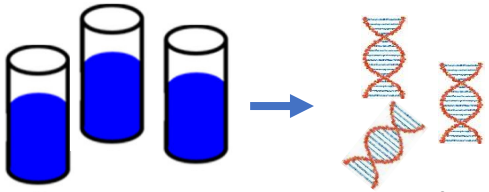
Addressing fuel biocontamination through the development of highly specific neutralizing peptides and rapid bio-detection platforms

Principal Investigator – Prof. Susanna Leong

Co-Investigators – A/P Joy Pang, A/P Adison Wong

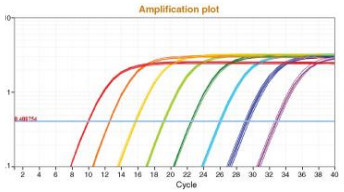
Thrust 1: Microbial profiling



Fuel samples

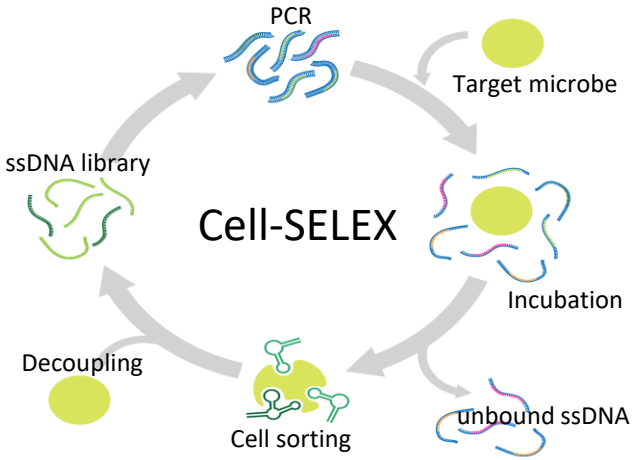
Microbial genetic material extraction

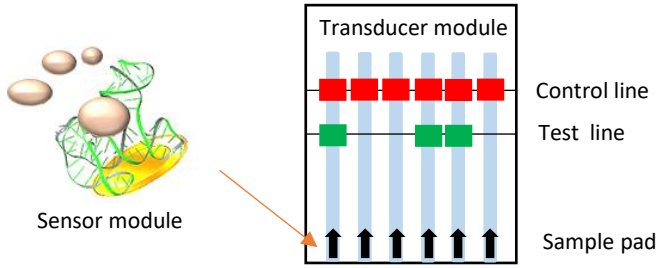
Next-Gen sequencing



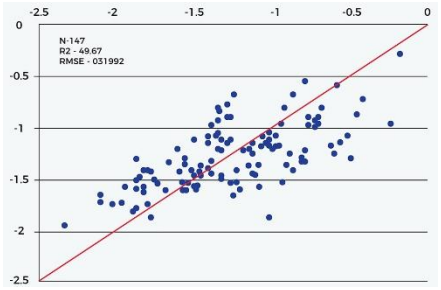
qPCR platform development for rapid quantitative microbial detection

