DNA

Contaminants Removal

Collect beads pellet
Wash & collect beads pellet

DNA Elution

Elution buffer raises pH
Collect elution buffer only



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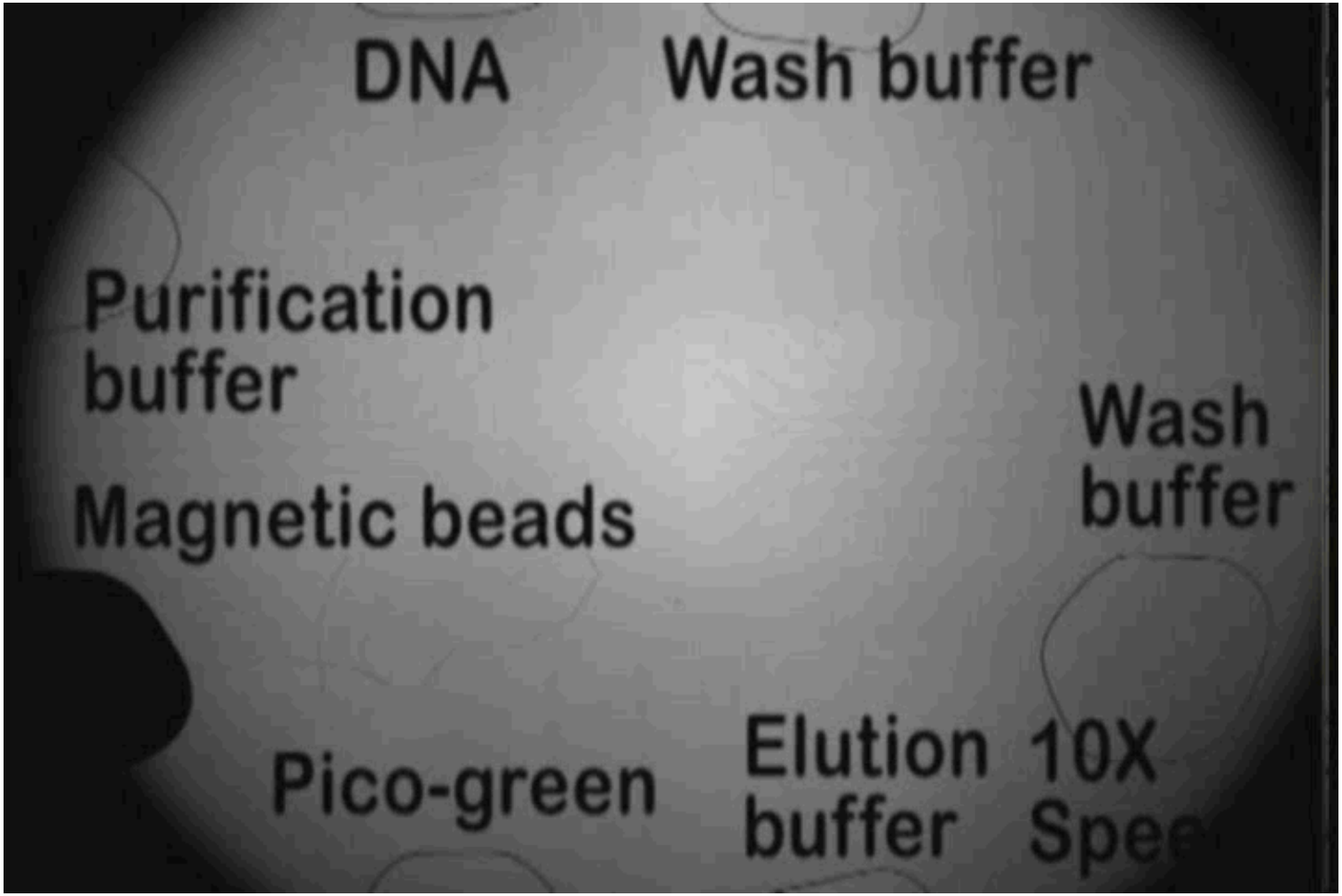
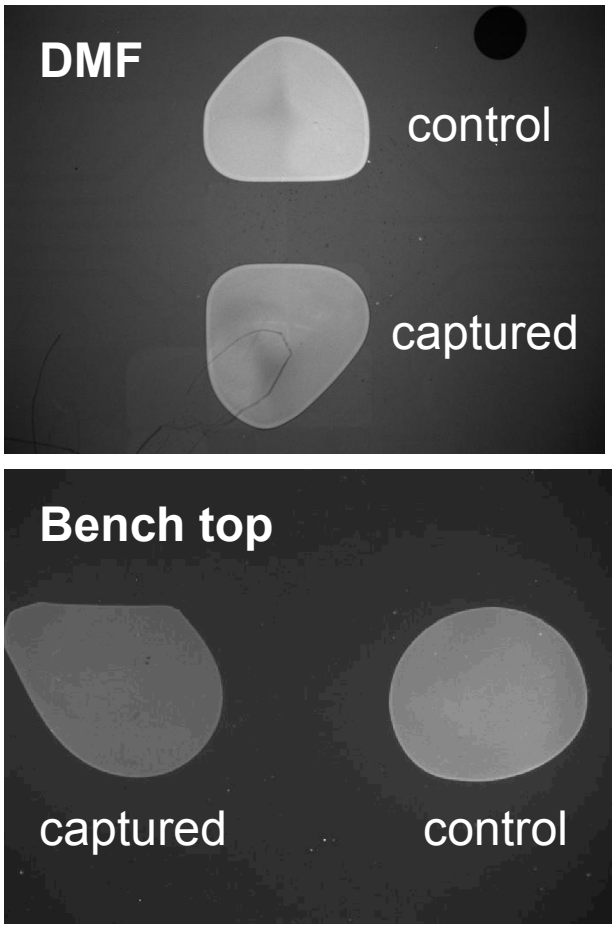
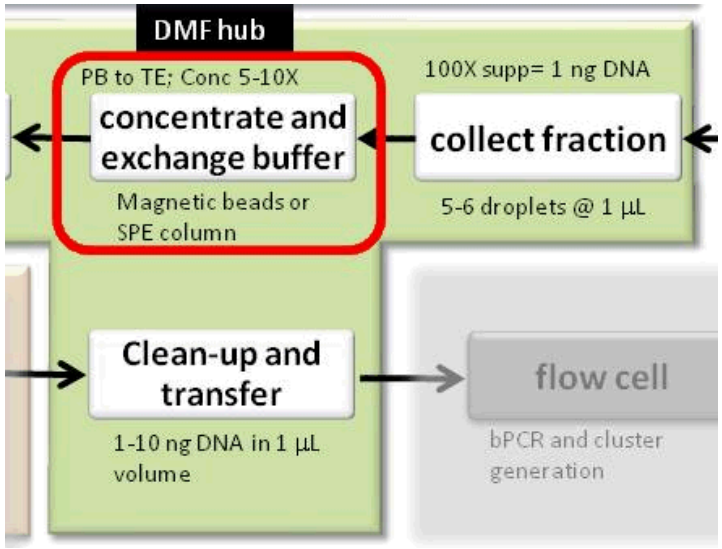


