Technologies for Healthcare Delivery

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Microsoft Research India

Joint work with Vaishnavi Ananthanarayanan, Michael Paik, Manish Bhardwaj, Emma Brunskill, Somani Patnaik, Nada Amin, Indrani Medhi, Kentaro Toyama, and Saman Amarasinghe

January 20, 2010
Microfluidic Chips for Rural Diagnostics

Disposable Enteric Card

*PATH,*
*Washington U.*
*Micronics, Inc., U. Washington*

Targets:
- E. coli, Shigella, Salmonella, C. jejuni

DxBox


Targets:
- malaria (done)
- dengue, influenza, Rickettsial diseases, typhoid, measles (under development)

CARD

*Rheonix, Inc.*

Targets:
- HPV diagnosis
- Detection of specific gene sequences
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)
Current Practice: Manage Gate-Level Details from Design to Operation

- For every change in the experiment or the chip design:
  1. Manually draw in AutoCAD
  2. Operate each gate from LabView
Abstraction Layers for Microfluidics

**Protocol Description Language**
- architecture-independent protocol description

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

**Fluidic Hardware Primitives**
- valves, multiplexers, mixers, latches

**Silicon Analog**
- C
- x86
- Pentium III, Pentium IV
- transistors, registers, …
Abstraction Layers for Microfluidics

**Protocol Description Language**
- architecture-independent protocol description

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

**Contributions**
- BioCoder Language [IWBDA 2009]
- Optimized Compilation [Natural Computing 2007]
- Demonstrate Portability [DNA 2006]
- Micado AutoCAD Plugin [MIT 2008, ICCD 2009]
- Digital Sample Control Using Soft Lithography [Lab on a Chip ‘06]

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**Fluidic Hardware Primitives**
- valves, multiplexers, mixers, latches

- chip 1
- chip 2
- chip 3
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);
}

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

450 Valve Operations
Implementation: Oil-Driven Chip

<table>
<thead>
<tr>
<th></th>
<th>Inputs</th>
<th>Storage Cells</th>
<th>Background Phase</th>
<th>Wash Phase</th>
<th>Mixing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chip 1</td>
<td>2</td>
<td>8</td>
<td>Oil</td>
<td>—</td>
<td>Rotary</td>
</tr>
</tbody>
</table>
Implementation: Oil-Driven Chip

\[
\text{mix } (S_1, S_2, D) \{ \\
\quad 1. \text{ Load } S_1 \\
\quad 2. \text{ Load } S_2 \\
\quad 3. \text{ Rotary mixing} \\
\quad 4. \text{ Store into } D \\
\}
\]

<table>
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**Implementation 2: Air-Driven Chip**

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<tr>
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<td>Oil</td>
<td>—</td>
<td>Rotary</td>
</tr>
<tr>
<td>Chip 2</td>
<td>4</td>
<td>32</td>
<td>Air</td>
<td>Water</td>
<td>In channels</td>
</tr>
</tbody>
</table>
**Implementation 2: Air-Driven Chip**

\[
\text{mix} \ (S_1, S_2, D) \{
1. \text{Load } S_1 \\
2. \text{Load } S_2 \\
3. \text{Mix / Store into } D \\
4. \text{Wash } S_1 \\
5. \text{Wash } S_2 \\
\}
\]

<table>
<thead>
<tr>
<th>Inputs</th>
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<td>Chip 2</td>
<td>4</td>
<td>32</td>
<td>Air</td>
<td>Water</td>
</tr>
</tbody>
</table>
BioCoder: A Language for Biology Protocols

In biology publications, can we replace the textual description of the methods used with a computer program?

Enable automation by mapping to microfluidic chips

Improve reproducibility by generating human-readable instructions
BioCoder Primitives

**Declaration of resources**
- new_fluid()
- new_solid()
- new_container()
- new_plate()
- new_slide()
- new_symbol()
- new_column()

**Temperature & storage**
- incubate()
- store_for()
- set_temp()
- store_plate()
- thermocycler()
- thermocycler_anneal()
- pcr_init_denat()
- pcr_final_ext()
- inoculation()
- incubate_plate()
- invert_dry()
- dry_pellet()
- dry_slide()

**Measurement**
- measure_fluid()
- measure_solid()
- measure_prop()
- add_to_column()
- add_to_slide()
- collect_tissue()
- plate_out()
- transfer()

