Abstraction Layers for Scalable Microfluidic Biocomputers

William Thies*, J.P. Urbanski[†],
Todd Thorsen[†] and Saman Amarasinghe*

* Computer Science and Artificial Intelligence Laboratory
[†]Hatsopoulos Microfluids Laboratory
Massachusetts Institute of Technology

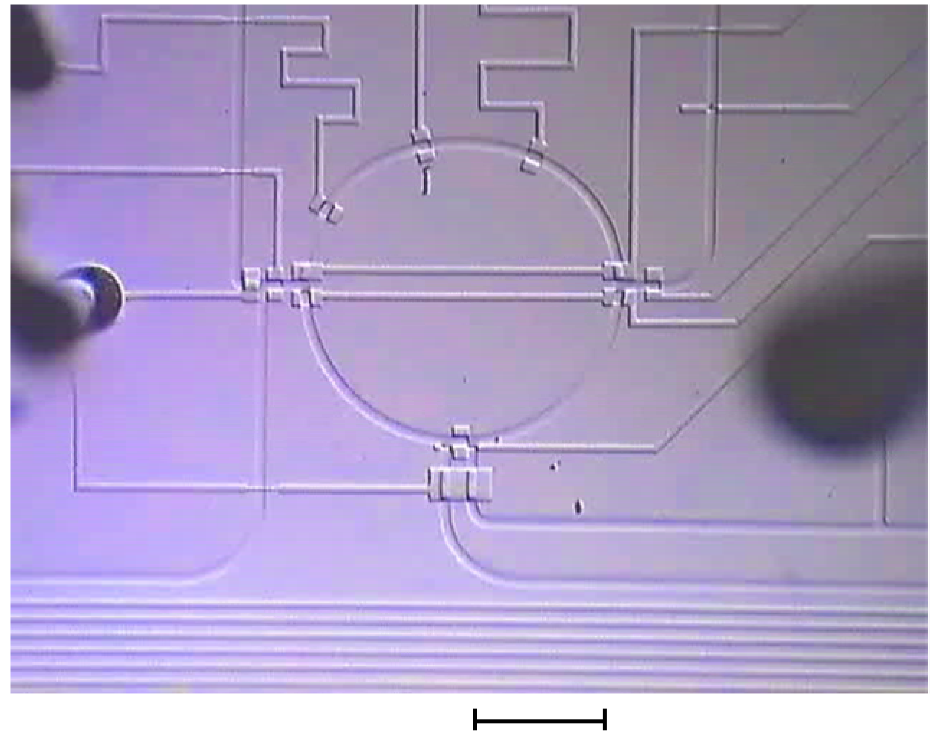
International Meeting on DNA Computing – June 8, 2006

Biocomputing Demands Complex Protocols

- **Complex biology experiments have many hazards**
  - Time-consuming laboratory protocols
  - Risk of human error
  - Expensive reagents and equipment
- **Biocomputing: complexity grows with problem size**
  - 20-variable 3-SAT problem: >96 hours to complete [Adleman02]
  - Larger instances will be even more challenging
  - Need a scalable approach
- **Our approach:**
  Write protocols as programs, run on microfluidic chips
  - Write once, run anywhere
  - This talk: how do you "program" a biological protocol?

Microfluidic Chips

- **Idea: a whole biology lab on a single chip**
  - Input/output
  - **Sensors**: luminescence, pH, glucose, etc.
  - **Actuators**: mixing, PCR, electrophoresis, cell lysis, etc.

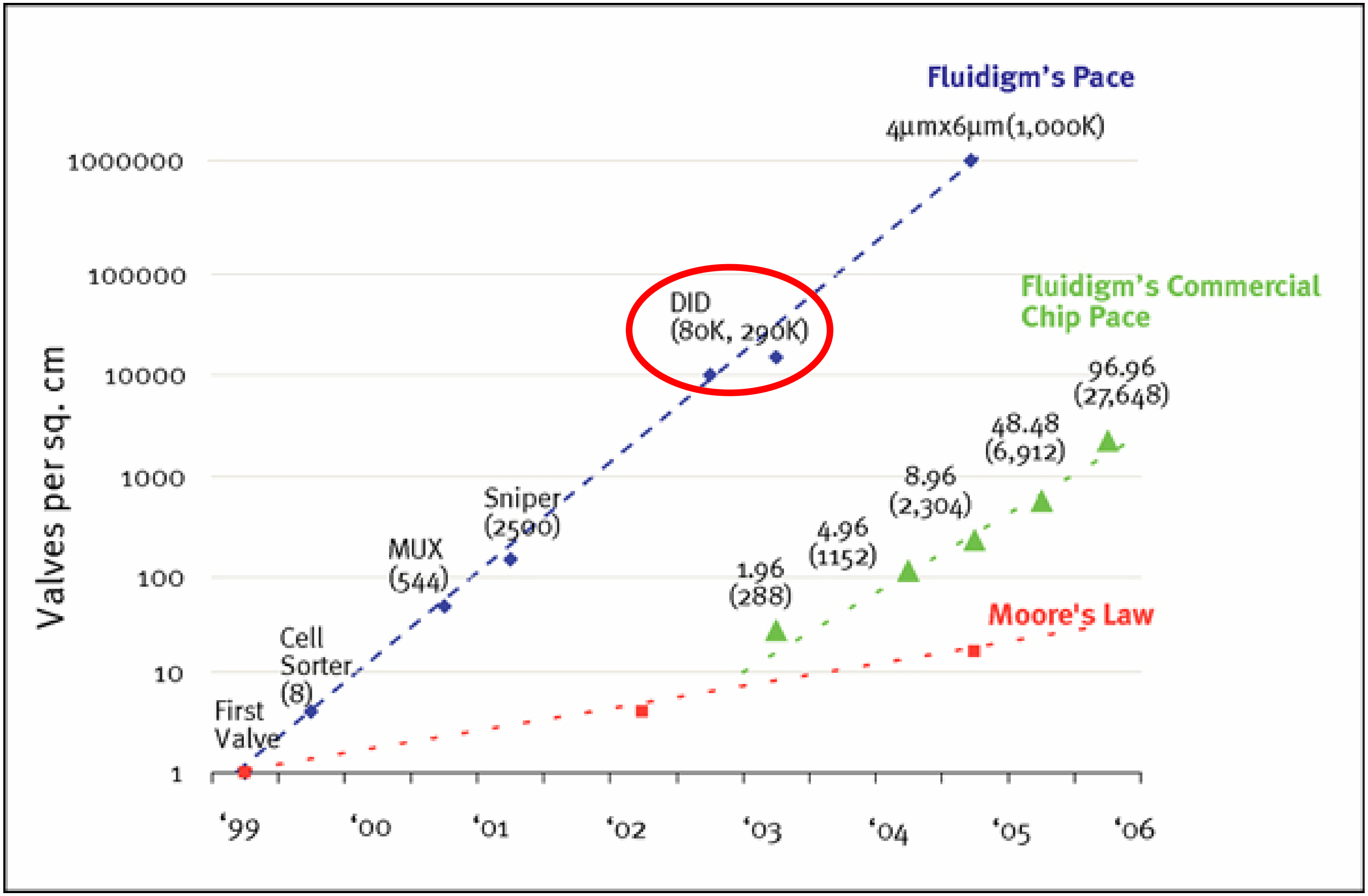


1 mm *10x real-time*

- **Benefits:**
  - Small sample volumes
  - High throughput
  - Geometrical manipulation
- **Applications:**
  - Biochemistry - Cell biology
  - Biological computing

