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Factorization Based Dilution of Biochemical Fluids with Micro-Electrode-Dot-Array Biochips

Sohini Saha (IIEST, Shibpur, India), Debraj Kundu, Sudip Roy (IIT Roorkee, India),
Sukanta Bhattacharjee (New York University Abu Dhabi, UAE), Krishnendu Chakrabarty (Duke Univ., U.S.A.),
Partha P. Chakrabarti (IIT Kharagpur, India), Bhargab B. Bhattacharya (ISI Kolkata, India)

Session 6C: New Trends in Biochips

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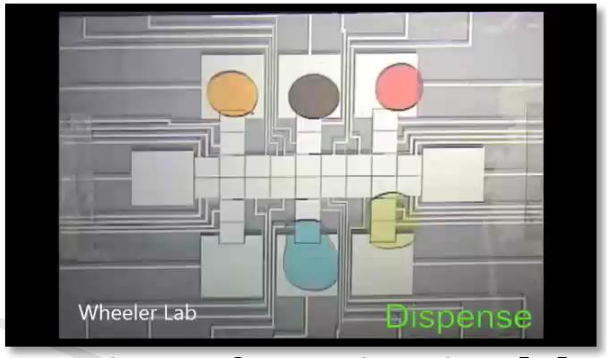
Outline

- Introduction to Microfluidic Biochips
- Sample Preparation using Microfluidic Biochips
- Proposed Approach: *FacDA*
- Conclusions

Introduction: Microfluidic Biochips

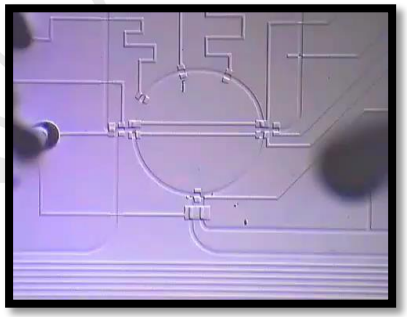
- Microfluidic biochips manipulate sub-milliliter scale volumes of fluid to perform different bioassays

- Microfluidic biochips are broadly classified as:
  - Digital Microfluidic biochips (DMF):
    - Electrowetting-on-dielectric (EWOD) based technology
    - Manipulates droplets of fluids



Working of DMF biochip [1]

  - Continuous-Flow Microfluidic biochips (CMF):
    - Micro-pump and micro-valve based technology
    - Manipulates fluids in micro-channels



Working of CMF biochip [2]

Sample Preparation: Automation and Optimization

- It refers to the generation of droplets of a particular concentration factor (*CF*) of a sample fluid OR volumetric ratio of some fluids
- In many biological assays, it is an inherent step that needs longer time using macroscopic biological systems
- Dilution: a particular biological sample and a buffer are involved
- Mixing: multiple sample and reagent fluids are involved
- **Inputs:** sample (S, 100% conc.), buffer (B, 0% conc.), target-concentration (OR volumetric ratio) C_t, concentration error-tolerance ($\mathcal{E}$), mixing volume constraint (*M*)
- **Objectives:** minimize sample or reagent count (n_s), waste count (n_w), mixing steps (n_m)

Sample Preparation using DMF and CMF

- Several algorithms proposed for both DMF and CMF biochips
- Different algorithms have different optimization criteria
- Dilution algorithms for CMF can be used for DMF as well, if (*n* : *n*) mixing model is followed
- Sample preparation algorithms:

DMF

- *twoWayMix* [2]
- *DMRW* [3]
- *GORMA* [4]
- *NFSP* [5]

CMF

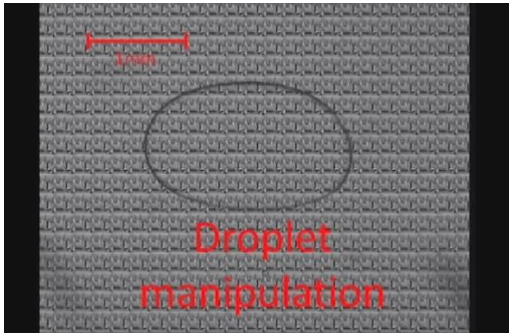
- *NWayMix* [2]
- *TPG* [6]
- *VOSPA* [7]
- *FloSPA* [8]

