Automated Detection and Quantification of Test and Control Strips in Lateral Flow Assays

August 19, 2025

1 Detection and Quantification

1.1 LFA strips mask area extraction

The core challenge in analyzing lateral flow assay (LFA) strips is to reliably detect the colored stripes that represent the control line or the test line. These regions are typically characterized by a red component relative to the surrounding. To exploit this property, we define a binary mask $M(x, y)$ by thresholding the red channel against the green and blue channels:

$$M(x,y) = \begin{cases} 1, & \text{if } \big(R(x,y) - G(x,y)\big) > \tau \ \wedge \ \big(R(x,y) - B(x,y)\big) > \tau \\ 0, & \text{otherwise,} \end{cases}$$

where τ is a user-defined *margin parameter*. This ensures that only pixels where red dominance exceeds both green and blue by at least τ are retained.

As shown in Figure 1. It robustly captures stripe regions even under variations in lighting and background. In practice, τ is restricted to a range (4–30) that balances sensitivity (capturing faint test lines) with specificity (excluding background noise).

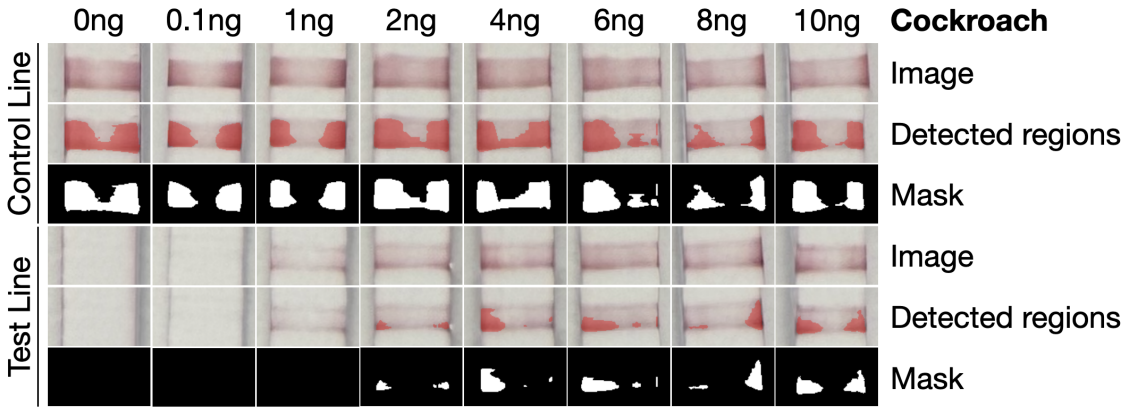

Figure 1: Example of masking for cockroach assay strips. Top three rows: control line region with raw image, detected pixels (overlay), and binary mask. Bottom three rows: corresponding test line region. The progression from 0 ng to 10 ng illustrates that the masking approach successfully highlights the line regions while excluding background noise, enabling consistent quantification across concentrations.

1.2 LFA strips color info extraction

For quantitative analysis, only the pixels retained in the mask are considered. We define a red-biased grayscale intensity to emphasize the contribution of the red channel:

$$G(x,y) = 0.05 \cdot B(x,y) + 0.05 \cdot G(x,y) + 0.90 \cdot R(x,y).$$

Over the set of masked pixels $\Omega = \{(x, y) \mid M(x, y) = 1\}$, we compute:

$$N = |\Omega|, \qquad S = \sum_{(x,y)\in\Omega} G(x, y).$$

Here N is the number of highlighted pixels (mask size), and S is the summed grayscale intensity over the mask. In subsequent steps, S serves as the key quantitative feature for both the test and control lines, while N can be used as an auxiliary descriptor of region size.

1.3 Normalization Against Control

To compare across strips, we normalize test-line intensities relative to their control-line counterpart. Let S_{test} and S_{con} denote the summed grayscale intensities for the test and control lines at a given concentration. We define the normalized value:

$$y = \frac{S_{\text{test}}}{S_{\text{con}}}, \qquad S_{\text{con}} > 0.$$

This ensures the control line is fixed as reference ($y = 1$), while test-line values scale accordingly.

1.4 Margin Optimization

The value of τ critically determines the mask size and, consequently, the quantitative results derived from it. A margin that is too small will include excessive background, artificially inflating measured intensities, while a margin that is too large will generate only faint signals and underestimate test-line strength. To resolve this, we create the following method to automatically evaluate candidate τ values and select the one that best preserves a smooth and interpretable response relationship.

