Towards Programmable Microfluidics

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Microfluidic Chips

- Idea: a whole biological lab on a single chip
  - Input channels for reagants
  - Chambers for mixing fluids
  - Actuators for modifying fluids
    - Temperature - Ultraviolet radiation
    - Light/dark - Electrophoresis
  - Sensors for reading properties
    - Luminescence - Immunosensors
    - pH - Glucose
- Starting to be manufactured and used today
- Active area of research

Microfluidic Applications

- Biochemistry
  - Enzymatic assays
  - The Polymerase Chain Reaction
  - Nucleic acid arrays
  - Biomolecular separations
  - Immunohybridization reactions
  - Piercing structures for DNA injection

Microfluidic Applications

- Biochemistry
- Cell biology
  - Flow cytometry / sorting
  - Sperm/embryo tools: sperm motility, in vitro fertilization, embryo branding
  - Force measurements with bending cantilevers
  - Dialectrophoresis / electrorotation
  - Impedance monitoring for cell motility and micromotion
  - Chemical / physical substrate patterning

Microfluidic Applications

- Biochemistry
- Cell biology
- General-Purpose Computing
  - Compute with fluids
  - Not our current interest

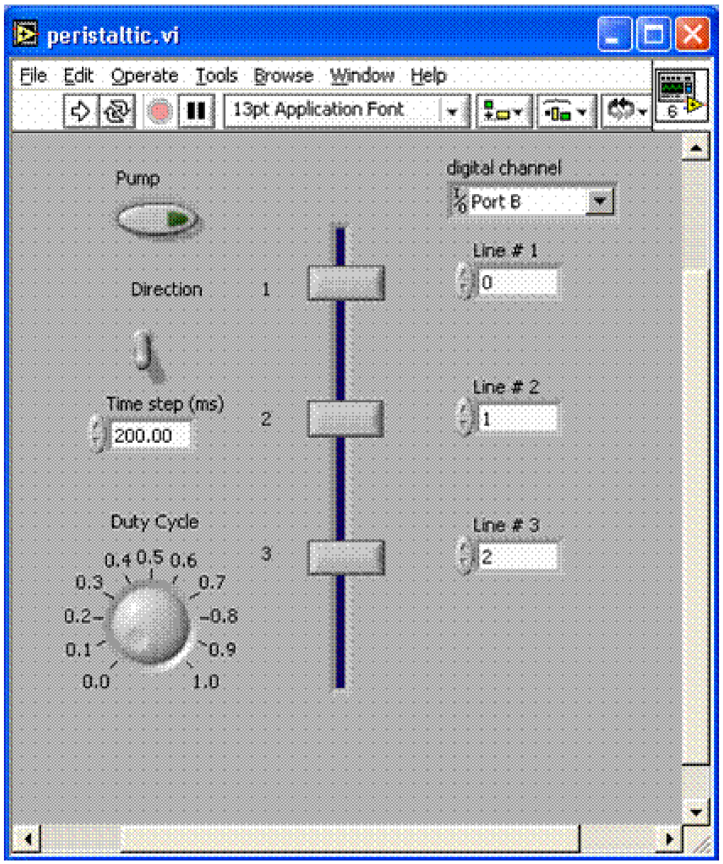
Microfluidic Applications

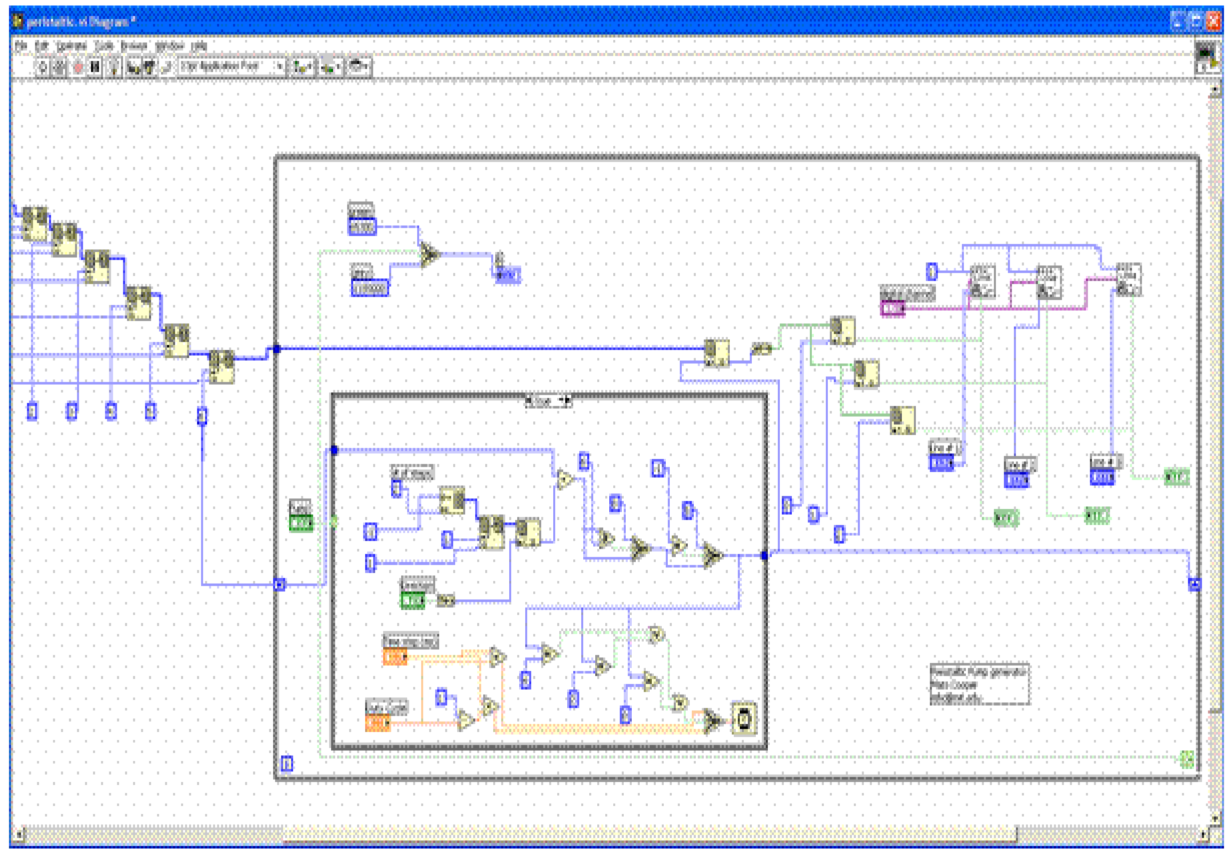
- Biochemistry
- Cell biology
- General-Purpose Computing

- Summary of Benefits:
  - High throughput
  - Small sample volumes
  - Geometric manipulation
  - Portable devices
  - Automatic Control

Our Goal:
Provide Abstraction Layers for this Domain

- Current interface: gate-level control (e.g., Labview)



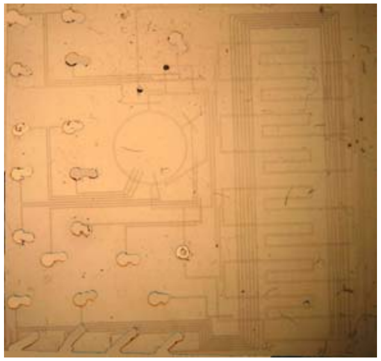


Our Goal:
Provide Abstraction Layers for this Domain

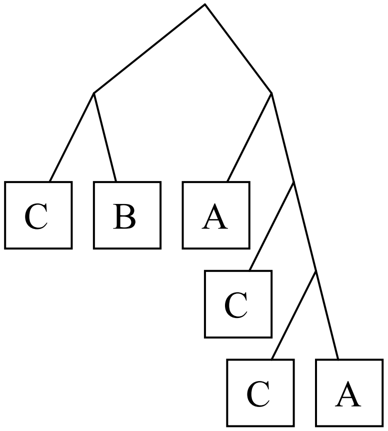
- Current interface: gate-level control (e.g., Labview)
- New abstraction layers will enable:
  - Scalability
    - Currently have 1,000 storage cells, can manage resources by hand
    - Soon will have 1,000,000: how to manage complexity?
  - Portability
    - Hide architecture-specific details from programmer
    - Same experiment works on successive generations of chips
  - Modularity
    - Create reusable components
    - Enable large and complex procedures
  - Adaptivity
    - Use real-time sensor feedback to guide experiment
    - Adjust procedure to suite field conditions

Our Contributions

1. End-to-end programmable system
   - General-purpose microfluidic chip
   - High-level software control



2. Novel mixing algorithms
   - Mix k fluids in any concentration (± 1/n)
   - Guarantees minimal number of mixes: O(k log n)



Outline

- Introduction
- Mixing algorithms
- General-purpose microfluidic chip
- Portable programming system
- Implementation
- Related Work
- Conclusions

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Mixing in Microfluidics

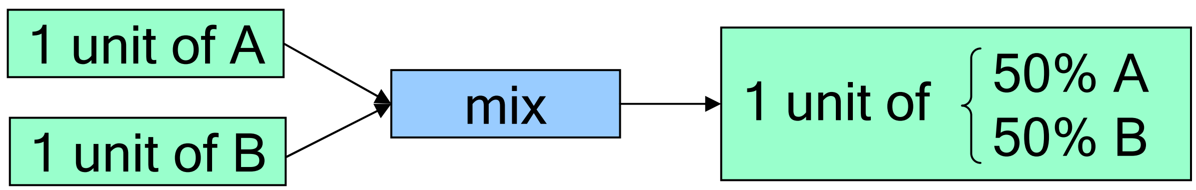
- Mixing is fundamental operation of microfluidics
  - Prepare samples for analysis
  - Dilute concentrated substances
  - Control reagant volumes

- Important to mix on-chip
  - Otherwise reagants leave system whenever mix needed
  - Enables large, self-directing experiments

➡ Analogous to ALU operations on microprocessors

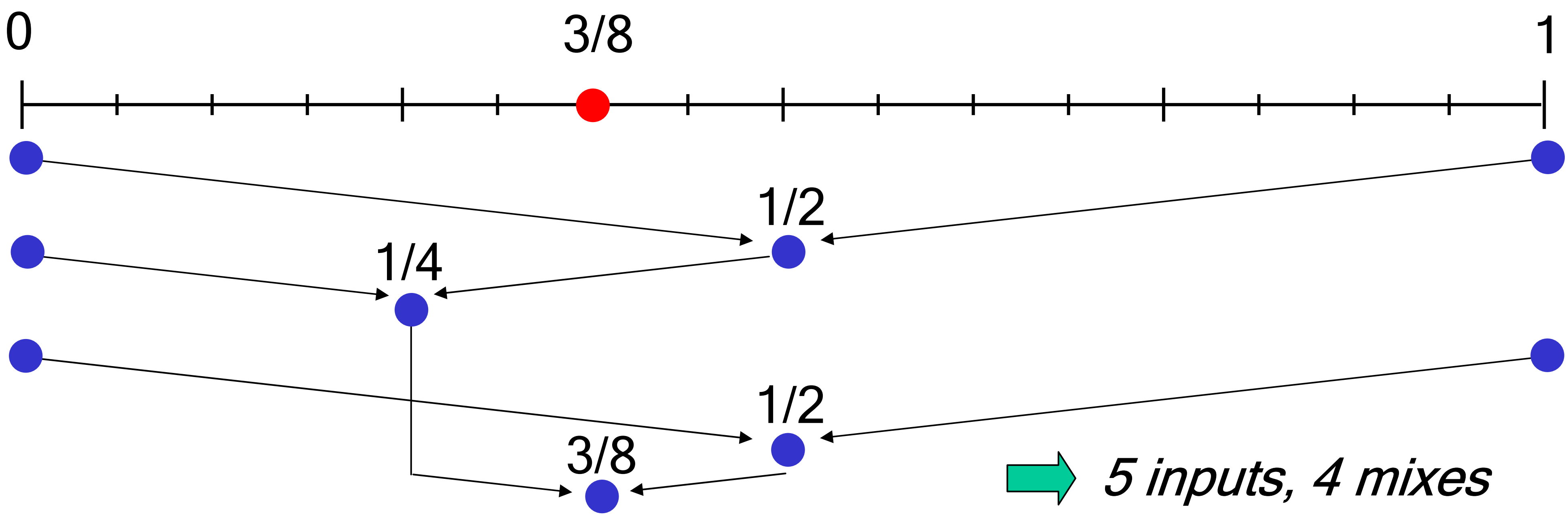
The Mixing Problem

- Experiments demand mixing in arbitrary proportions
  - For example, mix 15% reagant / 85% buffer
  - Users should operate at this level of abstraction
- However, microfluidic hardware lacks arbitrary mixers
  - Most common model: 1-to-1 mixer

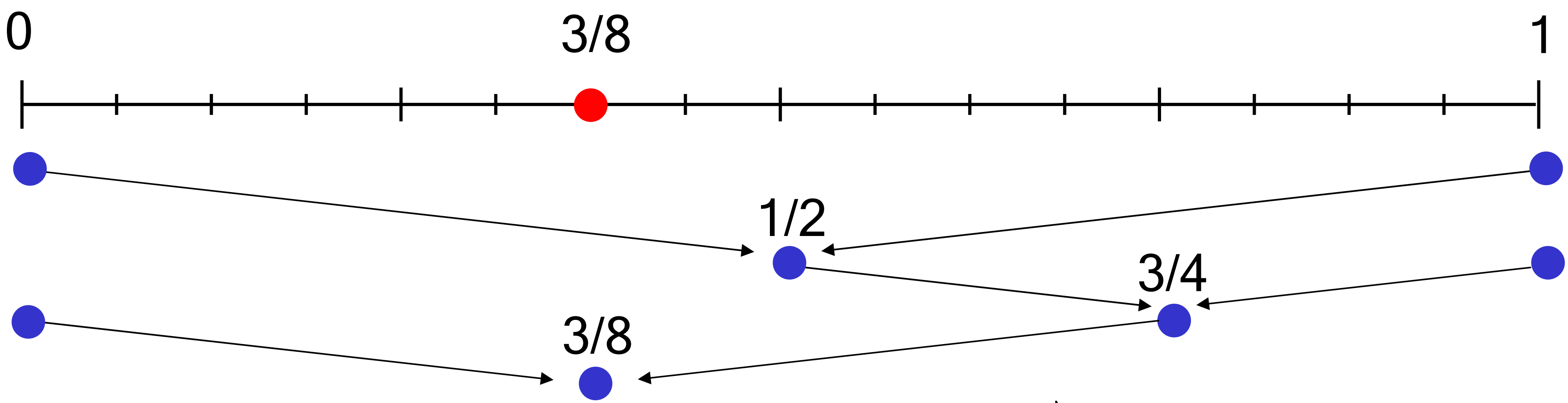


- Important optimization questions:
  - What mixtures are reachable?
  - How to minimize reagant consumption?
  - How to minimize number of mixes?

