Parallel Processing Of Multi-Functional, Point-Of-Care Bio-Applications On Electrowetting Chips

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Outline of Presentation

- Drivers for our EWD research:
  - Need for portable, point-of-care (POC) platform for bio-molecular detection and disease diagnosis:
  - Requirements:
    - on-chip sample preparation and molecular extraction
    - low I/O
    - parallel or multiplexed operation for throughput
    - on-chip resource sharing
    - scalability
- Technology for parallel assay POC lab-on-a-chip
  - Architectural choices
  - Digital microfluidics
- Summary and conclusions

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Parallel POC Microfluidic Systems

- Goal: extend the reach of lab-based microfluidics to multiple-assay, POC diagnostic devices
- Approaches to parallel microfluidics:
  - Fixed channels and functions
  - Fixed fluidic functions and programmable fluidic connections
    - Dynamically switchable fluidic connections require many valves
  - Programmable fluidic processors and fixed fluidic connections
    - Dynamically reconfigurable processors need to support numerous fluidic operations with a common set of reusable components
  - Programmable processors and connections and programmable control layer

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Fixed Fluidic Processors and Programmable Fluidic Connections

- Difficult to implement stand-alone, parallel continuous flow automated microfluidic systems for POC applications
    - Need for many valves and external pneumatic control box
    - Difficult to stage samples
    - Need for high I/O ports
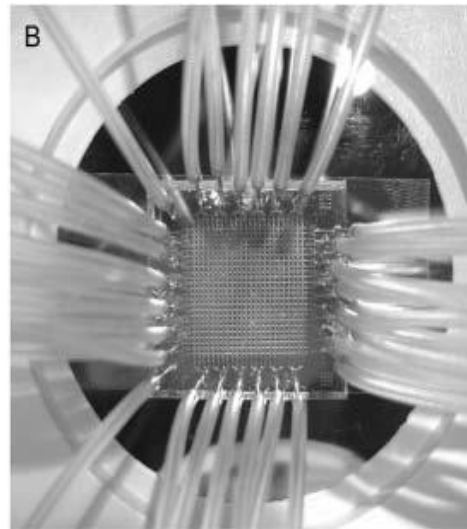   - Requires off-chip sample preparation

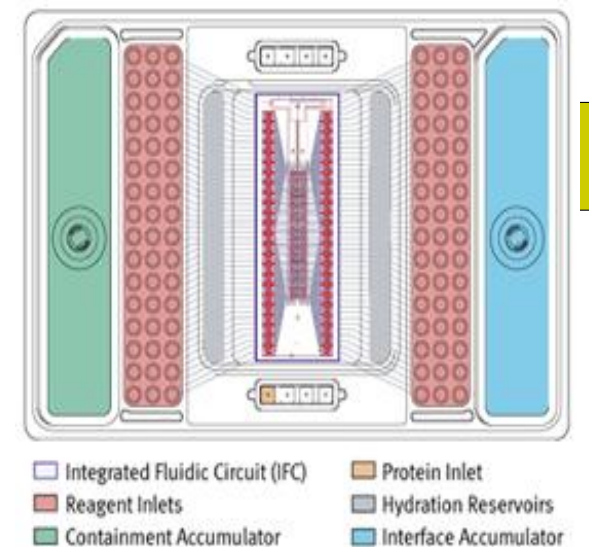# I/O Problem of Fixed Fluidic Processors and Programmable Fluidic Connections

I/O = 80
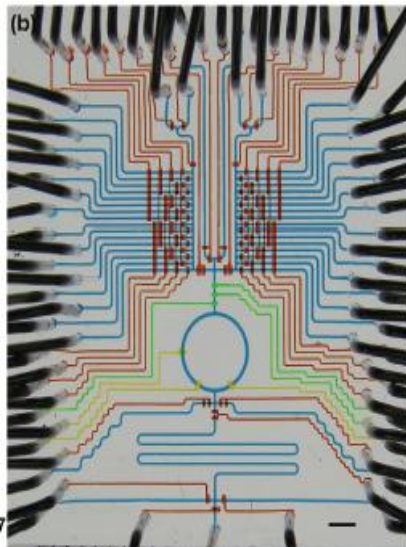
I/O = 32

I/O = 104

Parallel Reaction Chip
(Y. Wang, 2009)

Cytotoxicity Screening Chip
(Z. Wang, 2007)

Protein Crystallization Chip
(Fluidigm, 2005)

☐ Integrated Fluidic Circuit (IFC)   ☐ Protein Inlet
☐ Reagent Inlets   ☐ Hydration Reservoirs
☐ Containment Accumulator   ☐ Interface Accumulator

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Programmable Fluidic Processors and Fixed Fluidic Connections

Monarch Microfluidic Architecture

(Duke University – 2000)

