Details of Experimental Setup

(Refer to Table IV, Table V, and Table VI of the paper)
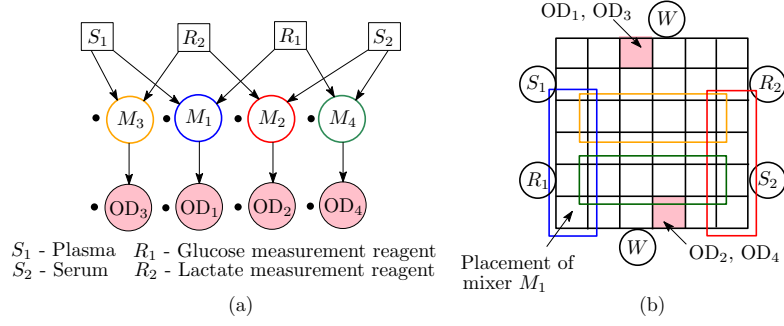
Contents

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1 In-vitro multiplexed assay

Figure A.1: (a) Sequencing graph of the in-vitro multiplexed assay ($s = 2, r = 2$) (b) Old DMF platform of size 6×6 and the corresponding mixer placements.

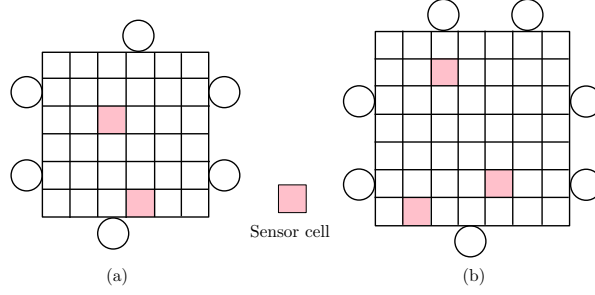

Figure A.2: Enhanced DMFB platform of dimensions (a) 6×6, and (b) chip of size 7×7 with sensors.
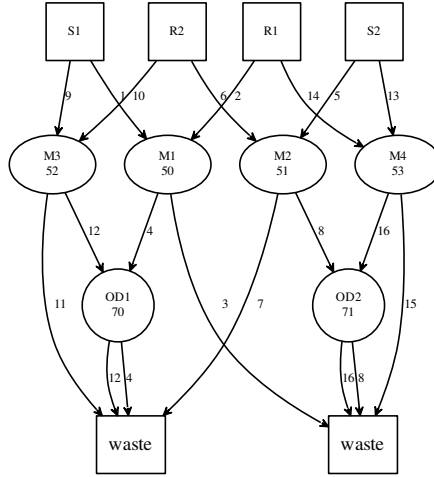

Figure A.3: Sequencing graph extracted from old actuation sequence using *SimBioSys* and assignment of $\mathcal{ID}$s to routing paths, mixers, and sensors.

Table A.1: Routing paths extracted from the old actuation sequence.

Routing paths	
Path ID	Path
$\mathcal{P}_1$	$[(2, 1), (3, 1)]$
$\mathcal{P}_2$	$[(5, 1), (6, 1)]$
$\mathcal{P}_3$	$[(6, 1), (6, 2), (6, 3)]$
$\mathcal{P}_4$	$[(3, 1), (3, 2), (2, 2), (1, 2), (1, 3), (1, 4)]$
$\mathcal{P}_5$	$[(5, 6), (6, 6)]$
$\mathcal{P}_6$	$[(2, 6), (3, 6)]$
$\mathcal{P}_7$	$[(3, 6), (3, 5), (3, 4), (2, 4), (1, 4)]$
$\mathcal{P}_8$	$[(6, 6), (6, 5), (6, 4), (6, 3)]$
$\mathcal{P}_9$	$[(2, 1), (3, 1), (3, 2)]$
$\mathcal{P}_{10}$	$[(2, 6), (3, 6), (3, 5)]$
$\mathcal{P}_{11}$	$[(3, 5), (2, 5), (1, 5), (1, 4)]$
$\mathcal{P}_{12}$	$[(3, 2), (2, 2), (1, 2), (1, 3), (1, 4)]$
$\mathcal{P}_{13}$	$[(5, 6), (5, 5)]$
$\mathcal{P}_{14}$	$[(5, 1), (5, 2)]$
$\mathcal{P}_{15}$	$[(5, 2), (6, 2), (6, 3)]$
$\mathcal{P}_{16}$	$[(5, 5), (6, 5), (6, 4), (6, 3)]$

Table A.2: Mixer and detector locations and their types extracted from the old actuation sequence.

Mixers		
Mixer ID	Mixer Type	Mixer location
50	4×1	$[(3, 1), (6, 1)]$
51	4×1	$[(3, 6), (6, 6)]$
52	1×4	$[(3, 2), (3, 5)]$
53	1×4	$[(5, 2), (5, 5)]$
Detectors		
Detector ID	Detector Type	Detector location
70	Optical	$(1, 3)$
71	Optical	$(6, 4)$

2 Dilution of a sample using *REMIA*

R_1 - Sample
R_2 - Buffer
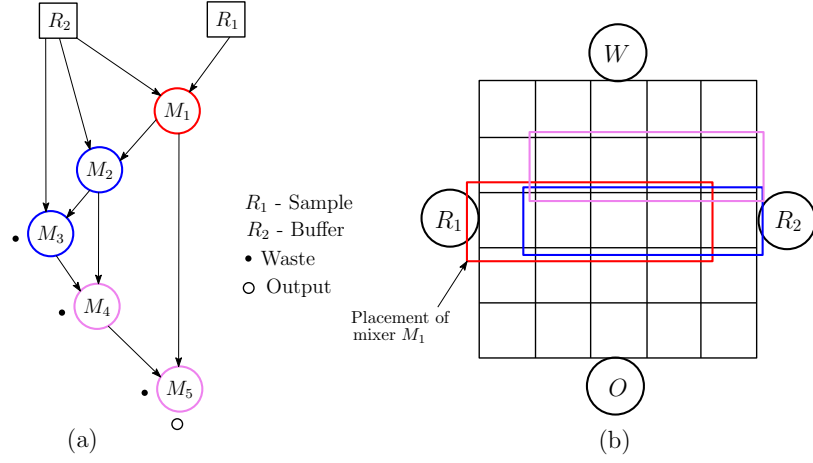• Waste
○ Output