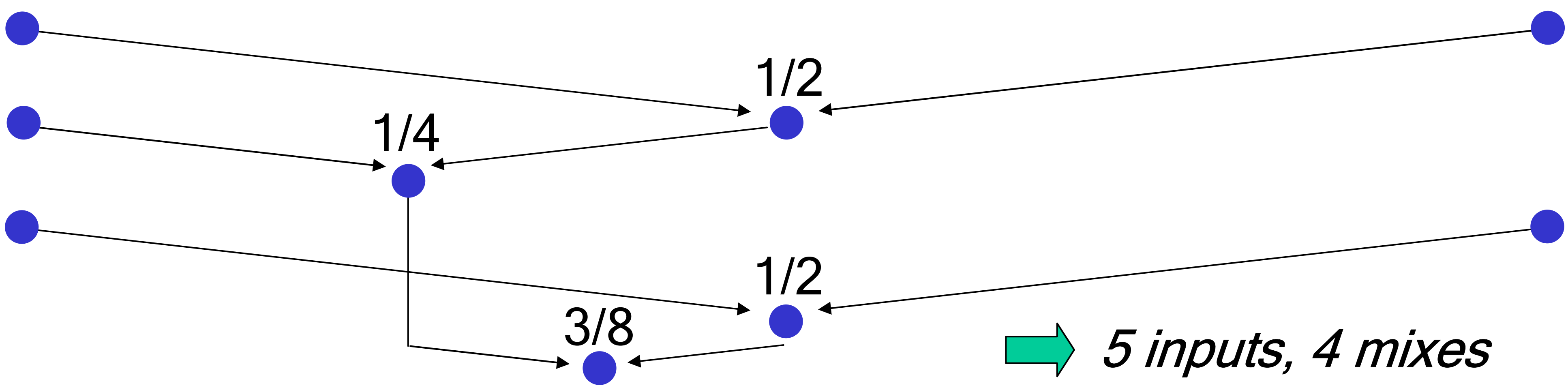
Why Not Binary Search?



Why Not Binary Search?

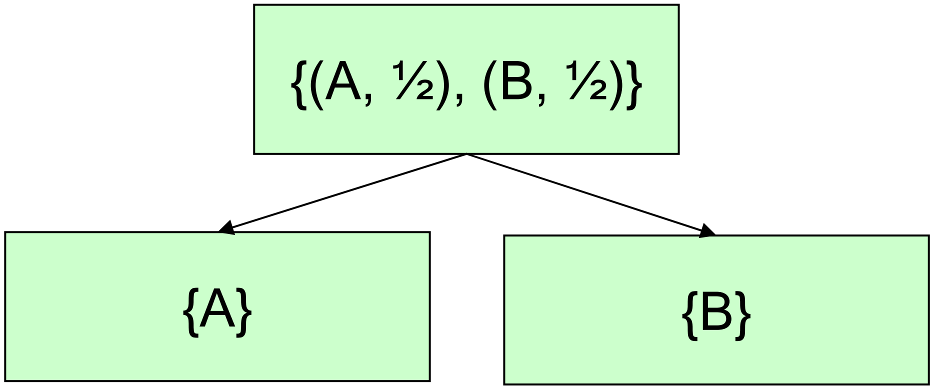


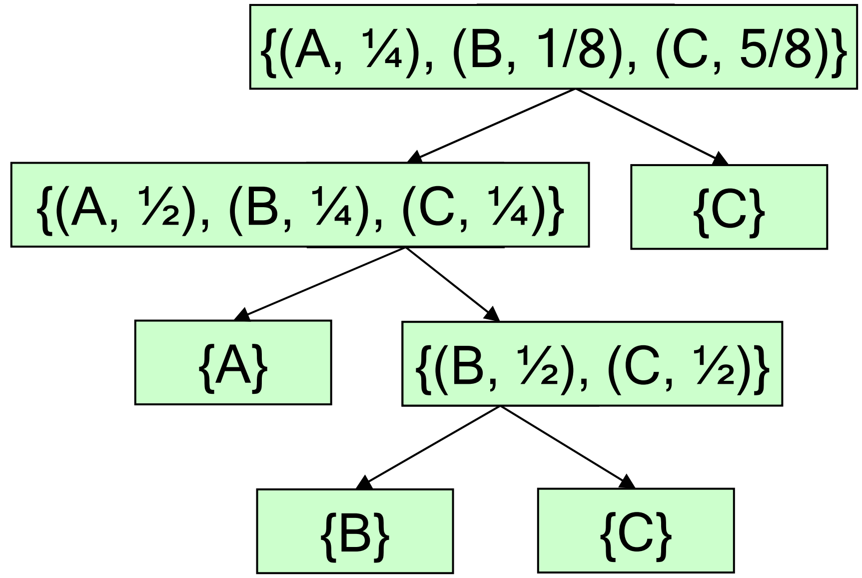
⇨ *4 inputs, 3 mixes*



⇨ *5 inputs, 4 mixes*

Mixing Trees





- Properties:
  - Mixing trees are binary trees
  - Leaf nodes: unit sample of an input fluid
  - Internal nodes: result of 1-to-1 mix of children
  - Evaluate from bottom to top
- Observation:
  - # leaf nodes = # internal nodes + 1 (induction on # nodes)

    # reagants used = # mixes + 1
  - Minimizing mixes and reagant usage is equivalent

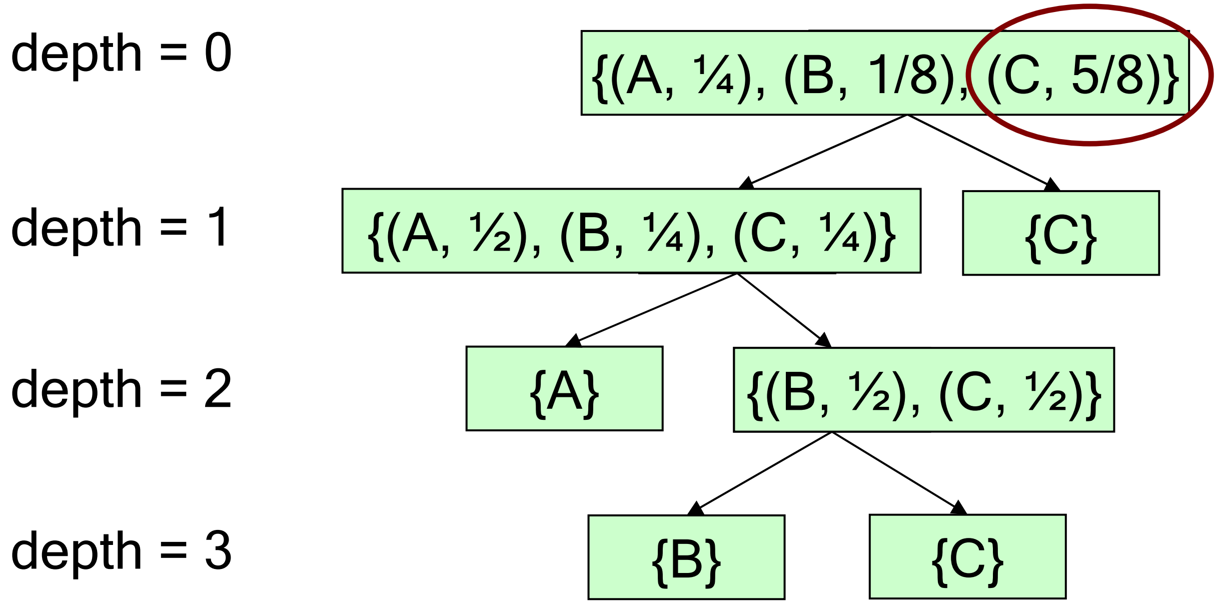
Mixing Trees

Example: {C}

conc = $2^{-1} + 2^{-3}$

conc = 1/2 + 1/8

conc = 5/8



Theorem: For substance S, let n_d denote number of leaf nodes at depth d. Then overall concentration for S is $\sum_d n_d * 2^{-d}$

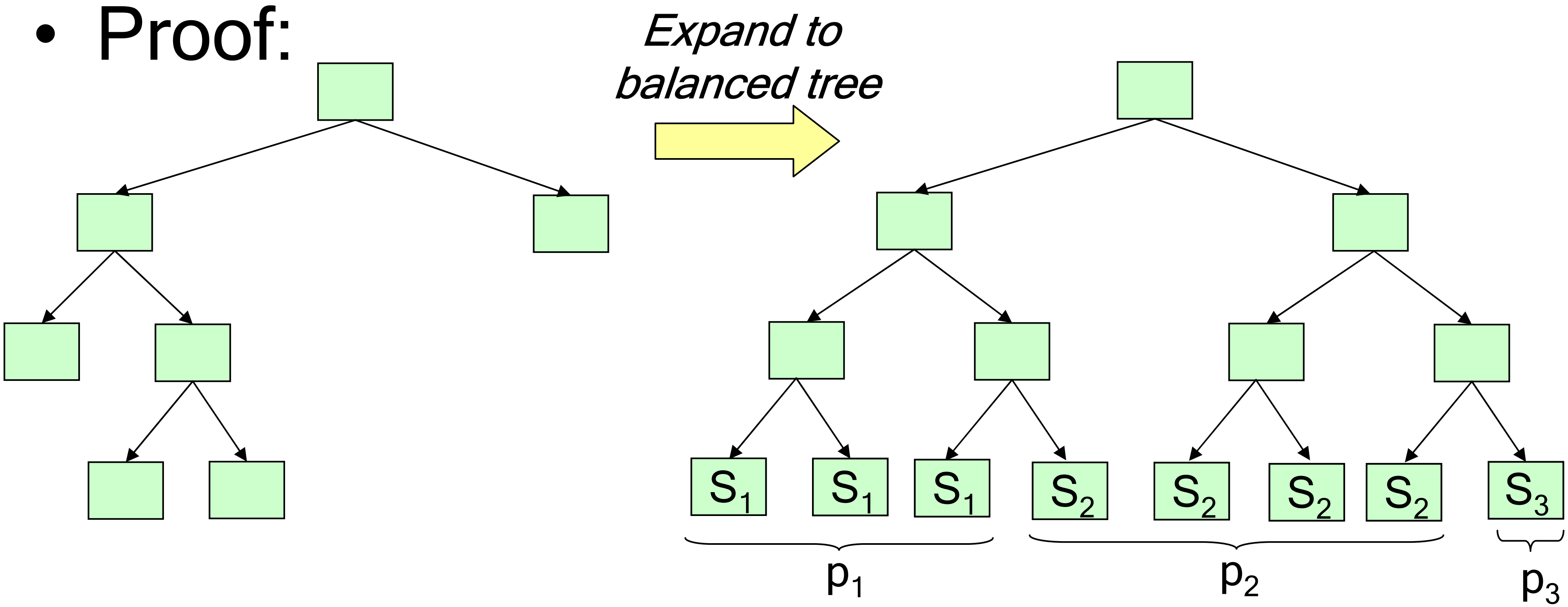
Proof: Substance is diluted 2X at each step, and final mixture is sum over all child nodes.

Reachable Mixtures

- Theorem: A mixture is reachable if and only if it can be written: $\boxed{\sum_i p_i = 2^d}$

$$\{(S_1, p_1/2^d), (S_2, p_2/2^d), \ldots, (S_k, p_k/2^d)\}$$

- Proof:



Must be mixing tree for mixture

Each leaf node contributes $1/2^d$

Min-Mix Example

- Recall example: mixture {(A, 3/8), (B, 5/8)}

bins

A=3 =0011

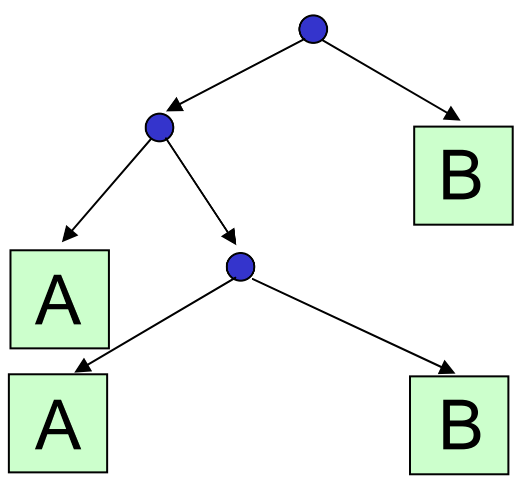
B=5 =0101

$2^3 = 8$

$2^2 = 4$

$2^1 = 2$

$2^0 = 1$



depth = 0

depth = 1

depth = 2

depth = 3

$c = 2^{-2} + 2^{-3}$
$= 1/4 + 1/8$
$= 3/8$

$c = 2^{-1}+2^{-3}$
$= 1/2 + 1/8$
$= 5/8$

3 mixes

Same as optimal

Min-Mix Example 2

- Mixture {(A, 5/16), (B, 7/16), (C, 4/16)}

bins	A=5 =00101	B=7 =00111	C=4 =00100

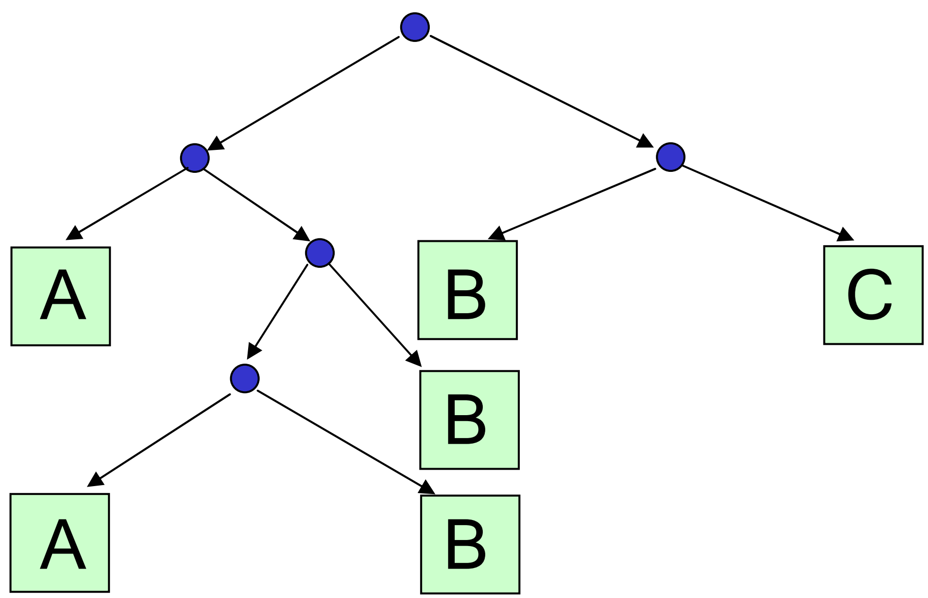
$2^4 = 16$

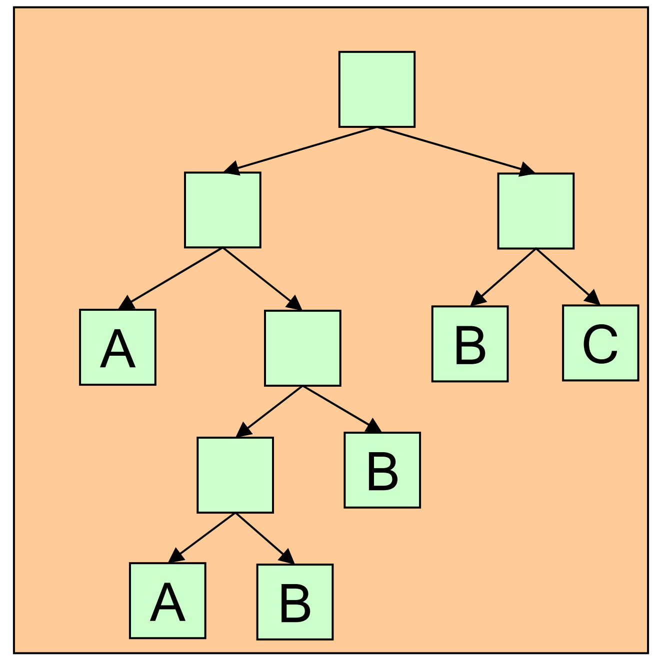
$2^3 = 8$

$2^2 = 4$

$2^1 = 2$

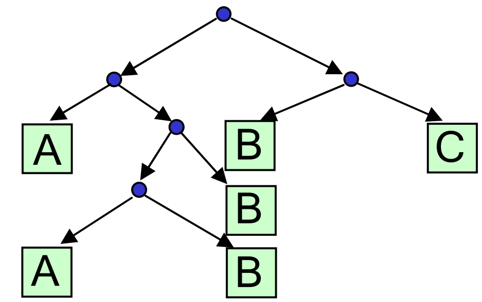
$2^0 = 1$





- Correctness intuition: put d’th most significant bit at depth d
- Can always build tree: induction on # bits at depth d

Min-Mix Algorithm

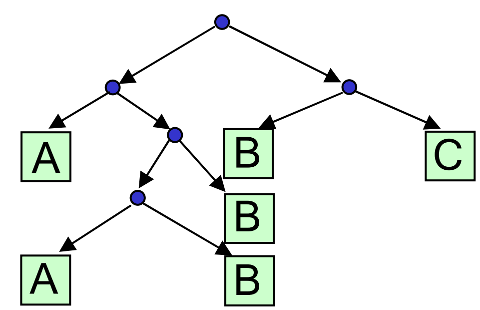



```
node buildMixingTree(mixture {(S_1, p_1/n), ..., (S_k, p_k/n)}) {
  depth = lg(n)
  bins = new stack[depth+1]
  for i = 1 to k
     for j = 0 to depth-1
        if (j'th least significant bit of p_i =1) {
           bins[j].push(S_i)
        }
  return buildMixingHelper(bins, depth)
}
```

bins[4] = { }
bins[3] = { }
bins[2] = { ~~A~~, ~~B~~, ~~C~~}
bins[1] = { ~~B~~ }
bins[0] = { ~~A~~, ~~B~~ }

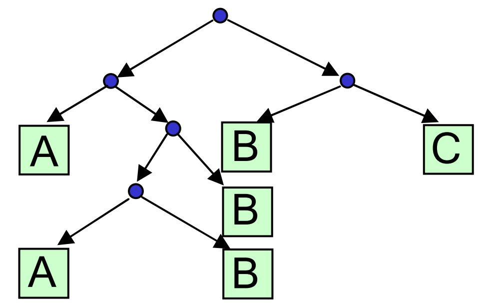
```
node buildMixingHelper(stack[] bins, int pow) {
  if bins[pow].empty() then
    node child1 = buildMixingHelper(bins, pow-1)
    node child2 = buildMixingHelper(bins, pow-1)
    return <child1, child2> as internal node;
  else
    return bins[pow].pop() as leaf node;
  endif
}
```

pow
4
3
2
1
0



Optimality of Min-Mix

- Consider mixture:
  $\{(S_1, p_1/n), \ldots, (S_k, p_k/n)\}$



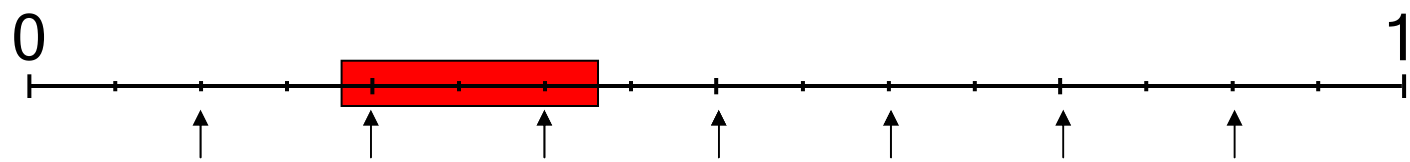
- Number of input samples used
  = number of bits in representation of inputs
- Theorem: this is optimal reagant usage
  - Implies optimal number of mixes
- Proof: otherwise some p_i/n is unattainable
- Asymptotic reagant usage: $O(k \lg n)$
  - This is also runtime of Min-Mix (visits nodes once)

Supporting Error Tolerances

- What if user wants to mix {(A, 1/3), (B, 2/3)}?
  - Impossible to obtain exactly with 1-to-1 mixer
  - However, can approximate within tolerance, $\pm\ \varepsilon$
  - Error bounds are natural part of all experiments

Supporting Error Tolerances

- Method: increase mixing depth d until some mix $p_1/2^d$ … $p_k/2^d$ falls within desired ranges
  - Example: mix {(A, 1/3), (B, 1/3), (C, 1/3)} ± 0.05?
    - Each substance should fall in range [0.23, 0.43]



Depth	Concentrations	
1	0.5	- Out of range
2	0.25,0.5,0.75	- In range, but infeasible: 0.25 + 0.25 + 0.25 < 1
3	…, 0.25, 0.375, …	- In range and feasible: 0.25 + 0.375 + 0.375 = 1

- Could be multiple solutions; we choose greedily

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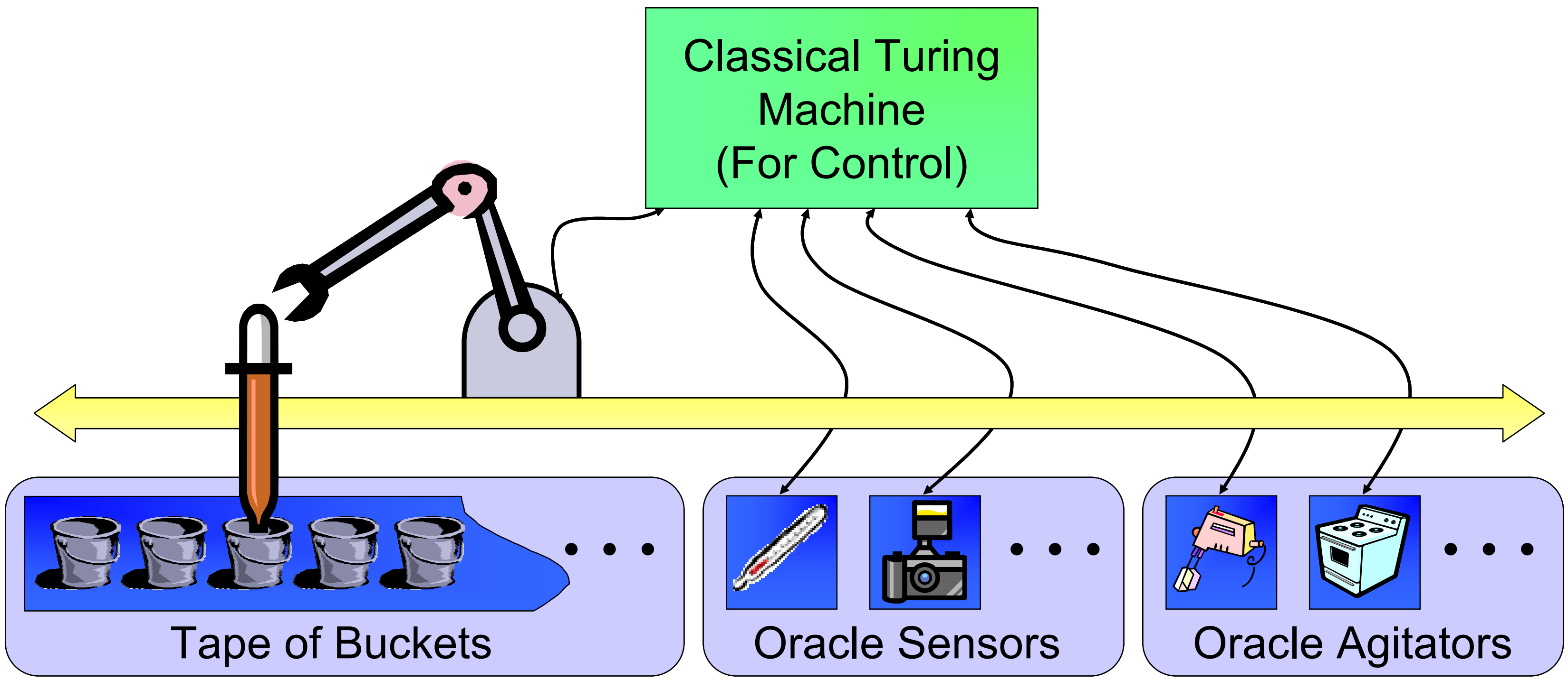
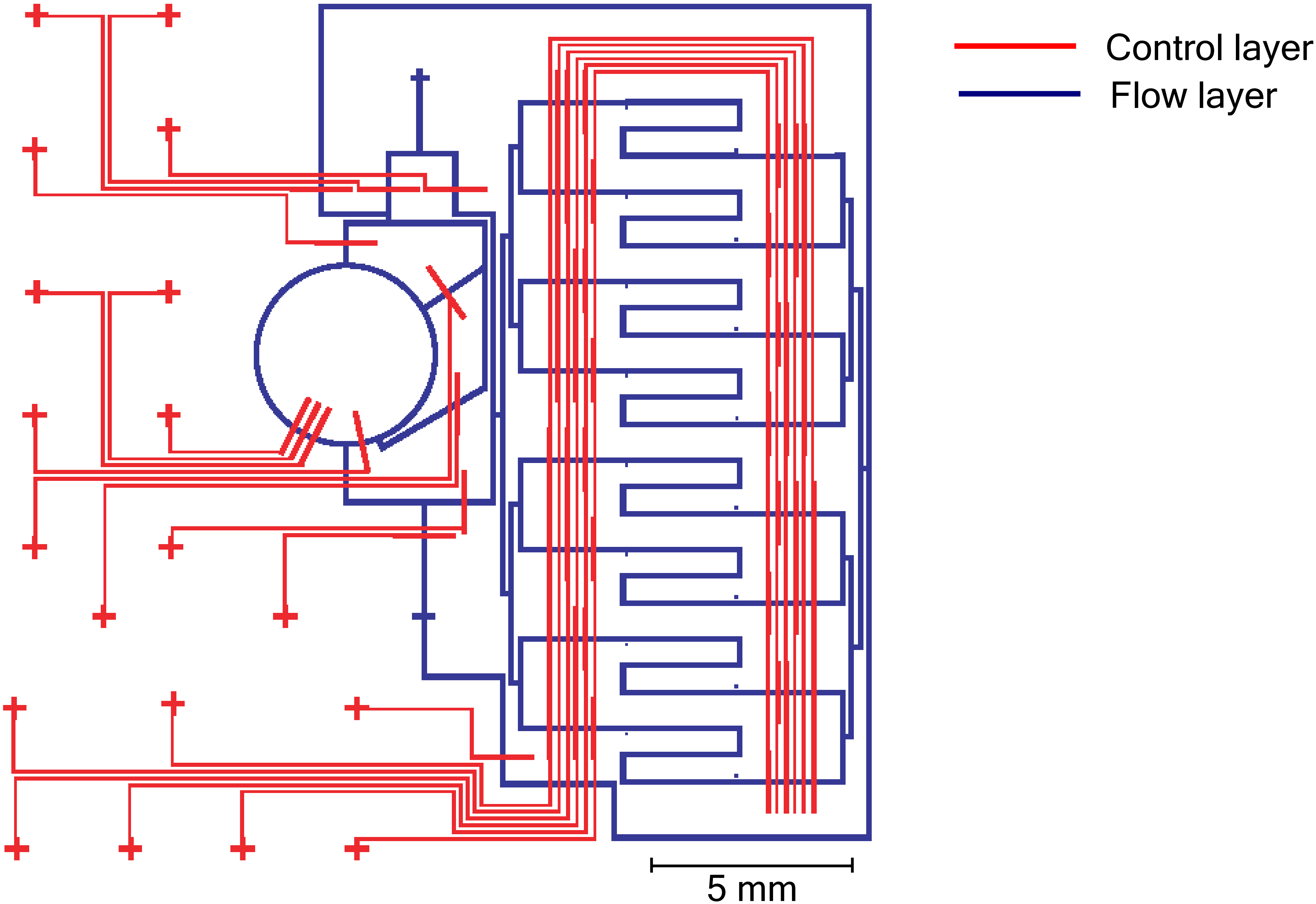
What Does General-Purpose Mean?