**Disposal**
- discard()
- drain()

**Centrifugation**
- centrifuge()
- centrifuge_pellet()
- centrifuge_phases_top()
- centrifuge_phases_bottom()
- centrifuge_column()
- centrifuge_flow_through()

**Combination & mixing**
- combine()
- combine_and_mix()
- dissolve()
- invert()
- pipet()
- resuspend()
- tap()
- vortex()
- vortex_column()
- incubate_and_mix()
- mixing_table()
- mixing_table_pcr()
- immerse_slide()
- remove_slide()
- wash_slide()
- homogenize_tissue()
- wash_tissue()

**Detection & analysis**
- ce_detect()
- electrophoresis()
- facs()
- measure_fluorescence()
- mount_observe_slide()
- sequencing()
- electroporate()
- weigh()
- cell_culture()
- transfection()

**Symbolic manipulation**
- set_value()
- assign()
- add()
- divide()
- subtract()
- multiply()
1. Standardizing Ad-Hoc Language

- Need to convert qualitative words to quantitative scale
- **Example: a common scale for mixing**
  - When a protocol says “mix”, it could mean many things
  - Level 1: tap
  - Level 2: stir
  - Level 3: invert
  - Level 4: vortex / resuspend / dissolve
- **Similar issues with temperature, timing, opacity, …**
2. Timing Constraints

• Precise timing is critical for many biology protocols
  – Minimum delay: cell growth, enzyme digest, denaturing, etc.
  – Maximum delay: avoid precipitation, photobleaching, etc.
  – Exact delay: regular measurements, synchronized steps, etc.

• May require parallel execution
  – Fluid f1 = mix(…); useBetween(f1, 10, 10);
  – Fluid f2 = mix(…); useBetween(f2, 10, 10);
  – Fluid f3 = mix(f1, f2);

• Addressed via lazy execution
### Benchmark Suite

<table>
<thead>
<tr>
<th>Source: Academic Laboratory</th>
<th>INSTR'S</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTAB DNA Plant Miniprep (Utpal Nath Lab)</td>
<td>225</td>
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<tr>
<td>2ab Assembly Protocol (Douglas Densmore)</td>
<td>90</td>
</tr>
<tr>
<td>DNA Miniprep (Eric Klavins Lab)</td>
<td>50</td>
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<tr>
<td>Ligation (Eric Klavins Lab)</td>
<td>21</td>
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<tr>
<td>Protein in situ localization (Utpal Nath Lab)</td>
<td>81</td>
</tr>
<tr>
<td>Restriction Digestion (Eric Klavins Lab)</td>
<td>20</td>
</tr>
<tr>
<td>Transformation (Eric Klavins Lab)</td>
<td>33</td>
</tr>
</tbody>
</table>

<table>
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<tr>
<th>Source: Textbook</th>
<th>INSTR'S</th>
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<tbody>
<tr>
<td>Plasmid DNA extraction - miniprep (Molec. Cloning)</td>
<td>60</td>
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<tr>
<td>Yeast transformation (Methods in Yeast Genetics)</td>
<td>89</td>
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<tr>
<th>Source: Published Paper</th>
<th>INSTR'S</th>
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<tbody>
<tr>
<td>Molecular barcodes (Miner et al., 2004)</td>
<td>75</td>
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<td>Plant RNA isolation (Bilgin et al., 2009)</td>
<td>66</td>
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<td>SIRT1 redistribution (Oberdoerffer et al., 2008)</td>
<td>107</td>
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<tr>
<td>Splinkerette PCR (Uren et al., 2009)</td>
<td>80</td>
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<tr>
<td>Touchdown PCR (Korbie &amp; Mattick, 2008)</td>
<td>21</td>
</tr>
<tr>
<td>Transcriptional instability (Warren et al., 2007)</td>
<td>84</td>
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</table>

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<th>Source: Commercial Kit</th>
<th>INSTR'S</th>
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<tbody>
<tr>
<td>AllPrep RNA protein protocol (Qiagen)</td>
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<tr>
<td>Miniprep (Qiagen)</td>
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<tr>
<td>Plasmid purification high yield (Qiagen)</td>
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<tr>
<td>Plasmid purification standard (Qiagen)</td>
<td>81</td>
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<tr>
<td>DNA sequencing by capillary elec. (Applied Biosys.)</td>
<td>60</td>
</tr>
</tbody>
</table>