- Continuous Flow

- Segregated Processing Units

- Shared Bus Architecture

- I/O to/from outside world

- Pressure driven

- Rigid functional units defined at assembly

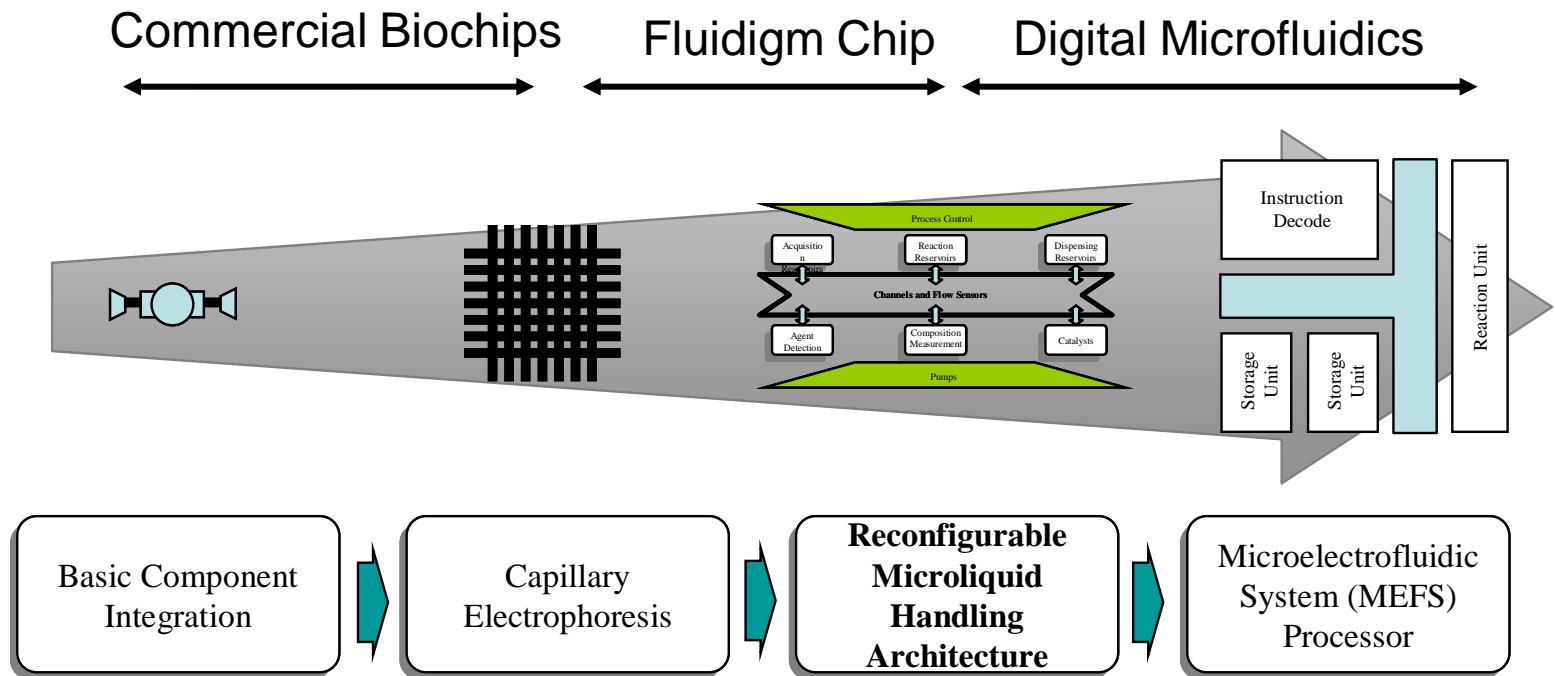Department of Electrical and Computer Engineering
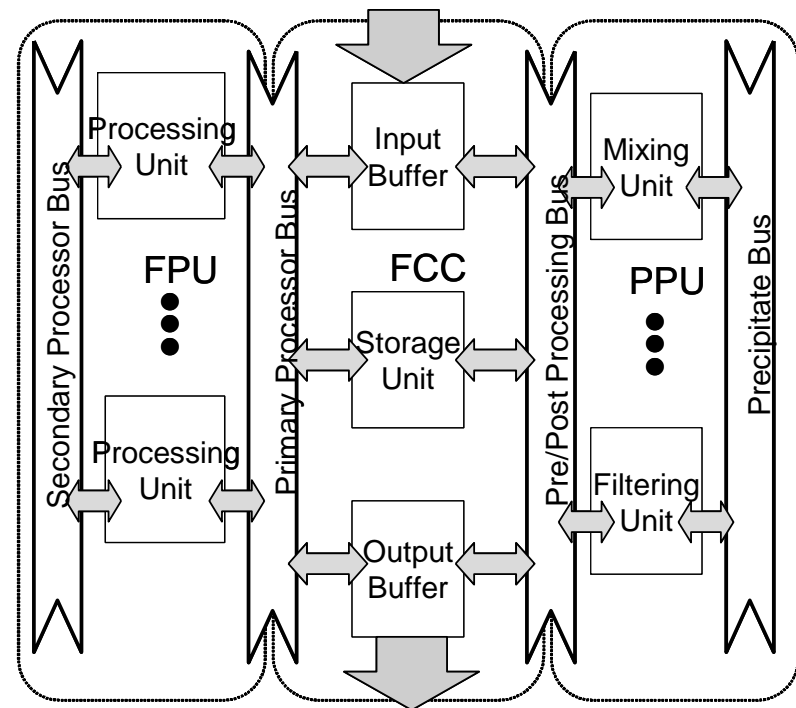
Programmable Processors and Connections and Programmable Control Layer

- Integration on single chip
- Programmable
  - Programmable at electronic control level
  - Fluidic operations performed on "assembled", configured components, not fixed components
    - Requires elemental components that can be assembled under electronic control to perform a fluidic operation
    - Reconfigurable fluidic processors and fluidic connections
  - No molecular cross-contamination of components
    - Reusable components
  - Multitasking
  - Adapts around processing bottlenecks
- Integrated sensing for adaptive behavior

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Road to Microfluidic Programmable Systems

- ## Where are we?

Commercial Biochips   Fluidigm Chip   Digital Microfluidics

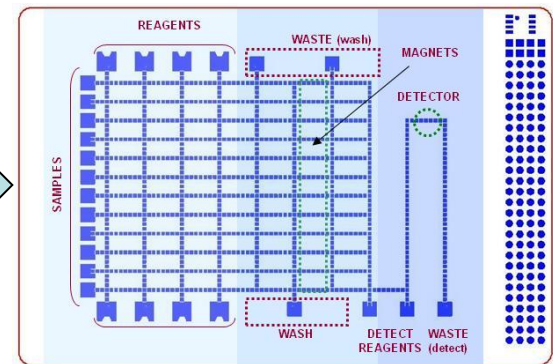| Basic Component Integration | Capillary Electrophoresis | **Reconfigurable Microliquid Handling Architecture** | Microelectrofluidic System (MEFS) Processor |

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Where Are We?

- Programmable electronic control demonstrated

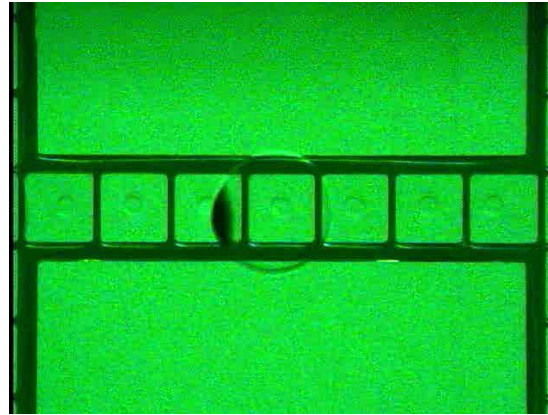- Multiplexed operation of single and multiple assays demonstrated