- Computing: Turing Machine
  - Implementation parameter: memory size
- Microfluidics: “Universal Fluidic Machine”

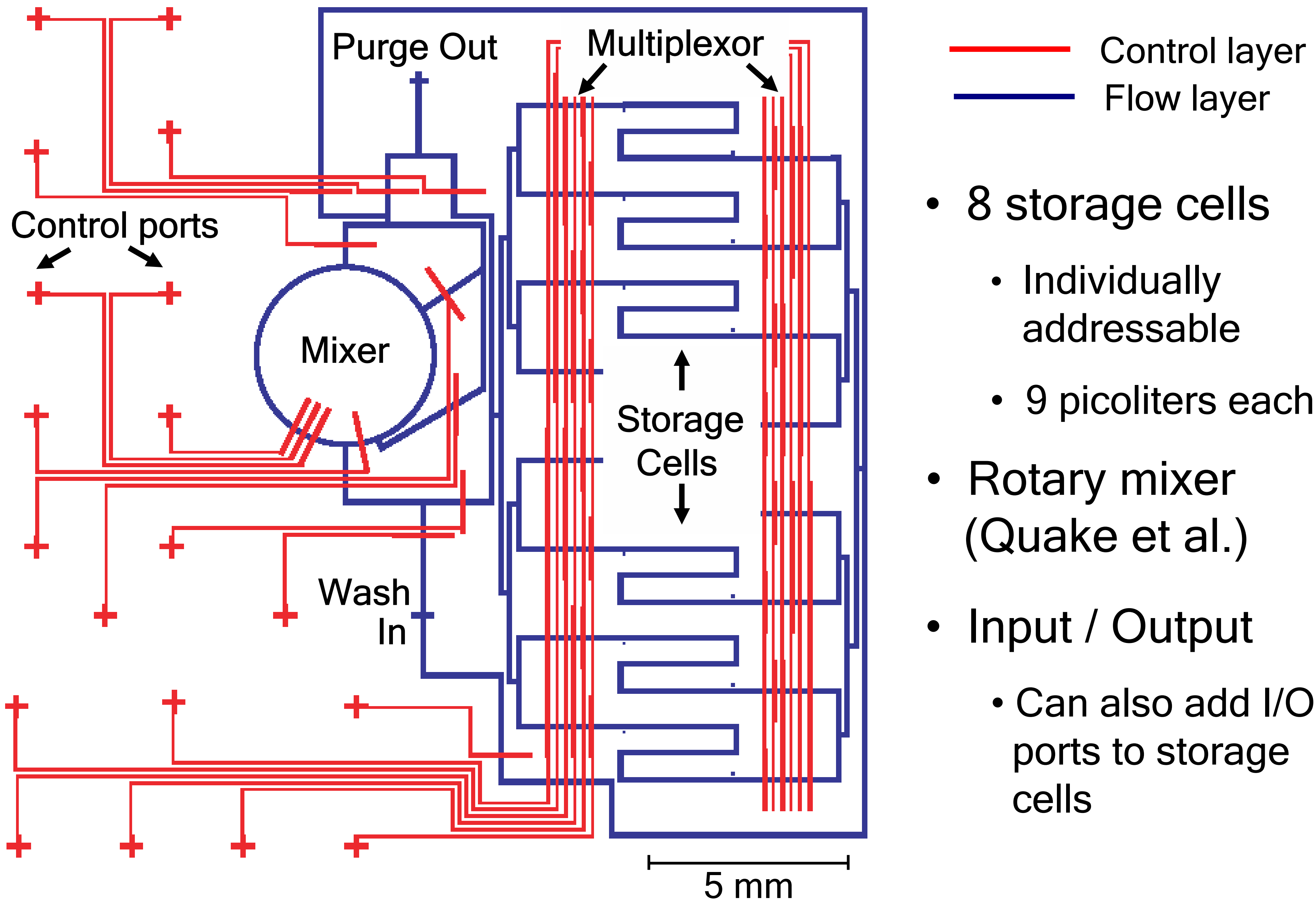


  - Implementation parameters:
    - memory size
    - precision
    - sensors/agitators

Our General-Purpose Chip (April 2004)

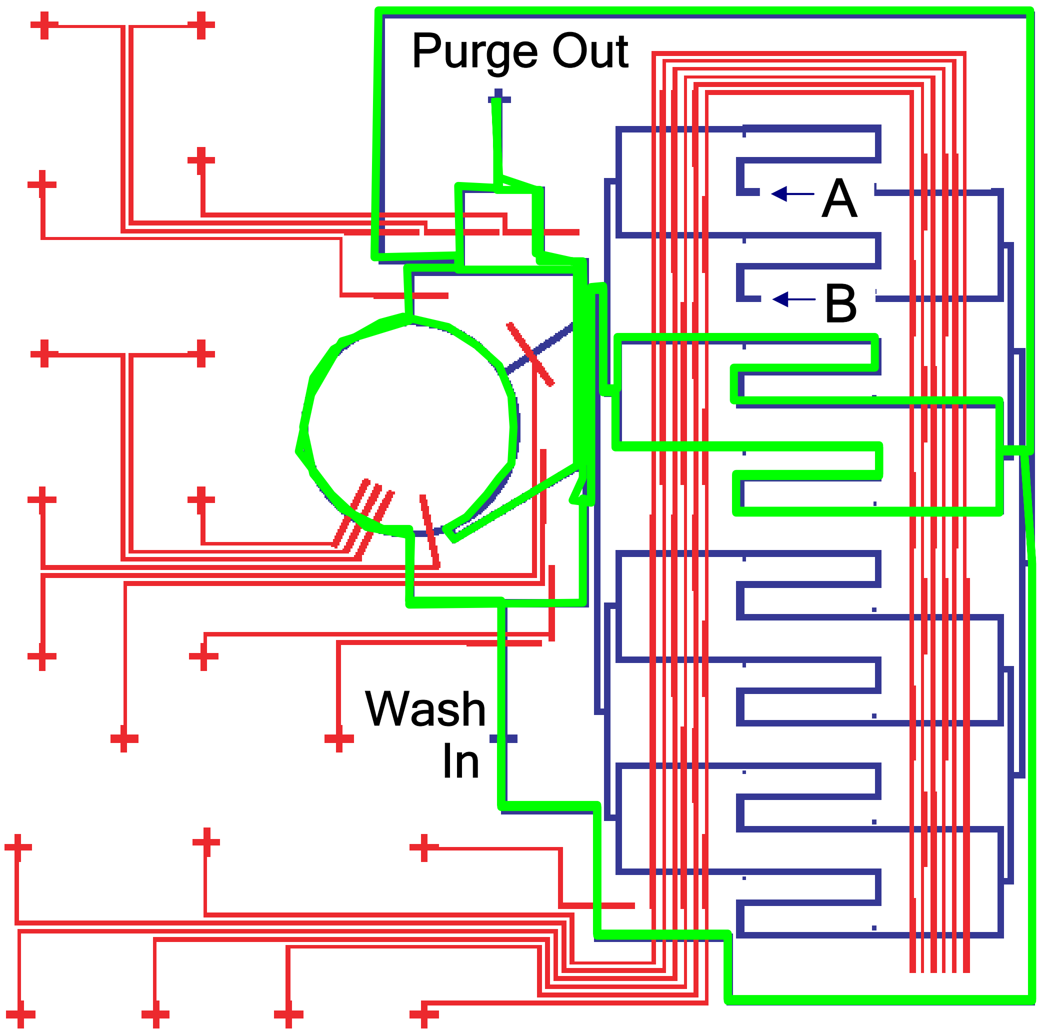


Our General-Purpose Chip (April 2004)



- 8 storage cells
  - Individually addressable
  - 9 picoliters each
- Rotary mixer (Quake et al.)
- Input / Output
  - Can also add I/O ports to storage cells

Chip-Level Operations



- All operations are "pushed" by input, flow to purge out
- Extra inputs / outputs attached to storage cells
- Basic operations:
  - storage → output
  - storage → mixer
  - mixer → storage
- Due to precision limits, output of mixer only fills one storage cell
- storage → storage is "mix 2 of same fluid and store"

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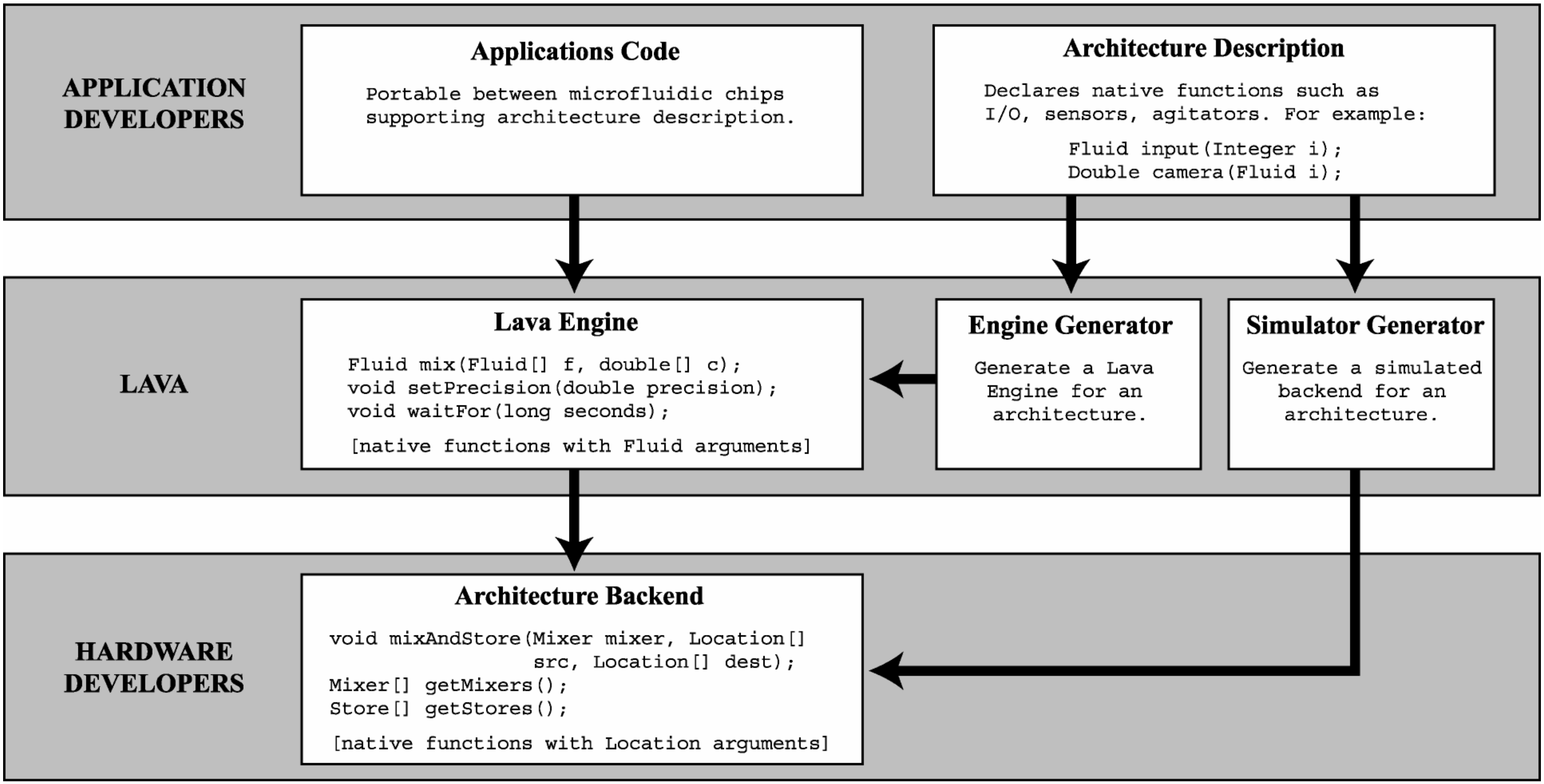
A Portable Machine Language (PML)

- C is PML for von-Neumann machines
  - Hides idiosyncratic differences
  - Exposes important properties
  - Enables portability
- Things to virtualize in microfluidic realm:
  - # of chambers, pipes, mixing reservoirs, etc.
  - Location of fluids on the chip
  - Precision of mixing and routing hardware
  - Timing of events
- Our solution: Lava
  - A Java library with first-class Fluid objects
  - Virtualizes basic resources
  - Provides native hooks for common agitators / sensors

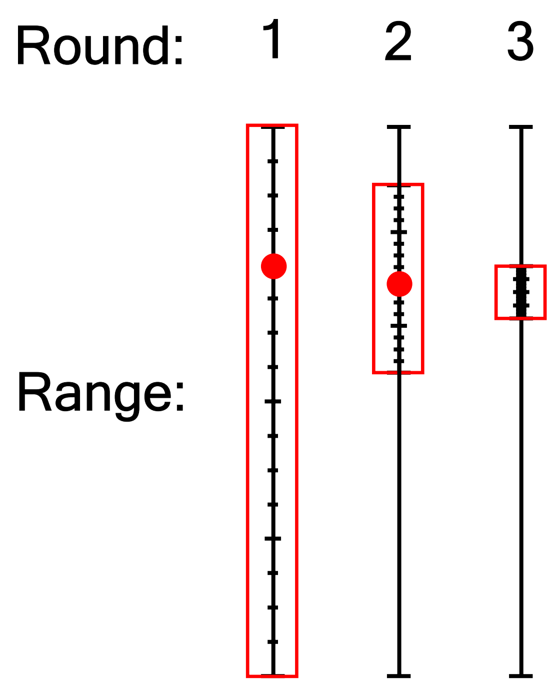
Lava System Architecture



Example: Recursive Descent Search

- Goal: find ratio of two fluids with highest activity
  - Common question in biology
    - Modeling activators / inhibitors
    - Understanding signaling pathways
    - Drug discovery
- Method: zoom in on area of interest

```
set range = [0,1]
for each round {
   for each point p in range {
     measure activity at p
   }
   adjust range around highest activity
}
report range and activity
```