Placement of mixer M_1

(a)

(b)

Figure A.4: (a) Sequencing graph of the concentration factor $\left(\frac{11}{32}\right)$ using *REMIA* (b) Old DMF platform of size 5×5 and the corresponding mixer placements.
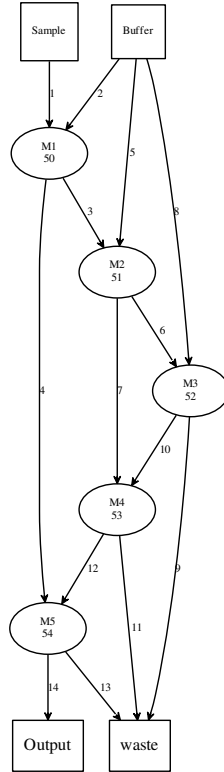

Figure A.5: Sequencing graph extracted from old actuation sequence using *SimBioSys* and assignment of $\mathcal{ID}$s to extracted routing paths and mixers.

Table A.3: Routing paths extracted from the old actuation sequence.

Routing paths	
Path ID	Path
$\mathcal{P}_1$	$[(3,1)]$
$\mathcal{P}_2$	$[(3,5),(3,4)]$
$\mathcal{P}_3$	$[(3,1),(3,2)]$
$\mathcal{P}_4$	$[(3,4),(4,4),(5,4),(5,3),(5,2),(4,2),(3,2),(2,2)]$
$\mathcal{P}_5$	$[(3,5)]$
$\mathcal{P}_6$	$[(3,2)]$
$\mathcal{P}_7$	$[(3,5),(4,5),(5,5),(5,4),(4,4),(3,4),(2,4),(2,5)]$
$\mathcal{P}_8$	$[(3,5)]$
$\mathcal{P}_9$	$[(3,5),(2,5),(1,5),(1,4),(1,3)]$
$\mathcal{P}_{10}$	$[(3,2),(2,2)]$
$\mathcal{P}_{11}$	$[(2,2),(1,2),(1,3)]$
$\mathcal{P}_{12}$	$[(2,5)]$
$\mathcal{P}_{13}$	$[(2,2),(1,2),(1,3)]$
$\mathcal{P}_{14}$	$[(2,5),(3,5),(4,5),(5,5),(5,4),(5,3)]$

Table A.4: Mixer locations and types extracted from the old actuation sequence.

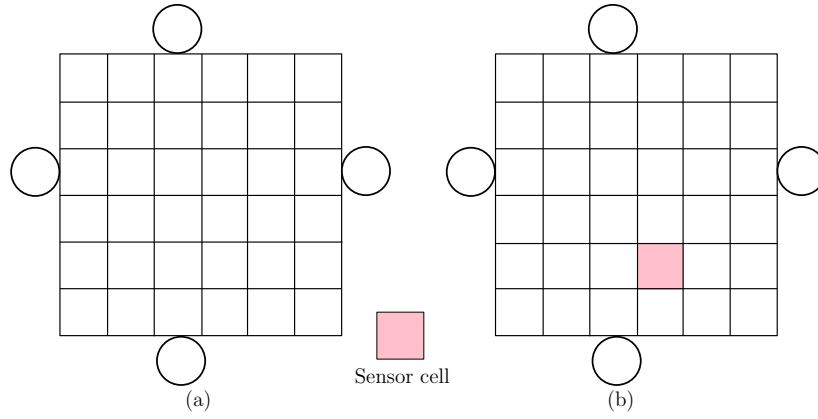| Mixers		
Mixer ID	Mixer Type	Mixer location
50	1×4	$[(3,1),(3,4)]$
51	1×4	$[(3,2),(3,5)]$
52	1×4	$[(3,2),(3,5)]$
53	1×4	$[(2,2),(2,5)]$
54	1×4	$[(2,2),(2,5)]$

Figure A.6: Enhanced DMFB platform of dimensions (a) 6×6, and (b) chip of size 6×6 with sensor.
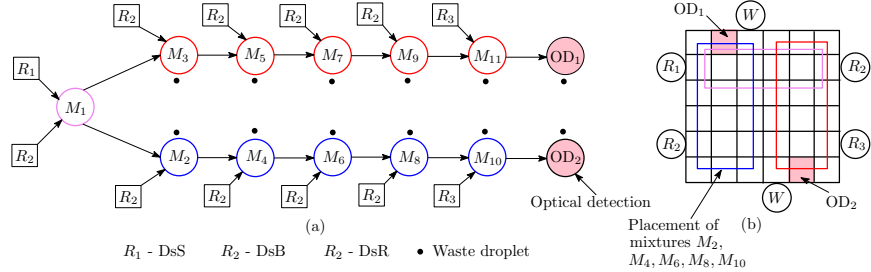
3 Protein assay