- Architectural flexibility demonstrated

- Magnetic bead assays demonstrated

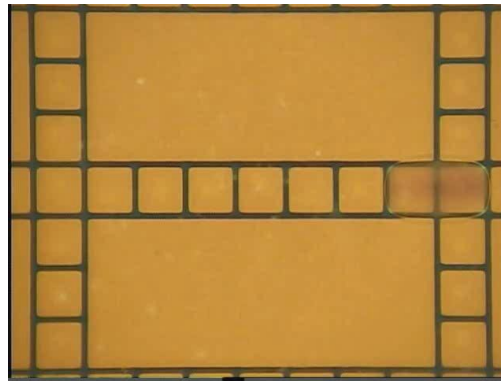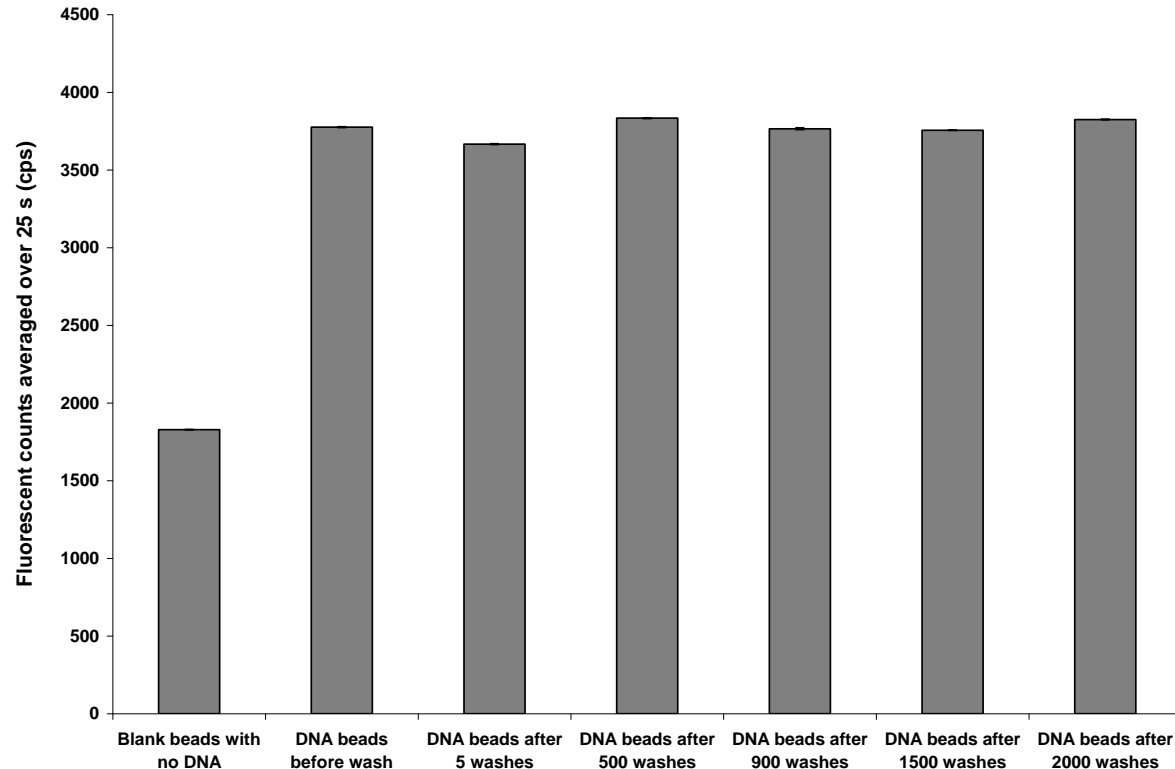Department of Electrical and
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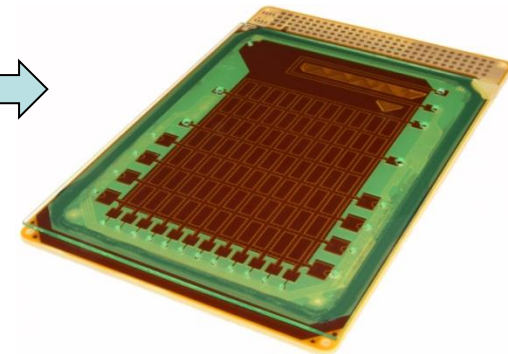
Key Elements of EWD Processor

- Magnetic bead-based assays with dilutional washing:

- Bead-based assays with replacement washing (phase gate):
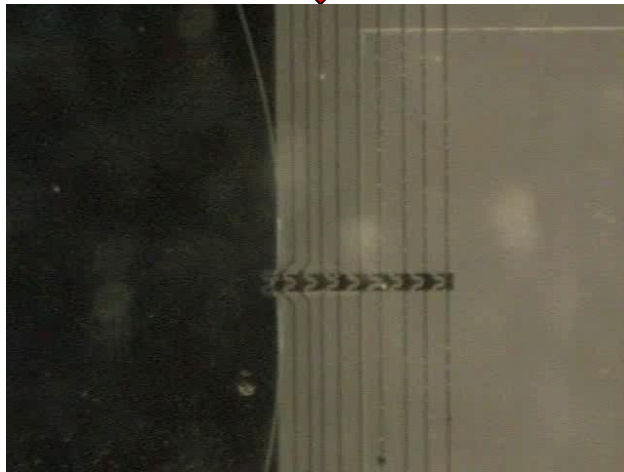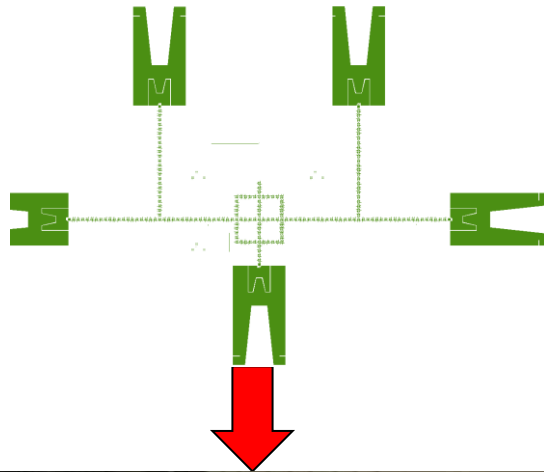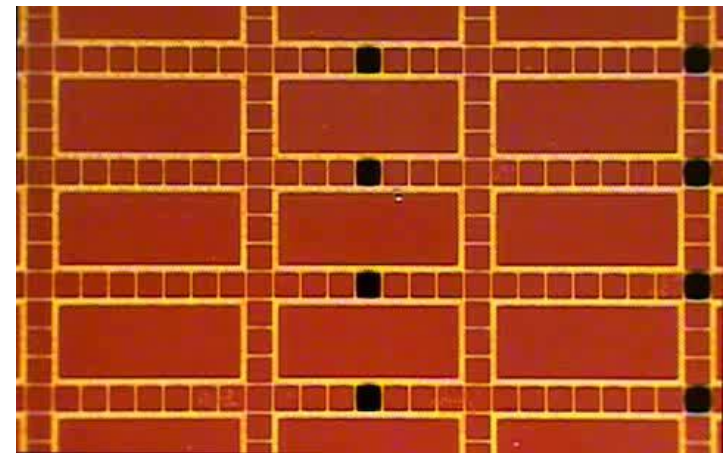
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Magnetic Bead Washing