**53 protocols; 2850 instructions**
Example: Plasmid DNA Extraction

I. Original protocol *(Source: Klavins Lab)*

Add 100 ul of 7X Lysis Buffer (Blue) and mix by inverting the tube 4-6 times. *Proceed to step 3 within 2 minutes.*

II. BioCoder code

```python
FluidSample f1 = measure_and_add(f0, lysis_buffer, 100*uL);
FluidSample f2 = mix(f1, INVERT, 4, 6);
time_constraint(f1, 2*MINUTES, next_step);
```

III. Auto-generated text output

Add 100 ul of 7X Lysis Buffer (Blue).
Invert the tube 4-6 times.
*NOTE: Proceed to the next step within 2 mins.*
Example: Plasmid DNA Extraction

DNA Miniprep Protocol

Solutions/reagents:
- bacterial culture grown in LB medium
- 7X Lysis Buffer (Blue)
- Neutralization Buffer (Yellow)
- Endo-Wash Buffer
- Zyppy™ Wash Buffer
- Zyppy™ Elution Buffer
- Zymo-Spin™ II Column

Equipment:
- Centrifuge
- Microfuge

Steps:
1. Measure out 600 μl of bacterial culture grown in LB medium into a 1.5ml- reaction tube.
2. Add 100 μl of 7X Lysis Buffer (Blue).
   Invert the tube 4-6 times.
   NOTE: Proceed to the next step within 2 mins.
3. Add 350 μl of Neutralization Buffer (Yellow).
   Vortex the mixture for a few secs.
Genetic Control of Surface Curvature

Utpal Nath, Brian C. W. Crawford, Rosemary Carpenter, Enrico Coen*
Genetic Control of Surface Curvature

Utpal Nath, Brian C. W. Crawford, Rosemary Carpenter, Enrico Coen*

Material and Methods

In situ Hybridization. The methods used for tissue preparation, digoxigenin-labelling of RNA probes, and in situ hybridisation were as described previously (S13). The probe used

floricaula: A Homeotic Gene Required for Flower Development in Antirrhinum majus

Enrico S. Coen, José M. Romero,* Sandra Doyle, Robert Elliott, George Murphy, and Rosemary Carpenter

“Immunological detection ... was carried out as described in the Boehringer digoxigenin-nucleic acid detection kit with some modifications.”
RNA probes, and in situ hybridisation were as described previously (S13). The probe used to detect the CIN transcript was a 1048 bp fragment from the cDNA clone, covering the entire ORF. For H4, the probe consisted of the entire cDNA (S14). For CYCLIN D3b, a 3′-
Genetic Control of Surface Curvature

Utpal Nath, Brian C. W. Crawford, Rosemary Carpenter, Enrico Coen*
Growing a Community

OpenWetWare is an effort to promote the sharing of information, know-how, and wisdom among researchers and groups who are working in biology & biological engineering. Learn more about us. If you would like edit access, would be interested in helping out, or want your lab website hosted on OpenWetWare, please join us.

Welcome new OWW users!


See all new users.
Growing a Community

One step 'miniprep' method for the isolation of plasmid DNA

plasmid miniprep

All 'miniprep' methods reported so far for the isolation of plasmid DNA involve multiple pipetting, extraction, centrifugation and changes of minifuge tubes. For screening large number of samples, they are therefore cumbersome, time consuming and not economical.

The technical report below by Chowdhury, K. (1991) is a very fast, simple and one step 'miniprep' procedure. The quality and quantity of DNA obtained by using this procedure is similar to those obtained by the other commonly used procedures of Sarghini et al. (1) or Birkofer and Doly (2). According to this procedure, the bacterial culture is directly extracted with a mixture of phenol:chloroform-isomycyl alcohol and the liberated DNA is precipitated with isopropanol. This method is now being used routinely in our laboratory for isolating plasmids upto 12kb in size. A detailed description of this method is presented below:

1. Take 0.5ml of overnight E coli culture in a microfuge tube. We routinely grow our cells in standard T bacteriological media supplied by Merck, Germany.
2. Add 0.5ml of phenol:chloroform-isomycyl alcohol (25:24:1). The phenol was saturated with TE (10mM Tris, 7.5, 1mM EDTA) prior to mixing with chloroform and isomycyl alcohol.
3. Mix by vortexing at the maximum speed for 1 minute. Alternatively, vortex for 10 seconds and then transfer to an overhead rotator for 5 minutes.
4. Spin at 12,000g for 5 minutes. During the spin, prepare microfuge tubes with 0.5ml of isopropanol. After the spin, remove carefully about 0.45ml of the upper aqueous phase leaving the interphase undisturbed and add it to the isopropanol. Mix well and spin immediately at 12,000g for 5 minutes. Addition of salt and cooling is unnecessary.
5. Pour off the supernatant, add carefully 0.5ml of 70% ethanol to the side of the tube, pour off. Repeat the washing once more. Vacuum dry the pellet and suspend in 100ul of RNase (About 5-10ul of this DNA can now be cleaved with appropriate restriction enzyme(s) for analysis).

Additional Notes
- Starlab LB broth works very well in this protocol
- In step 2, one can pipette 1.5ml of broth spin the microfuge tube, decant 1ml and leave behind 500ul to resuspend the pellet and continue as from step 2. This maximizes the total yield of plasmid.

References

BioStream version

Following is the One step 'miniprep' method for the isolation of plasmid DNA protocol in BioStream, a high-level programming language for expressing biology protocols. What you see here is the auto-generated text output of the protocol that was coded up in BioStream (see Source code). More information about BioStream can be found on my home page. Feel free to mail me your comments/suggestions Vaishnavi.

Text Output
One step 'miniprep' method for the isolation of plasmid DNA protocol

Source Code
One step 'miniprep' method for the isolation of plasmid DNA protocol - source code
Growing a Community

One step 'miniprep' method for the isolation of plasmid DNA protocol

Solutions/reagents:
- overnight E. coli culture
- phenol : chloroform : isoamyl alcohol (25:24:1)
  (phenol saturated with TE(10mM Tris, 7.5, 1mM EDTA) prior to mixing with chloroform and
  isoamyl/alc.</p>
- isopropanol
- 70% ethanol
- 100 μl RNAase

Equipment:
- Centrifuge
- Flasks of appropriate volumes
- Sterile 1.5-ml microcentrifuge tubes

Steps:
1. Measure out 0.5 ml of overnight E. coli culture into a sterile 1.5-ml microcentrifuge tubes.
   We routinely grow our cells in 'standard' bacteriological media supplied by Merck, Germany.
2. Add 0.5 ml of phenol : chloroform : isoamyl alcohol (25:24:1).
3. Vortex the mixture for 1 min.
   Vortex at maximum speed.
   Alternatively, vortex for 10 seconds and then transfer to an Eppendorf mixer or an over-the-top rotator for 5 minutes.
4. Centrifuge at a speed of 12000 Xg for 5 mins at room temperature.
5. Meanwhile:
   Set aside a fresh sterile 1.5-ml microcentrifuge tube. Call it Tube I.
   Measure out 0.5 ml of isopropanol into Tube I.
   Measure out 0.45 ml of top aqueous phase obtained after centrifugation into Tube I.
   Vortex the mixture for a few secs.
   Centrifuge at a speed of 12000 Xg for 5 mins at room temperature, gently aspirate out the supernatant and discard it.
   Addition of salt and cooling is unnecessary.
7. Add 0.5 ml of 70% ethanol.
   Add ethanol carefully to the side of the tube.
   Discard solution.
8. Repeat Step 7: Add 100 μl RNAase to solution.
   Resuspend the pellet by vortexing by shaking vigorously.
   About 5-10ul of this DNA can now be cleaved with appropriate restriction enzyme(s) for analysis.
Health Challenges in India
## Health Challenges in India