Magnetic beads assay performed on DMF captures DNA effectively

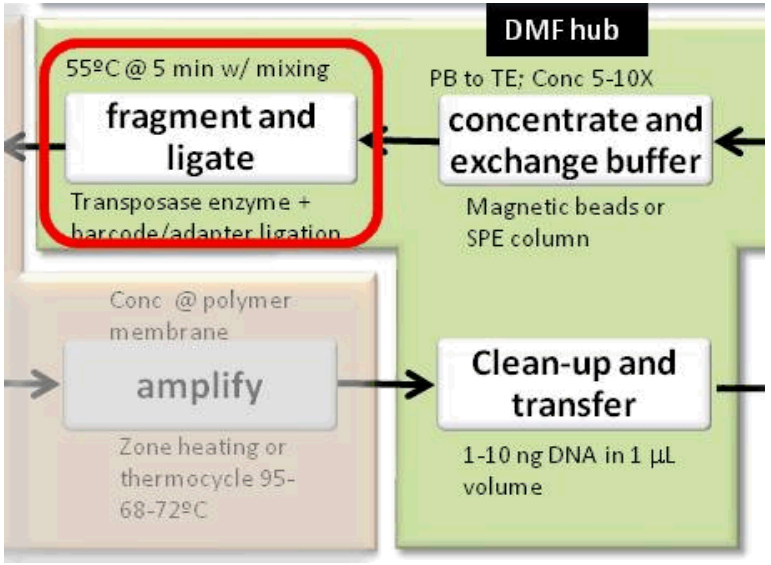
Assay on DMF hub shows comparable capturing efficiency of DNA to Bench top process