Properties	DMF	CMF
Mixing model	(1:1)	Depends on the size of the mixer
Reconfigurability	Yes	No
Mixing time	Relatively more	Less

Micro-Electrode-Dot-Array (MEDA) DMF

- MEDA-DMF [9, 10] is EWOD-based, sea-of-microelectrodes technology

- Many advantages over DMF and CMF:
  - Manipulates droplets with higher granularity
  - More reconfigurable on-chip components
  - Addition droplet routability and more flexibility in droplet-shape
  - Equipped with in-built sensors and hence suitable for cyber-physical error management



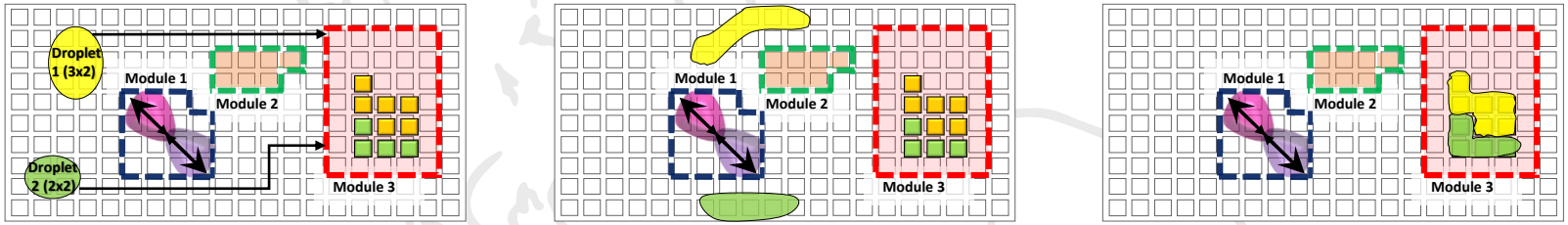
Working of MEDA biochip [9]

- New EDA tools needed for mapping biochemical assays on MEDA-DMF

- Sample preparation with MEDA-DMF possesses new challenges

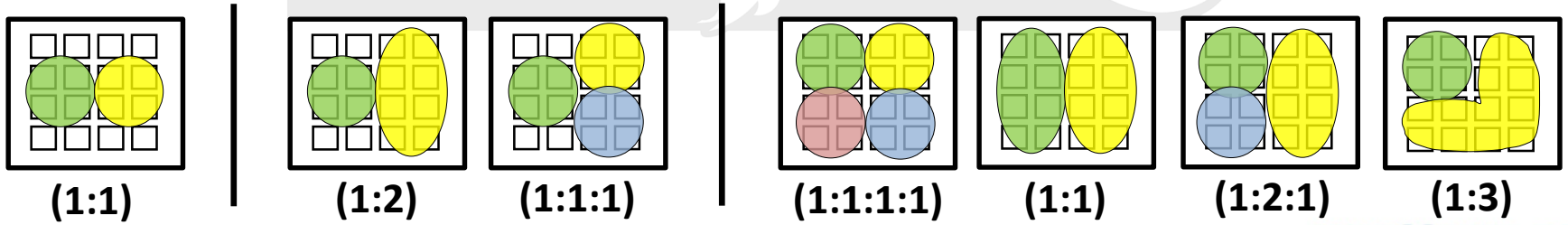
Sample Preparation in MEDA

- Advantages of sample preparation in MEDA-DMF biochips:
  - Significant reduce in mixing time due to SAR (split-and-rotate) based laminar mixing
  - More mixing models that can reduce mixing steps/time
  - More reliable sample preparation due to integrated sensors with each micro-electrode



Fluid flow on MEDA

- Mixing models with a mixing volume constraint, $M = 4$ for MEDA



Previous Work

- ***WSPM* [11]:** – Dilution algorithm exploiting various mixing models of MEDA

  Weighted Sample Preparation Method

  – *WSPM* works as follows:
    - Generates a set of primary droplets (*PD*s) of intermediate *CF*s
    - Determines dilution graph for all *PD*s
    - Obtain minimum cost dilution graph

- ***FloSPA-D* [8]**: – Dilution algorithm for CMF biochip that can be tuned for MEDA