Example: Recursive Descent Search

```
interface SimpleEngine extends FluidEngine {
  Fluid input(Integer i);                          // require array of fluid inputs
  Double luminescence(Fluid f);                    // require luminescence camera
}

class RecursiveDescent {
  public static void main(String[] args) {
    SimpleEngine engine = (SimpleEngine)           // build engine for interface
      EngineFactory.buildEngine("SimpleEngine", MY_BACKEND);
    run(engine);
  }

  static void run(SimpleEngine engine) {
    …
  }
}
```

Example: Recursive Descent Search

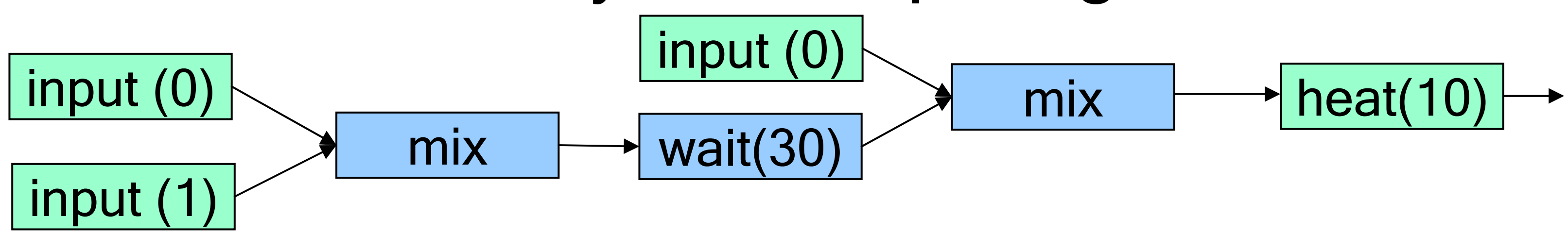
```
static void run(SimpleEngine engine) {
   Fluid A = engine.input(new Integer(0));                      // input Fluids
   Fluid B = engine.input(new Integer(1));
   double center = 0.5, radius = 0.5;                           // set range of interest
   double act, bestActivity = -1;

   for (int i=0; i<ROUNDS; i++) {                               // repeat a number of rounds
     int bestJ = 0;
     for (int j=0; j<10; j++) {                                 // try 10 samples
       double target = center+radius*(1-2*(double)j/10);
       Fluid f = engine.mix(A, target, B, 1-target);            // prepare mixture
       engine.waitFor(30);

       act = engine.luminescence(f).doubleValue();              // measure activity
       if (act > bestActivity) {
           bestActivity = act;  bestJ = j;                      // remember highest activity
     }}
     center = center+radius*(1-2*(double)bestJ/10);             // zoom in on highest activity
     radius = radius / 2;
   }
   System.out.println("Highest activity at: " + center);
}
```

Providing Digital Abstraction

- Challenge: Fluid variables used multiple times
  - But once a fluid is used on-chip, it is gone!
  - This is a lossy system
  - Need to provide some notion of GAIN

Providing Digital Abstraction

- Challenge: Fluid variables used multiple times
- Solution: re-generate fluids on demand
  - Lava traces history for computing each Fluid



  - Current model: stateless mixing, native functions
  - If unavailable fluid referenced, re-evaluate history
- Optimizations
  - Lazy evaluation
  - Evaluate in order that minimizes temporaries

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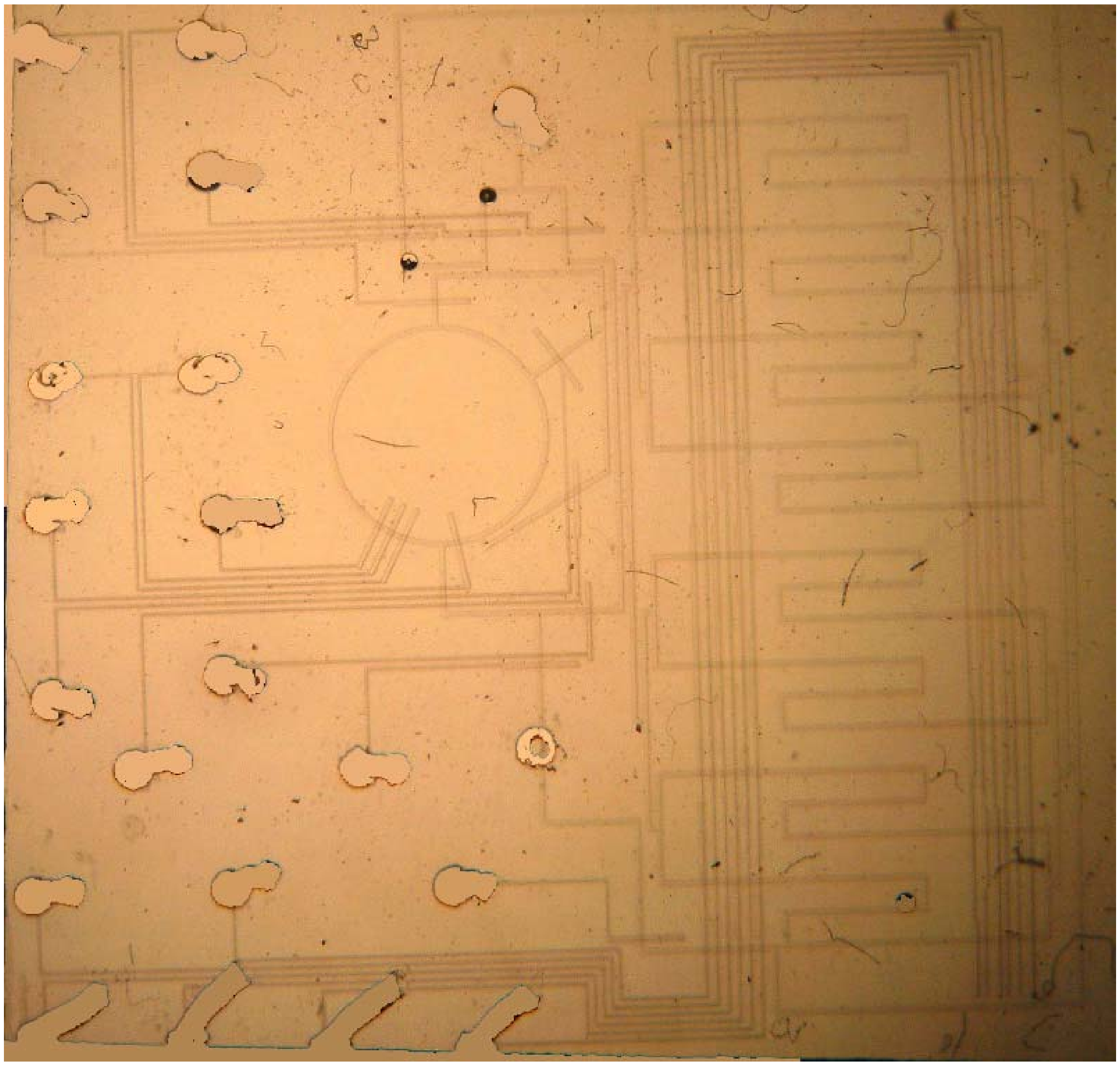
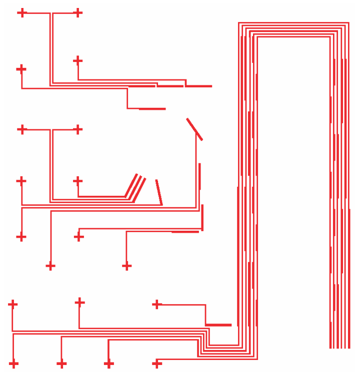
Implementation Status (April 2004)



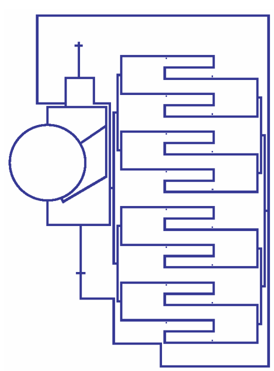
- Prototype chip fabricated
- Demonstrated I/O, moving fluids, mixing
- Current focus:
  - Robustness
    - Air bubbles
    - Diffusion
  - Calibration
    - Need to determine timing for automatic control

Fabrication Process (Quake et al.)



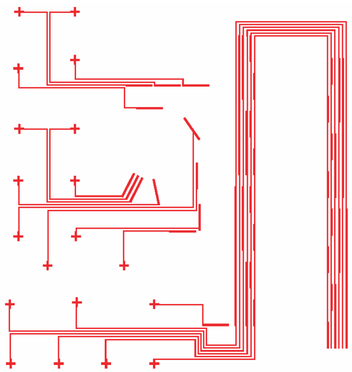
Control Layer

0. Start with mask of channels



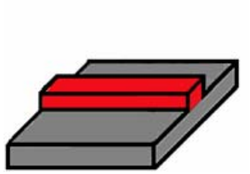
Flow Layer

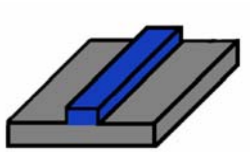
Fabrication Process (Quake et al.)

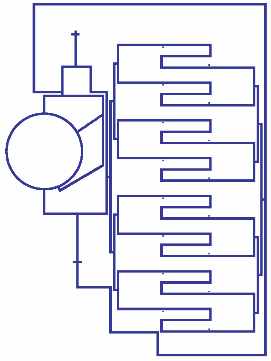


Control Layer

1. Deposit pattern on silicon wafer

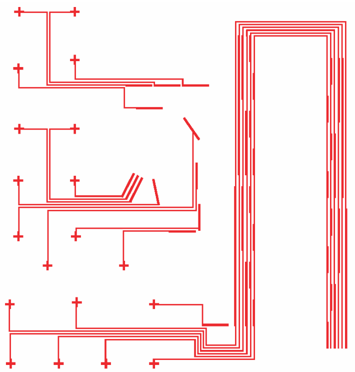






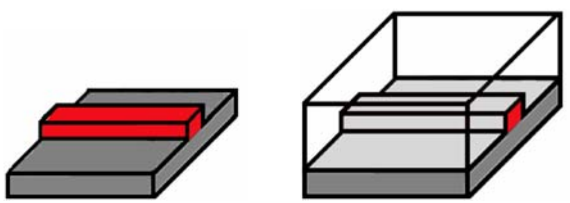
Flow Layer

Fabrication Process (Quake et al.)

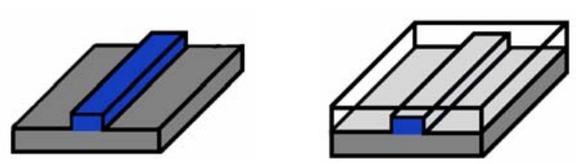


Control
Layer

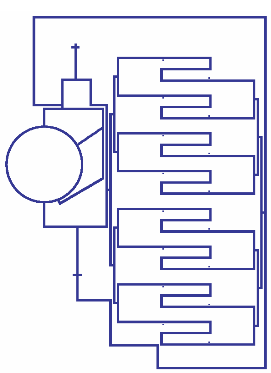
2. Pour PDMS over mold
 - polydimexylsiloxane: “soft lithography”



Thick layer (poured)

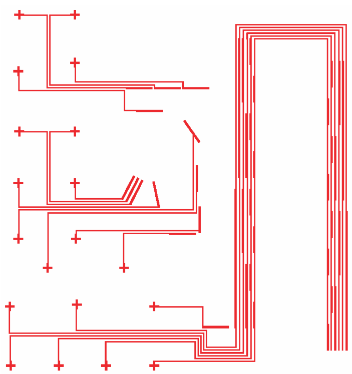


Thin layer (spin-coated)



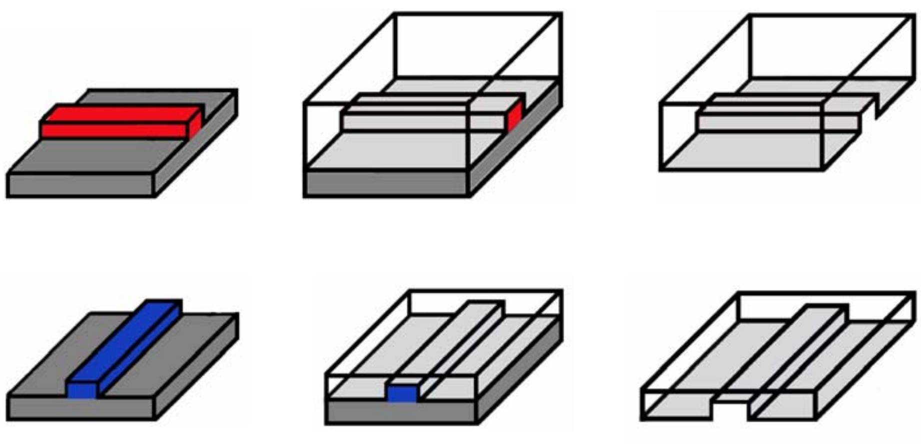
Flow
Layer

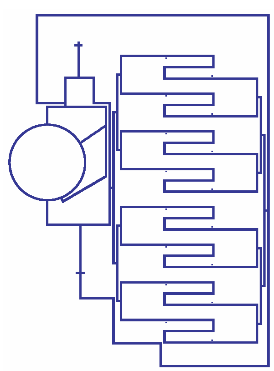
Fabrication Process (Quake et al.)