Figure A.7: (a) Sequencing graph of the protein assay (b) Old DMF platform of size 6×6 and the corresponding mixer and sensor placements.
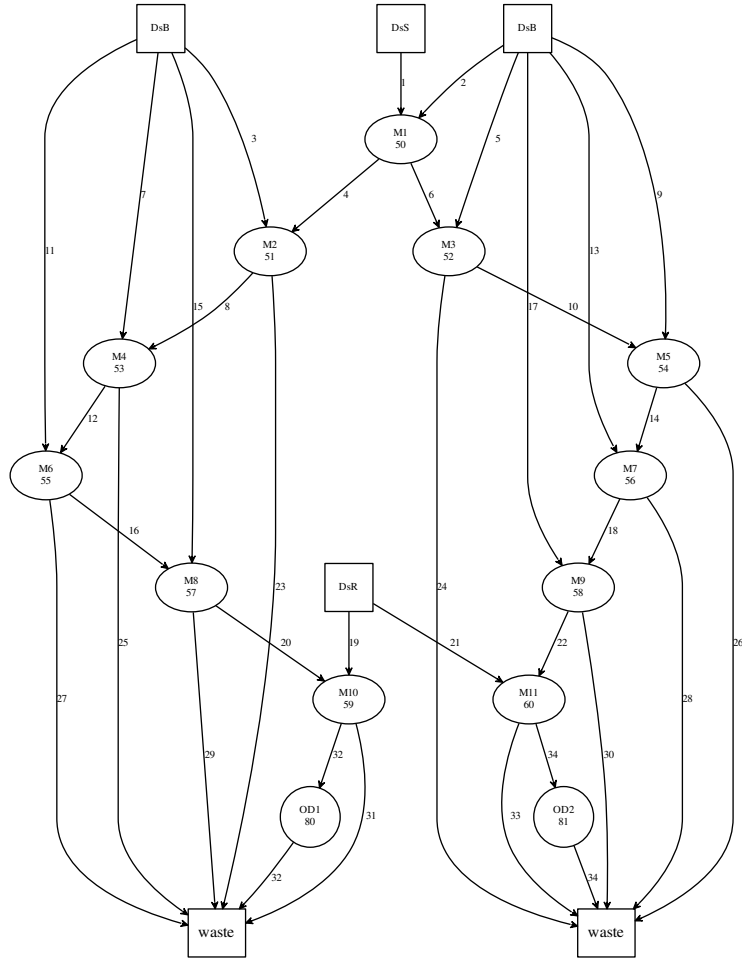

Figure A.8: Sequencing graph extracted from old actuation sequence using *SimBioSys* and assignment of $\mathcal{ID}$s to routing paths, sensors, and mixers.

Table A.5: Routing paths extracted from the old actuation sequence.

Path ID	Path
$\mathcal{P}_1$	$[(2,1),(2,2)]$
$\mathcal{P}_2$	$[(2,6),(2,5)]$
$\mathcal{P}_3$	$[(5,1),(5,2)]$
$\mathcal{P}_4$	$[(2,2)]$
$\mathcal{P}_5$	$[(2,6),(2,5)]$
$\mathcal{P}_6$	$[(2,5),(3,5),(4,5),(5,5)]$
$\mathcal{P}_7$	$[(5,1),(5,2)]$
$\mathcal{P}_8$	$[(5,2),(4,2),(3,2),(2,2)]$
$\mathcal{P}_9$	$[(2,6),(2,5)]$
$\mathcal{P}_{10}$	$[(2,5),(3,5),(4,5),(5,5)]$
$\mathcal{P}_{11}$	$[(5,1),(5,2)]$
$\mathcal{P}_{12}$	$[(5,2),(4,2),(3,2),(2,2)]$
$\mathcal{P}_{13}$	$[(2,6),(2,5)]$
$\mathcal{P}_{14}$	$[(2,5),(3,5),(4,5),(5,5)]$
$\mathcal{P}_{15}$	$[(5,1),(5,2)]$
$\mathcal{P}_{16}$	$[(5,2),(4,2),(3,2),(2,2)]$
$\mathcal{P}_{17}$	$[(2,6),(2,5)]$
$\mathcal{P}_{18}$	$[(2,5),(3,5),(4,5),(5,5)]$
$\mathcal{P}_{19}$	$[(5,6),(5,5),(5,4),(5,3),(5,2)]$
$\mathcal{P}_{20}$	$[(5,2),(4,2),(3,2),(2,2)]$
$\mathcal{P}_{21}$	$[(5,6),(5,5)]$
$\mathcal{P}_{22}$	$[(2,5)]$
$\mathcal{P}_{23}$	$[(2,2),(1,2),(1,3)]$
$\mathcal{P}_{24}$	$[(5,5),(6,5),(6,4)]$
$\mathcal{P}_{25}$	$[(2,2),(1,2),(1,3)]$
$\mathcal{P}_{26}$	$[(5,5),(6,5),(6,4)]$
$\mathcal{P}_{27}$	$[(2,2),(1,2),(1,3)]$
$\mathcal{P}_{28}$	$[(5,5),(6,5),(6,4)]$
$\mathcal{P}_{29}$	$[(2,2),(1,2),(1,3)]$
$\mathcal{P}_{30}$	$[(5,5),(6,5),(6,4)]$
$\mathcal{P}_{31}$	$[(5,2),(4,2),(3,2),(2,2),(1,2),(1,3)]$
$\mathcal{P}_{32}$	$[(2,2),(1,2),(1,3)]$
$\mathcal{P}_{33}$	$[(2,5),(3,5),(4,5),(5,5),(6,5),(6,4)]$
$\mathcal{P}_{34}$	$[(5,5),(6,5),(6,4)]$

Table A.6: Mixer and detector locations and types extracted from the old actuation sequence.