  Flow-based Sample Preparation Algorithm for Dilution

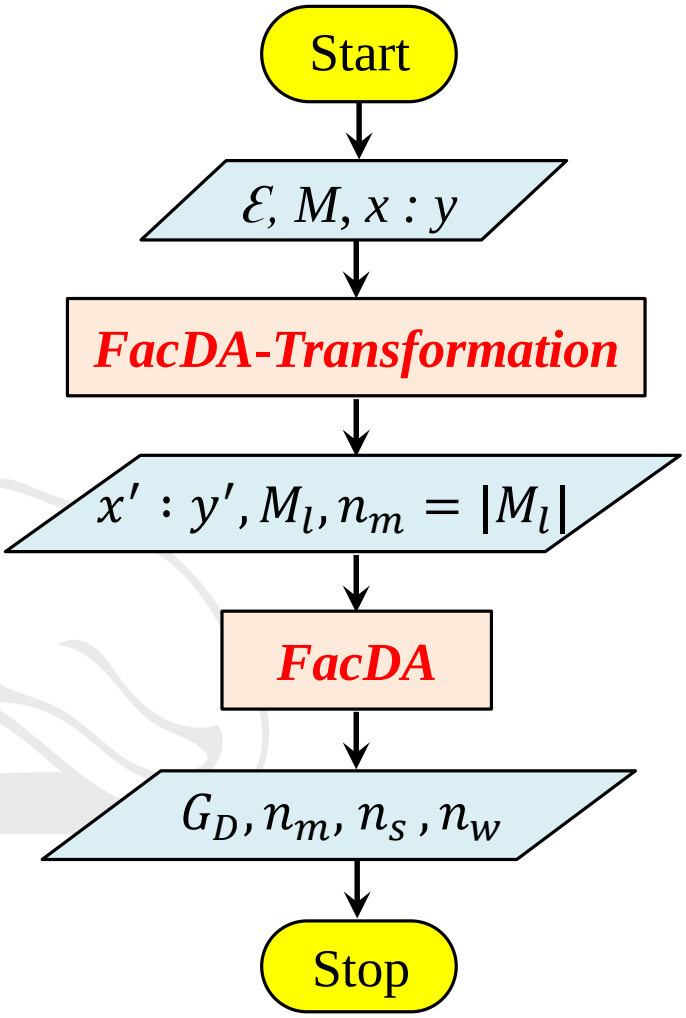
  – Mixing tree with minimum number of mixing operations generated by *NWayMix* [8]

  – SMT-based approach reduces the sample or reagent count, n_s

Proposed Approach

- **Fac**torization based **D**ilution **A**lgorithm for MEDA-DMF biochip (***FacDA***)

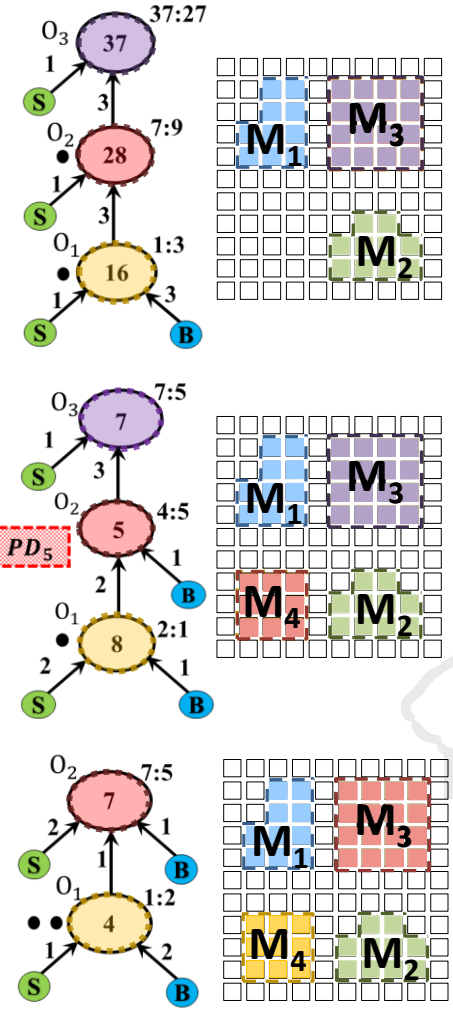
  - Exploits all possible mixing models for a mixer of size *M*
  - Assures a dilution graph with minimum height that reduces mixing steps/time, n_m
  - Uses a Satisfiability Modulo Theories (SMT) based approach to reduce the sample or reagent count, n_s



