**SUPPORTING INFORMATION**

# Capillary-Flow Driven Microfluidic Sensor Based on Tyrosinase for Fast User-friendly Assessment of Pesticide Exposure

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**Table of Contents**

## Color Analysis

To determine the optimal color channel for detecting ziram pesticide, the normalized signal against ziram concentration was plotted for each color channel: red (R), green (G), and blue (B). The green channel was selected for color analysis as it showed the highest overall signal (Fig. S1). Additionally, some samples developed a blueish tint after extended analysis time, so using the green channel helped avoid this interference in the signal.

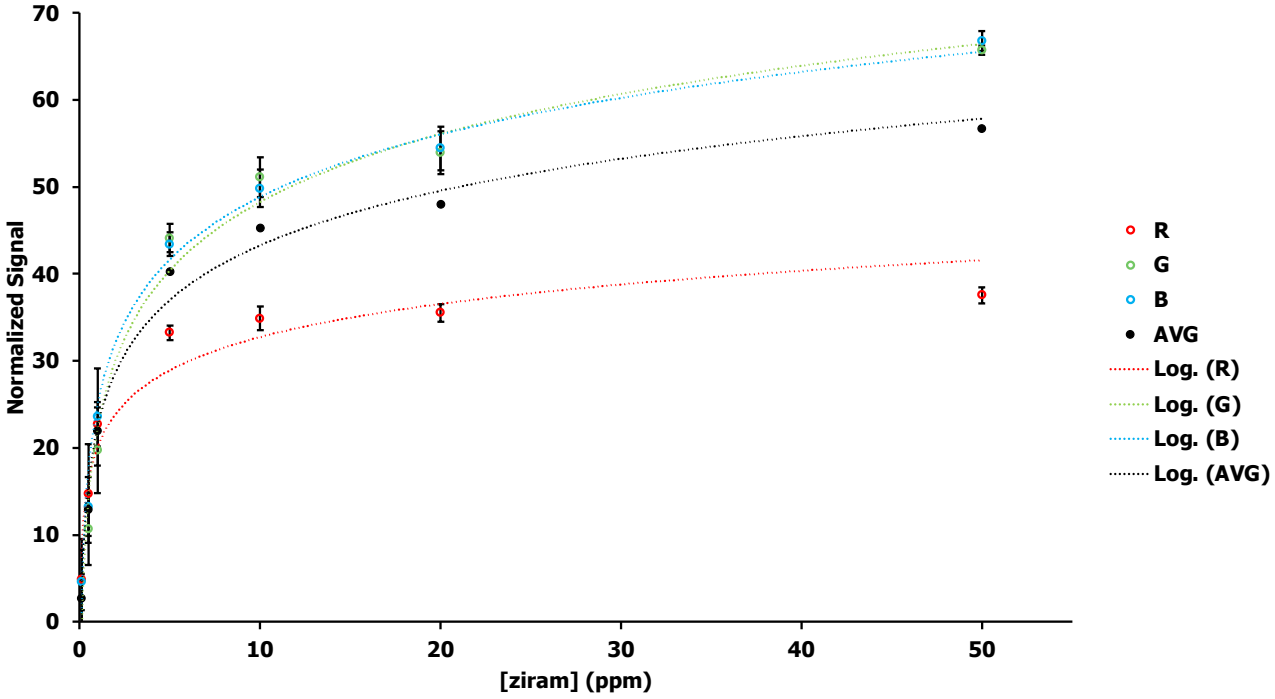


**Fig. S1.** Color analysis

## ImageJ analysis Step-by-Step

The steps to analyze an image of the fast-flow device on Image J are shown as follows (Fig. S2): To analyze an image of the fast-flow device using ImageJ, first import the captured image as a JPEG file. Next, split the image into its red, green, and blue channels, and isolate the green channel. Invert the image, then use the circle selector tool to define the region of interest (ROI) by dragging it over the circles in the image. Finally, record the mean grayscale value for each selected circle.