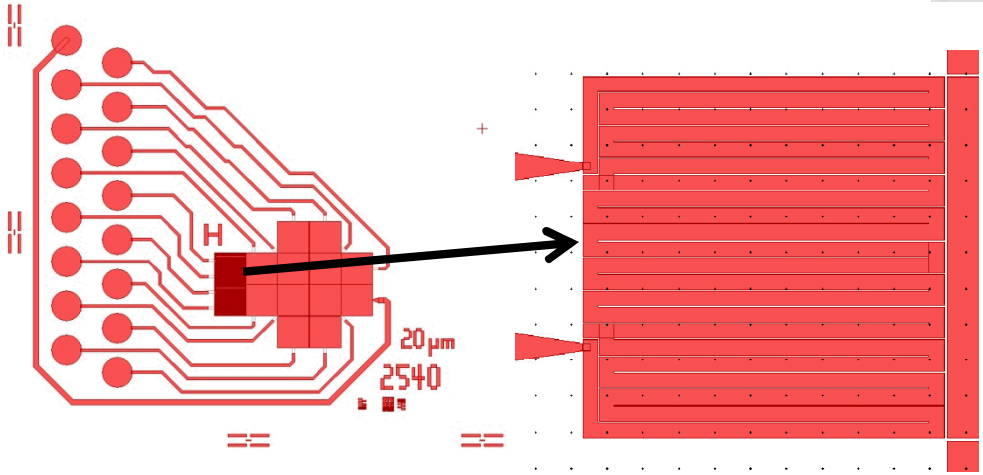


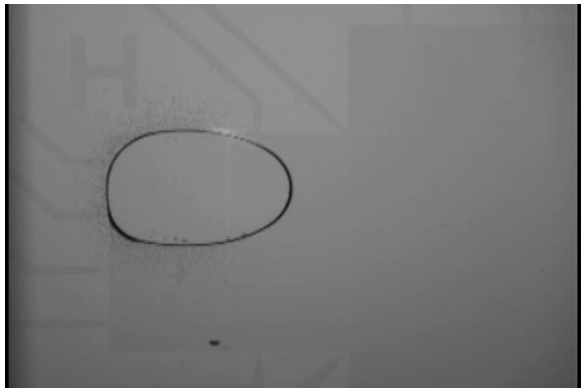


An integrated heating pad on DMF enables transposase reaction

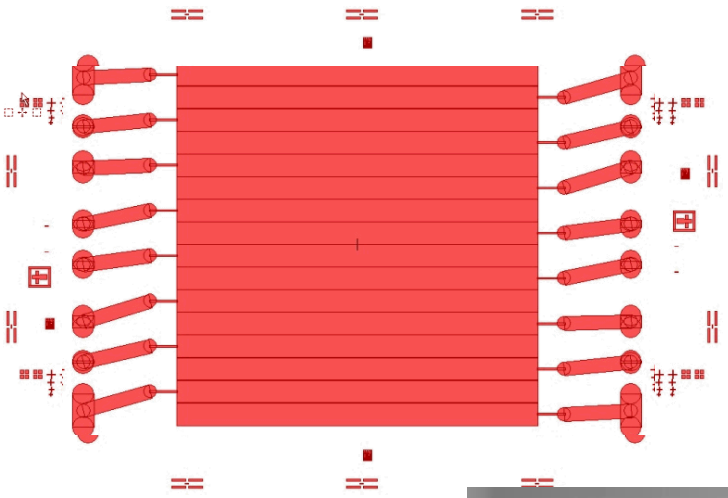
- Serpentine heater was built in DMF pad
  - Heating tested up to 55°C
  - Heating pad is also actuatable