Fluorescence of beads with FAM-labeled primer

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Scalability

35pl

300nl

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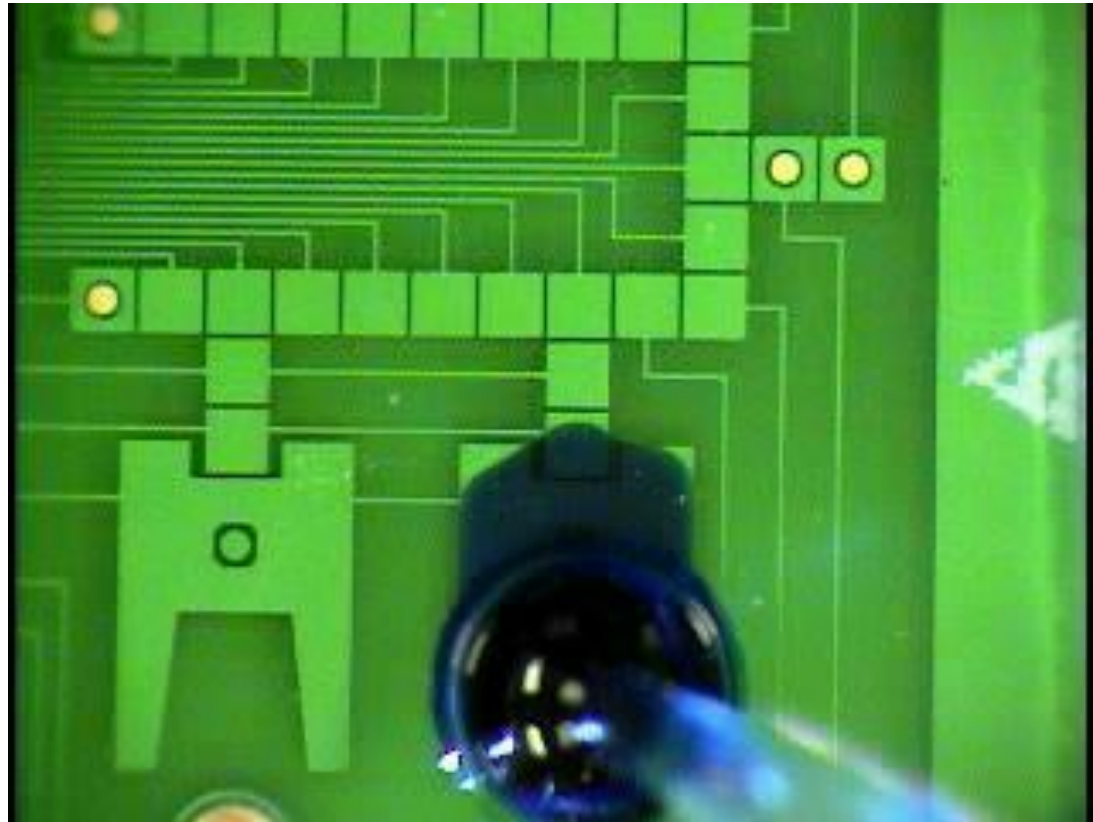
Reduced I/O

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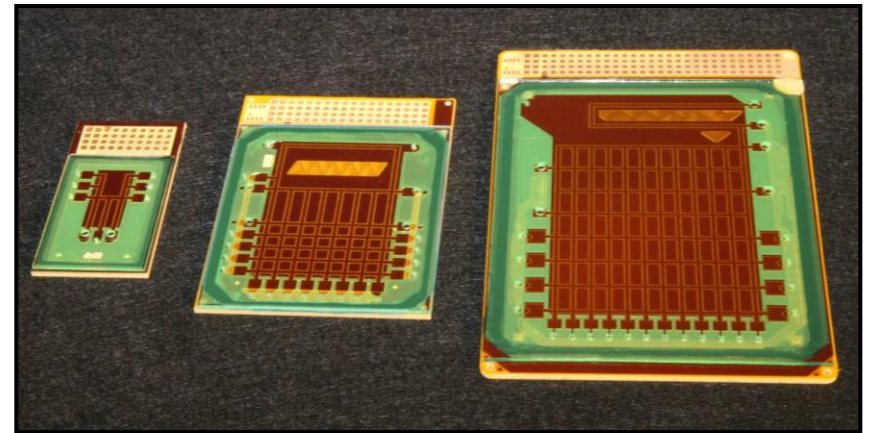
Advanced Liquid Logic, Inc.
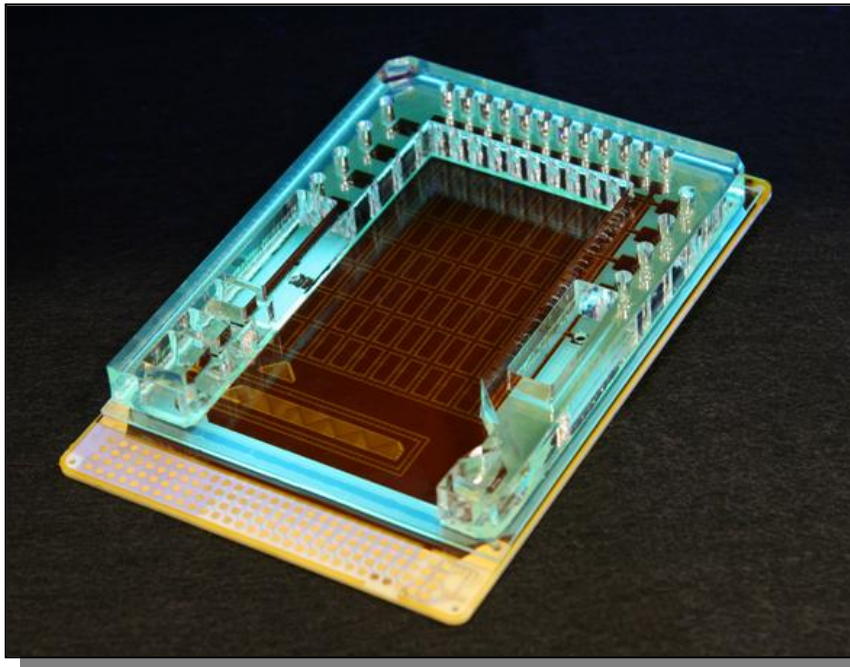
World to Chip Interface

- Well-plate interface
  - Easy and familiar loading
  - 384-well spacing
  - Inputs from microliters to milliliters