Motivating Example

$C_t = \frac{7}{12}$, $\mathcal{E} = 0.02$, $M = 4$, transformed $C_t = \frac{37}{64}$

● : waste droplet



Previous Operations

Time

$A_1 \rightarrow M_1$

$A_2 \rightarrow M_2$

$A_3 \rightarrow M_3$

FloSPA-D

WSPM

FacDA

Module	Size	*M*
M_1	10	3
M_2	10	3
M_3	16	4
M_4	9	3

FacDA-Transformation

Problem Formulation:

Inputs: Target ratio *sample* : *buffer* = x : y, where $x + y = N$, concentration error-tolerance $\mathcal{E}$, mixing volume constraint M.

Output: Transformed ratio x' : y', feasible mixer-set (M_ℓ).

- Find all possible factors of N
- Sort the factor sets in non-increasing order of set-cardinalities
- Choose the first factor set whose every element is less than M, in many cases this will give us an exact solution

$x : y = 233:7,$
$\epsilon = 0.000005,$
$M = 5$

240 = { …, 12*4*5, … , 2*4*5*6, 3*4*4*5, … }

× × ✓ M_ℓ

- If no such factor set exists, then approximate the given ratio maintaining the error-tolerance by increasing N and repeating the above steps

FacDA

- Order of the factors in the final mixer-set decided by a greedy approach called as *Division-by-Factor* method
- A set of constraints is imposed on the skeleton of the final dilution graph and a *SMT-based approach* reuses the fluids of intermediate *CF*s

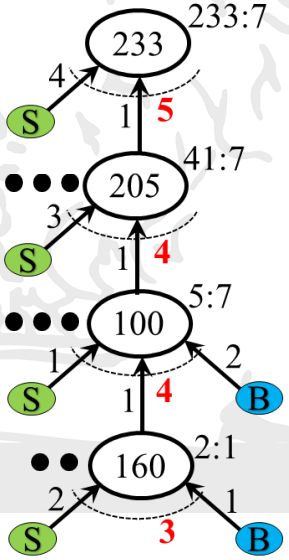
$$C_t = \frac{233}{240}$$

$$M_\ell = \{3, 4, 4, 5\}$$

Factor ($\in M_l$)	Reagent		Target Ratio	
	#S	#B	233	7
3	2	1	77	2
4	1	2	19	0
4	3	0	4	0
5	4	0	0	0

Remainder Quotient

Total = 10

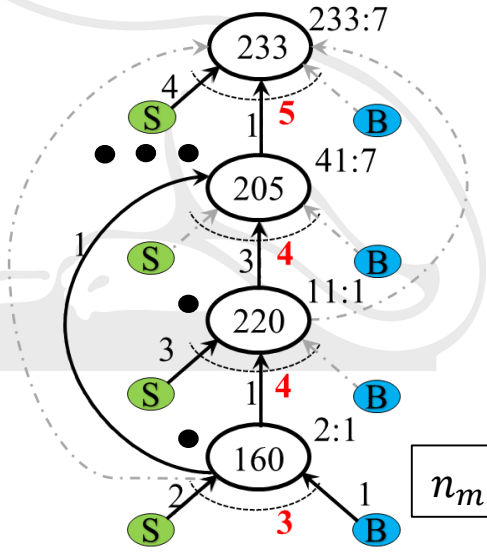


$n_m = 4,\ n_s = 10,\ n_w = 8$

Division-by-Factor

$$X_i = \frac{\frac{X_{i+1}}{\prod_{j=d}^{i+1} f_j} + k + (\dots)}{f_i} = \frac{X_{i+1} + k \prod_{j=d}^{i+1} f_j + (\dots)}{\prod_{j=d}^{i} f_j}$$

$$X_i = \frac{\frac{X_{i+1}}{\prod_{j=d}^{i+1} f_j} + k \frac{X_p}{\prod_{j=d}^{p} f_j} + (\dots)}{f_i} = \frac{X_{i+1} + k X_p \prod_{j=p-1}^{i+1} f_j + (\dots)}{\prod_{j=d}^{i} f_j}$$