Mixers		
Mixer ID	Mixer Type	Mixer location
50	1×4	$[(2,2),(2,5)]$
51	4×1	$[(2,2),(5,2)]$
52	4×1	$[(2,5),(5,5)]$
53	4×1	$[(2,2),(5,2)]$
54	4×1	$[(2,5),(5,5)]$
55	4×1	$[(2,2),(5,2)]$
56	4×1	$[(2,5),(5,5)]$
57	4×1	$[(2,2),(5,2)]$
58	4×1	$[(2,5),(5,5)]$
59	4×1	$[(2,2),(5,2)]$
60	4×1	$[(2,5),(5,5)]$

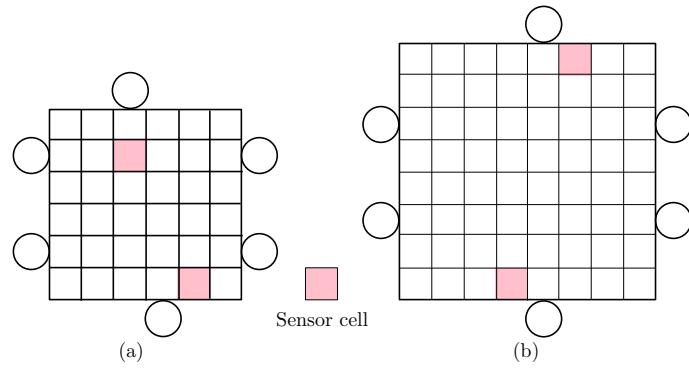| Detectors		
Detector ID	Detector Type	Detector location
80	Optical	$(1,2)$
81	Optical	$(6,5)$

Figure A.9: Enhanced DMFB platform of dimensions (a) 6×6, and (b) chip of size 8×8 with sensors.
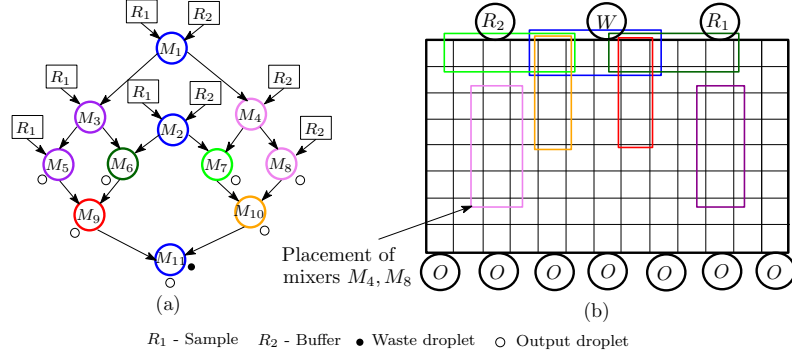
4 Linear dilution gradient

Figure A.10: (a) Sequencing graph of the linear dilution gradients (b) Old DMF platform of size 8×13 and the corresponding mixer placements.
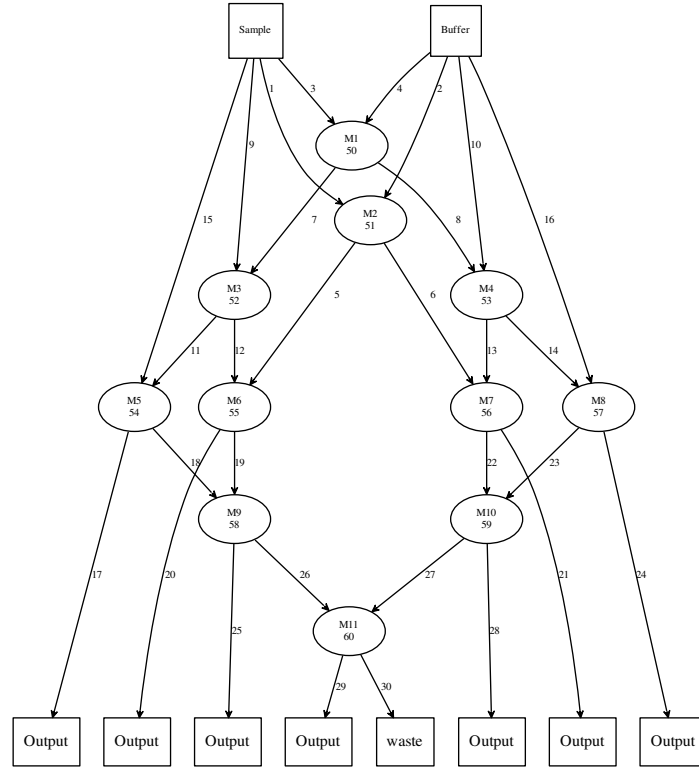

Figure A.11: Sequencing graph extracted from old actuation sequence using *SimBioSys* and assignment of $\mathcal{ID}$s to extracted routing paths and mixers.

Table A.7: Routing paths extracted from the old actuation sequence.