Control
Layer

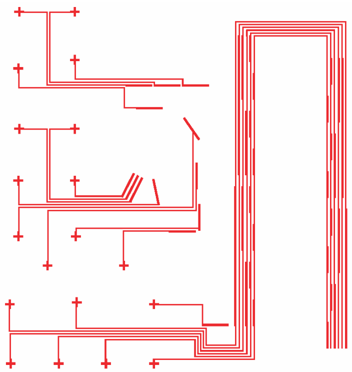
3. Bake at 80° C (primary cure),
   then release PDMS from mold





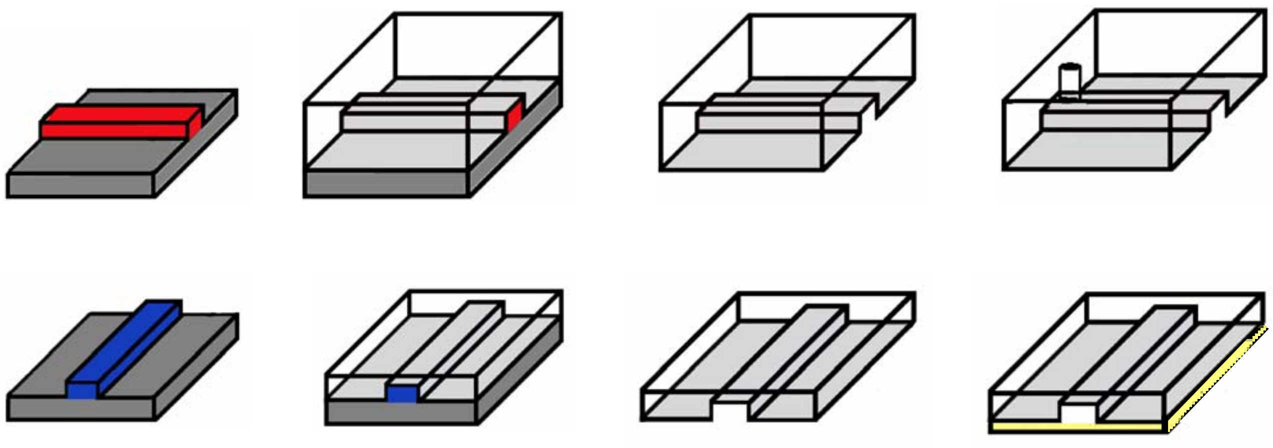
Flow
Layer

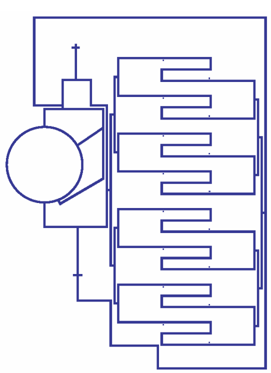
Fabrication Process (Quake et al.)



Control Layer

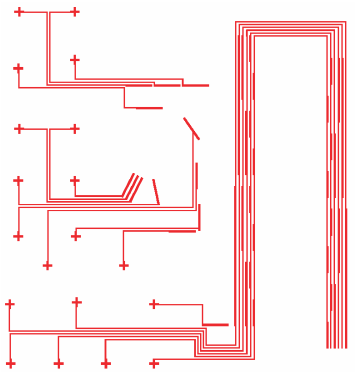
4a. Punch hole in control channel
4b. Attach flow layer to glass slide





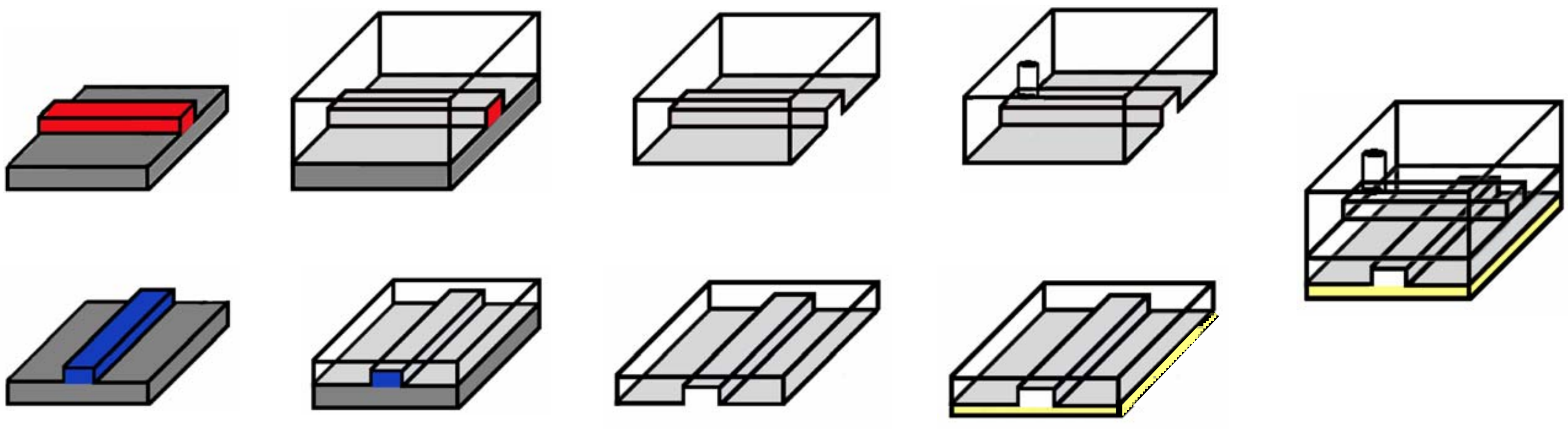
Flow Layer

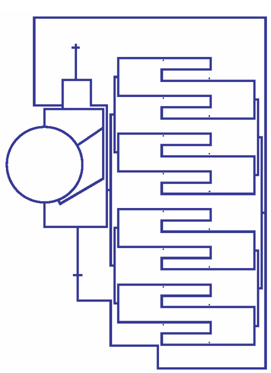
Fabrication Process (Quake et al.)



Control Layer

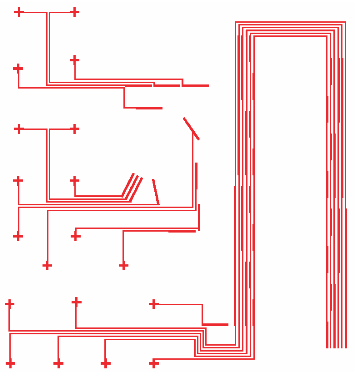
5. Align flow layer over control layer





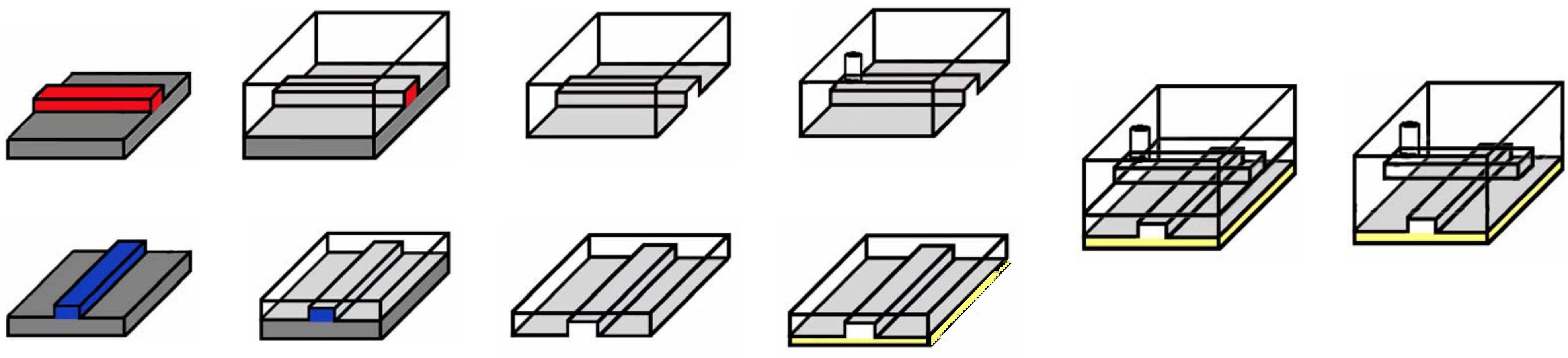
Flow Layer

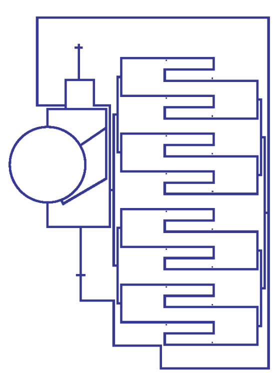
Fabrication Process (Quake et al.)



Control Layer

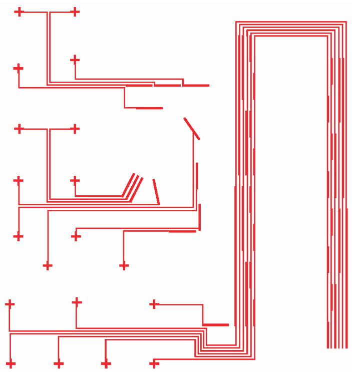
6. Bake at 80° C (secondary cure)





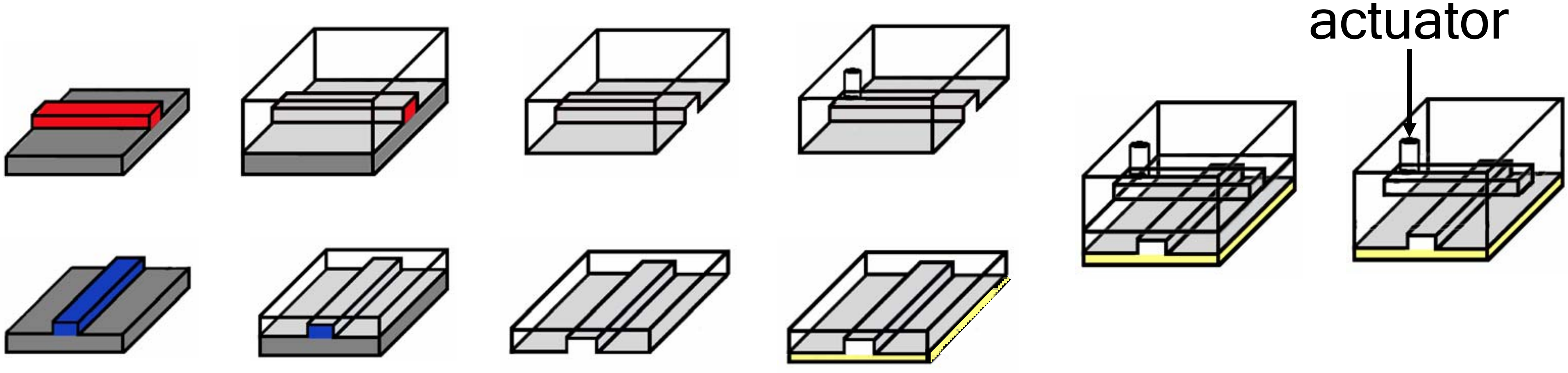
Flow Layer

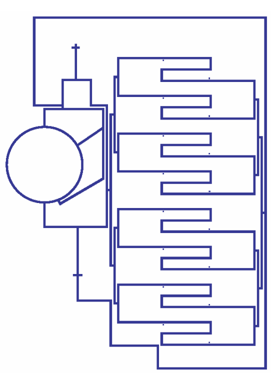
Fabrication Process (Quake et al.)



Control Layer

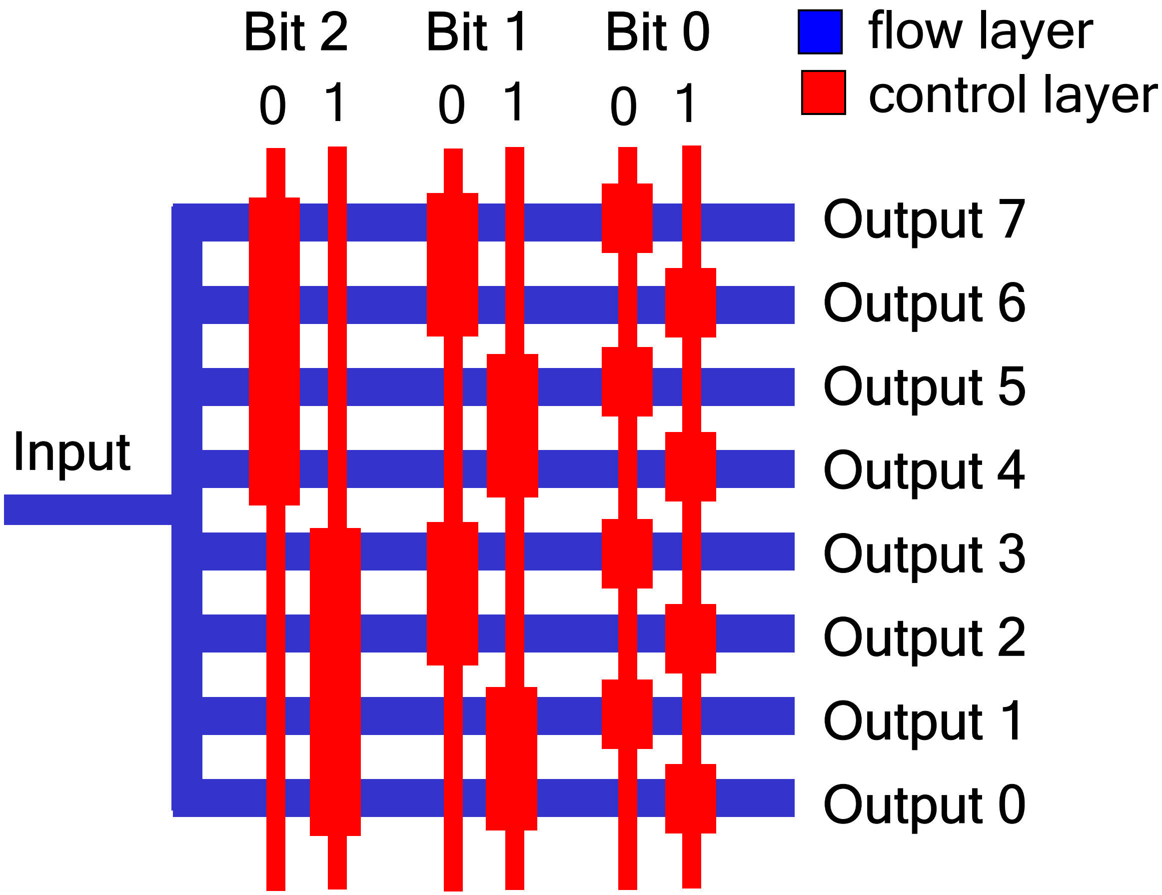
7. When pressure is high, control channel pinches flow channel to form a valve