<table>
<thead>
<tr>
<th>Deaths in India (expect. 70 years)</th>
<th>Deaths in USA (expect. 78 years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart disease (15%)</td>
<td>Heart disease (26%)</td>
</tr>
<tr>
<td>Lower respiratory infections (11%)</td>
<td>Cancer (23%)</td>
</tr>
<tr>
<td>Cerebrovascular disease (7%)</td>
<td>Stroke (6%)</td>
</tr>
<tr>
<td>Perinatal conditions (7%)</td>
<td>Lower respiratory infections (5%)</td>
</tr>
<tr>
<td>Bronchitis and emphysema (5%)</td>
<td>Accidents (5%)</td>
</tr>
<tr>
<td>Diarrhoeal diseases (4%)</td>
<td>Diabetes (3%)</td>
</tr>
<tr>
<td>Tuberculosis (4%)</td>
<td>Alzheimer's disease (3%)</td>
</tr>
<tr>
<td>HIV/AIDS (3%)</td>
<td>Influenza and pneumonia (2%)</td>
</tr>
</tbody>
</table>

- Half of children are underweight
- Only 1 in 3 have access to improved sanitation such as toilets
- 900,000 die each year from contaminated water, polluted air
- Yet $2B medical tourism industry (doctors sparser in rural areas)

Sources: WHO, CDC
Focus on Tuberculosis

Global TB Statistics
- $4B/yr. is spent on TB control
- 14M patients worldwide
- 9M new cases/yr.
- India has highest burden
  - 3M existing cases
  - 300K deaths/yr.

Tuberculosis in India
- New cases: 1.9M/yr.
- Actively infectious: 850K/yr
- Current reach of care providers: 450K/yr
Challenge: Medication Adherence

- Tuberculosis patients must adhere to a strict drug regimen
  - 4 drugs, 3 days / week, for 6 months

- Consequences of missed doses
  - Not cured
  - Develop drug resistance

- Barriers to adherence:
  - Side effects - Lack of education
  - Stigma - Expense of medicines
  - Travel - Forget / too busy
Directly Observed Therapy (DOT)

- Relies on *providers* to observe each dose
  - Public hospitals, private businesses, traditional healers…

- Protocol
  - Government supplies box of medication for a patient
  - Patient travels to provider
    - 3 times per week (first 2 months)
    - 1 time per week (last 4 months)
  - Provider should fetch patients who miss doses
  - Providers get $5 per “successful outcome”
Cornerstone: TB Treatment Cards

- **Drawbacks**
  - Hard to verify if visits happened
  - Hard to quickly interpret
  - Hard to aggregate

- **Treatment programs operate in the dark**
  - Are drugs reaching patients?
  - Are patients taking medication?
  - Are patients getting better?
A Biometric Terminal for TB Clinics

- For verifying that patient and health worker interacted

- Consists of:
  - Low-cost netbook
  - Fingerprint reader
  - Low-cost cell phone for data upload

- Usage model:
  - Patient scans fingerprint upon each visit to the clinic
  - At the end of the day, visit logs uploaded over SMS
  - Data visualized by supervisors at central offices

- Benefits:
  - Immediate response to missed doses
  - Incentives for workers, accountability to donors
  - Estimated cost: < $2 / patient
Initial Trials in Tuberculosis Clinics
with Innovators In Health & Operation ASHA in Delhi, October 2009

- 4-day trial with 30 patients
- Overwhelmingly positive response
- Refinements:
  - Don’t use thumb print
  - Add incentives for providers, who sometimes relied on intermediaries to deliver drugs
- Larger deployment in clinics planned for Spring 2010
Extension to HIV/AIDS Clinics
by Julie Weber (U. Michigan) with Swathi Mahila Sangha

- **Project Pragati**
  - Promotes health of 16,500 sex workers in Bangalore
  - Via education, medical assistance, drop-in facilities

- **Challenges with records**
  - Inconsistent ID from visitors
  - Managing paperwork

- **Biometrics deployed for two months**
  - In 2 clinics; hundreds of patients and thousands of visits
  - About 1% of patients unable to register
  - Recognition speed is a challenge at scale (10s / 100 patients)
Recurring theme: Automation may not be cheaper or better

- Example: mobile data collection
  - Lots of excitement about using mobile phones to collect data

- Benefits of using a *live operator*?
  - Lowest error rate
  - Less education and training needed
  - Most flexible interface
  - Surprisingly cost effective!

- Research opportunity: incorporate more, rather than fewer, human actors
Conclusions: Technologies for Healthcare Delivery

- Philosophy: identify technical areas that have particular impact on the developing world
  - In microfluidics, technology research may be bottleneck to impact
  - In computer technology, bottleneck is often in the application
  - Opportunity: matching the technology with socio-cultural context