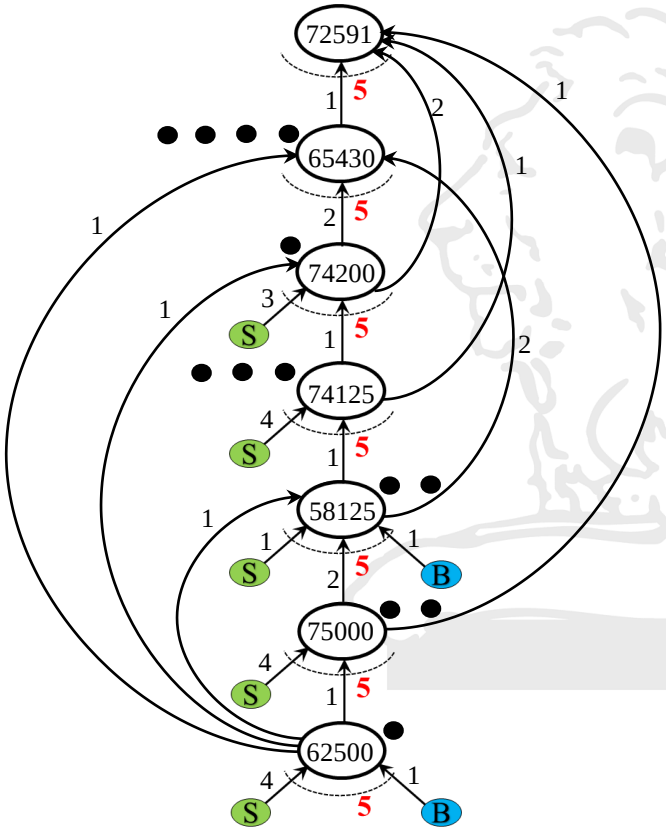
$n_m = 4,\ n_s = 9,\ n_w = 5$

SMT-based Approach

Comparative Example

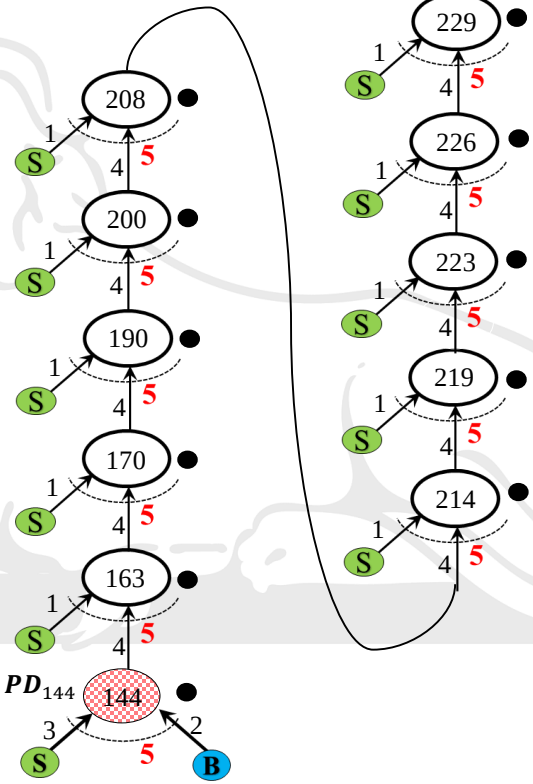
$C_t = \frac{233}{240}$, $\mathcal{E} = 0.000005$, $M = 5$, transformed $C_t = \frac{72591}{5^7}$ ● : waste droplet