Routing paths	
Path ID	Path
$\mathcal{P}_1$	$[(1, 11), (1, 10), (1, 9), (1, 8)]$
$\mathcal{P}_2$	$[(1, 3), (1, 4), (1, 5)]$
$\mathcal{P}_3$	$[(1, 11), (1, 10), (1, 9), (1, 8)]$
$\mathcal{P}_4$	$[(1, 3), (1, 4), (1, 5)]$
$\mathcal{P}_5$	$[(1, 8)]$
$\mathcal{P}_6$	$[(1, 5)]$
$\mathcal{P}_7$	$[(1, 8), (1, 9), (1, 10), (1, 11), (2, 11), (3, 11)]$
$\mathcal{P}_8$	$[(1, 5), (1, 4), (1, 3), (2, 3), (3, 3)]$
$\mathcal{P}_9$	$[(1, 11), (2, 11), (3, 11), (4, 11), (5, 11), (6, 11)]$
$\mathcal{P}_{10}$	$[(1, 3), (2, 3), (3, 3), (4, 3), (5, 3), (6, 3)]$
$\mathcal{P}_{11}$	$[(6, 11)]$
$\mathcal{P}_{12}$	$[(3, 11), (3, 12), (3, 13), (2, 13), (1, 13), (1, 12), (1, 11)]$
$\mathcal{P}_{13}$	$[(3, 3), (3, 2), (3, 1), (2, 1), (1, 1), (1, 2)]$
$\mathcal{P}_{14}$	$[(6, 3)]$
$\mathcal{P}_{15}$	$[(1, 11), (2, 11), (3, 11)]$
$\mathcal{P}_{16}$	$[(1, 3), (2, 3), (3, 3)]$
$\mathcal{P}_{17}$	$[(6, 11), (7, 11), (8, 11)]$
$\mathcal{P}_{18}$	$[(3, 11), (3, 10), (3, 9), (4, 9), (4, 8)]$
$\mathcal{P}_{19}$	$[(1, 8)]$
$\mathcal{P}_{20}$	$[(1, 11), (2, 11), (3, 11), (4, 11), (5, 11), (6, 11), (7, 11), (8, 11), (8, 10), (8, 9)]$
$\mathcal{P}_{21}$	$[(1, 2), (2, 2), (3, 2), (4, 2), (5, 2), (6, 2), (6, 3), (7, 3), (7, 4), (7, 5), (8, 5)]$
$\mathcal{P}_{22}$	$[(1, 5)]$
$\mathcal{P}_{23}$	$[(3, 3), (3, 4), (3, 5), (4, 5)]$
$\mathcal{P}_{24}$	$[(6, 3), (7, 3), (8, 3), (8, 2), (8, 1)]$
$\mathcal{P}_{25}$	$[(4, 8), (5, 8), (5, 9), (5, 10), (5, 11), (6, 11), (7, 11), (8, 11)]$
$\mathcal{P}_{26}$	$[(1, 8)]$
$\mathcal{P}_{27}$	$[(1, 5)]$
$\mathcal{P}_{28}$	$[(4, 5), (5, 5), (5, 4), (5, 3), (6, 3), (7, 3), (8, 3)]$
$\mathcal{P}_{29}$	$[(1, 8), (1, 7), (2, 7), (3, 7), (4, 7), (5, 7), (6, 7), (7, 7), (8, 7)]$
$\mathcal{P}_{30}$	$[(1, 5), (1, 6), (1, 7)]$

Table A.8: Mixer locations and types extracted from the old actuation sequence.

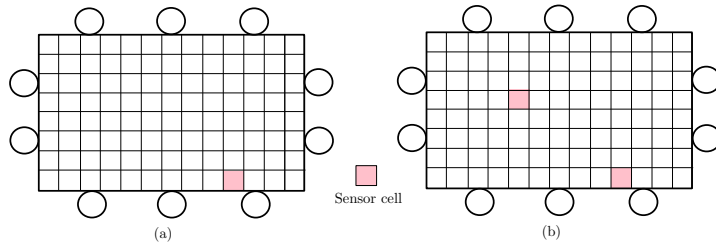| Mixers		
Mixer ID	Mixer Type	Mixer location
50	1×4	$[(1, 5), (1, 8)]$
51	4×1	$[(1, 5), (1, 8)]$
52	4×1	$[(3, 11), (6, 11)]$
53	4×1	$[(3, 3), (6, 3)]$
54	4×1	$[(3, 11), (6, 11)]$
55	1×4	$[(1, 8), (1, 11)]$
56	1×4	$[(1, 2), (1, 5)]$
57	4×1	$[(3, 3), (6, 3)]$
58	4×1	$[(1, 8), (4, 8)]$
59	4×1	$[(1, 5), (4, 5)]$
60	1×4	$[(1, 5), (1, 8)]$

Figure A.12: Enhanced DMFB platform of dimensions (a) 8×13, and (b) chip of size 8×13 with sensors.

5 PCR mixing and streaming of PCR mixture droplet

5.1 PCR mixing

R_1 - Reactant buffer R_2 - dNTP R_3 - Forward primer R_4 - Reverse primer R_5 - DNA template R_6 - Optimase R_7 - Water

$R_1 : R_2 : R_3 : R_4 : R_5 : R_6 : R_7 = 2 : 1 : 1 : 1 : 1 : 1 : 9$
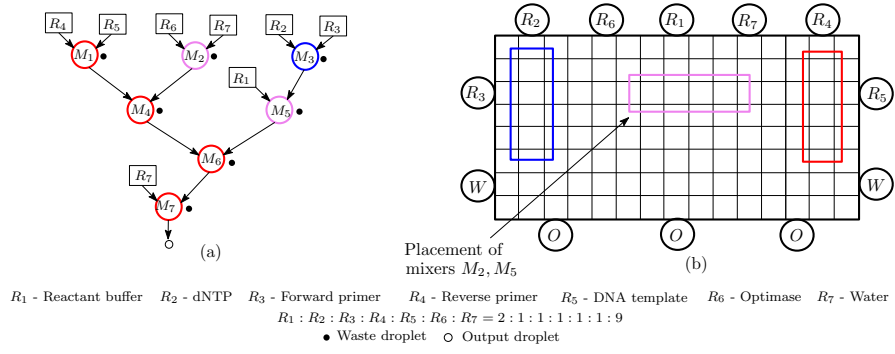
● Waste droplet   ○ Output droplet