- Wash/waste reservoirs support 48+ tests
  - Load and go

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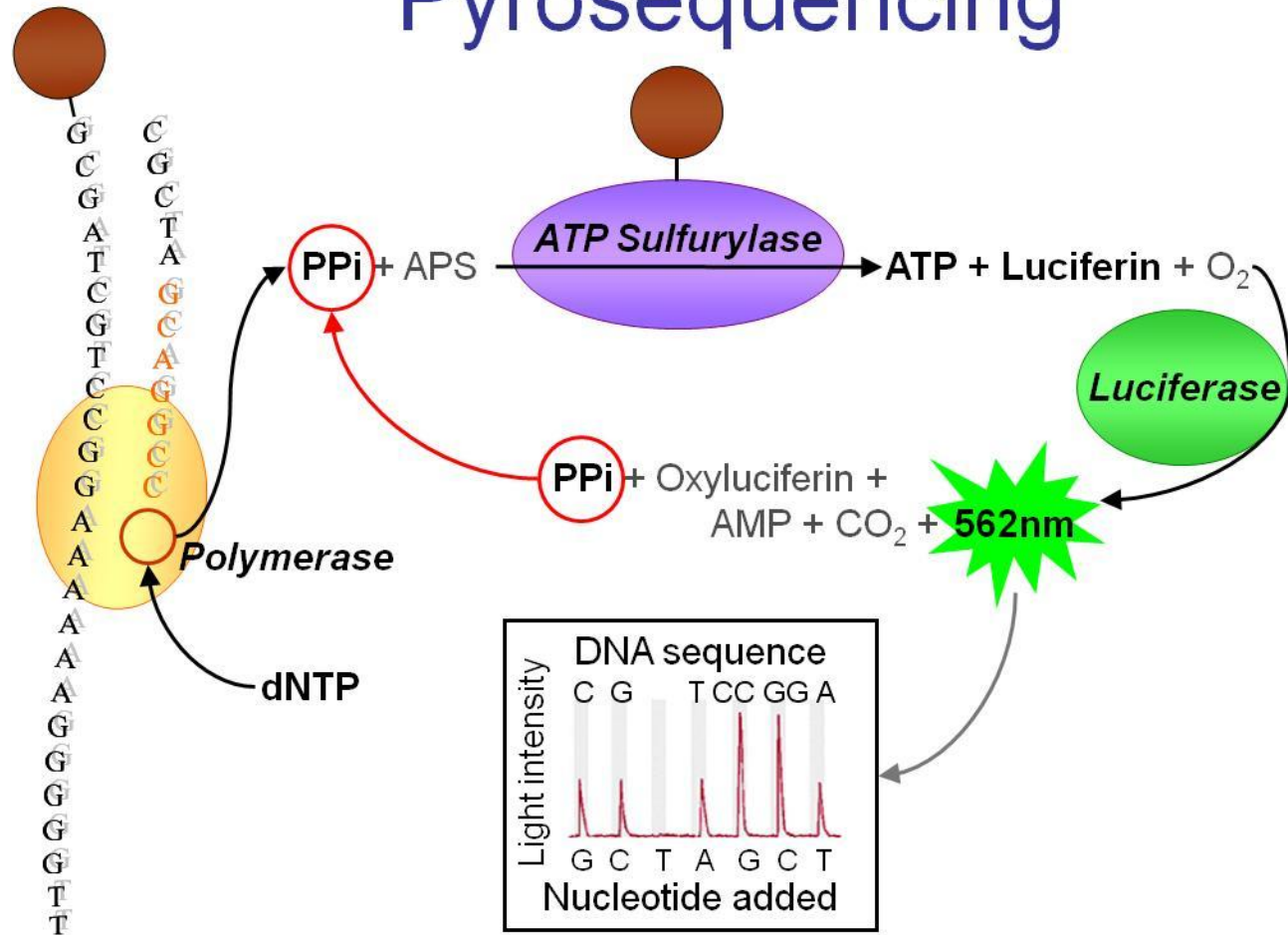
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Current Application Drivers

- Multiplexed DNA sequencing by synthesis
- Bead-based assays
- Cytotoxicity screening
- On-chip sample preparation and DNA extraction
- Malaria detection
- Integrated on-chip sensing

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Pyrosequencing

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Multiplexed Pyrosequencing

Enzyme mix 2   Wash reservoir   Nucleotide reservoirs

Waste

Reservoirs containing beads attached to different DNA sequences

Common detection spot

Magnets to hold beads while washing   Waste   Enzyme mix 1

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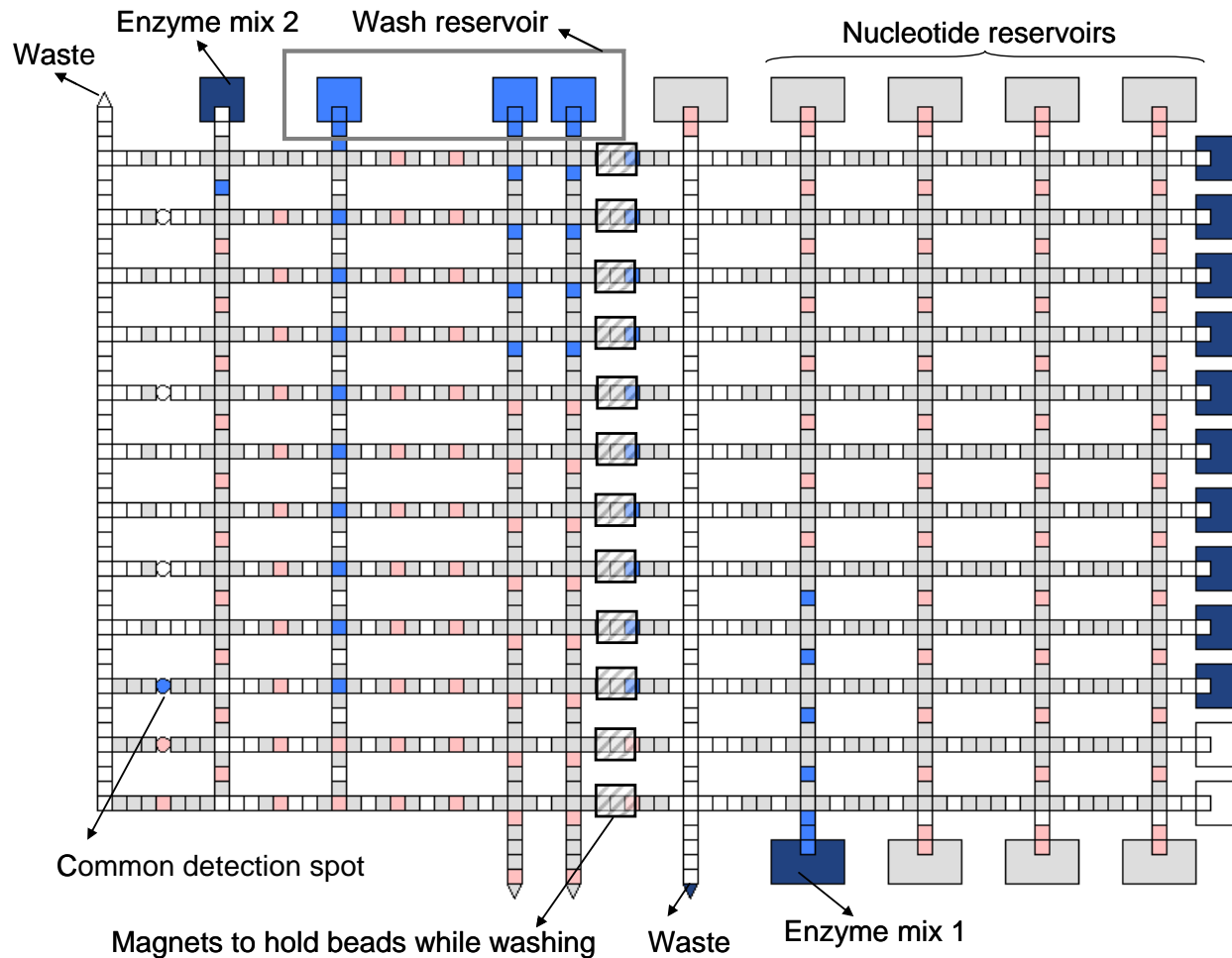
4-plex Sequencing Using Decoupled PPi Assay Format