S = {78125/5^7}, B = {0/5^7}
n_m = 7
n_s = 16, n_w = 13



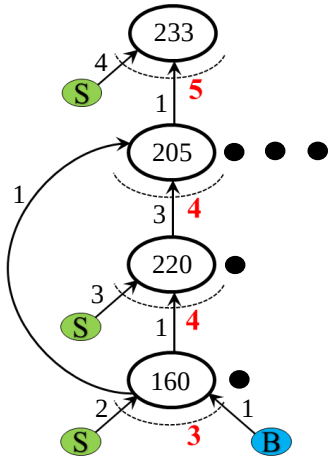
FloSPA-D

S = {240/240}
B = {0/240}
n_m = 13
n_s = 15, n_w = 12



WSPM

S = {240/240}
B = {0/240}
n_m = 4
n_s = 9, n_w = 5



FacDA

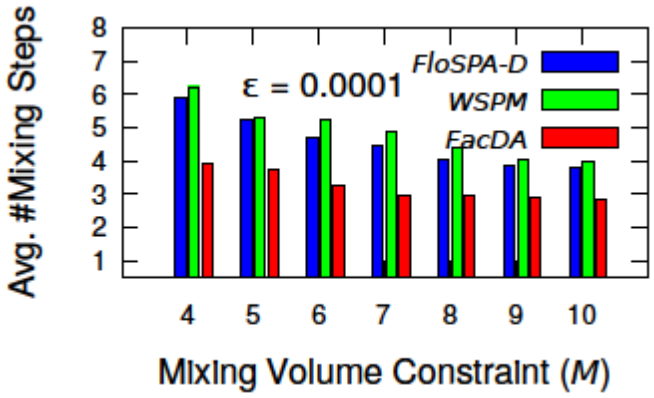
Simulation Results

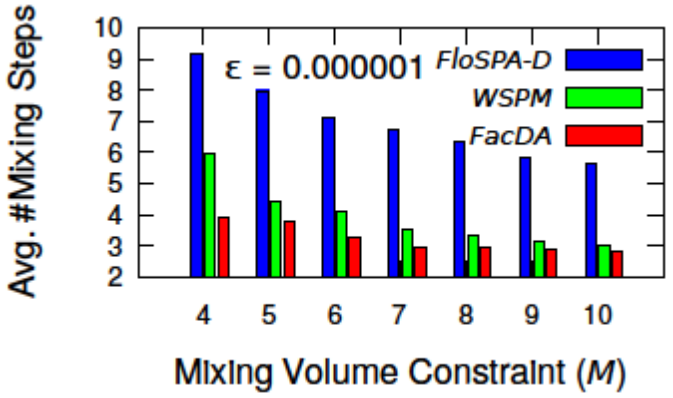
- A set of total 19,947 unique target ratios of *sample* and *buffer* considered, where the ratio-sum varies between 2 and 256

(i.e., 1 : 1, 1 : 2, 2 : 1, 1 : 3, 3 : 1, 1 : 4, 2 : 3, 3 : 2, 4 : 1, … , 1 : 255, … , 255 : 1)

2 3 4 5 … 256

- *M* varies in the range of 4 to 10
- Average number of mixing steps compared among three methods



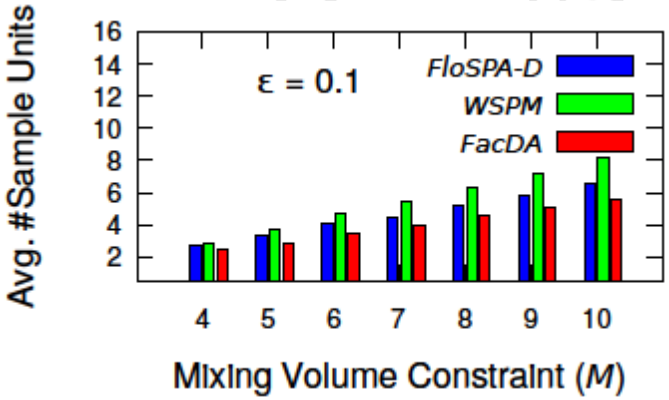


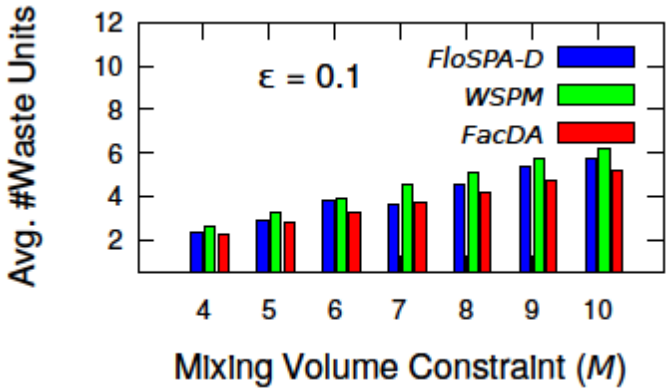
Distributions of avg. #mixing steps for three algorithms with $\mathcal{E}$ = 0.0001 and $\mathcal{E}$ = 0.000001