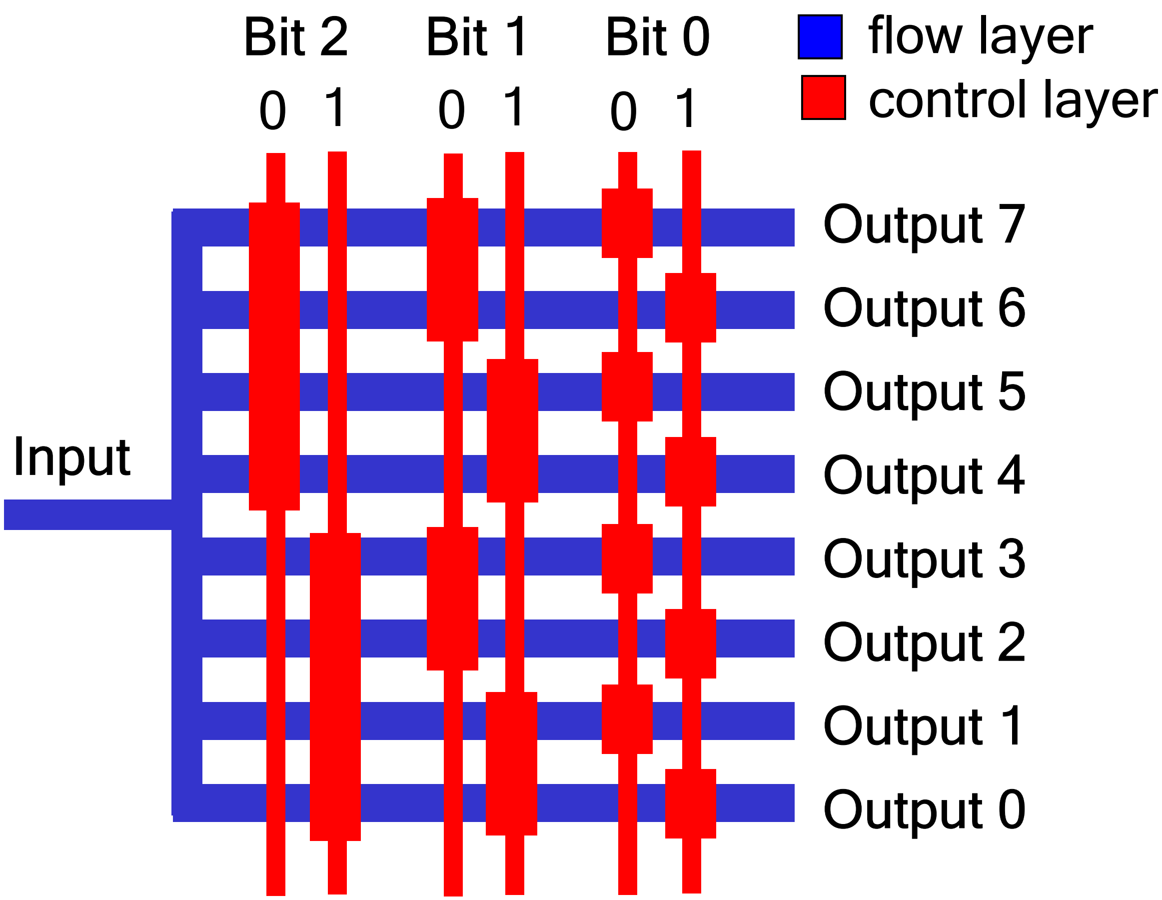
Flow Layer

Making a Multiplexor (Thorsen et al.)



- Control lines can cross flow lines
  - Only thick parts make valves
- Logic is not complimentary
- To control n flow lines, need 2 $\log_2$ n control lines

Making a Multiplexor (Thorsen et al.)



Example: select 3 = 011

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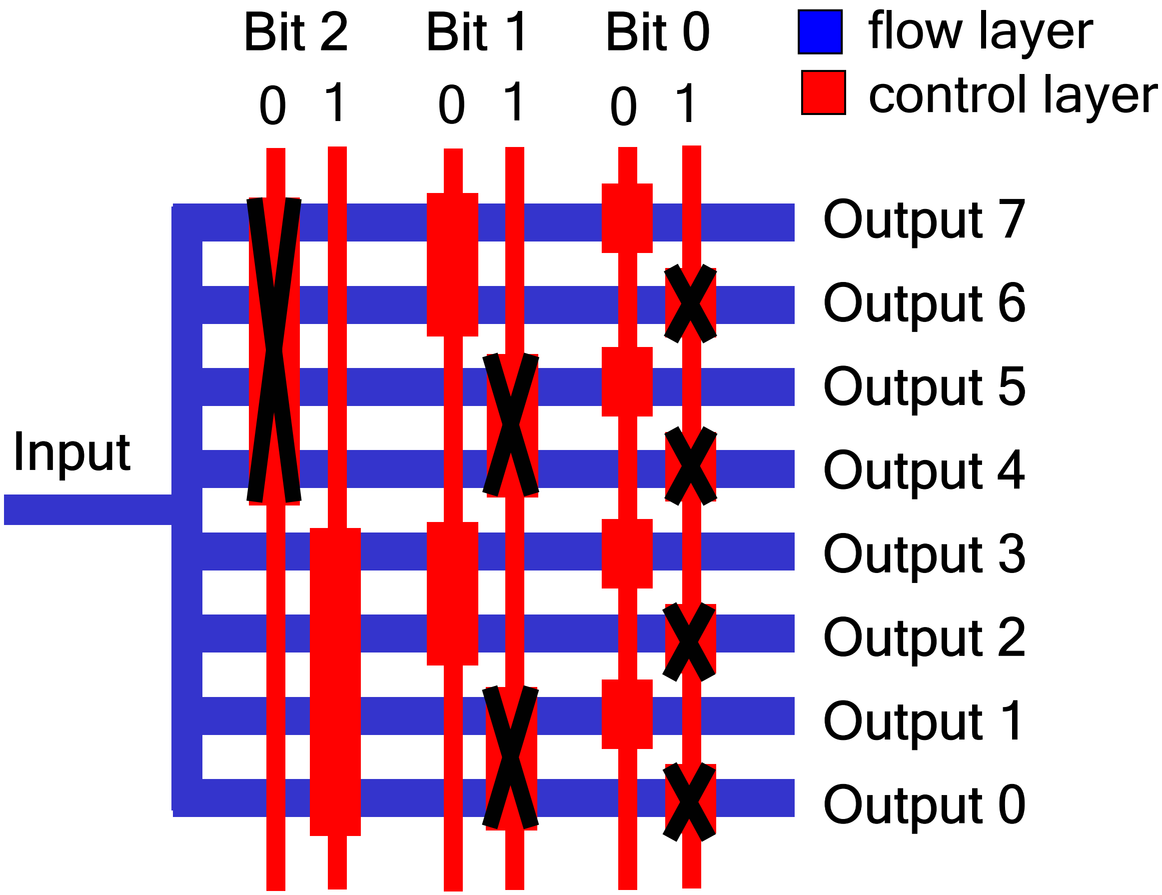
Making a Multiplexor (Thorsen et al.)



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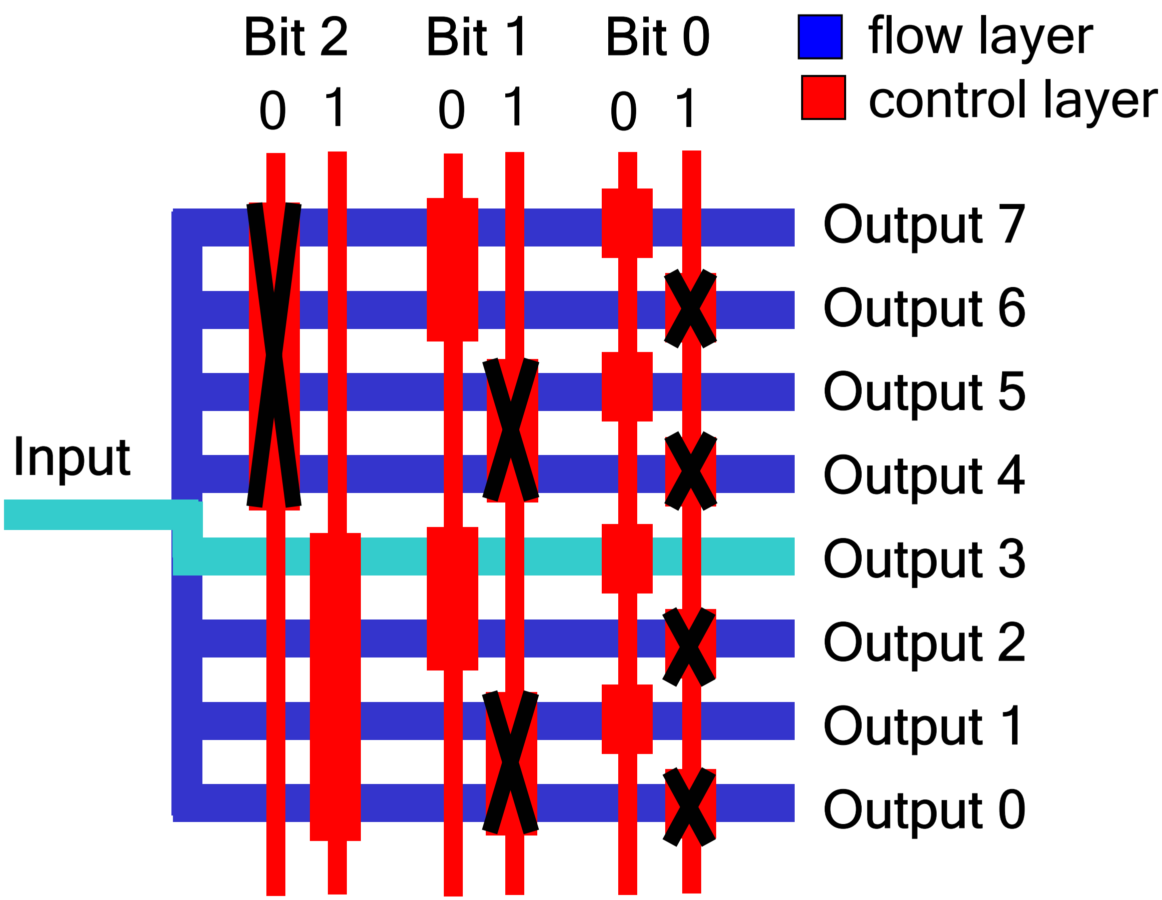