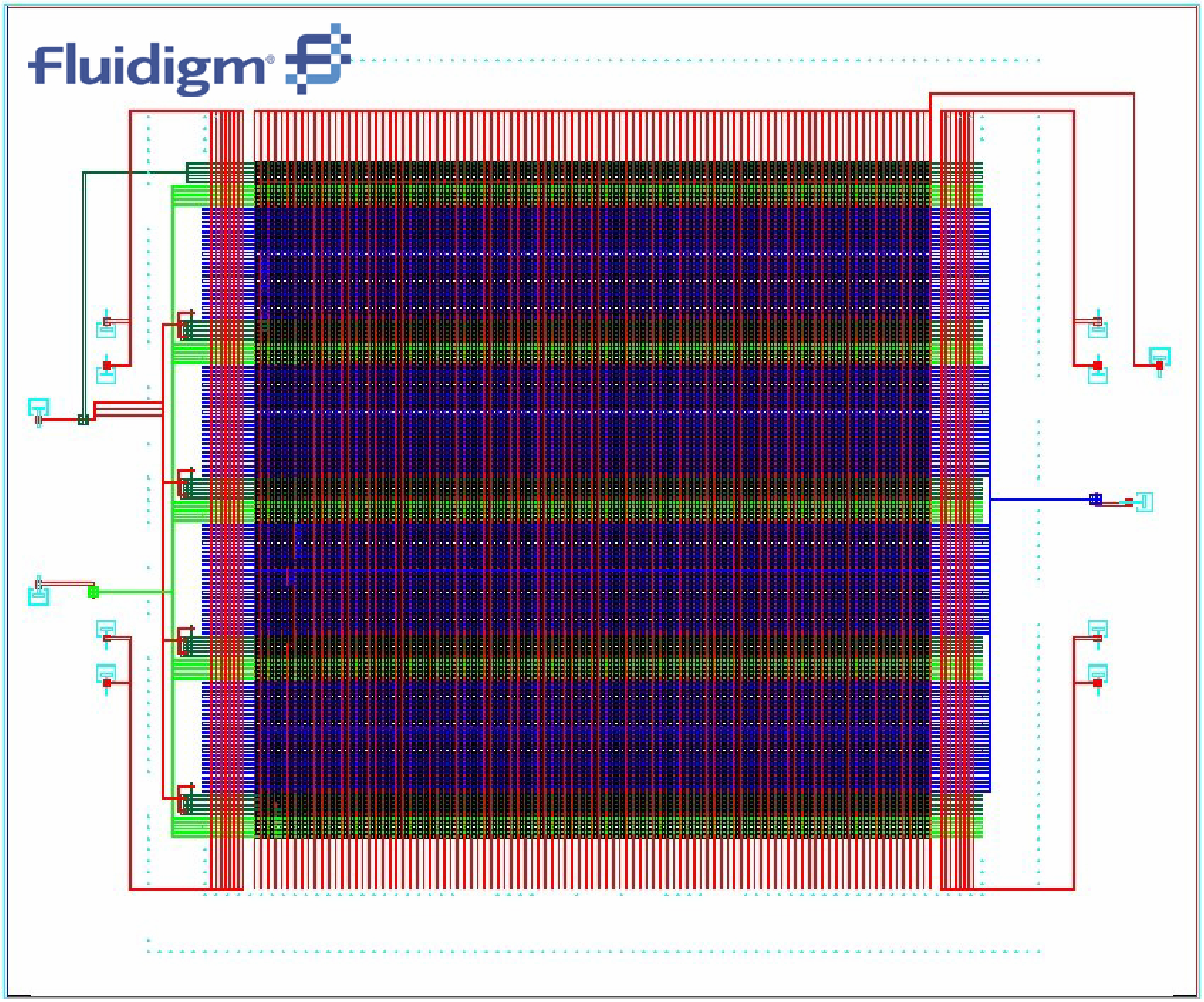
  [Livstone/Landweber] [van Noort] [Grover/Mathies] [McCaskill] [Gehani/Reif] [Farfel/Stefanovic] [Somei/Kaneda/Fujii/Murata]

Moore's Law of Microfluidics: Valve Density Doubles Every 4 Months



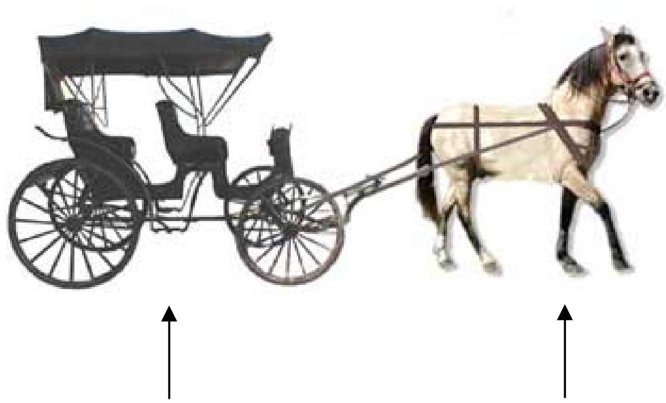
Source: Fluidigm Corporation (http://www.fluidigm.com/images/mlaw_lg.jpg)

Moore's Law of Microfluidics: Valve Density Doubles Every 4 Months



Source: *Fluidigm Corporation (http://www.fluidigm.com/didIFC.htm)*

How to Conduct Experiments?



Human director ⟺ Lab bench

Lab-on-a-chip

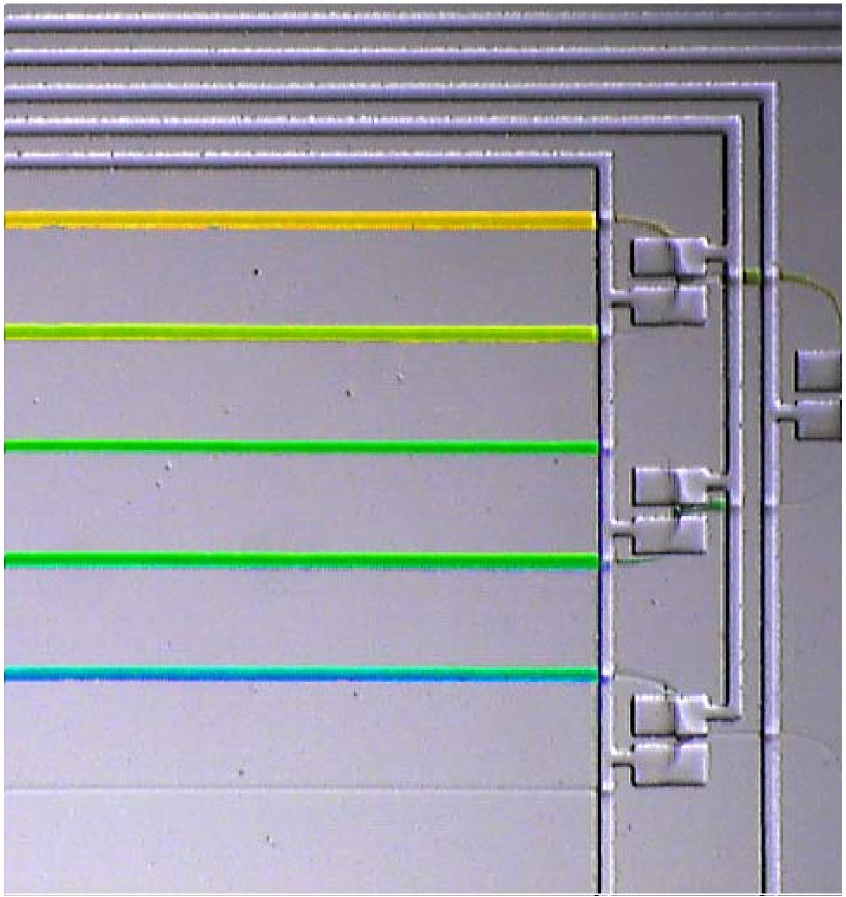
- **Two choices:**
  - Design custom microfluidic chip to automatically perform your experiment

  - Orchestrate every valve operation by hand (e.g., using Labview)

Programmable Solution

- **Example: Gradient generation**

```
Fluid yellow = input (0);
Fluid blue = input(1);
for (int i=0; i<=4; i++) {
   mix(yellow, 1-i/4, blue, i/4);
}
```



450 Valve Operations

- **Hidden from programmer:**
  - Location of fluids
  - Details of mixing, I/O
  - Logic of valve control
  - Timing of chip operations