Figure A.13: (a) Sequencing graph of the PCR mixture (b) Old DMF platform of size 8×15 and the corresponding mixer placements.
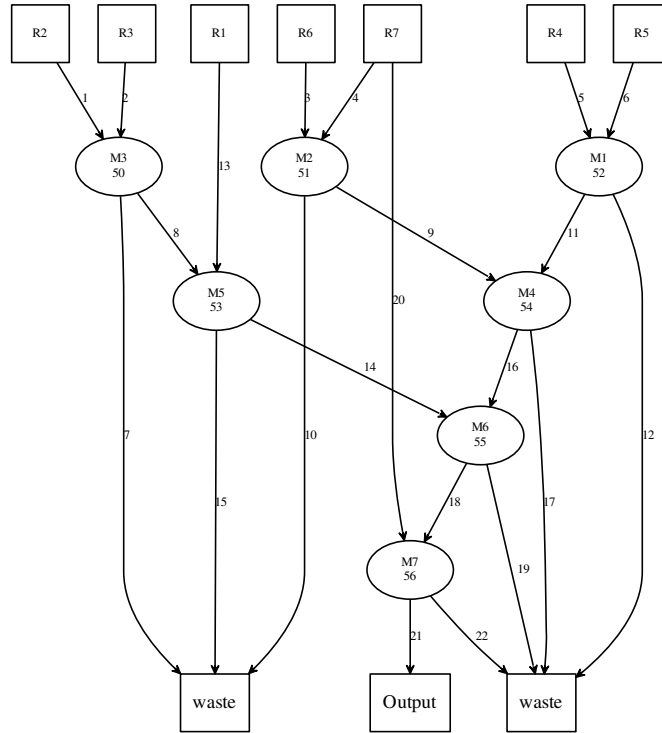

Figure A.14: Sequencing graph extracted from old actuation sequence using *SimBioSys* and assignment of $\mathcal{ID}$s to extracted routing paths and mixers.

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Table A.9: Routing paths extracted from the old actuation sequence.

Routing paths	
Path ID	Path
$\mathcal{P}_1$	$[(1,2),(2,2)]$
$\mathcal{P}_2$	$[(3,1),(4,1),(5,1),(5,2)]$
$\mathcal{P}_3$	$[(1,5),(2,5),(3,5),(3,6),(3,7)]$
$\mathcal{P}_4$	$[(1,11),(2,11),(3,11),(3,10)]$
$\mathcal{P}_5$	$[(1,14),(2,14)]$
$\mathcal{P}_6$	$[(3,15),(4,15),(5,15),(5,14)]$
$\mathcal{P}_7$	$[(5,2),(6,2),(7,2),(7,1)]$
$\mathcal{P}_8$	$[(2,2),(2,3),(2,4),(3,4),(3,5),(3,6),(3,7)]$
$\mathcal{P}_9$	$[(3,10),(4,10),(5,10),(5,11),(5,12),(5,13),(5,14)]$
$\mathcal{P}_{10}$	$[(3,7),(4,7),(5,7),(6,7),(7,7),(7,6),(7,5),(7,4),(7,3),(7,2),(7,1)]$
$\mathcal{P}_{11}$	$[(2,14)]$
$\mathcal{P}_{12}$	$[(5,14),(6,14),(7,14),(7,15)]$
$\mathcal{P}_{13}$	$[(1,8),(1,9),(1,10),(2,10),(3,10)]$
$\mathcal{P}_{14}$	$[(3,10),(4,10),(5,10),(5,11),(5,12),(5,13),(5,14)]$
$\mathcal{P}_{15}$	$[(3,7),(4,7),(5,7),(6,7),(7,7),(7,6),(7,5),(7,4),(7,3),(7,2),(7,1)]$
$\mathcal{P}_{16}$	$[(2,14)]$
$\mathcal{P}_{17}$	$[(5,14),(6,14),(7,14),(7,15)]$
$\mathcal{P}_{18}$	$[(2,14)]$
$\mathcal{P}_{19}$	$[(5,14),(6,14),(7,14),(7,15)]$
$\mathcal{P}_{20}$	$[(1,11),(1,12),(2,12),(3,12),(4,12),(5,12),(5,13),(5,14)]$
$\mathcal{P}_{21}$	$[(5,14),(6,14),(7,14),(8,14),(8,13)]$
$\mathcal{P}_{22}$	$[(2,14),(3,14),(4,14),(5,14),(6,14),(6,15),(7,15)]$

Table A.10: Mixer locations and types extracted from the old actuation sequence.

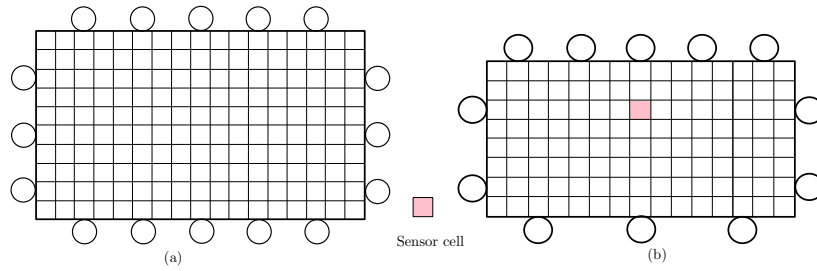| Mixers		
Mixer ID	Mixer Type	Mixer location
50	4×1	$[(2,2),(5,2)]$
51	1×4	$[(3,7),(3,10)]$
52	4×1	$[(2,14),(5,14)]$
53	1×4	$[(3,7),(3,10)]$
54	4×1	$[(2,14),(5,14)]$
55	4×1	$[(2,14),(5,14)]$
56	4×1	$[(2,14),(5,14)]$

Figure A.15: Enhanced DMFB platform of dimensions (a) 10×17, and (b) chip of size 8×15 with sensor.
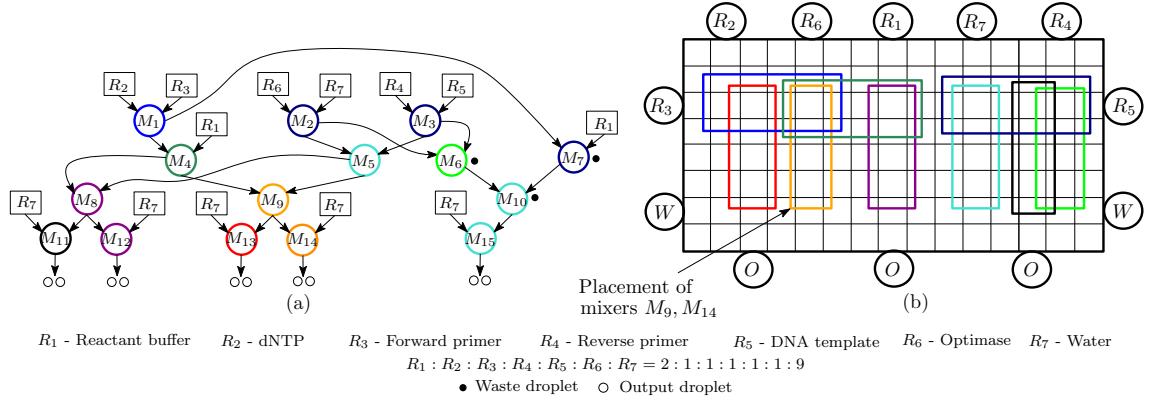
5.2 Streaming of PCR mixture droplets