Correct sequence: TAAG GCGG GATC GCTT TGAC

Lane 9
Lane 7
Lane 5 (blank)
Lane 3

Luminescent peak intensities

G T AA C C GG C

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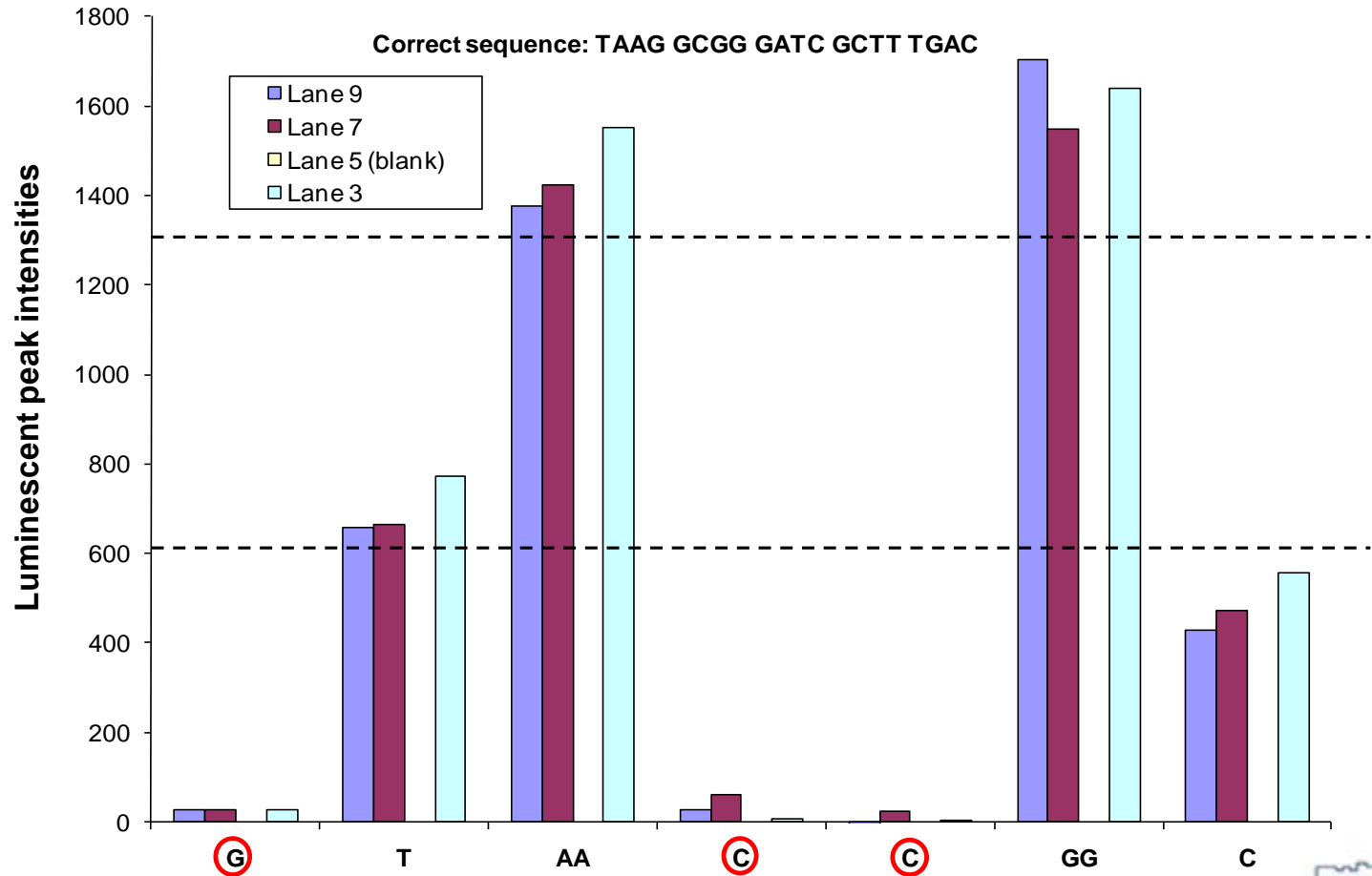
Parallel Processing with Feedback

Look-ahead Sequencing With Voting – Beads Don't Pass Magnet

1. Pre-load array with nucleotides and luciferase
2. Dispense 4 droplets of identical DNA on beads
3. Transport samples and nucleotides to magnet

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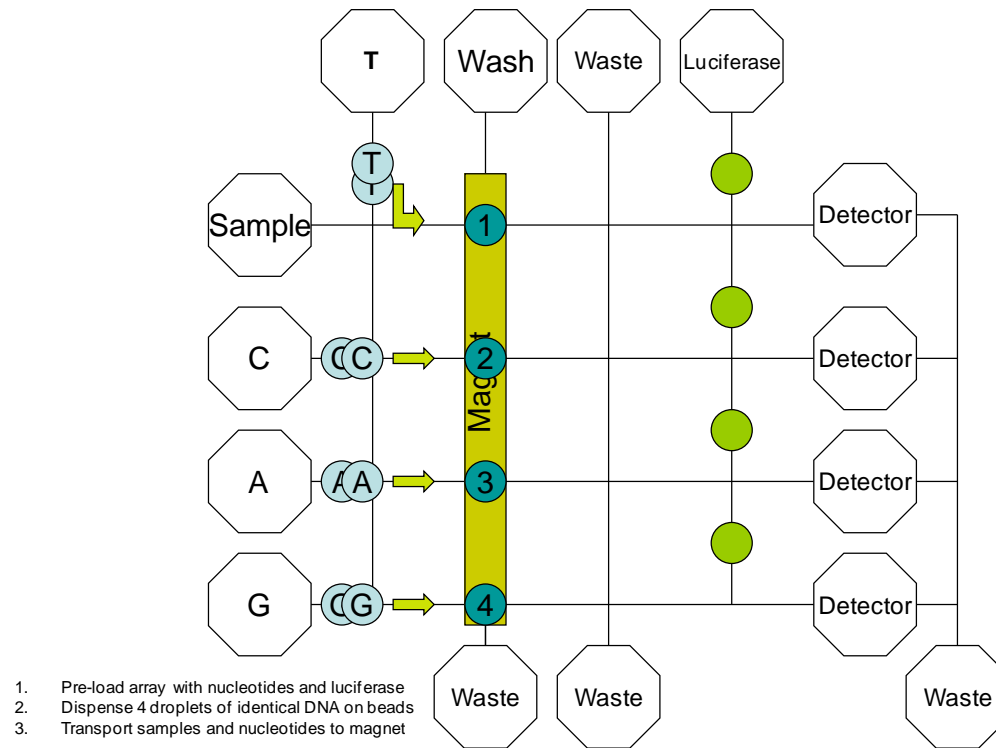
Parallel Processing with Feedback