Programmable Solution

- **Example: Gradient generation**

```
Fluid yellow = input (0);
Fluid blue = input(1);
for (int i=0; i<=4; i++) {
   mix(yellow, 1-i/4, blue, i/4);
}
```

```
setValve(0, HIGH);   setValve(1, HIGH);
setValve(2, LOW);    setValve(3, HIGH);
setValve(4, LOW);    setValve(5, LOW);
setValve(6, HIGH);   setValve(7, LOW);
setValve(8, LOW);    setValve(9, HIGH);
setValve(10, LOW);  setValve(11, HIGH);
setValve(12, LOW);  setValve(13, HIGH);
setValve(14, LOW);  setValve(15, HIGH);
setValve(16, LOW);  setValve(17, LOW);
setValve(18, LOW);  setValve(19, LOW);
wait(2000);
setValve(14, HIGH); setValve(2, LOW);
wait(1000);
setValve(4, HIGH);   setValve(12, LOW);
setValve(16, HIGH); setValve(18, HIGH);
setValve(19, LOW);
wait(2000);
```

450 Valve Operations

- **Hidden from programmer:**
  - Location of fluids
  - Details of mixing, I/O
  - Logic of valve control
  - Timing of chip operations

Programmable Solution

- **Example: Gradient generation**

```
Fluid yellow = input (0);
Fluid blue = input(1);
for (int i=0; i<=4; i++) {
    mix(yellow, 1-i/4, blue, i/4);
}
```

→

```
wait(2000);
setValve(14, HIGH); setValve(2, LOW);
wait(1000);
setValve(4, HIGH);   setValve(12, LOW);
setValve(16, HIGH); setValve(18, HIGH);
setValve(19, LOW);
wait(2000);
setValve(0, LOW);    setValve(1, LOW);
setValve(2, LOW);    setValve(3, HIGH);
setValve(4, LOW);    setValve(5, HIGH);
setValve(6, HIGH);   setValve(7, LOW);
setValve(8, LOW);    setValve(9, HIGH);
setValve(10, HIGH); setValve(11, LOW);
setValve(12, LOW);  setValve(13, LOW);
setValve(14, LOW);  setValve(15, HIGH);
setValve(16, HIGH); setValve(17, LOW);
setValve(18, HIGH); setValve(19, LOW);
```

- **Hidden from programmer:**
  - Location of fluids
  - Details of mixing, I/O
  - Logic of valve control
  - Timing of chip operations

Fluidic Abstraction Layers

Abstract Computational Problem
- SAT formula, max-clique graph

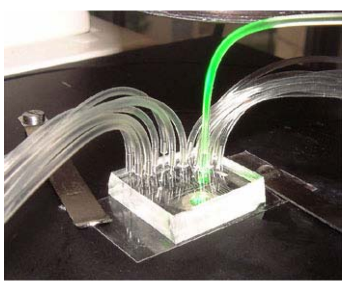
↓

Protocol Description Language
- readable code with high-level mixing ops

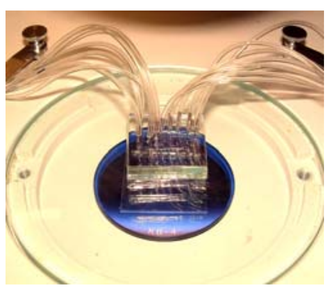
↓

Fluidic Instruction Set Architecture (ISA)
- primitives for I/O, storage, transport, mixing

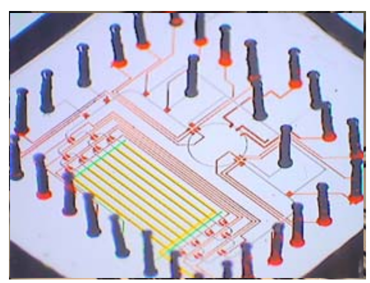
↓ ↓ ↓



chip 1



chip 2



chip 3

Silicon Analog

Mathematica

↓

C

↓

x86

↓

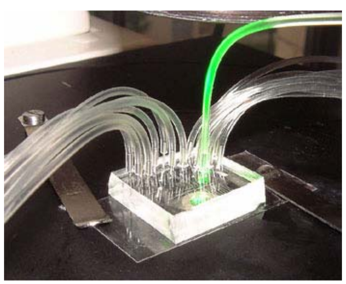
Pentium III,
Pentium IV

Fluidic Abstraction Layers

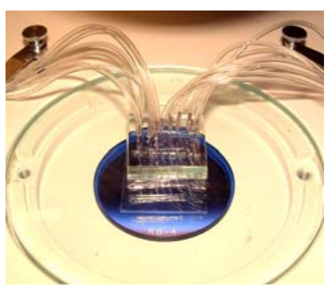
Abstract Computational Problem
- SAT formula, max-clique graph

Protocol Description Language
- readable code with high-level mixing ops

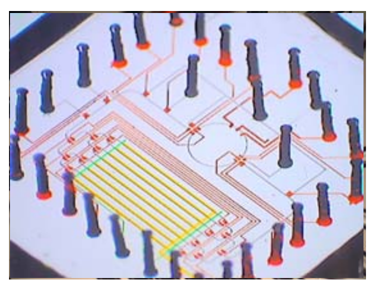
Fluidic Instruction Set Architecture (ISA)
- primitives for I/O, storage, transport, mixing



chip 1



chip 2



chip 3

Benefits:

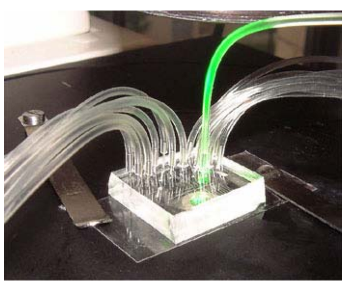
– Portability
– Division of labor
– Scalability
– Expressivity

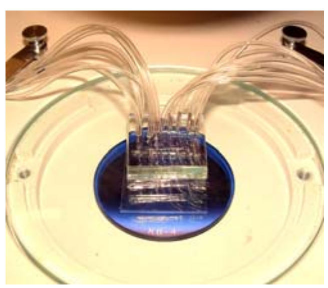
Fluidic Abstraction Layers

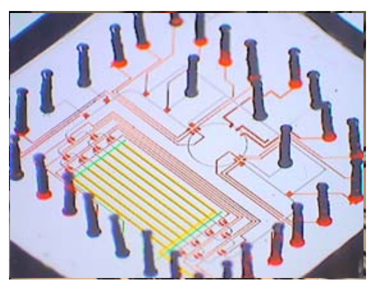
Abstract Computational Problem
- SAT formula, max-clique graph

Protocol Description Language
- readable code with high-level mixing ops

Fluidic Instruction Set Architecture (ISA)
- primitives for I/O, storage, transport, mixing


chip 1


chip 2

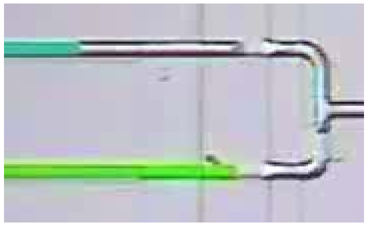

chip 3

Benefits:

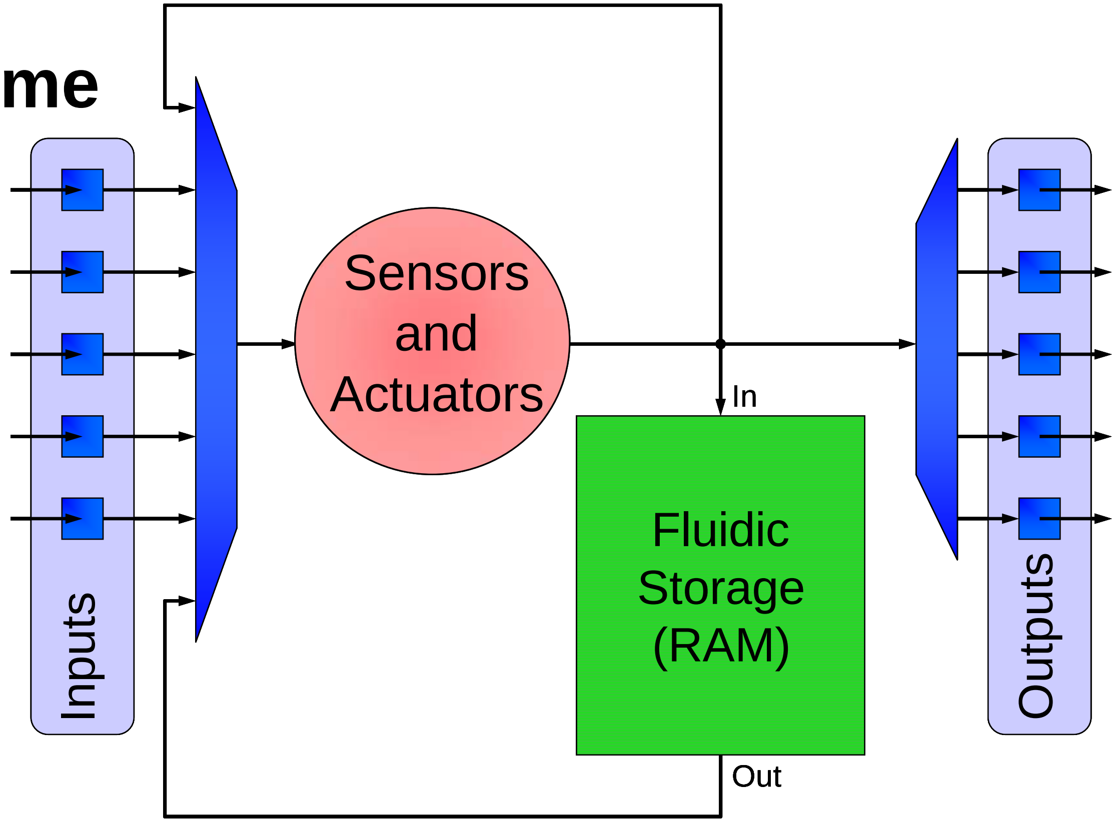
– Portability
– Division of labor
– Scalability
– Expressivity

Abstraction 1: Digital Architecture

- **Recent chips can control independent fluid samples**
  - Droplet-based samples [Fair et al.]
  - Continuous-flow samples [Urbanski et al.]
  - Microfluidic latches [Urbanski et al.]

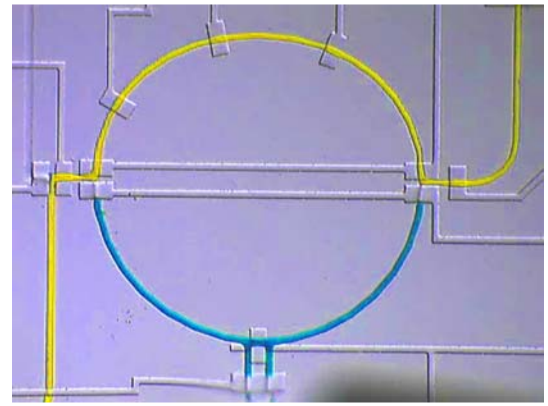


- **In abstract machine, all samples have unit volume**
  - Input/output a sample
  - Store a sample
  - Operate on a sample



Abstraction 2: Mix Instruction

- **Microfluidic chips have various mixing technologies**
  - Electrokinetic mixing [Levitan et al.]
  - Droplet mixing [Fair et al.]
  - Rotary mixing [Quake et al.]



- **Common attributes:**
  - Ability to mix two samples in equal proportions, store result
- **Fluidic ISA:** **mix** (int src_1, int src_2, int dst)
  - Ex: mix(1, 2, 3)

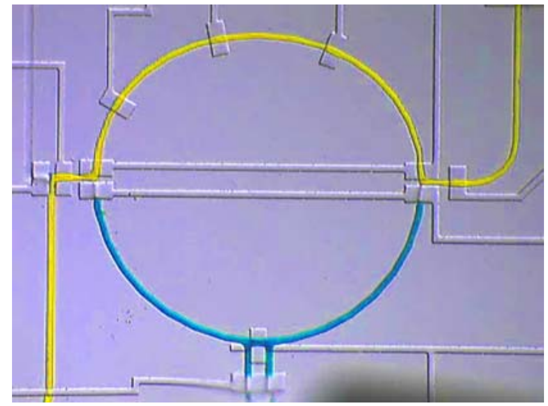
Storage Cells	
1	
2	
3	
4	

Mixer

  - To allow for lossy transport, only 1 unit of mixture retained

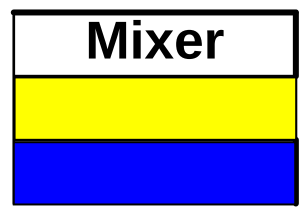
Abstraction 2: Mix Instruction

- **Microfluidic chips have various mixing technologies**
  - Electrokinetic mixing [Levitan et al.]
  - Droplet mixing [Fair et al.]
  - Rotary mixing [Quake et al.]



- **Common attributes:**
  - Ability to mix two samples in equal proportions, store result
- **Fluidic ISA:** **mix** (int src_1, int src_2, int dst)
  - Ex: mix(1, 2, 3)

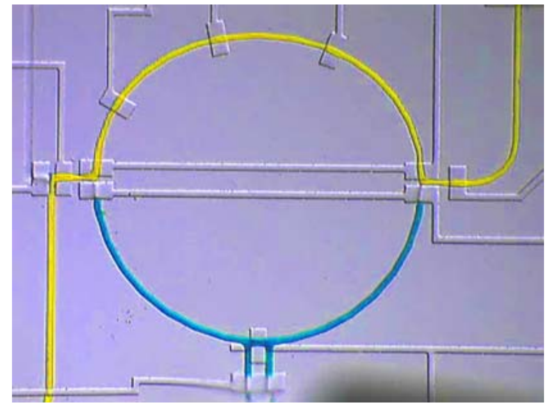
Storage Cells	
1	
2	
3	
4	



  - To allow for lossy transport, only 1 unit of mixture retained

Abstraction 2: Mix Instruction

- **Microfluidic chips have various mixing technologies**
  - Electrokinetic mixing [Levitan et al.]
  - Droplet mixing [Fair et al.]
  - Rotary mixing [Quake et al.]



- **Common attributes:**
  - Ability to mix two samples in equal proportions, store result
- **Fluidic ISA: mix** (int src_1, int src_2, int dst)
  - Ex: mix(1, 2, 3)

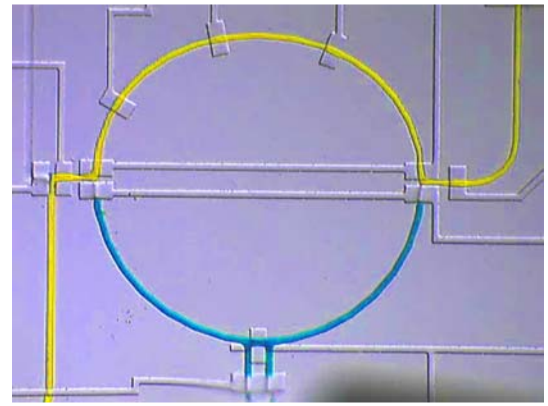
Storage Cells	
1	
2	
3	
4	

Mixer

  - To allow for lossy transport, only 1 unit of mixture retained

Abstraction 2: Mix Instruction

- **Microfluidic chips have various mixing technologies**
  - Electrokinetic mixing [Levitan et al.]
  - Droplet mixing [Fair et al.]
  - Rotary mixing [Quake et al.]