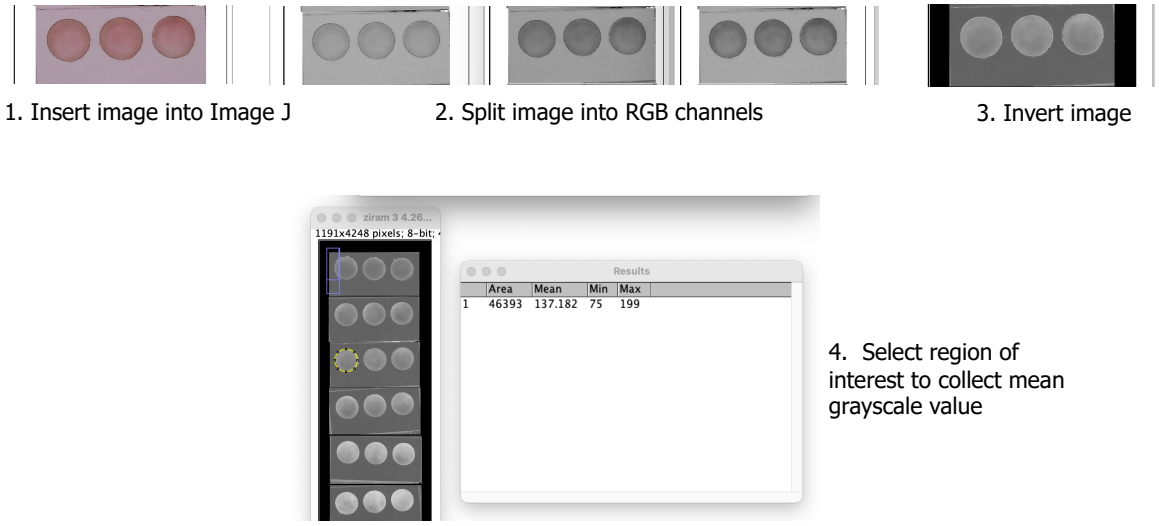


**Fig. S2.** ImageJ step-by-step analysis for capturing mean grayscale value.

**Solvent Optimization**

Four different solvents were tested in a tyrosinase assay at varying ratios (100%, 70%, 50%, and 30%) using a microwell plate reader with absorbance measured at 450 nm. Initially, no ziram was present in these samples to determine which solvent produced the highest blank signal (Fig. S3). Methanol and ethanol both showed the highest initial signals, indicating they had the least impact on the tyrosinase enzyme.

Based on these results, 50% and 30% concentrations of methanol and ethanol were selected for further testing with ziram pesticide in solution. The experiment was then repeated with 5 ppm ziram samples, comparing the results to the blanks to identify which solvent ratio provided the largest difference between blank and sample signals, along with the lowest standard deviation (SD) (Fig. S4). Ethanol outperformed methanol based on these criteria, leading to the selection of 50% and 30% ethanol for further testing on devices.

When the assays were tested on the devices, a similar trend was observed, with 30% ethanol emerging as the optimal solvent (Fig. S5). Consequently, 30% ethanol was used as the standard solvent for all of the subsequent studies unless otherwise noted.