Thrust 2: Biosensor development



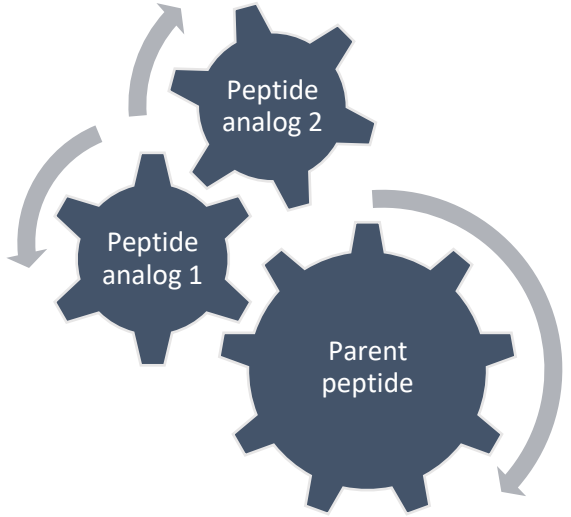


Device Fabrication and Testing

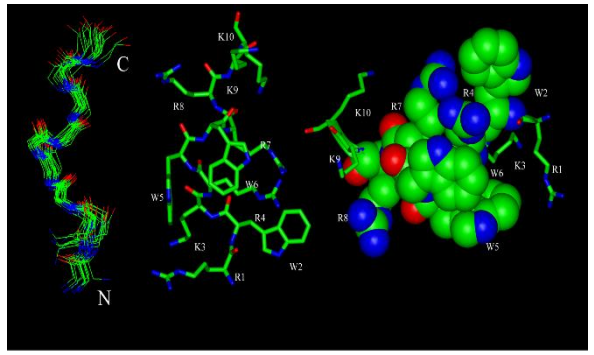


Efficacy validation

Thrust 3: Antimicrobial peptide development



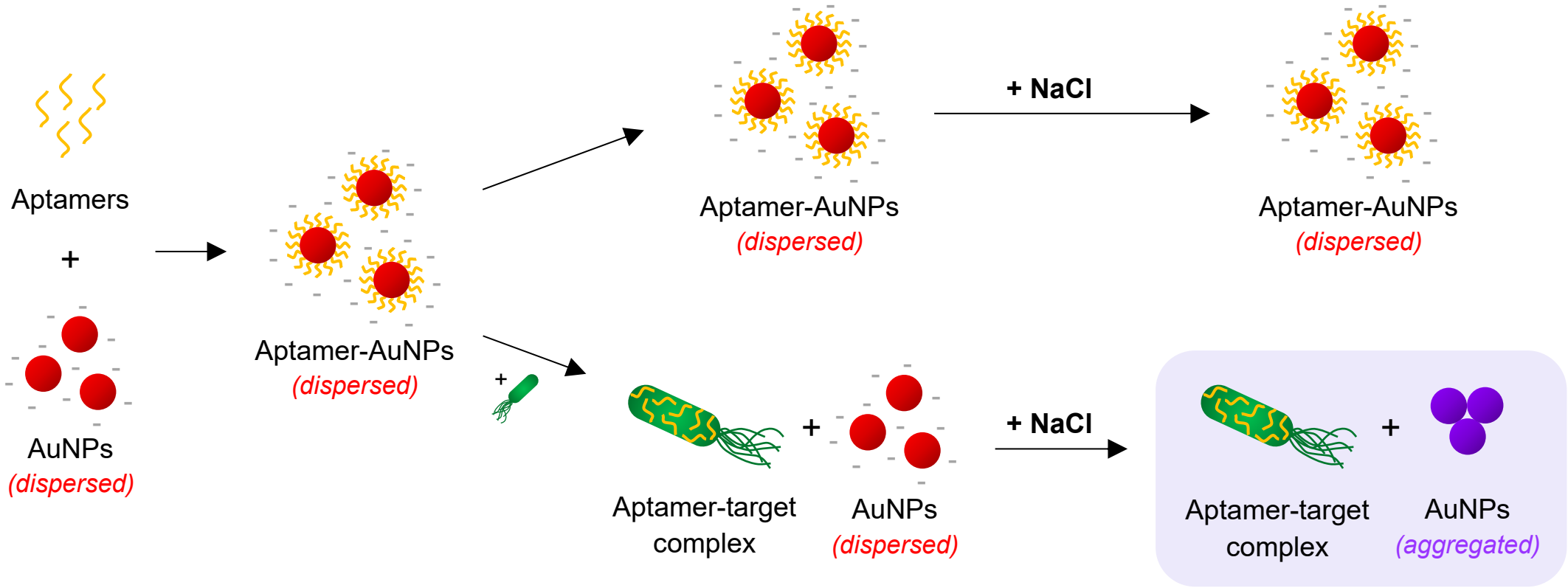
Peptide engineering



Structural characterization

Colorimetric assay development

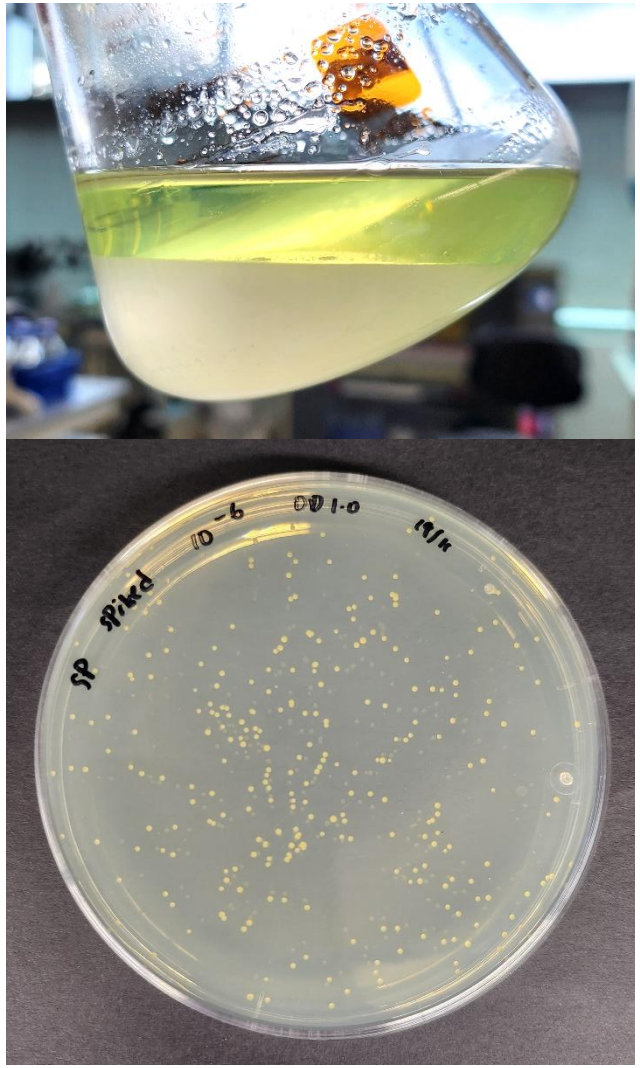
- **Detection principle:**
  - ➢ Functionalization of AuNPs with DNA aptamers provides target specificity



Detection in spiked fuel samples (SP10-B)

- **Method:**
  - 50 mL diesel + 50 mL deionised water (sterile-filtered)