- **Common attributes:**
  - Ability to mix two samples in equal proportions, store result
- **Fluidic ISA:** **mix** (int src_1, int src_2, int dst)
  - Ex: mix(1, 2, 3)

Storage Cells	
1	
2	
3	
4	

Mixer

  - To allow for lossy transport, only 1 unit of mixture retained

Gradient Generation in Fluidic ISA

```
wait(2000);
setValve(14, HIGH); setValve(2, LOW);
wait(1000);
setValve(4, HIGH);   setValve(12, LOW);
setValve(16, HIGH); setValve(18, HIGH);
setValve(19, LOW);
wait(2000);
setValve(0, LOW);    setValve(1, LOW);
setValve(2, LOW);    setValve(3, HIGH);
setValve(4, LOW);    setValve(5, HIGH);
setValve(6, HIGH);   setValve(7, LOW);
setValve(8, LOW);    setValve(9, HIGH);
setValve(10, HIGH); setValve(11, LOW);
setValve(12, LOW);  setValve(13, LOW);
setValve(14, LOW);  setValve(15, HIGH);
setValve(16, HIGH); setValve(17, LOW);
setValve(18, HIGH); setValve(19, LOW);
```

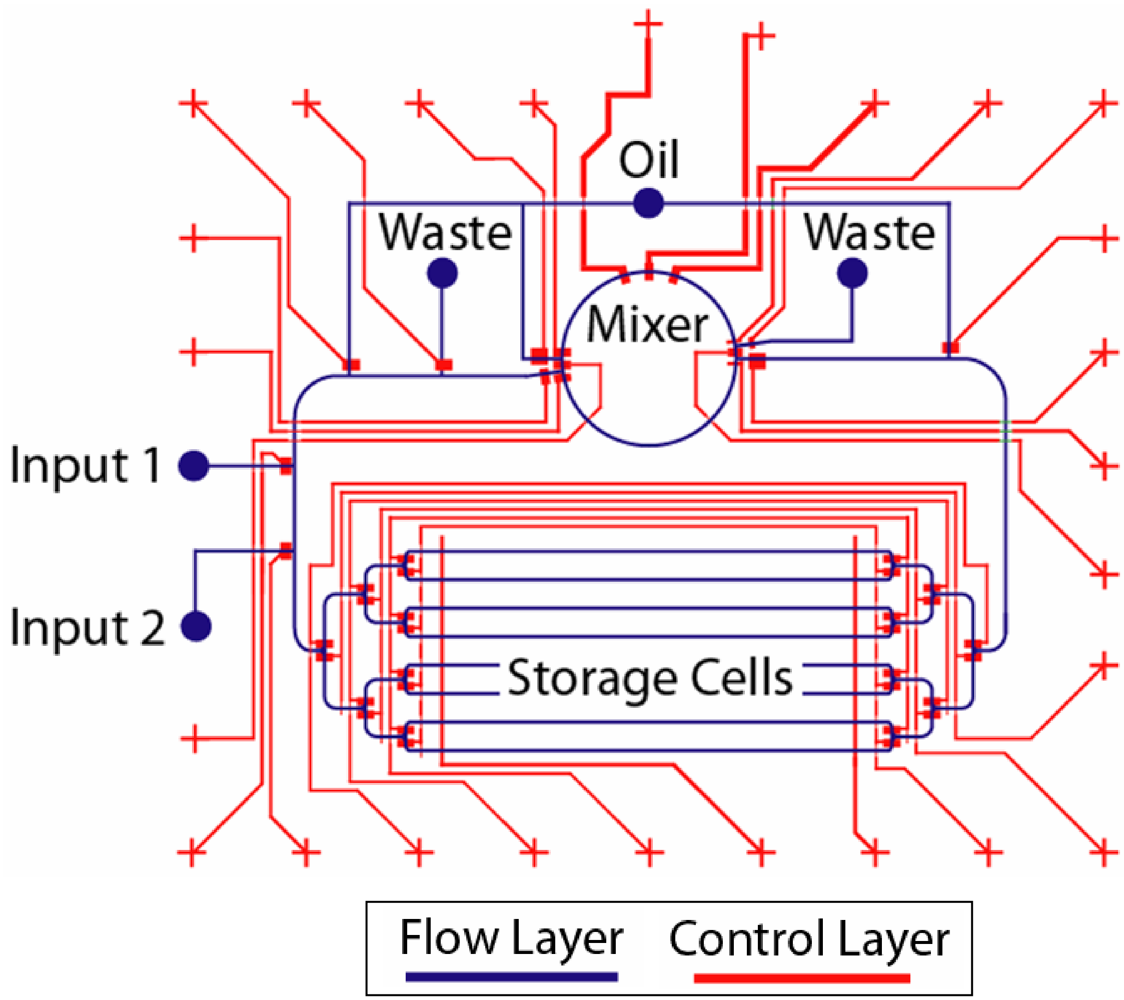
abstraction →

```
input(0, 0);
input(1, 1);
input(0, 2);
mix(1, 2, 3);
input(0, 2);
mix(2, 3, 1);
input(1, 3);
input(0, 4);
mix(3, 4, 2);
input(1, 3);
input(0, 4);
mix(3, 4, 5);
input(1, 4);
mix(4, 5, 3);
mix(0, 4);
```

Direct Control
- 450 valve actuations
- only works on 1 chip

Fluidic ISA
- 15 instructions
- portable across chips

Implementation: Oil-Driven Chip



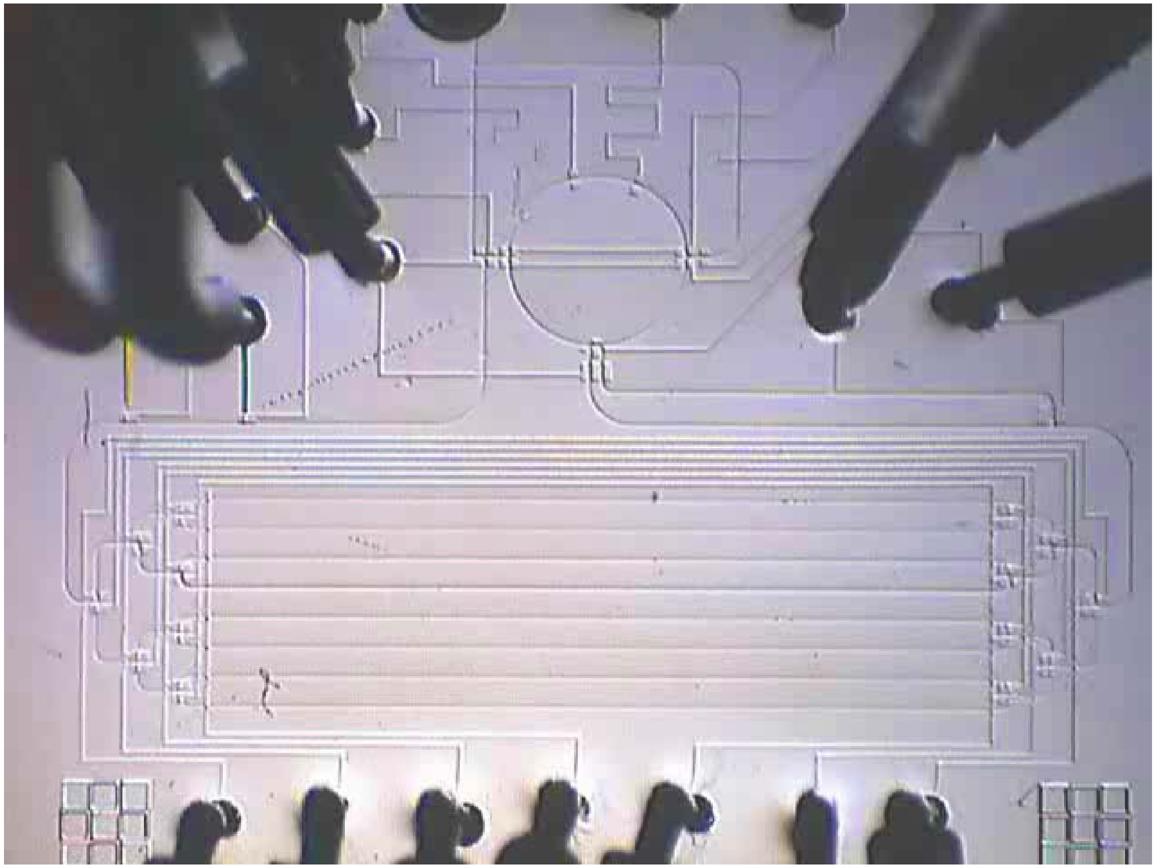
	Inputs	Storage Cells	Background Phase	Wash Phase	Mixing
Chip 1	2	8	Oil	—	Rotary