For each candidate τ, we extract intensities $\{(x_i, y_i)\}$, where x_i is the known concentration level (in ng) and y_i is the corresponding test-line intensity normalized by the control line. We then fit a global linear model:

$$y_i \approx a + bx_i,$$

and compute the following quantitative metrics:

- **Root Mean Squared Error (RMSE):**

$$\text{RMSE} = \sqrt{\tfrac{1}{n}\sum_i (y_i - \hat{y}_i)^2},$$

  where $\hat{y}_i$ is the predicted value from the fitted line. RMSE measures the absolute prediction error, penalizing deviations between observed and fitted values.

- **Consistency of Direction:** For each margin τ, consider the normalized sequence $\{y_i\}$ at concentrations $\{x_i\}$. Define the first-order differences:

$$d_i = y_{i+1} - y_i, \qquad i = 1, \ldots, n-1.$$

  The sign consistency is measured by

$$C(\tau) = \frac{1}{n-1}\sum_{i=1}^{n-1} \text{sgn}(d_i),$$

  where $\text{sgn}(\cdot)$ is the sign function. Margins with $|C(\tau)|$ close to 1 indicate strong monotonicity (all differences positive or all negative), while $C(\tau) \approx 0$ indicates inconsistency.

- **Separation of Neighboring Points:** For each consecutive triplet (y_{i-1}, y_i, y_{i+1}), we normalize:

$$\tilde{y}_i = \frac{y_i - y_{i-1}}{y_{i+1} - y_{i-1}}, \qquad 0 \le \tilde{y}_i \le 1.$$

If the values follow a consistent direction ($d_{i-1} \cdot d_i > 0$), then the score is assigned as

$$S_i = 1 - \left|\tilde{y}_i - 0.5\right|,$$

which rewards middle points that lie close to the normalized mean (balanced separation). If the direction reverses ($d_{i-1} \cdot d_i < 0$), then

$$S_i = -\big(1 - \left|\tilde{y}_i - 0.5\right|\big),$$

penalizing inconsistent or reversed trends. The overall separation score for margin τ is the average:

$$S(\tau) = \frac{1}{n-2} \sum_{i=2}^{n-1} S_i.$$

- **Angle Score:** The slope b defines an angle $\theta = \arctan(b)$ relative to the x-axis. To avoid trivial slopes (too flat or nearly vertical), we define:

$$\text{AngleScore} = \frac{2|b|}{1+b^2}.$$

  This score approaches zero for $b \to 0$ (flat line) or $b \to \infty$ (vertical line), and reaches its maximum when the slope is balanced, thereby favoring interpretable dose–response curves.

- **Dynamic Range:** The difference $y_{\max} - y_{\min}$ indicates the spread of measured values, normalized by their mean $\bar{y}$. A larger dynamic range is desirable as it improves discriminability between concentrations.

These terms are integrated into a composite margin score:

$$\text{Score}(\tau) = w_C \cdot C(\tau) + w_S \cdot S(\tau) + w_A \cdot \text{AngleScore} - w_E \cdot \frac{\text{RMSE}}{y_{\max} - y_{\min}},$$

where w_C, w_S, w_A, w_E are tunable weights that balance direction consistency, neighbor separation, monotonicity, dynamic range and prediction error, respectively.

By including $C(\tau)$ and $S(\tau)$, the scoring function explicitly rewards margins that produce (i) globally consistent increasing or decreasing trends, and (ii) locally well-separated neighboring points. This prevents degenerate solutions where values fluctuate irregularly or collapse to nearly identical magnitudes. The additional terms ensure that the selected τ reflects both overall monotonicity and sufficient discriminatory power between consecutive concentrations.

1.5 Curve Fitting and Interpolation

To allow continuous interpolation between measured concentrations, we fit candidate models (linear, polynomial, exponential, logarithmic, power law, reciprocal). For each model $f(x)$, we compute RMSE:

$$\text{RMSE}(f) = \sqrt{\frac{1}{n} \sum_i \big(y_i - f(x_i)\big)^2}.$$

As shown in Fugre 2, the model with the lowest RMSE is selected as the best-fit curve.