  - Aqueous phase spiked with 100 µL fuel culture of *S. paucimobilis*
  - Incubated for 15 days at 30°C, 120 rpm

- *S. paucimobilis* concentration measured daily via **biosensor vs plate count**
  - **Negative control: 0 CFU/mL**
  - **Negligible contamination: < 10^5 CFU/mL**
  - **Moderate contamination: 10^5–10^6 CFU/mL**
  - **Heavy contamination: > 10^6 CFU/mL**



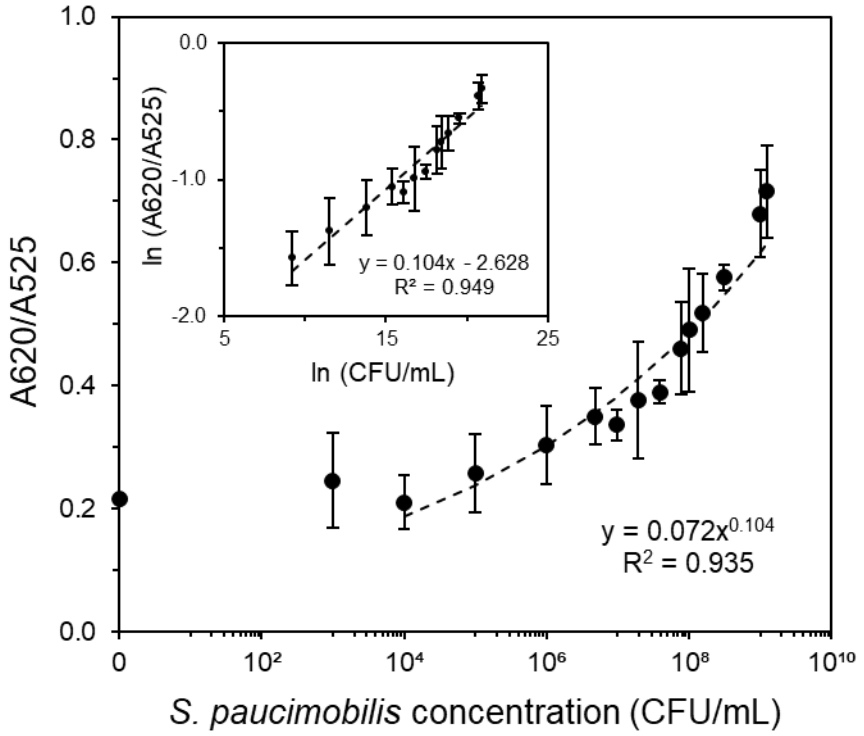
Detection in spiked fuel samples (SP10-B)

- Quantify using **calibration curve** obtained previously:
  - Linear range: **10^4–10^9 CFU/mL**
  - Detection limit