Implementation: Oil-Driven Chip

mix (S_1, S_2, D) {
1. Load S_1
2. Load S_2
3. Rotary mixing
4. Store into D

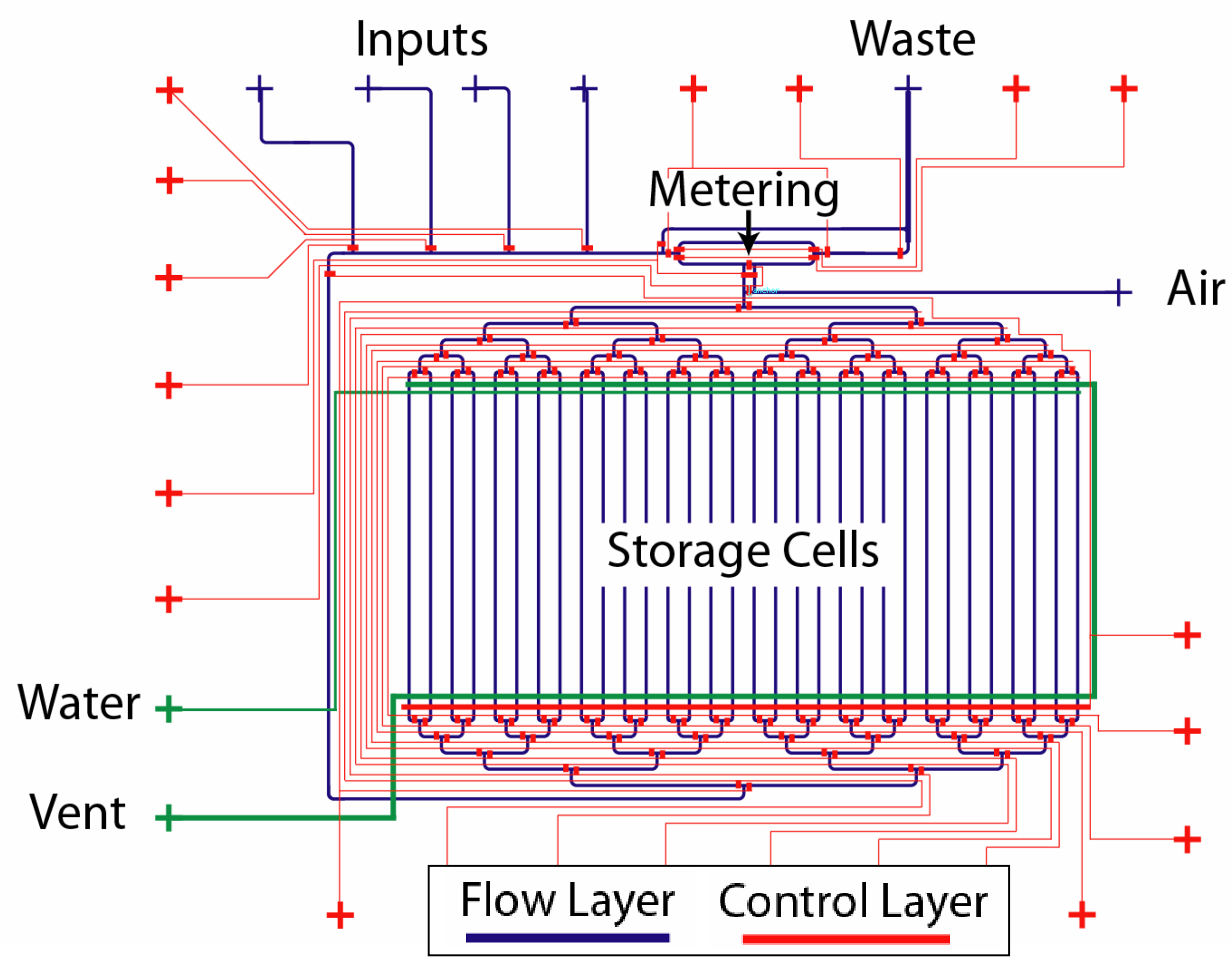
}

50x real-time



	Inputs	Storage Cells	Background Phase	Wash Phase	Mixing
Chip 1	2	8	Oil	—	Rotary

Implementation 2: Air-Driven Chip



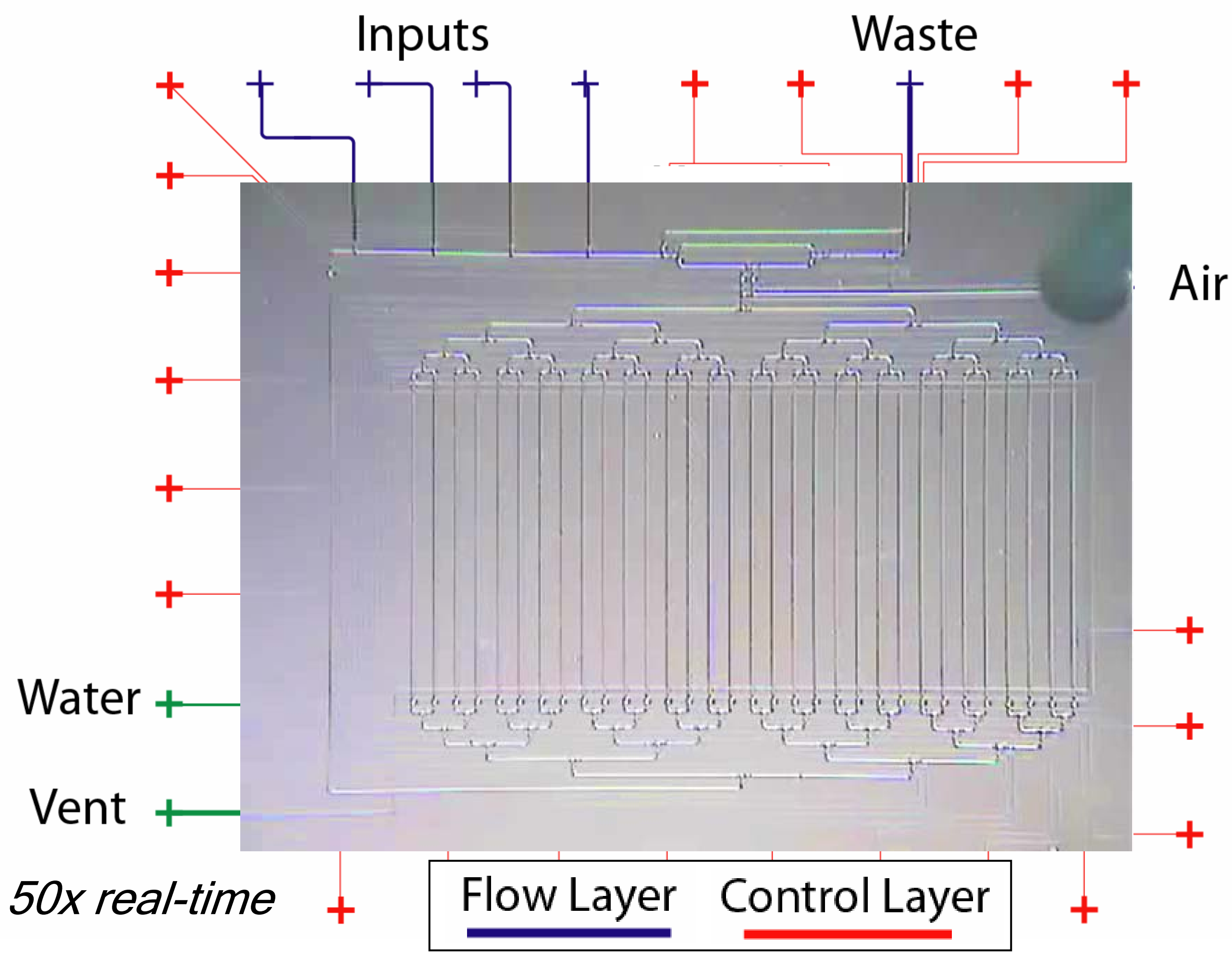
	Inputs	Storage Cells	Background Phase	Wash Phase	Mixing
Chip 1	2	8	Oil	—	Rotary
Chip 2	4	32	Air	Water	In channels