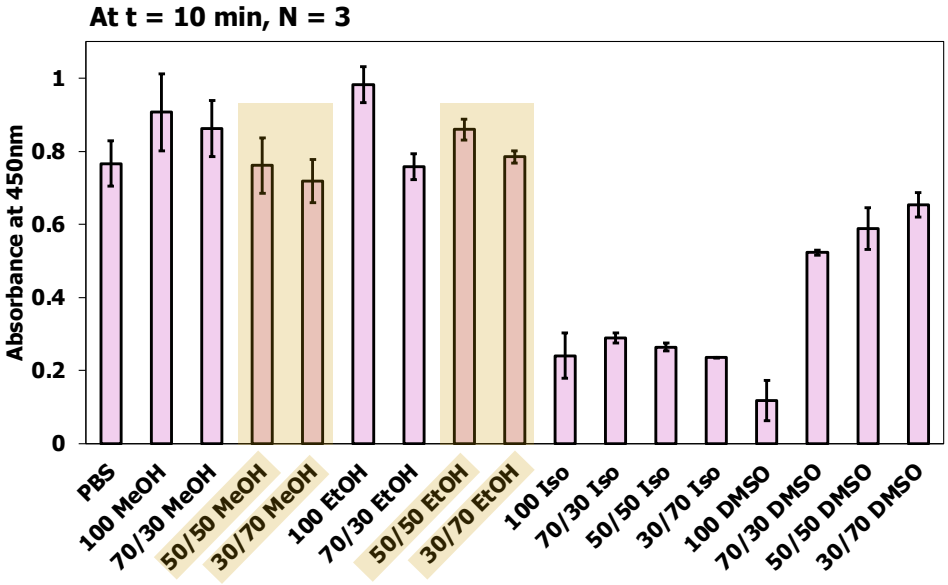


**Fig. S3.** Solvent optimization in solution using plate reader

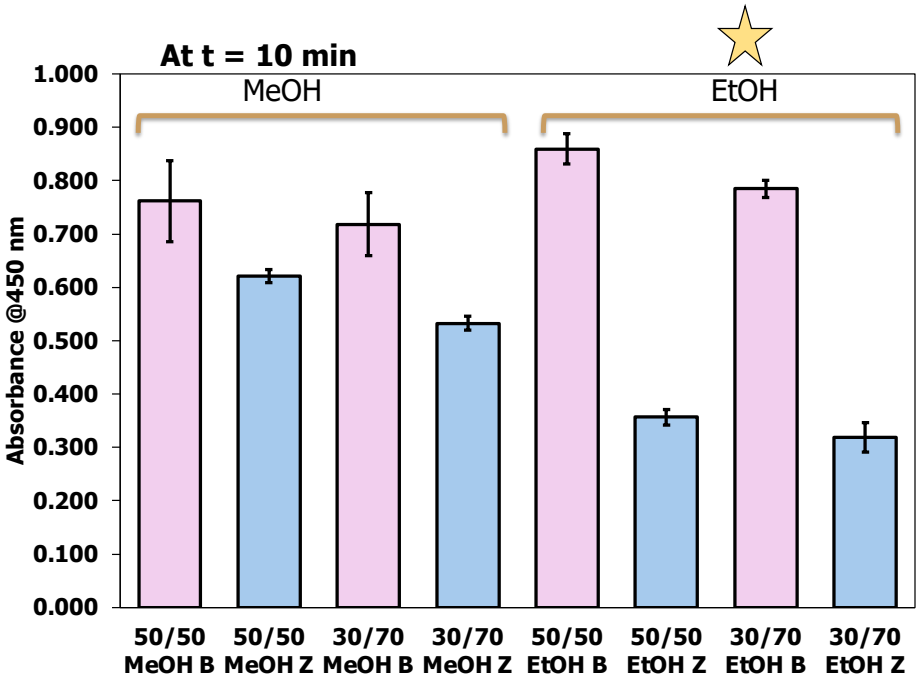


**Fig. S4.** Solvent optimization using plate reader (blank vs ziram) comparison

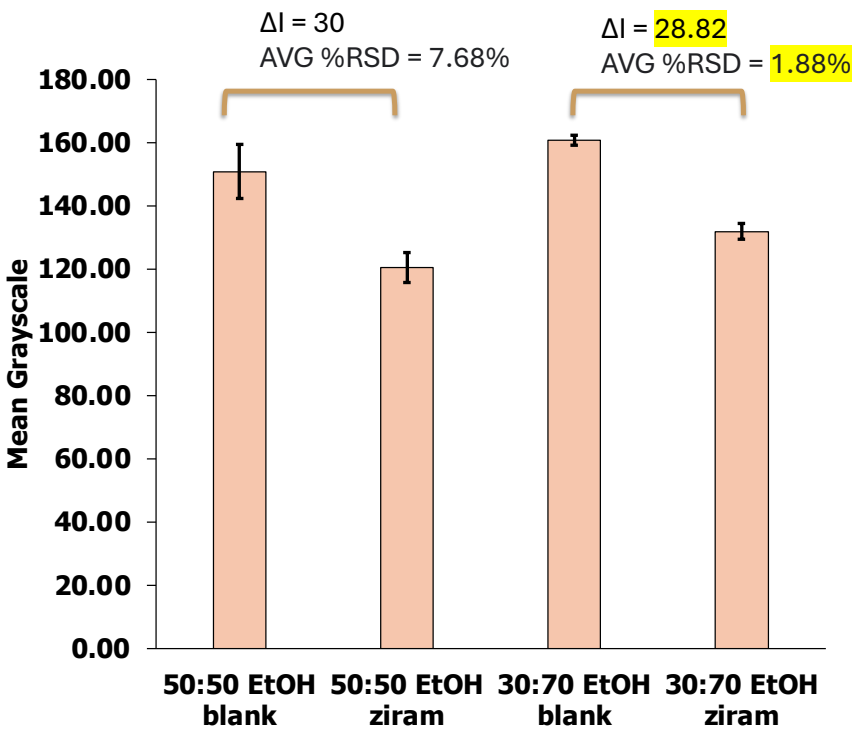


**Fig. S5.** Solvent optimization on devices (with vs without ziram)

**Paper Optimization**

Four paper types were evaluated for the fast-flow device: Whatmann 1, Whatmann 3, Whatmann 4, and Ahlstrom blotting paper (Table S1). Although Whatmann 3 paper delivered the highest overall signal, it exhibited greater variability between circles. In contrast, Whatmann 1 paper provided a high overall signal and demonstrated lower circle variability. Therefore, Whatmann 1 was selected as the optimal paper type due to its balanced performance and consistency.

**Table 1.** Paper type optimization.

| | w3 a | w3 b | | w4 a | | w4 b | | al a | | al b | | w1 a | | w1 b | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **min** | **avg.** | **avg.** | **sd** | **avg.** | **sd** | **avg.** | **sd** | **avg.** | **sd** | **avg.** | **sd** | **avg.** | **sd** | **avg.** | **sd** |
| **10** | 110 | 109 | 7.9 | 102 | 9 | 103 | 6 | 99 | 4 | 94 | 5 | 107 | 4 | 109 | 6 |