    = Mean A_{620}/A_{525} of blank + stdev of blank

    = 0.215 + 0.007

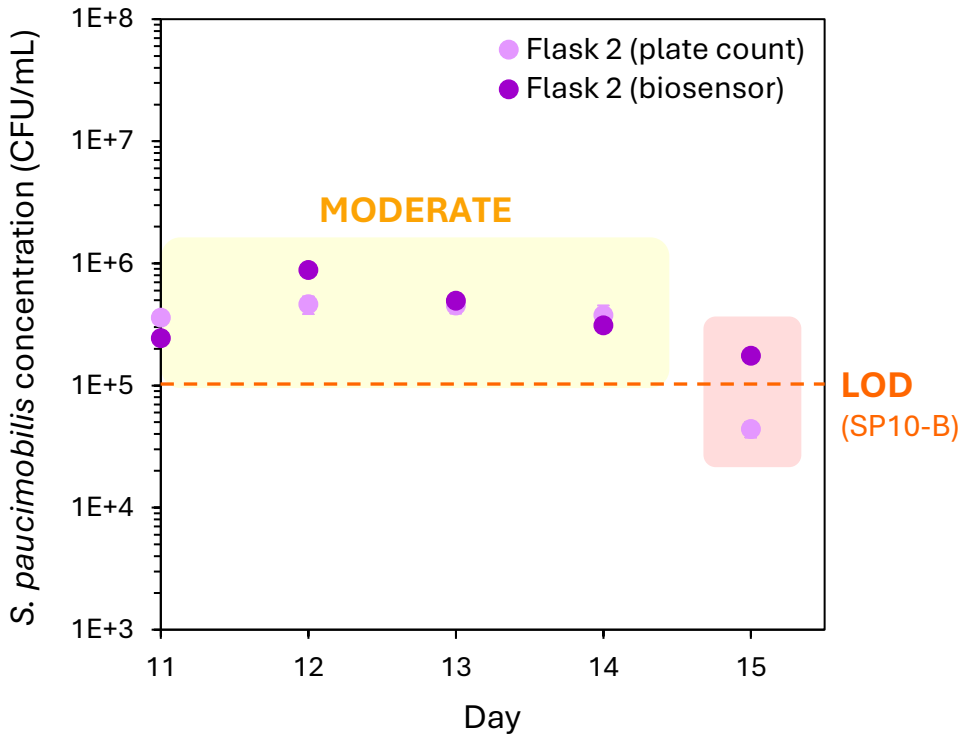
    = **0.237**
  - Detection limit: **1.0 × 10^5 CFU/mL** (from linear equation)



- **Biosensor measurement:**
  - A_{620}/A_{525} **< 0.237** = **Negligible** (microbe concentration **< 10^5 CFU/mL**)
  - A_{620}/A_{525} **≥ 0.237** = **Positive** (microbe concentration **≥ 10^5 CFU/mL**)

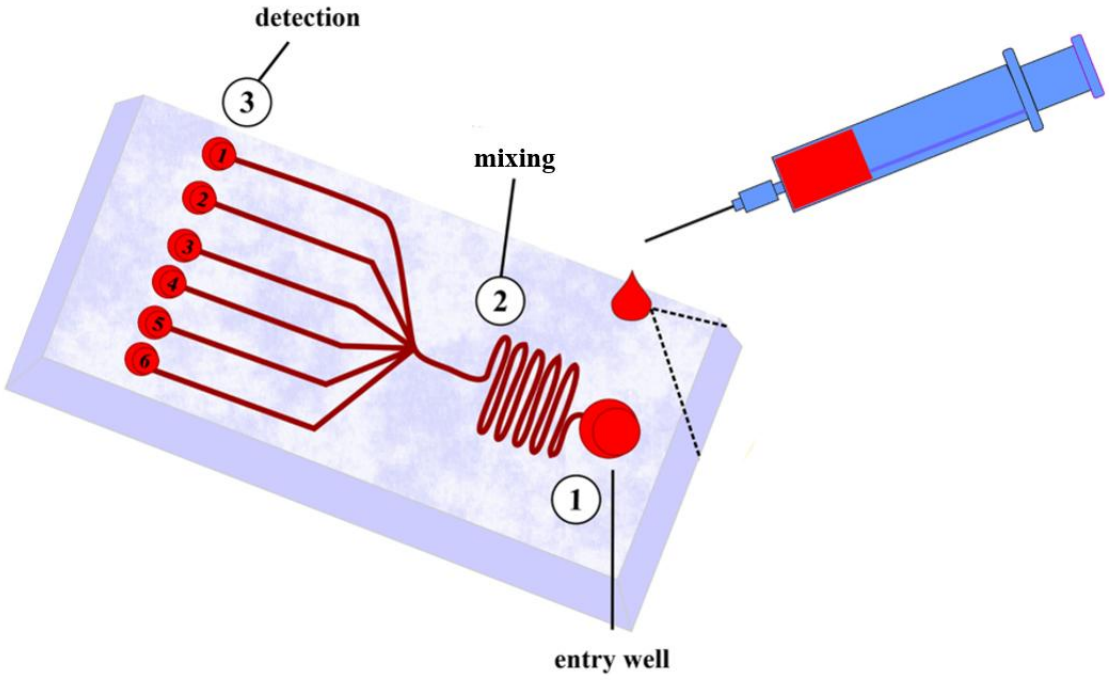
Detection in spiked fuel samples (SP10-B)

- For **MODERATE** contamination level:
  - ➢ Microbe quantitation using biosensor yields **similar range as plate count**
- But **some false-positive results** noted:
  - ➢ Presence of **fuel deposits** in drawn samples occasionally causes higher A_{620}/A_{525}
- Currently investigating **buffer exchange** methods to remove fuel deposits before biosensing



Biocide Dispensing System – Overview

- Compatible with **single** or **multi-species** detection
- Based on an input database of **microbe species** and their respective **MIC values**



Biosensor channel	Microbe species detected	MIC (µg/mL)
1	*Sphingomonas paucimobilis*	1.78
2	*Pseudomonas aeruginosa*	3.75
3	*Micrococcus yunnanensis*	1.78
4	*Dermacoccus nishinomiyaensis*	1.78
5	*Kocuria rosea*	3.75
6	*Staphylococcus equorum*	1.78
7	*Bacillus licheniformis*	1.78
8	*Hormoconis resinae*	3.85

Biocide Dispensing System – Workflow

Step 1: System receives input signal via MQTT

- Biosensor channel: **1**
- Detected microbe concentration (CFU/mL): **From app**

Step 2: Decide whether to administer biocide, determine MIC

Input interpreted as **MODERATE** contamination of the fuel sample with ***S. paucimobilis*** (Channel 1), corresponding to a MIC value of **1.78 µg/mL**.