Implementation 2: Air-Driven Chip

mix (S_1, S_2, D) {

1. Load S_1
2. Load S_2
3. Mix / Store into D
4. Wash S_1
5. Wash S_2

}



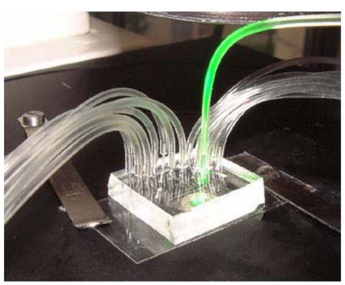
	Inputs	Storage Cells	Background Phase	Wash Phase	Mixing
Chip 1	2	8	Oil	—	Rotary
Chip 2	4	32	Air	Water	In channels

Abstraction Layers

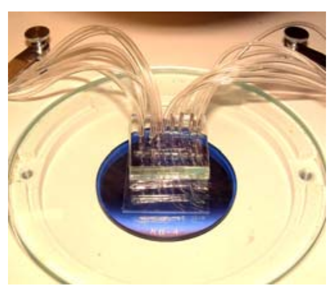
Abstract Computational Problem
- SAT formula, max-clique graph

Protocol Description Language
- readable code with high-level mixing ops

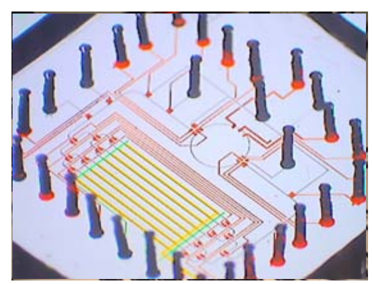
Fluidic Instruction Set Architecture (ISA)
- primitives for I/O, storage, transport, mixing



chip 1



chip 2



chip 3

Abstraction 1: Managing Fluid Storage

Fluidic ISA

```
input(0, 0);
input(1, 1);
input(0, 2);
mix(1, 2, 3);
input(0, 2);
mix(2, 3, 1);
input(1, 3);
input(0, 4);
mix(3, 4, 2);
input(1, 3);
input(0, 4);
mix(3, 4, 5);
input(1, 4);
mix(4, 5, 3);
mix(0, 4);
```

→

```
Fluid[] out = new Fluid[8];
Fluid yellow, blue, green;
out[0] = input(0);
yellow = input(0);
blue = input(1);
green = mix(yellow, blue);
yellow = input(0);
out[1] = mix(yellow, green);
yellow = input(0);
blue = input(1);
out[2] = mix(yellow, blue);
yellow = input(0);
blue = input(1);
green = mix(yellow, blue);
blue = input(1);
out[3] = mix(blue, green);
out[4] = input(1);
```

1. Storage Management

- **Programmer uses location-independent Fluid variables**
  - Runtime system assigns & tracks location of each Fluid
  - Comparable to automatic memory management (e.g., Java)

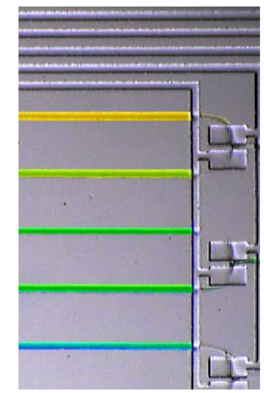
Abstraction 2: Fluid Re-Generation

```
Fluid[] out = new Fluid[8];
Fluid yellow, blue, green;
out[0] = input(0);
yellow = input(0);
blue = input(1);
green = mix(yellow, blue);
yellow = input(0);
out[1] = mix(yellow, green);
yellow = input(0);
blue = input(1);
out[2] = mix(yellow, blue);
yellow = input(0);
blue = input(1);
green = mix(yellow, blue);
blue = input(1);
out[3] = mix(blue, green);
out[4] = input(1);
```

```
Fluid[] out = new Fluid[8];
Fluid yellow = input(0);
Fluid blue = input(1);
Fluid green = mix(yellow, blue);

out[0] = yellow;
out[1] = mix(yellow, green);
out[2] = green;
out[3] = mix(blue, green);
out[4] = blue;
```

2. Fluid Re-Generation



- **Programmer may use a Fluid variable multiple times**
  - Each time, a physical Fluid is consumed on-chip
  - Runtime system re-generates Fluids from computation history

Abstraction 3: Arbitrary Mixing

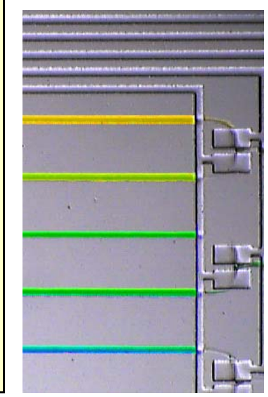
```
Fluid[] out = new Fluid[8];
Fluid yellow = input(0);
Fluid blue = input(1);
Fluid green = mix(yellow, blue);

out[0] = yellow;
out[1] = mix(yellow, green);
out[2] = green;
out[3] = mix(blue, green);
out[4] = blue;
```

2. Fluid Re-Generation

```
Fluid[] out = new Fluid[8];
Fluid yellow = input (0);
Fluid blue = input (1);

out[0] = yellow;
out[1] = mix(yellow, 3/4, blue, 1/4);
out[2] = mix(yellow, 1/2, blue, 1/2);
out[3] = mix(yellow, 1/4, blue, 3/4);
out[4] = blue;
```



3. Arbitrary Mixing

- **Allows mixing fluids in any proportion, not just 50/50**
  - Fluid **mix** (Fluid F_1, float p_1, Fluid f_2, float F_2)

    → Returns Fluid that is p_1 parts $\mathbf{F_1}$ and $\mathbf{p_2}$ parts $\mathbf{F_2}$
  - Runtime system translates to 50/50 mixes in Fluidic ISA
  - Note: some mixtures only reachable within error tolerance ε

Abstraction 3: Arbitrary Mixing

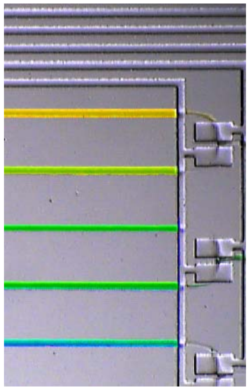
```
Fluid[] out = new Fluid[8];
Fluid yellow = input (0);
Fluid blue = input (1);

out[0] = yellow;
out[1] = mix(yellow, 3/4, blue, 1/4);
out[2] = mix(yellow, 1/2, blue, 1/2);
out[3] = mix(yellow, 1/4, blue, 3/4);
out[4] = blue;
```

3. Arbitrary Mixing

```
Fluid[] out = new Fluid[8];
Fluid yellow = input (0);
Fluid blue = input (1);

for (int i=0; i<=4; i++) {
  out[i] = mix(yellow, 1-i/4, blue, i/4);
}
```



4. Parameterized Mixing

- **Allows mixing fluids in any proportion, not just 50/50**
  - Fluid **mix** (Fluid F_1, float p_1, Fluid f_2, float F_2)
    → Returns Fluid that is p_1 parts $\mathbf{F_1}$ and $\mathbf{p_2}$ parts $\mathbf{F_2}$
  - Runtime system translates to 50/50 mixes in Fluidic ISA
  - Note: some mixtures only reachable within error tolerance ε