Simulation Results

- Target volume V_t is one of the input parameters for *WSPM* [11]
- Average sample and waste count compared for two cases:
  - I. V_t = 2
  - II. V_t > 2 (final volume of target concentration is same as of *FacDA*)

- Case I: V_t = 2

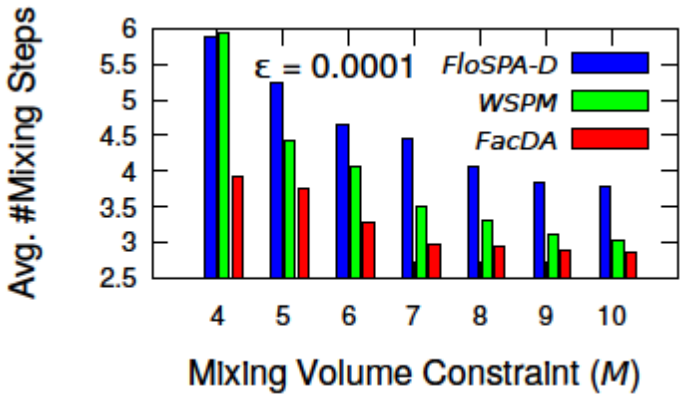


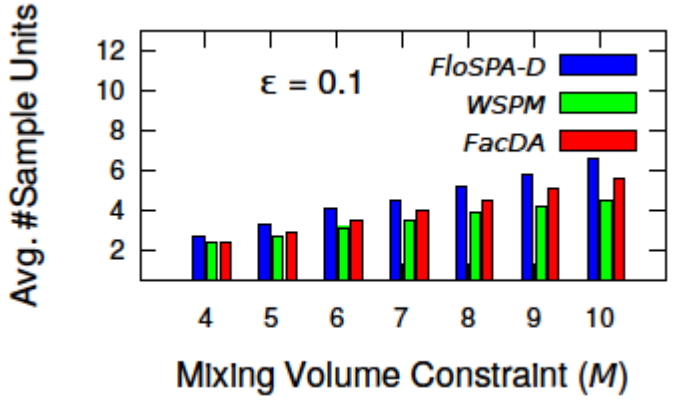


Distributions of avg. #*sample units* and avg. #*waste units* by three algorithms with $\mathcal{E}$ = 0.1

Simulation Results

- Case II: $V_t > 2$





Distributions of n_m and n_s, when V_t is same as the output volume by *FacDA* with $\mathcal{E}$ = 0.0001 and $\mathcal{E}$ = 0.1

- Average CPU time comparison among three algorithms, where *M* varies in the range of 4 to 8 and $\mathcal{E}$ = 0.05

Algorithm	Avg. CPU time (in seconds)				
	***M* = 4**	***M* = 5**	***M* = 6**	***M* = 7**	***M* = 8**
FloSPA-D	0.0173	0.0288	0.0231	0.0166	0.0126
WSPM	0.0085	0.0320	0.0732	0.1708	0.2816
FacDA	0.0745	0.0919	0.0505	0.0253	0.0267

Simulation Results

- Comparison between *FloSPA-D* [8] and *FacDA* for the number of test-cases without ratio transformation
- Considered a subset of the target ratio set that includes the ratios whose ratio-sum lies between M^{d-1} to M^{d}

ε	M	#Test-cases	#Test-cases without transformation by *FloSPA-D*	#Test-cases without transformation by *FacDA*	% Improvement of *FacDA* over *FloSPA-D*
0.001	3	2914	105	193	29.53
	4	30624	255	1069	61.4
	5	187250	625	4762	76.82
	6	250042	0	9540	100.0
0.0001	3	2914	105	193	29.53
	4	30624	591	1069	28.79
	5	187250	776	4762	71.97
	6	250042	255	9540	94.79

Conclusions

- Sample preparation on MEDA-DMF based architecture has many new scopes due to the availability of wide range of mixer models
- Unlike the conventional approach of (m_1 : m_2) here we assume the mixer models to be (m_1 : m_2 : … : m_n) for n distinct input fluids in case of MEDA-DMF
- *FacDA* manages to exploit the architectural advantages of MEDA-DMF and assures a dilution graph with minimum height
- The theory behind earlier methods *twoWayMix* and *FloSPA-D* follows as corollary to *FacDA*
- As a future work mixing algorithm can be designed for MEDA-DMF

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Thank you for your attention!