Step 3: Calculate biocide dosage required

$$= MIC \times Fuel\ tank\ size \times Multiplication\ factor$$

$$= 1.78\ \mu g/mL \times 5000\ mL \times 5 = \mathbf{44.5\ mg}$$

Step 4: Calculate volume of biocide stock to dispense

$$= \frac{Biocide\ dosage\ (mg)}{Biocide\ stock\ concentration\ (mg/mL)}$$

$$= \frac{44.5\ mg}{1\ mg/mL} = \mathbf{44.5\ mL}$$

Step 5: Calculate dispense time

$$= \frac{Biocide\ stock\ volume\ (mL)}{Pump\ flowrate\ (mL/s)}$$

$$= \frac{44.5\ mL}{1.17\ mL} = \mathbf{38.14\ s}$$

Step 6: Round up dispense time

$$= 38.14\ s\ \rightarrow \mathbf{38.2\ s}$$

Step 7: Initiate pump

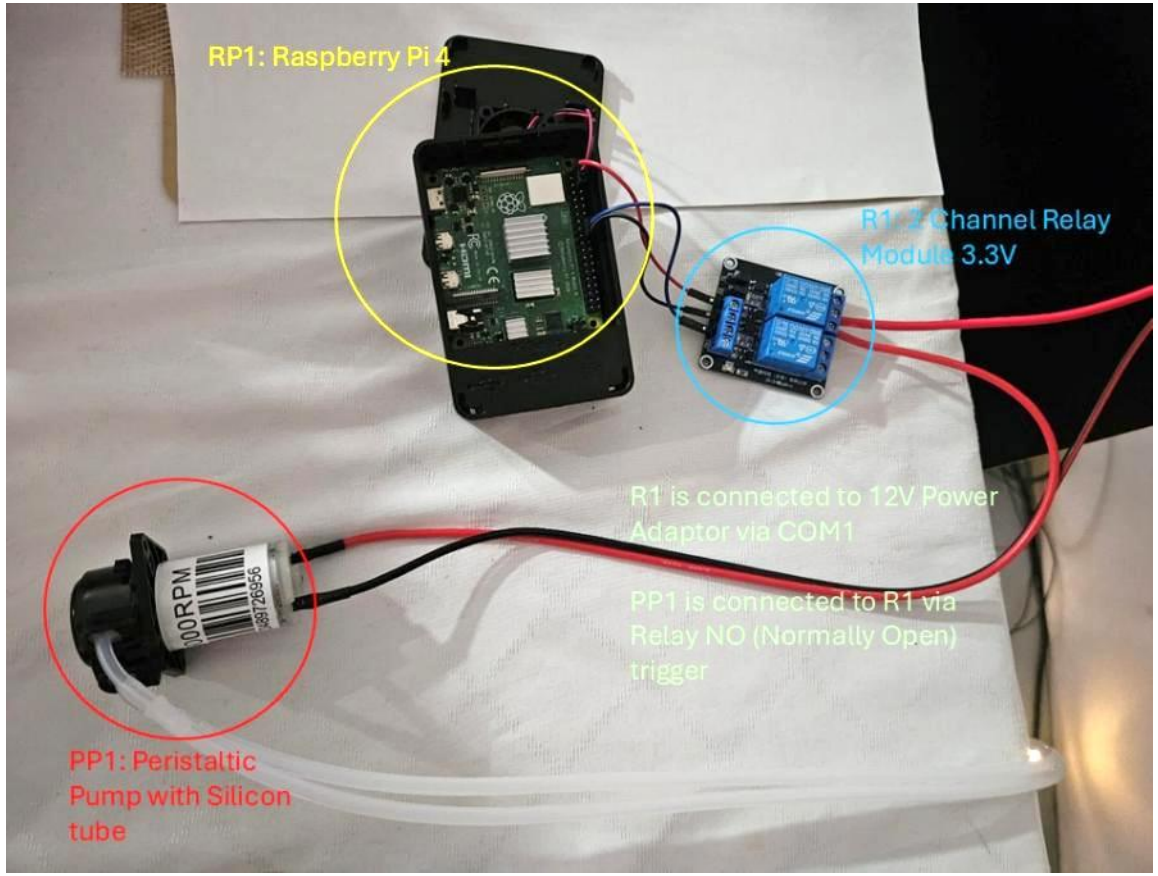
$$Actual\ biocide\ volume\ dispensed$$

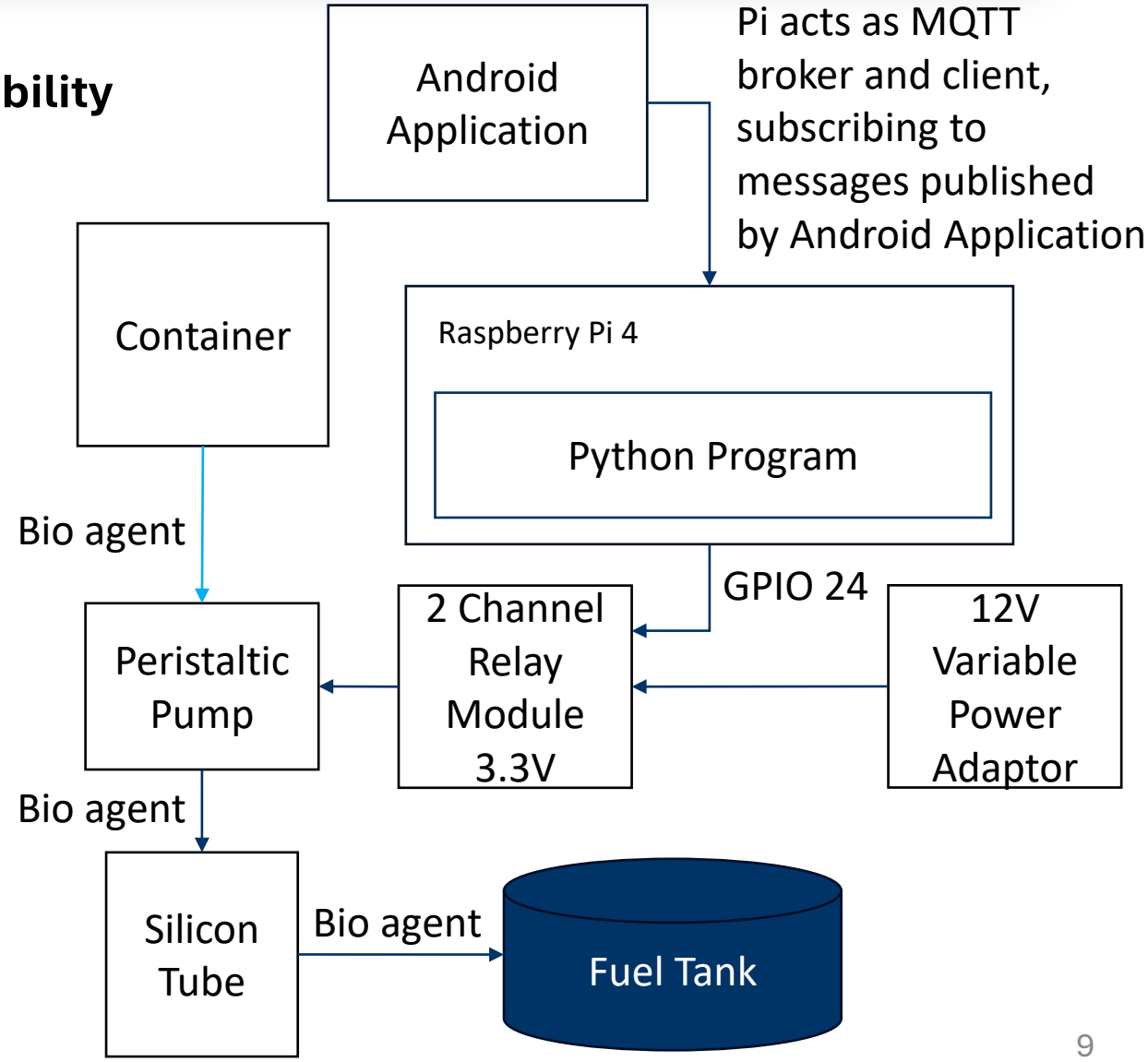
$$= Pump\ flowrate\ (mL/s) \times Dispense\ time_{roundup}(s)$$

$$= 1.17\ mL/s \times 38.2\ s = \mathbf{44.6\ mL}$$

Biocide Dispensing System – Hardware

- Developed on **Raspberry Pi 4** for **Wi-Fi compatibility**





Biocide Dispensing System – Operation

4.3 Adding or Modifying Bacterial Strains

To add a new strain, modify the CHANNELS dictionary:

```
CHANNELS = {
    1: {"name": "Sphingomonas paucimobilis", "mic": 1.78},
    2: {"name": "Pseudomonas aeruginosa", "mic": 3.75},
    3: {"name": "Micrococcus yunnanensis", "mic": 1.78},
    4: {"name": "Dermacoccus nishinomiyaensis", "mic": 1.78},
    5: {"name": "Kocuria rosea", "mic": 3.75},
    6: {"name": "Staphylococcus equorum", "mic": 1.78},
    7: {"name": "Bacillus licheniformis", "mic": 1.78},
    8: {"name": "Hormoconis resinae", "mic": 3.85},
    9: {"name": "Test Strain", "mic": 20.0}
}
```

Fixed variables:

Pump flowrate (mL/min): 70

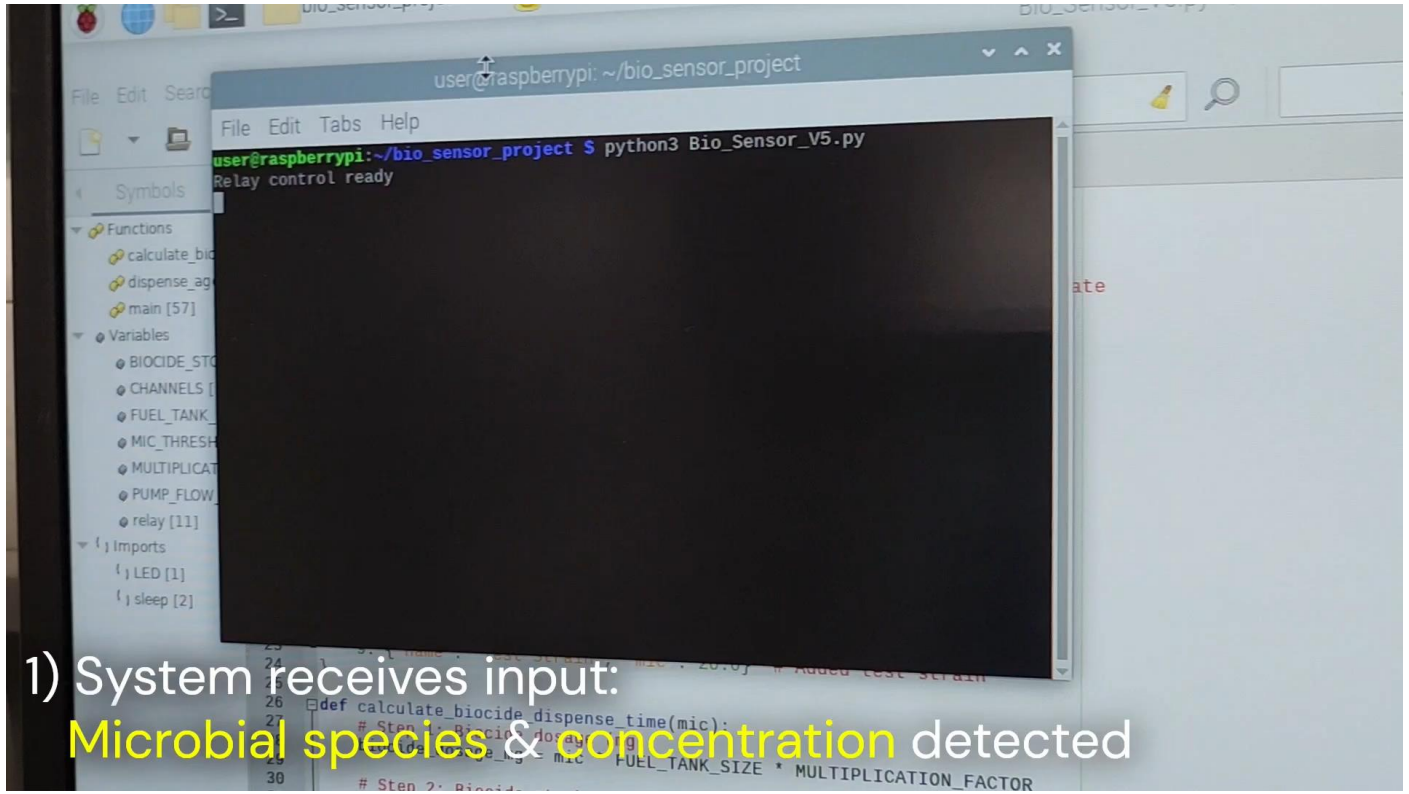
Multiplication factor: 5

Fuel tank size (L): 5

Biocide stock concentration (mg/mL): 1

Biocide Dispensing System – Validation

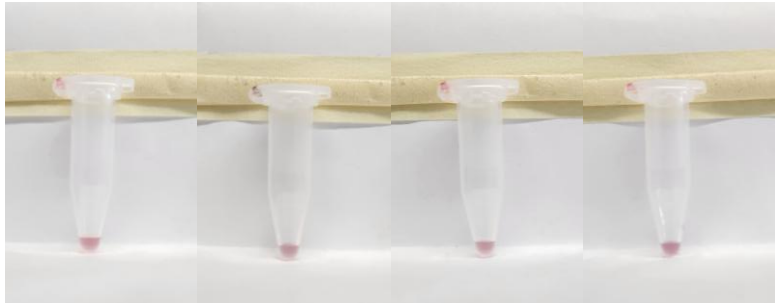
- Biocide dispensed with **high volume accuracy**



Channel no.	1	1	5	8
Detected conc. (CFU/mL)	**5E+03**	**5E+05**	**1E+05**	**5E+06**
Detected species	-	SP	KR	HR
Contamination level	**NEG**	**MOD**	**MOD**	**HVY**
Biocide required?	NO	YES	YES	YES
MIC (µg/mL)	-	1.78	3.75	3.85
Fuel tank size (L)	1	1	1	5
Biocide conc. (mg/mL)	1	1	1	1
Biocide required (mL)	**0.0**	**8.88**	**18.75**	**96.20**
Dispense time (s)	-	5.4	11.3	57.8
Biocide dispensed (mL)	**0.0**	**9.0**	**18.8**	**96.6**
Difference	**-**	**+1.1%**	**+0.3%**	**+0.4%**

ML Model – Progress

- Started with ~1500 photos



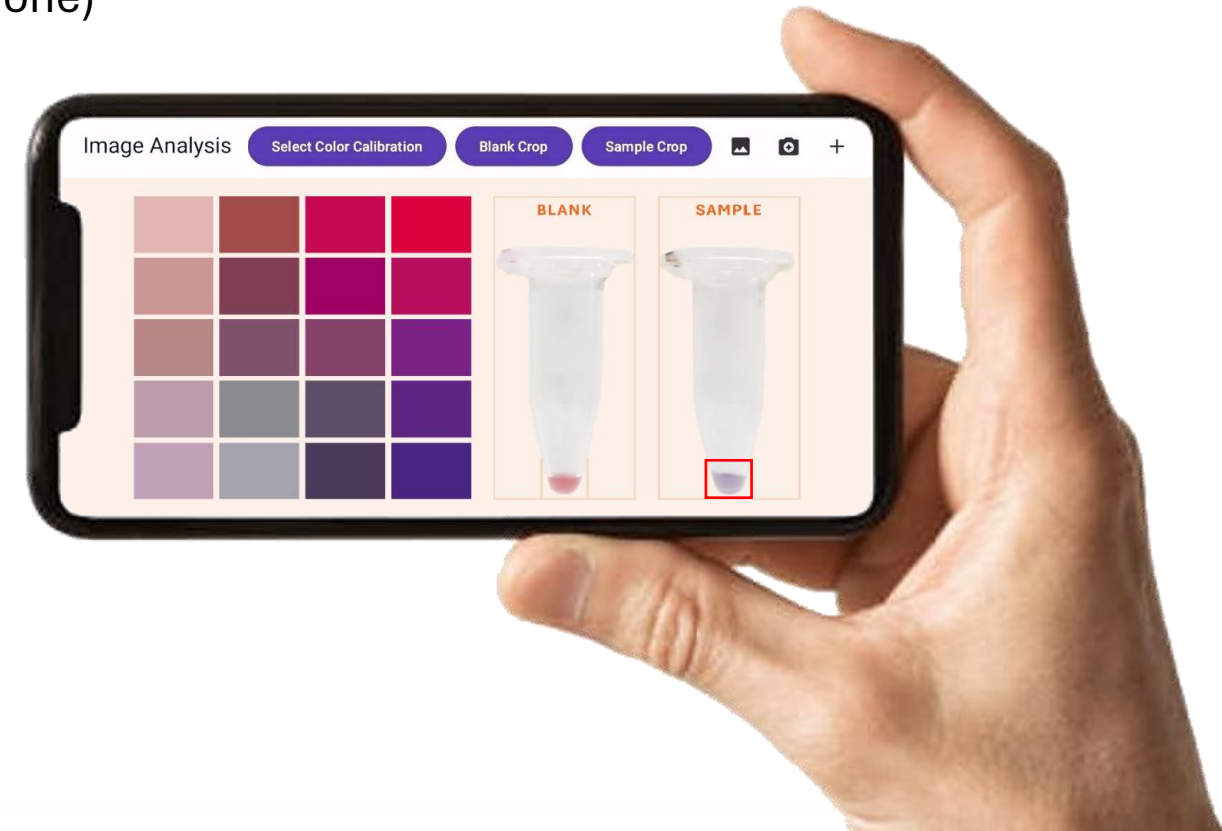
- Superimposed onto card photo

- Lighting and phone differences

Colour Analysis App

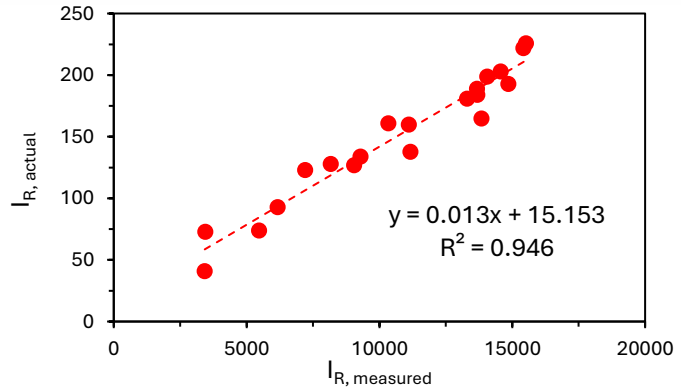
- Added **image calibration** function to correct for variations in:
  - ➢ Lighting condition (e.g., bright, dark, warm)
  - ➢ Phone brand and model
  - ➢ Auto-focus effects (photo replicates by the same phone)