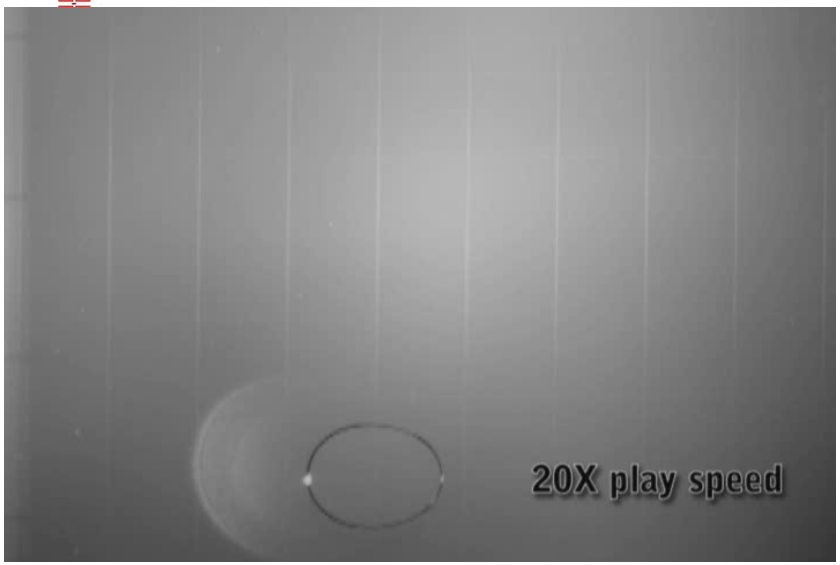


DMF designs with enhanced flexibility



Matrix DMF

- Superposition of 2 strip electrode devices
- 256 (16 x 16) addressable pads
- True flexibility in sample volume
- Parallel processing of samples

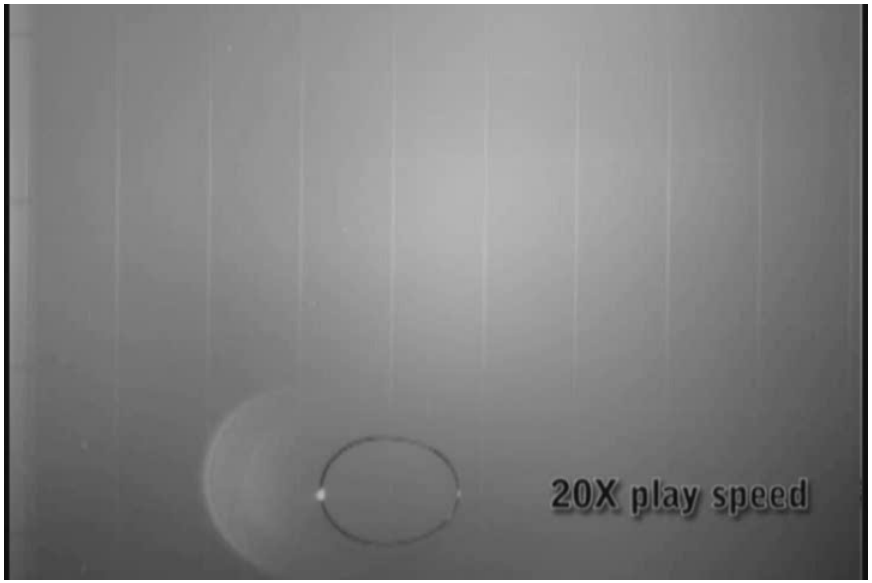


Summary and Plan

1. DMF hub platform engineered for reliability and flexibility
2. Established transparent DMF devices fabrication processes
3. DMF compatible with sample processing buffers
4. Carry-over is well controlled below 10^{-6}
5. Demonstrated Fraction collection and Buffer exchange
6. Built-in heating pad on DMF usable for enzyme based processing
7. Matrix DMF shows enhanced droplet manipulation flexibility

1. Implementation of transposase enzyme processing on DMF
2. Test of DMF hub fluidic interface with normalization unit
3. Application of Matrix DMF

Question?



Matrix DMF

Carry over experiment