Example: select 3 = 011

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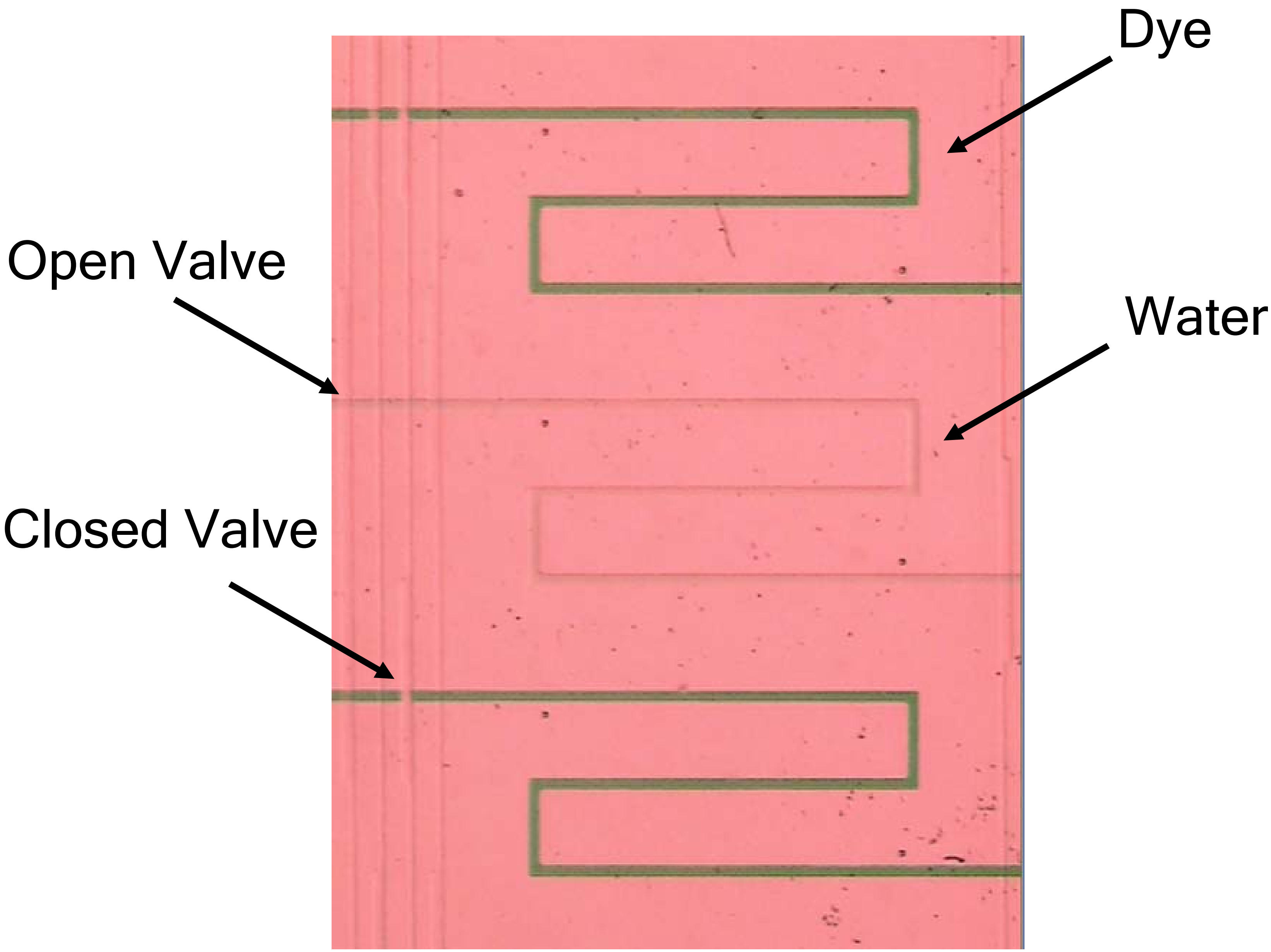
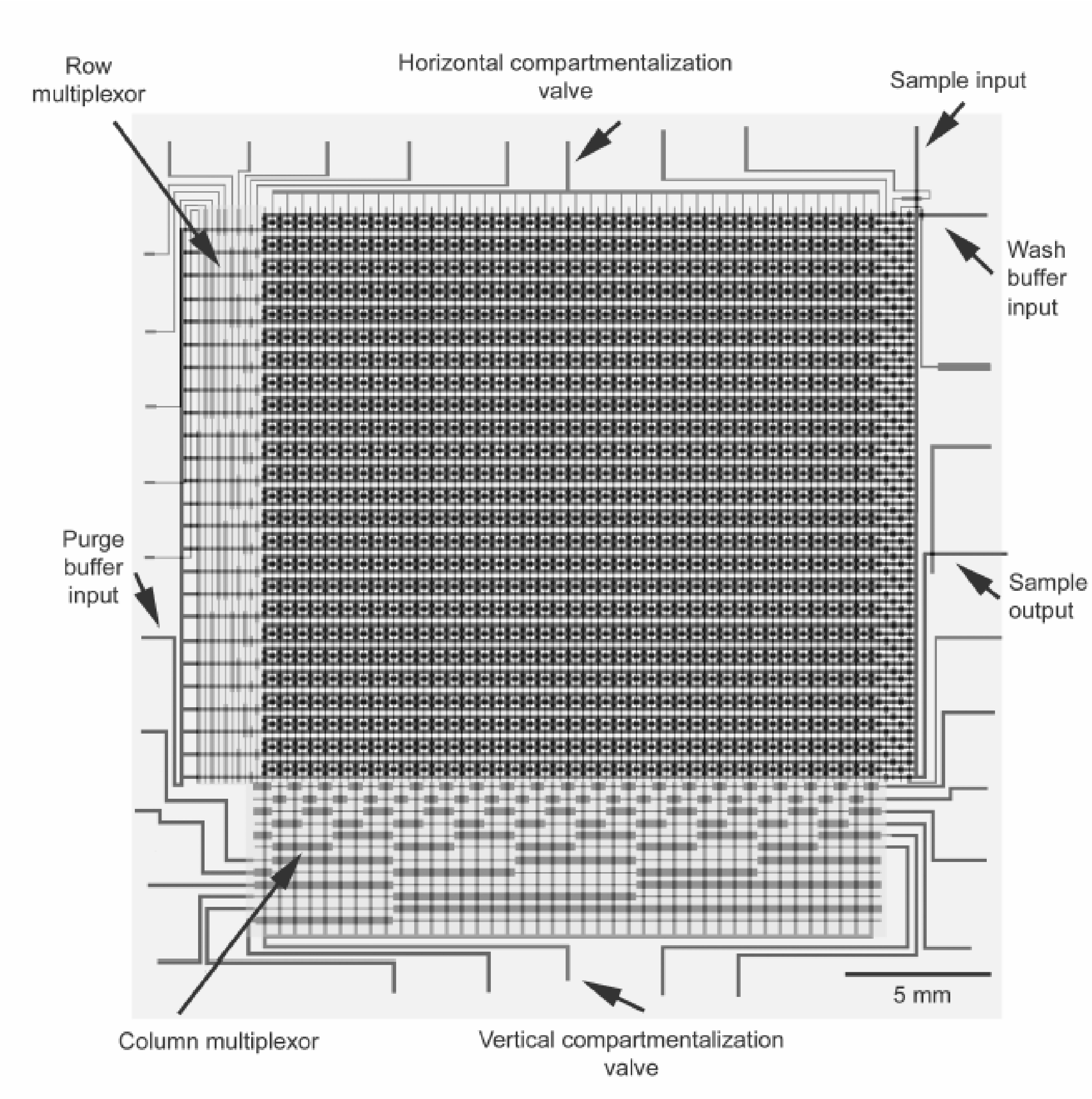
Our Multiplexor in Operation

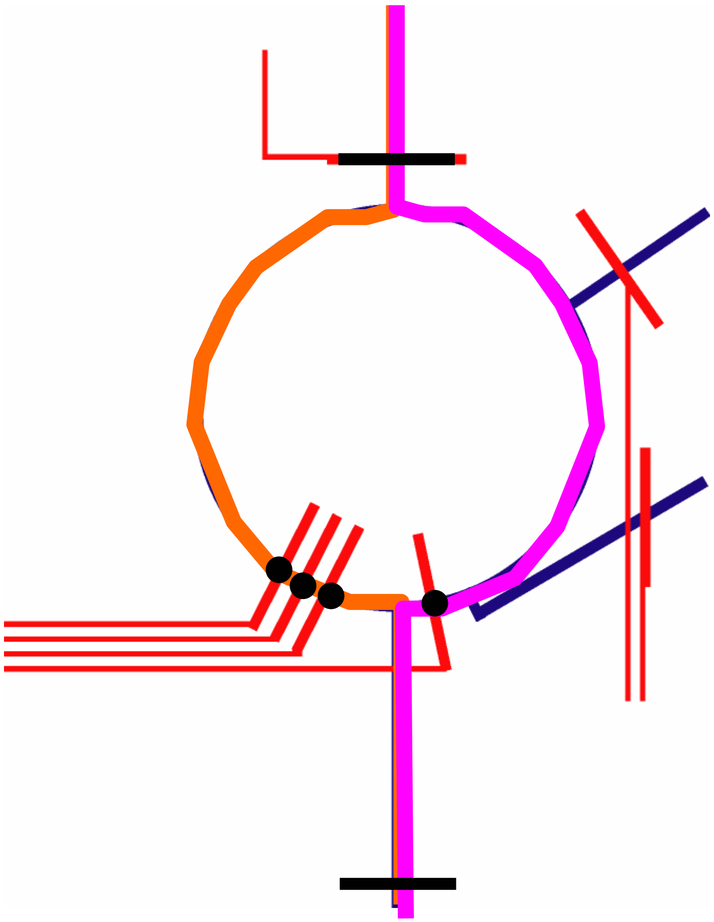


Scaling to Large Chips (Thorsen et al.)



- 1000 individually addressable chambers
- Uses row multiplexor, column multiplexor
- With industrial fabrication processes, will be possible to scale much further

Rotary Mixer (Quake et al.)

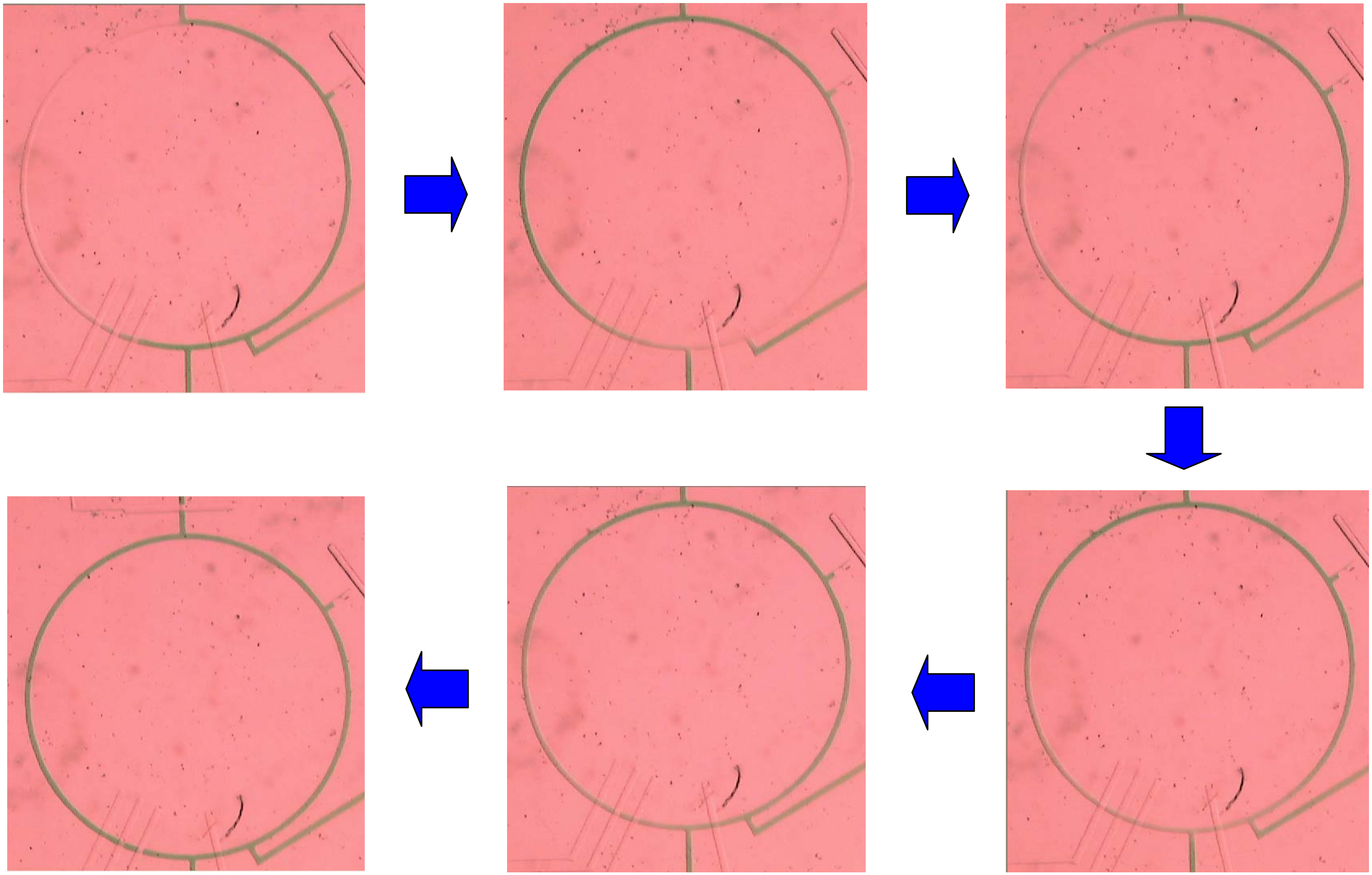


Mode of operation:

1. Fill left with reagant A
2. Fill right with reagant B
3. Lock down I/O
4. Use mixer valves as peristaltic pump

Channel mixes due to difference in inner / outer rotational velocities

Our Mixer in Operation

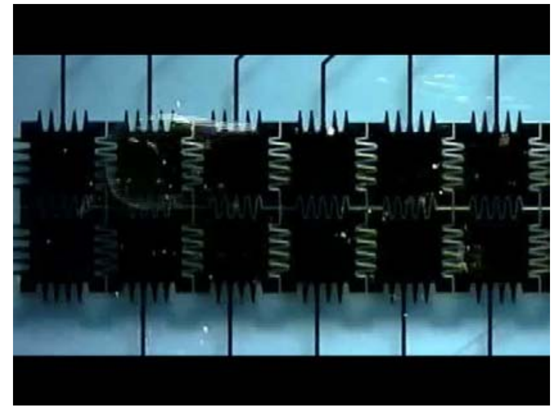


Outline

- Introduction
- General-purpose microfluidic chip
- Portable programming system
- Mixing algorithms
- Related work
- Conclusions

Related Work

- Droplet-based microfluidics (Fair et al.)
  - Manipulate discrete droplets using electrowetting
  - Pro:
    - Flexible grid of cells
    - No diffusion
    - Conventional fabrication process



  - Con:
    - Unclear if droplets can scale down (currently 100X larger than our storage chambers)
    - Non-polar reagants cannot be manipulated
    - Imprecise dispensing and splitting of droplets
  - Droplets vs. continuous flow will be ongoing debate
- Lava can target a droplet-based machine
  - Easy to emulate mixer, storage on chip

Related Work

- Mixing for droplets (Fair et al.)
  - Seems to suggest binary-search procedure
    - O(n) mixes to obtain concentration p/n
    - Only deals with two fluids
    - Slightly different model of computation
  - Our algorithm is improvement: O(k lg n)
- Quake et al. – continuous flow microfluidics
  - Two-layer soft lithography, rotary mixer, PCR
  - Our work relies on these foundations

Outline

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Future Work

- Mixing Algorithms
  - Generalize 1-to-1 mixing model to N-to-M mixer
  - Find mixing tree with minimal storage
  - Exploit error tolerances to optimize mixing
- Software
  - Expand language to encompass broader idioms
    - Can we simulate an entire cell on-chip?
  - Scheduling optimizations: re-order computation
  - Verification of safety properties
- Hardware
  - Integrate sensors / agitators on chip
  - Develop CAD tools for micofluidic domain
  - Explore parallel hardware constructs

Conclusions

- Microfluidic is the next big thing in biology
- Many opportunities for computer scientists
- Our contributions:

1. End-to-end programmable system
    - Universal Fluidic Machine
    - General-purpose microfluidic chip
    - Lava: portable, high-level language
2. Novel mixing algorithm
    - Mix k fluids with precision ± 1/n: O(k lg n) mixes
    - Guarantees optimal reagant usage, # of mixes

- Vision: create de-facto language for experimental scientists
  - Replicate a published experiment on your own microfluidic chip