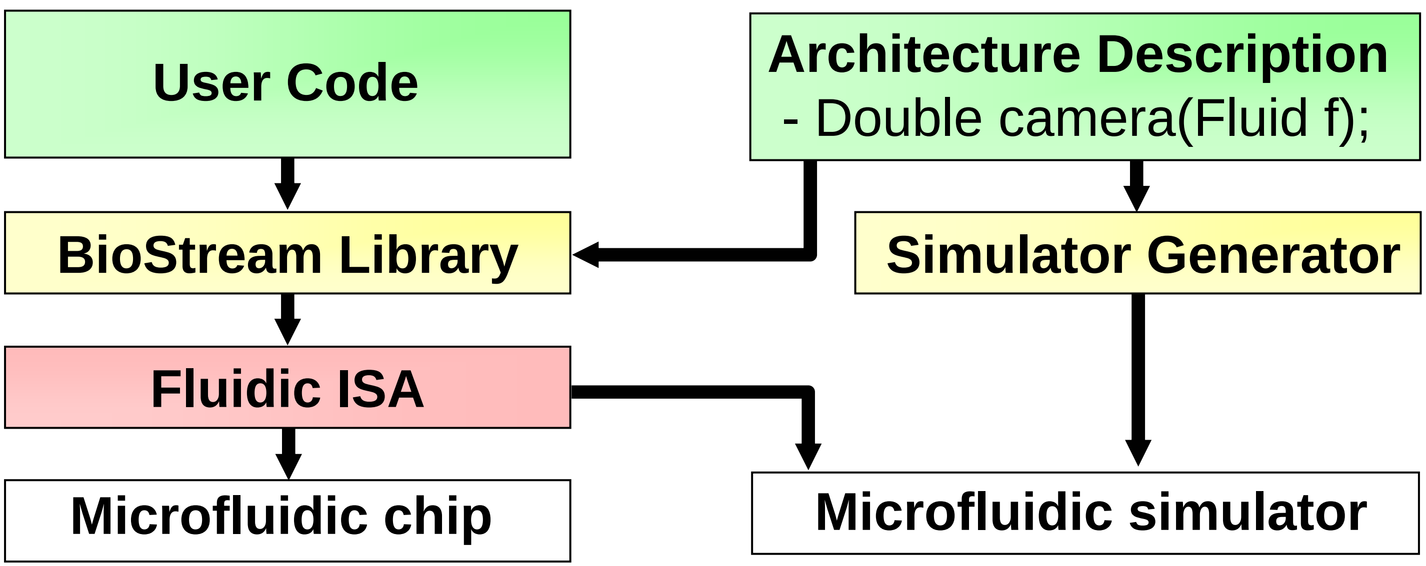
BioStream Protocol Language

- **Supports all the abstractions**
  - Automatic storage management
  - Re-generation of fluids
  - Arbitrary mixing

```
Fluid yellow = input (0);
Fluid blue = input (1);
Fluid[] out = new Fluid[8];

for (int i=0; i<=4; i++) {
  out[i] = mix(yellow, 1-i/4, blue, i/4);
}
```

- **Implemented as a Java library**
  - Allows flexible integration with general-purpose Java code
- **Targets microfluidic chips or auto-generated simulator**



Example: Fixed-pH Reaction

- **Goal: maintain given pH throughout reaction**
  - For in-vitro modeling of natural environment
- **Method: periodically test, adjust pH if needed**

```
Fluid sample = input (0);
Fluid acid = input(1);
Fluid base = input(2);
do {
    Fluid pH_test = mix(sample, 0.9, indicator, 0.1);    // test pH of sample
    double pH = test_luminescence(pH_test);
    if (pH > 7.5) {                                      // if pH too high, add acid
        sample = mix (sample, 0.9, acid, 0.1);
    } else if (pH < 6.5) {                               // if pH too low, add base
        sample = mix (sample, 0.9, base, 0.1);
    }
    wait(60);
} while (detect_activity(sample));
```

Example: Fixed-pH Reaction

- **Goal: maintain given pH throughout reaction**
  - For in-vitro modeling of natural environment
- **Method: periodically test, adjust pH if needed**

```
Fluid sample = input (0);
Fluid acid = input(1);
Fluid base = input(2);
do {
   Fluid pH_test = mix(sample, 0.9, indicator, 0.1);
   double pH = test_luminescence(pH_test);
   if (pH > 7.5) {
      sample = mix (sample, 0.9, acid, 0.1);
   } else if (pH < 6.5) {
      sample = mix (sample, 0.9, base, 0.1);
   }
   wait(60);
} while (detect_activity(sample));
```

Feedback-Intensive Applications:

- Recursive descent search
- Directed evolution
- Cell isolation and manipulation
- Dose/response curves
- Long, complex protocols

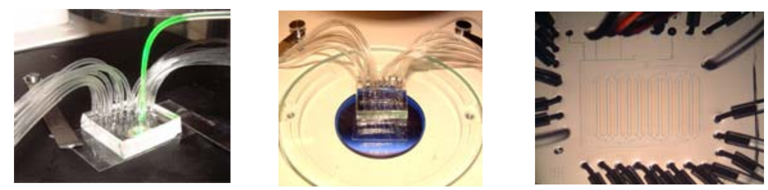
Big Picture

- **Abstraction layers enable simple, scalable hardware**
  - Instead of custom chips, build general-purpose devices
- **Vision for microfluidics: everyone uses a standard chip**
  - Thousands of storage cells
  - Dozens of parallel mixers
  - Integrated support for cell manipulation, PCR, readout, etc.

Abstract Computational Problem
- SAT formula, max-clique graph

BioStream Protocol Language
- readable code with high-level mixing ops

Fluidic Instruction Set Architecture
- primitives for storage, transport, mixing



chip 1 chip 2 chip 3

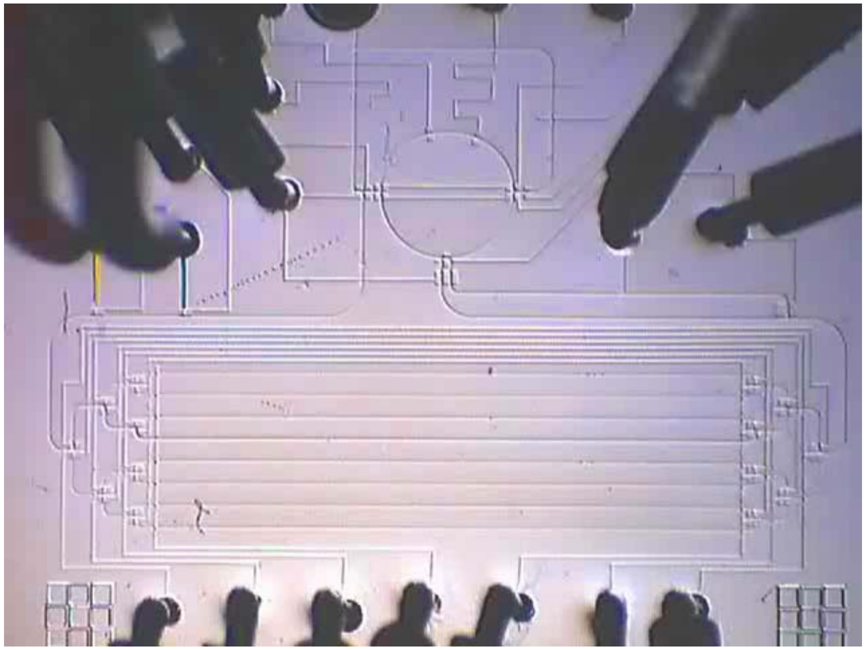
- **Vision for software: a defacto language for experimental science**
  - You can download a colleague's code, run it on your chip

Related Work

- **Automatic generation / scheduling of biology protocols**
  - EDNAC computer for automatically solving 3-SAT [Johnson]
  - Compile SAT to microfluidic chips [Landweber et al.] [van Noort]
  - Robot scientist: generates/tests genetic hypotheses [King et al.]
  - Mapping sequence graphs to grid-based chips [Su/Chakrabarty]
- **Custom microfluidic chips for biological computation**
  - DNA computing [Grover & Mathies] [van Noort et al.] [McCaskill] [Livstone, Weiss, & Landweber] [Gehani & Reif] [Farfel & Stefanovic]
  - Self-assembly [Somei, Kaneda, Fujii, & Murata] [Whitesides et al.]
- **General-purpose microfluidic chips**
  - Using electrowetting, with flexible mixing [Fair et al.]
  - Using dialectrophoresis, with retargettable GUI [Gascoyne et al.]
  - Using Braille displays as programmable actuators [Gu et al.]

Conclusions

- **End-to-end system for programmable microfluidics**
  – Usable: high-level code is natural expression of protocol
  – Portable: same code executes on diverse chips
  – Scalable: protocols automatically utilize parallel resources
- **Relies on abstraction layers**
  – Fluidic ISA
  – BioStream language
- **Future work**
  – Incorporate stateful molecules, cells into abstraction layers
  – Looking for killer applications!



http://cag.csail.mit.edu/biostream