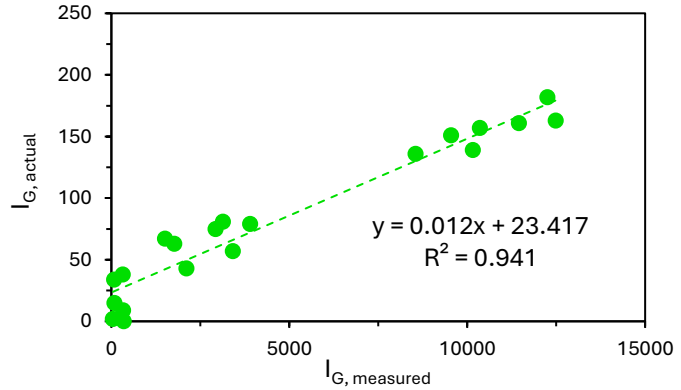
Column 1	Column 2	Column 3	Column 4
I_R = 226 I_G = 182 I_B = 178	I_R = 161 I_G = 75 I_B = 75	I_R = 199 I_G = 9 I_B = 81	I_R = 222 I_G = 2 I_B = 63
I_R = 203 I_G = 151 I_B = 149	I_R = 128 I_G = 63 I_B = 85	I_R = 160 I_G = 2 I_B = 100	I_R = 184 I_G = 15 I_B = 94
I_R = 181 I_G = 136 I_B = 134	I_R = 127 I_G = 81 I_B = 109	I_R = 134 I_G = 67 I_B = 106	I_R = 123 I_G = 34 I_B = 134
I_R = 189 I_G = 157 I_B = 170	I_R = 138 I_G = 139 I_B = 145	I_R = 93 I_G = 79 I_B = 104	I_R = 73 I_G = 38 I_B = 130
I_R = 193 I_G = 161 I_B = 183	I_R = 165 I_G = 163 I_B = 174	I_R = 74 I_G = 57 I_B = 89	I_R = 41 I_G = 43 I_B = 127

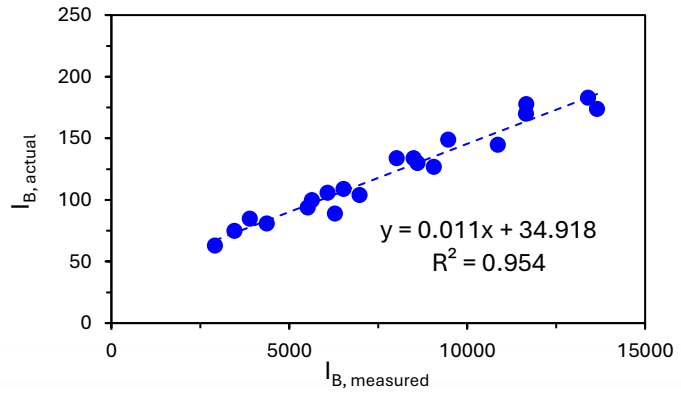


Colour Analysis App – RGB Correction

1. [USER] Perform colorimetric test
2. [USER] Paste blank and sample tubes on calibration card
3. [USER] Launch app, capture image/import from gallery
4. [APP] Locate calibration, blank, and sample areas
5. [APP] Measure RGB channel intensity (I_x) for each region of interest
6. [APP] Plot calibration curves for RGB channels using the calibration block values
7. [APP] Use channel equations to correct I_x values for the blank and sample tubes
8. [APP] Calculate A_B/A_R for blank and sample tubes using corrected I_x