**Color formation during stability study**

When catechol and 4-AAP are applied together on the same paper pad, they undergo a slower reaction without tyrosinase, yet still produce a noticeable color change over time. By day three of the stability test, the paper changed from white to light red/tan, even though the devices were stored in cooler conditions (Fig. S6). This indicates that the reaction will still occur despite low temperatures. It's important to consider this inevitable uncatalyzed reaction for future device designs and ensure the two components are separated during long-term storage to prevent premature color formation.

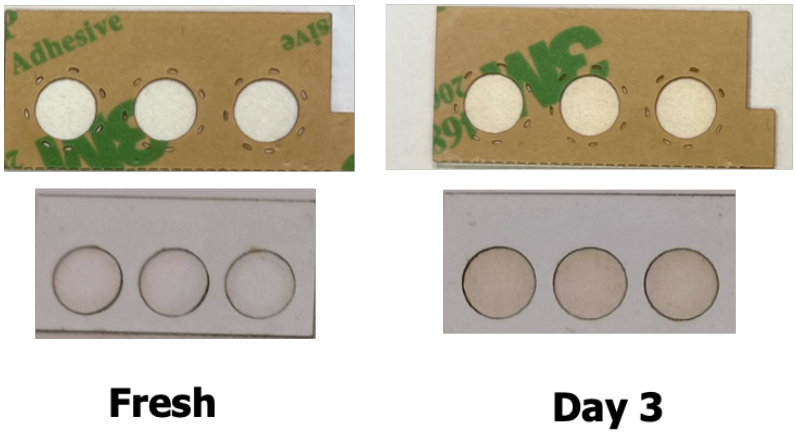


**Fig. S6.** Color formation on paper as a result of catechol and 4AAP reaction.

**Sustainability analysis – AGREE Report**

The Analytical GREEnness Metric Approach and Software was utilized to assess the sustainability of the assay and device. This tool evaluates 12 key areas where the technique or assay can impact environmental well-being, including factors like energy usage, sample size, and operator safety. With the exception of the reagents required for the assay, all other criteria received high scores, indicating that the device is quite sustainable and well-suited for on-site pesticide analysis (Fig. S7).