Figure A.16: (a) Sequencing graph of droplet streaming for the PCR mixture with waste droplet sharing (b) Old DMF platform of size 8×15 and the corresponding mixer placements.
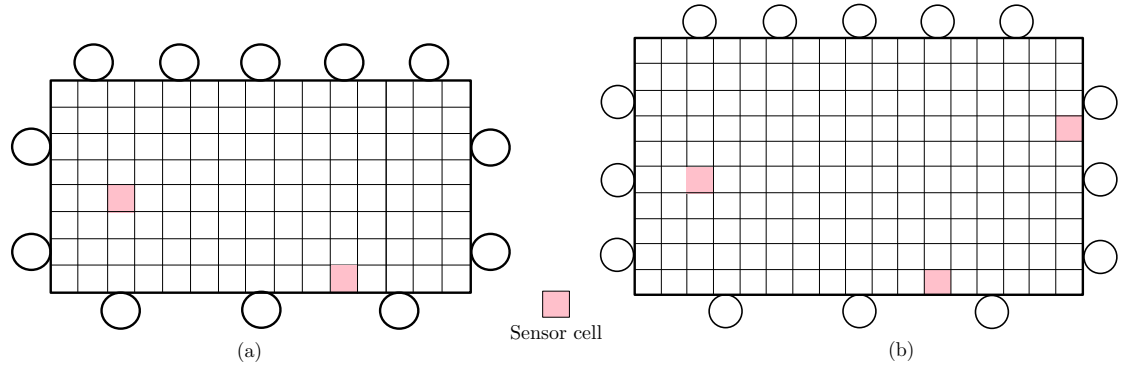

Figure A.17: Enhanced DMFB platform of dimensions (a) 8×15 with sensors, and (b) chip of size 10×17 with changes in reservoir and sensor geometry.
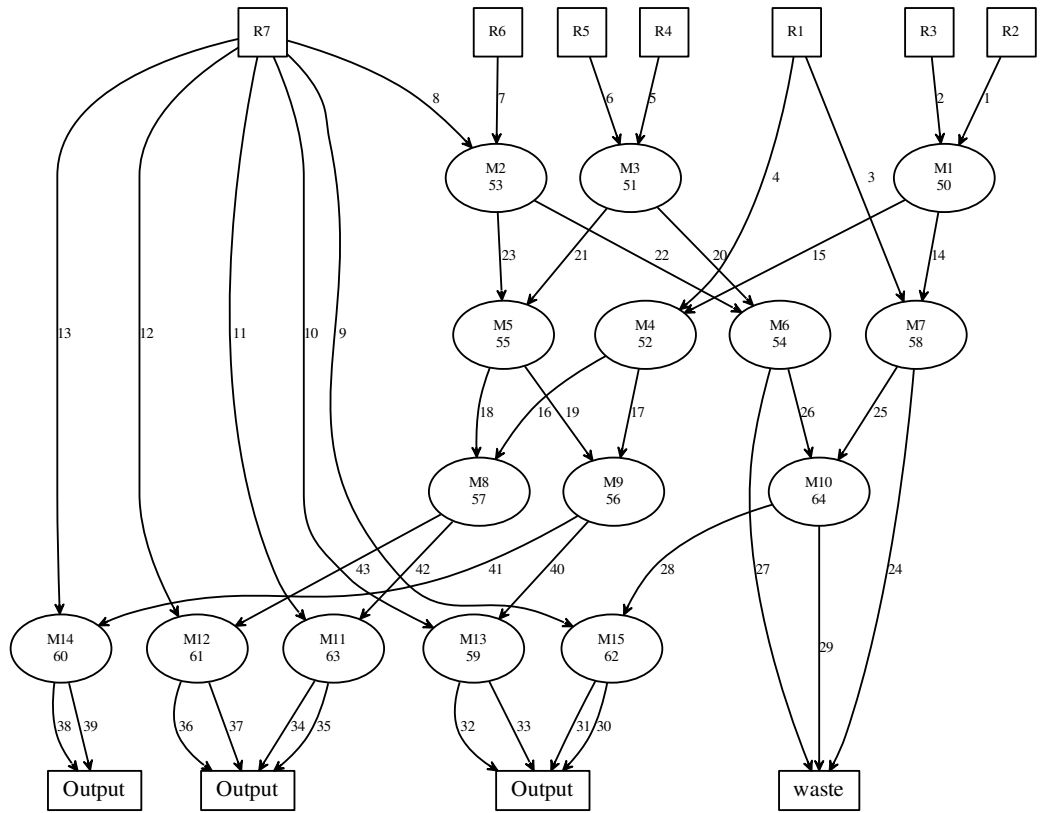
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Figure A.18: Sequencing graph extracted from old actuation sequence using *SimBioSys* and assignment of $\mathcal{ID}$s to extracted routing paths and mixers.

Table A.11: Routing paths extracted from the old actuation sequence.