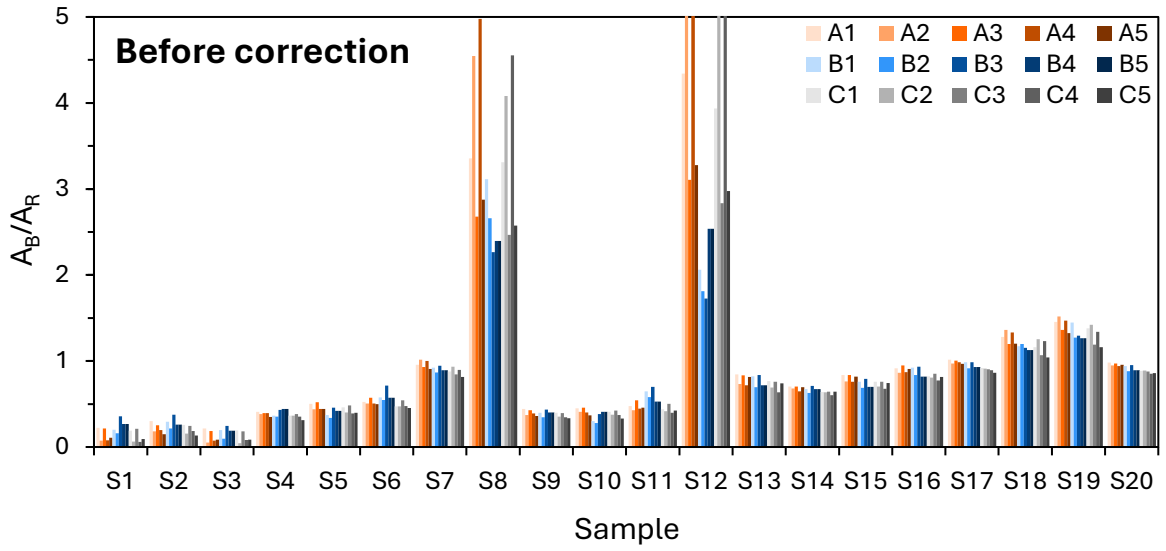


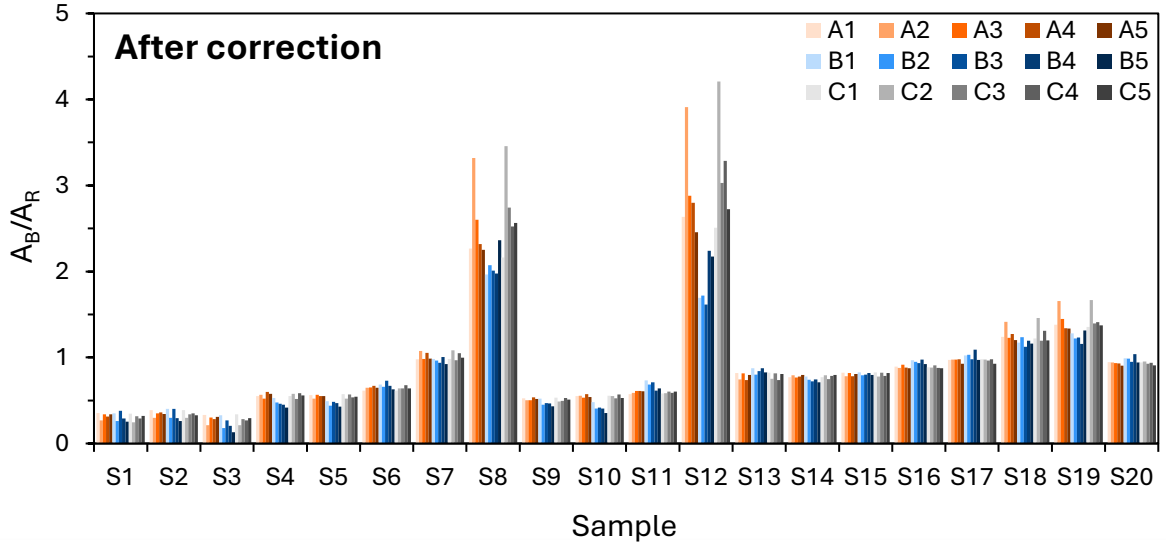
Colour Analysis App – RGB Correction

- Relative standard deviation (RSD) in A_B/A_R values across 5 phones and 3 lighting conditions lowered from **17.7%** to **9.7%**

Phone	Brand	Model
1	Samsung	Z Flip 4
2	iPhone	15 Pro
3	Redmi	Note 10 5G
4	iPhone	16
5	Samsung	Galaxy A03

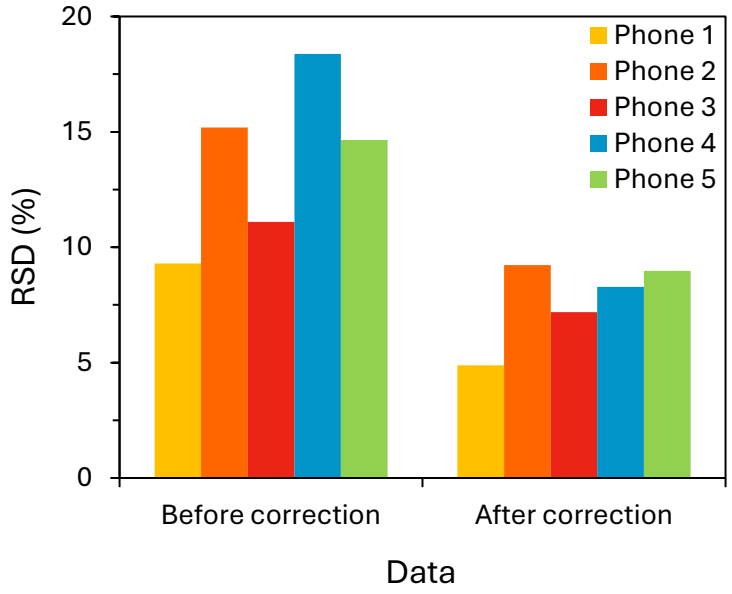
Condition	Location	Description
A	Jean's bench	Bright
B	Light box	Dark
C	Jean's bench	Warm

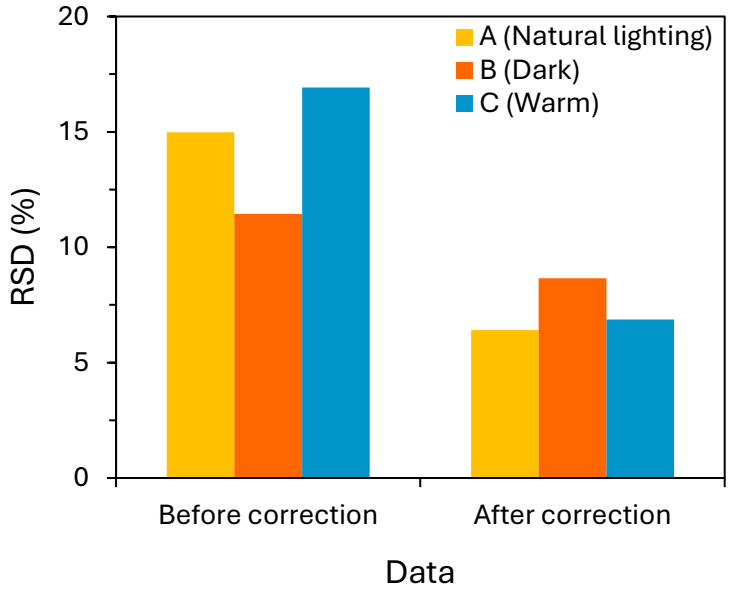




Colour Analysis App – RGB Correction

- Calibration efficiency:
  - Lighting condition: **13.7%** to **7.7%**
  - Phone model: **14.5%** to **7.3%**
  - Replicates by same phone: **2.5%** to **1.4%**





Phone	Brand	Model	RSD (%)	
			Before	After
1	Samsung	Z Flip 4	1.3	0.9
2	iPhone	15 Pro	3.5	1.6
3	Redmi	Note 10 5G	2.8	2.0
4	iPhone	16	3.3	1.4
5	Samsung	Galaxy A03	1.8	1.2

Colour Analysis App – Workflow

1) Image adjustment

Open camera OR import from gallery → Convert image format (e.g., .HEIC to .JPG)

2) Colour analysis

Split image into **RGB** channels → Crop out 3 areas from each region of interest (ROI) → Measure $\mathbf{I_R}$, $\mathbf{I_G}$, $\mathbf{I_B}$ for each ROI (card, blank, sample)

↓

Plot **actual vs measured** I_R, I_G, I_B for calibration block → Use curve equation to **correct $\mathbf{I_R}$, $\mathbf{I_G}$, $\mathbf{I_B}$** → Calculate $\mathbf{A_B/A_R}$ for blank and sample

3) Colour interpretation

Predict $\mathbf{A_{620}/A_{525}}$ from **ML model** generated from photo library → Calculate **microbe concentration** (CFU/mL)

$$= 0.071 \times (A_{620}/A_{525})^{0.104}$$

→ Indicate **contamination level:**

Negligible: $< 10^5$ CFU/mL

Moderate: $10^5 – 10^6$ CFU/mL

Heavy: $>10^6$ CFU/mL