Incorporation and Displacement

4. Mix and displace

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Parallel Processing with Feedback

Split and Mix

5. Split mixed 2x droplets and mix split solution droplet with luciferase.

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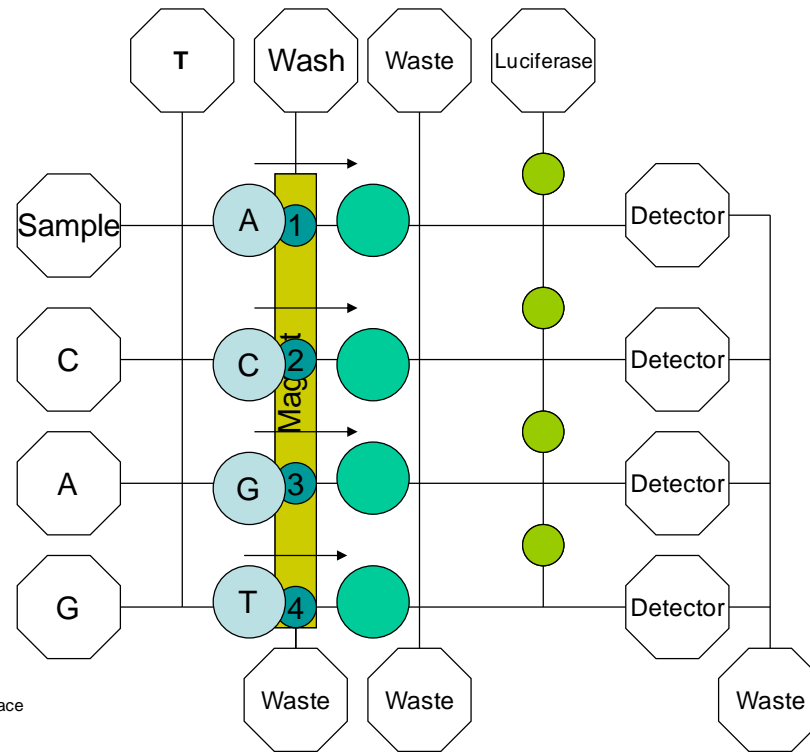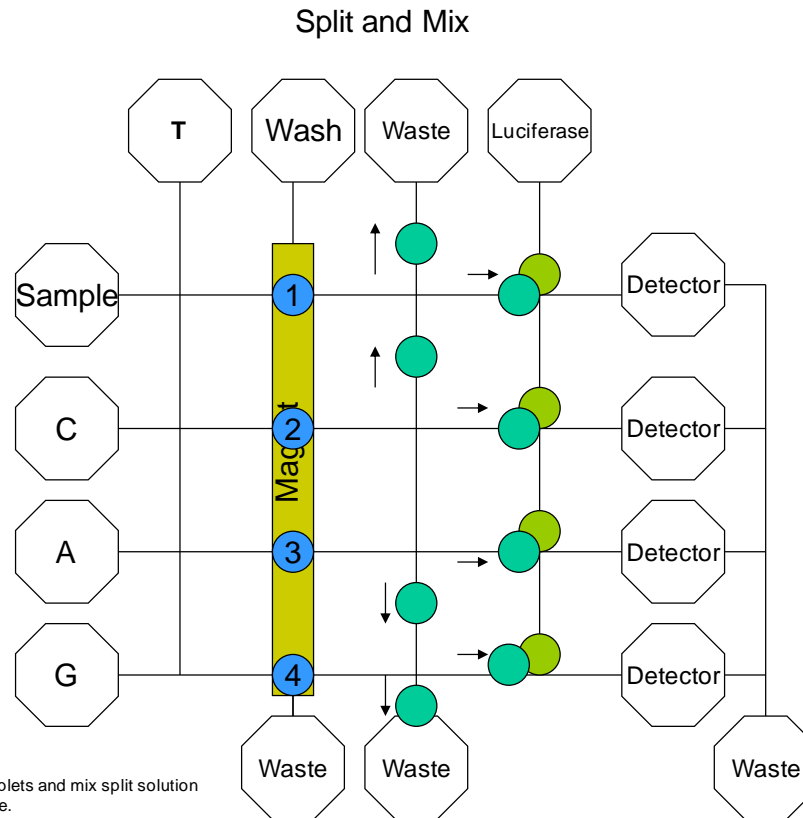
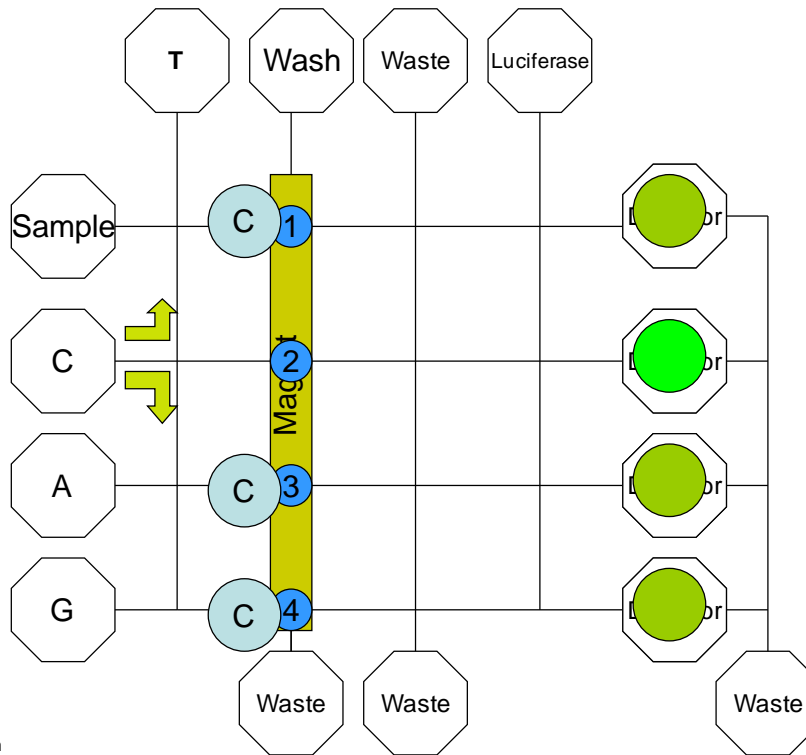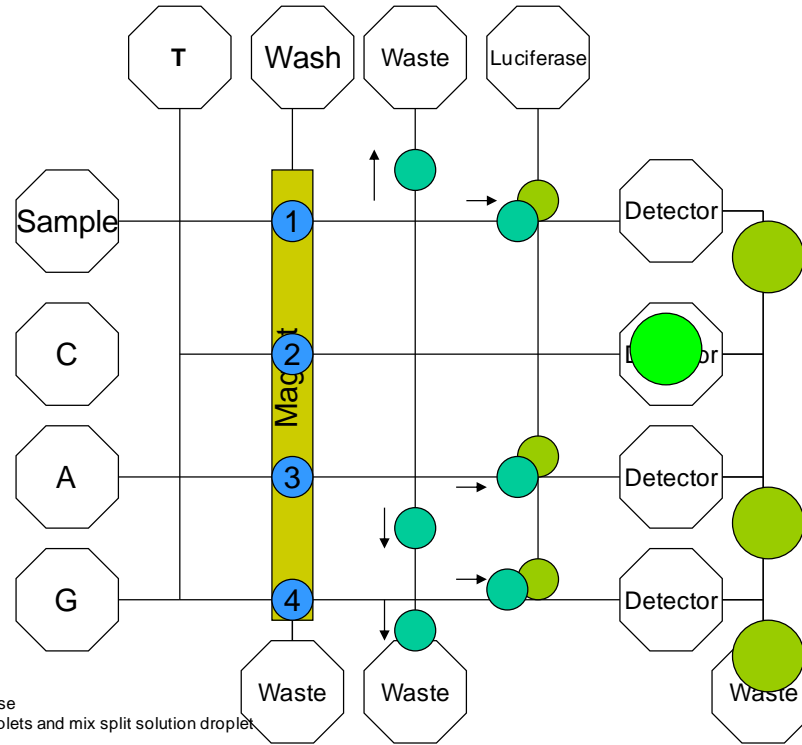# Parallel Processing with Feedback