Routing paths	
Path ID	Path
$\mathcal{P}_1$	$[(1,2),(2,2),(3,2)]$
$\mathcal{P}_2$	$[(3,1),(3,2),(3,3),(3,4),(3,5)]$
$\mathcal{P}_3$	$[(1,8),(1,9),(1,10),(1,11),(2,11),(3,11),(3,12),(3,13),(3,14)]$
$\mathcal{P}_4$	$[(1,8),(2,8),(3,8)]$
$\mathcal{P}_5$	$[(1,14),(2,14),(3,14)]$
$\mathcal{P}_6$	$[(3,15),(3,14),(3,13),(3,12),(3,11)]$
$\mathcal{P}_7$	$[(1,5),(2,5),(3,5),(3,6),(3,7),(3,8),(3,9),(3,10),(3,11)]$
$\mathcal{P}_8$	$[(1,11),(2,11),(3,11),(3,12),(3,13),(3,14)]$
$\mathcal{P}_9$	$[(1,11),(2,11),(3,11)]$
$\mathcal{P}_{10}$	$[(1,11),(2,11),(3,11),(3,10),(3,9),(3,8),(3,7),(3,6),(3,5),(3,4),(3,3)]$
$\mathcal{P}_{11}$	$[(1,11),(2,11),(3,11),(3,12),(3,13)]$
$\mathcal{P}_{12}$	$[(1,11),(2,11),(3,11),(3,10),(3,9),(3,8)]$
$\mathcal{P}_{13}$	$[(1,11),(2,11),(3,11),(3,10),(3,9),(3,8),(3,7),(3,6),(3,5)]$
$\mathcal{P}_{14}$	$[(3,2),(3,3),(2,3),(1,3),(1,4),(1,5),(1,6),(1,7),(1,8),(1,9),(1,10),(1,11),(2,11),(3,11)]$
$\mathcal{P}_{15}$	$[(3,5)]$
$\mathcal{P}_{16}$	$[(3,8),(4,8),(5,8),(6,8)]$
$\mathcal{P}_{17}$	$[(3,5),(4,5),(5,5),(6,5)]$
$\mathcal{P}_{18}$	$[(6,11),(5,11),(4,11),(3,11),(3,10),(3,9),(3,8)]$
$\mathcal{P}_{19}$	$[(3,11),(3,10),(3,9),(3,8),(3,7),(3,6),(3,5)]$
$\mathcal{P}_{20}$	$[(3,14),(4,14),(5,14),(6,14)]$
$\mathcal{P}_{21}$	$[(3,11),(4,11),(5,11),(6,11)]$
$\mathcal{P}_{22}$	$[(3,14)]$
$\mathcal{P}_{23}$	$[(3,11)]$
$\mathcal{P}_{24}$	$[(3,14),(4,14),(5,14),(6,14),(7,14),(7,15)]$
$\mathcal{P}_{25}$	$[(3,11)]$
$\mathcal{P}_{26}$	$[(3,14),(4,14),(5,14),(6,14),(6,13),(6,12),(6,11)]$
$\mathcal{P}_{27}$	$[(6,14),(7,14),(7,15)]$
$\mathcal{P}_{28}$	$[(3,11),(4,11),(5,11),(6,11)]$
$\mathcal{P}_{29}$	$[(6,11),(6,12),(6,13),(6,14),(6,15),(7,15)]$
$\mathcal{P}_{30}$	$[(6,11),(6,12),(6,13),(7,13),(8,13)]$
$\mathcal{P}_{31}$	$[(3,11),(3,12),(3,13),(4,13),(5,13),(6,13),(7,13),(8,13)]$
$\mathcal{P}_{32}$	$[(6,3),(7,3),(8,3)]$
$\mathcal{P}_{33}$	$[(3,3),(4,3),(5,3),(6,3),(7,3),(8,3)]$
$\mathcal{P}_{34}$	$[(3,13),(4,13),(5,13),(6,13),(7,13),(8,13)]$
$\mathcal{P}_{35}$	$[(6,13),(7,13),(8,13)]$
$\mathcal{P}_{36}$	$[(6,8),(7,8),(8,8)]$
$\mathcal{P}_{37}$	$[(3,8),(4,8),(5,8),(6,8),(7,8),(8,8)]$
$\mathcal{P}_{38}$	$[(6,5),(6,4),(6,3),(7,3),(8,3)]$
$\mathcal{P}_{39}$	$[(3,5),(3,4),(3,3),(4,3),(5,3),(6,3),(7,3),(8,3)]$
$\mathcal{P}_{40}$	$[(6,5),(6,4),(6,3)]$
$\mathcal{P}_{41}$	$[(3,5),(4,5),(5,5),(6,5)]$
$\mathcal{P}_{42}$	$[(6,8),(6,9),(6,10),(6,11),(6,12),(6,13)]$
$\mathcal{P}_{43}$	$[(3,8),(4,8),(5,8),(6,8)]$

Table A.12: Mixer locations and types extracted from the old actuation sequence.

Mixers		
Mixer ID	Mixer Type	Mixer location
50	1×4	$[(3, 2), (3, 5)]$
51	1×4	$[(3, 11), (3, 14)]$
52	1×4	$[(3, 5), (3, 8)]$
53	1×4	$[(3, 11), (3, 14)]$
54	4×1	$[(3, 14), (6, 14)]$
55	4×1	$[(3, 11), (6, 11)]$
56	4×1	$[(3, 5), (6, 5)]$
57	4×1	$[(3, 8), (6, 8)]$
58	1×4	$[(3, 11), (3, 14)]$
59	4×1	$[(3, 3), (6, 3)]$
60	4×1	$[(3, 5), (6, 5)]$
61	4×1	$[(3, 8), (6, 8)]$
62	4×1	$[(3, 11), (6, 11)]$
63	4×1	$[(3, 13), (6, 13)]$
64	4×1	$[(3, 11), (6, 11)]$