| Criteria | Score | Weight |
|---|---|---|
| 1. Direct analytical techniques should be applied to avoid sample treatment. | 0.85 | 2 |
| 2. Minimal sample size and minimal number of samples are goals. | 1.0 | 2 |
| 3. If possible, measurements should be performed in situ. | 1.0 | 2 |
| 4. Integration of analytical processes and operations saves energy and reduces the use of reagents. | 1.0 | 2 |
| 5. Automated and miniaturized methods should be selected. | 0.75 | 2 |
| 6. Derivatization should be avoided. | 1.0 | 2 |
| 7. Generation of a large volume of analytical waste should be avoided, and proper management of analytical waste should be provided. | 0.69 | 2 |
| 8. Multi-analyte or multi-parameter methods are preferred versus methods using one analyte at a time. | 0.77 | 2 |
| 9. The use of energy should be minimized. | 1.0 | 2 |
| 10. Reagents obtained from renewable sources should be preferred. | 0.5 | 2 |
| 11. Toxic reagents should be eliminated or replaced. | 1.0 | 2 |
| 12. Operator's safety should be increased. | 0.8 | 2 |

**Fig. S7.** AGREE (Analytical GREEnness Metric Approach and Software) results.

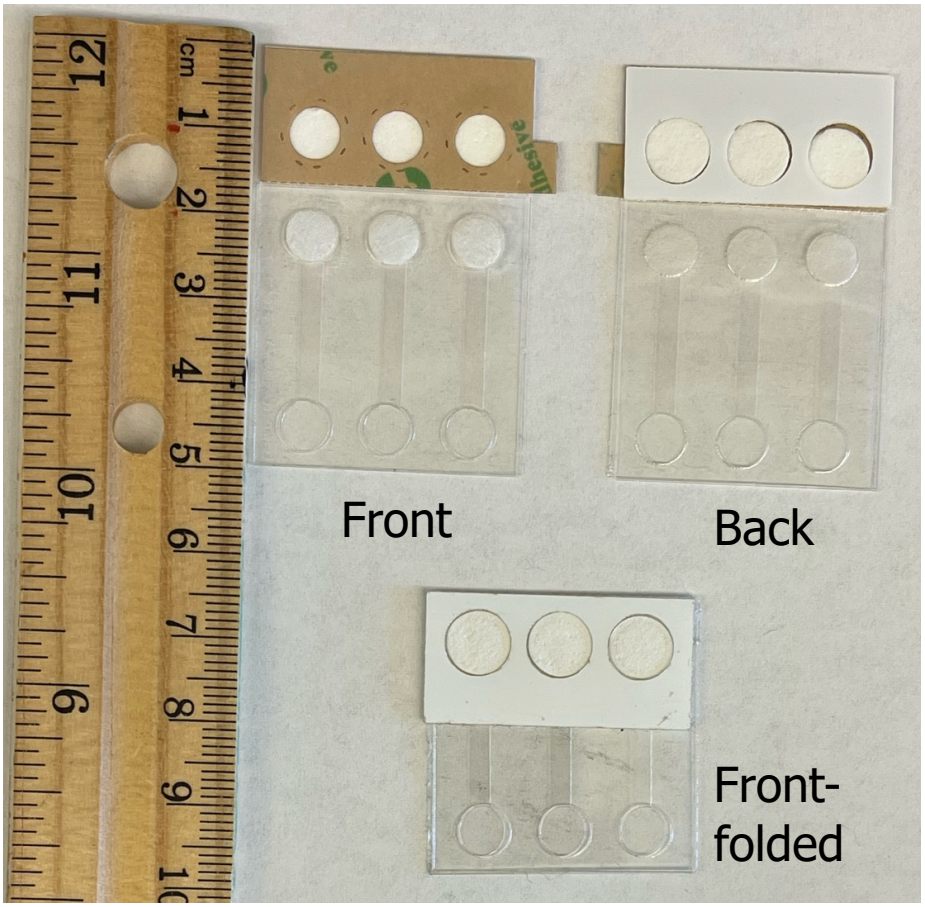


**Fig. S8.** Full assembled device.