Detect C Incorporation, Dispense dCTP

7. Detect "C" incorporation
8. Dispense dCTP drops and mix/displace with samples 1, 3, 4.

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Parallel Processing with Feedback

Dispense and Mix Luciferase

10. Dispense luciferase
11. Split mixed 2x droplets and mix split solution droplet
   with luciferase.

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Parallel Processing with Feedback

Detect and Vote, Initiate Sample Chain Wash

10. Detect and vote
11. Top-bottom chain wash samples 1-4

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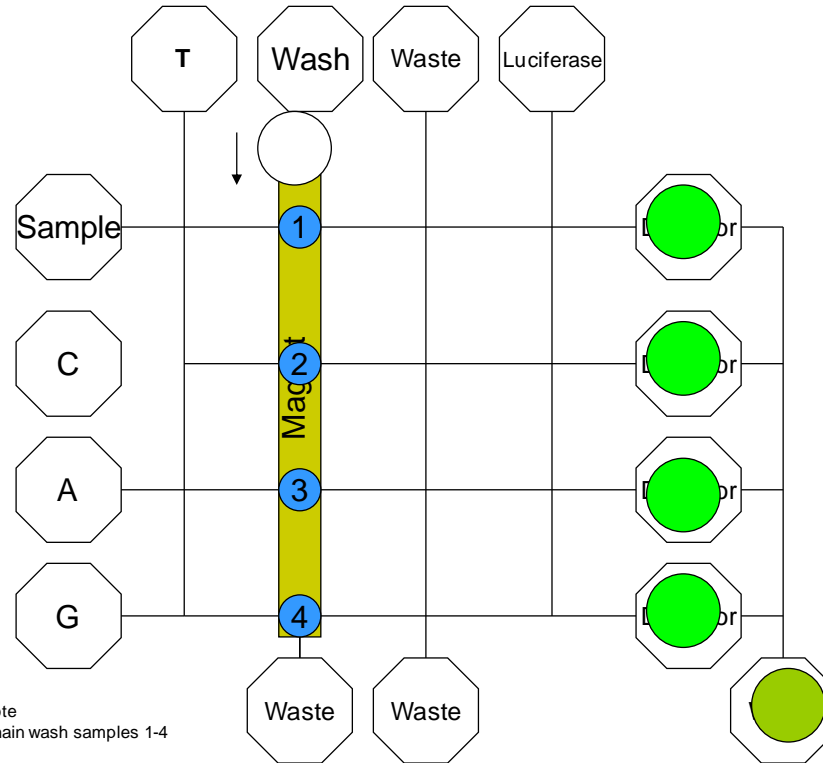
Parallel Processing with Feedback

Reload Array, Chain Wash Continuation

12. Reload array, clear detectors
13. Sample 1 washed, set aside

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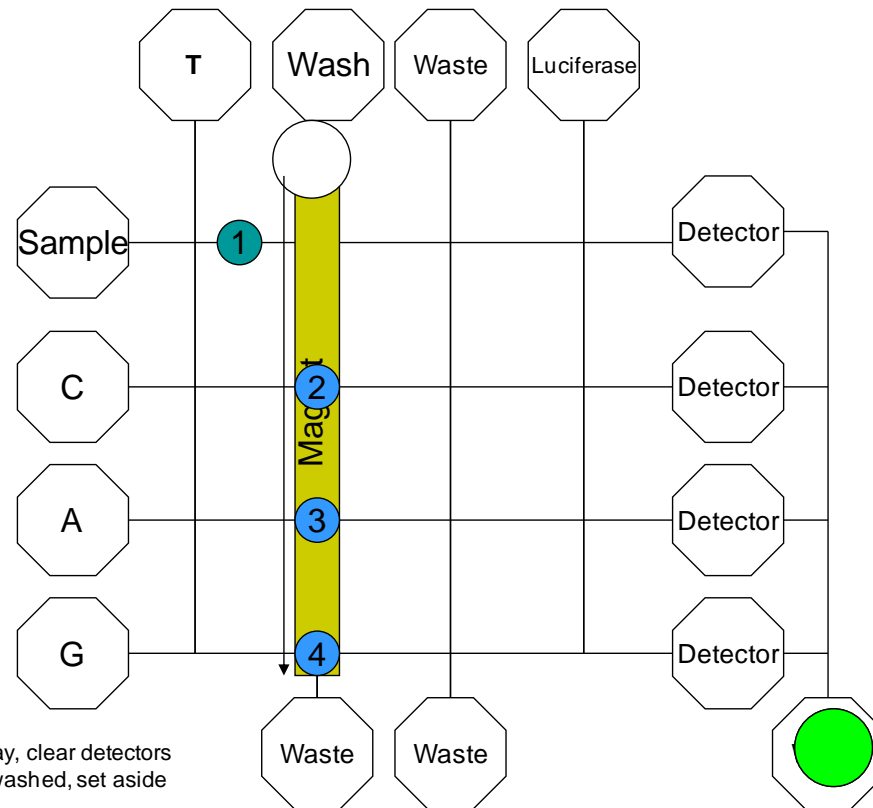
Programmable Parallel POC Chip Status

- Digital microfluidic toolkit demonstrated
  - Many individual fluidic functions demonstrated
  - Multiplexed assays demonstrated with shared on-chip resources and limited cross contamination
    - Multiple enzymatic assays
    - Common sequencing assays run in parallel
    - Magnetic bead washing for sample refresh
    - On-chip sample preparation development underway
    - Reliable I/O achieved
- Parallel multifunctional bio applications for POC
  - Program control capability exists
  - Digital microfluidic chip technology exists
  - Needs to be driven by an application

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