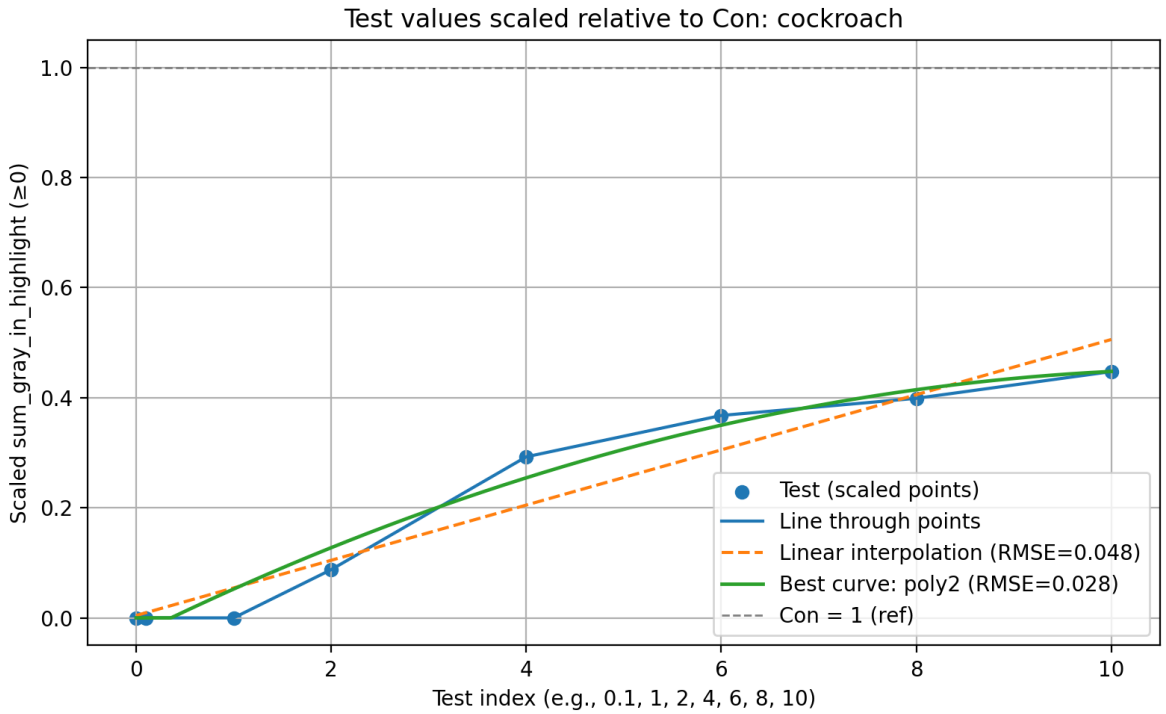

Figure 2: Scaled test-line intensities relative to the control (cockroach assay). Blue points represent measured values, connected by a line for visualization. The dashed orange line shows the linear interpolation (RMSE = 0.048), while the green curve represents the best-fit polynomial model (degree 2) with lower error (RMSE = 0.028). The gray dashed line indicates the normalized control reference ($y = 1$).

2 Supplementary

2.1 Der F

As shown in Figure 3, our method has been successfully differenciate the stripe and the background. For the interpolation, because the zero-concentration test line introduces strong non-monotonic deviations (Figure 4), we also evaluated the trend after excluding readings below 1 ng. This adjustment eliminates spurious fluctuations at the origin and produces a clearer monotonic dose–response curve. As shown in Figure 5, the revised data yields a smoother and more consistent interpolation, with substantially lower RMSE. This suggests that excluding unstable (hard to differentiate) points enhances interpretability and reliability of the fitted model.

2.2 Der P

For the Der P assay, the masking (Figure 6) successfully isolates both the control and test lines across a wide concentration range (0–80 ng). The detected regions and binary masks confirm that the method robustly excludes background and preserves the line signals, even at low concentrations.

When normalized test-line intensities are scaled against the control line, the resulting curve exhibits a clear monotonic trend that saturates at higher concentrations. As shown in Figure 7, direct linear interpolation yields a poor fit (RMSE = 0.27), underestimating curvature in the response. In contrast, a logarithmic model captures the rapid rise at low concentrations and the gradual plateau at higher concentrations more effectively, reducing RMSE to 0.082.

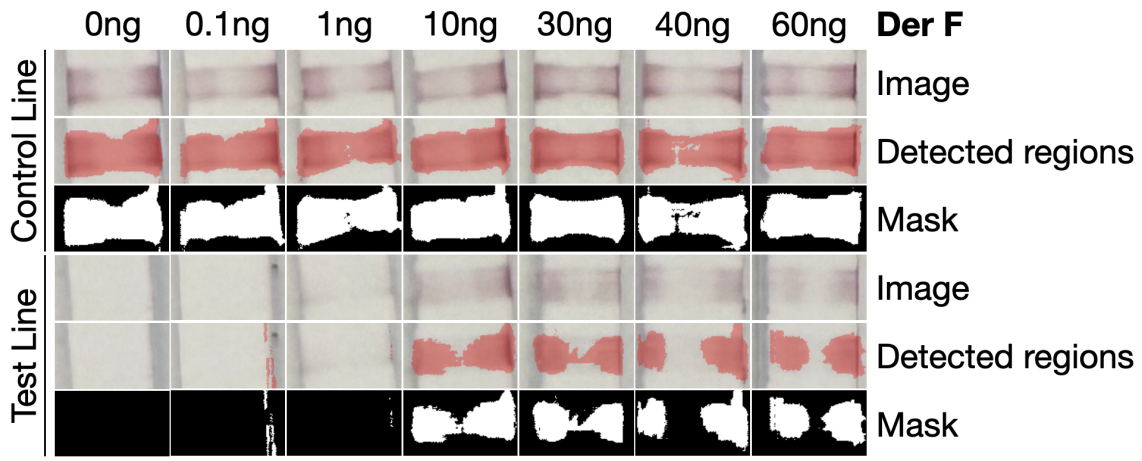

Figure 3: The mask for Der F assay strips.

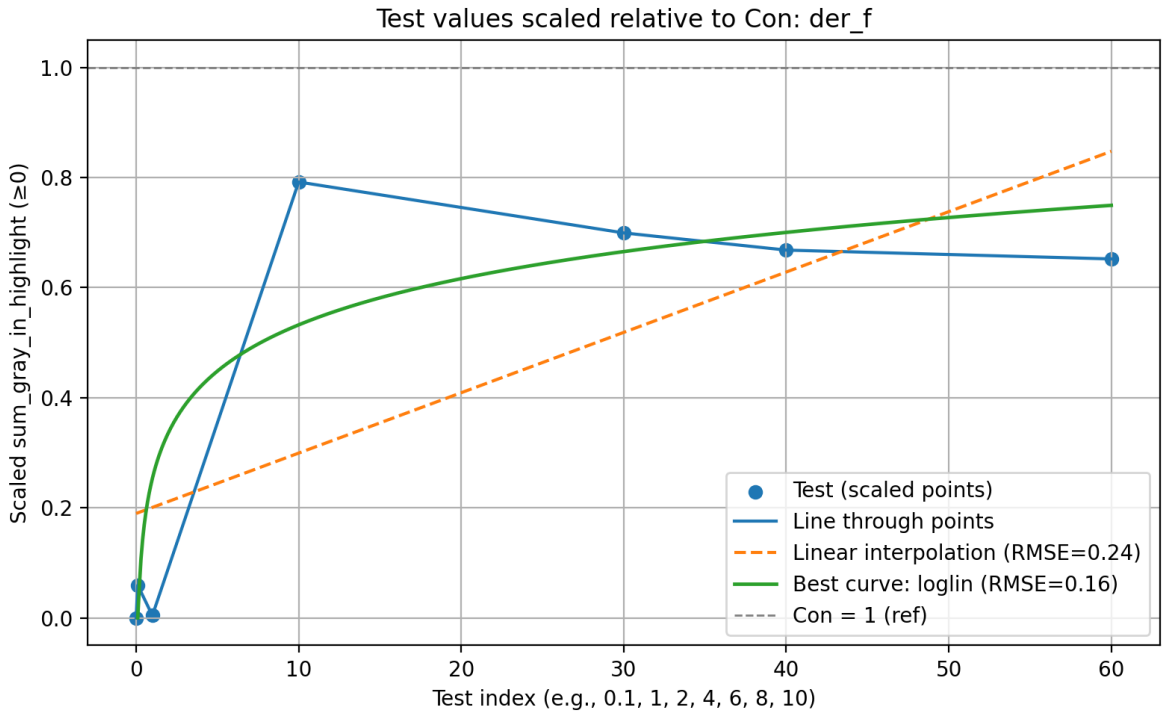

Figure 4: Scaled test-line intensities relative to the control (Der F assay).

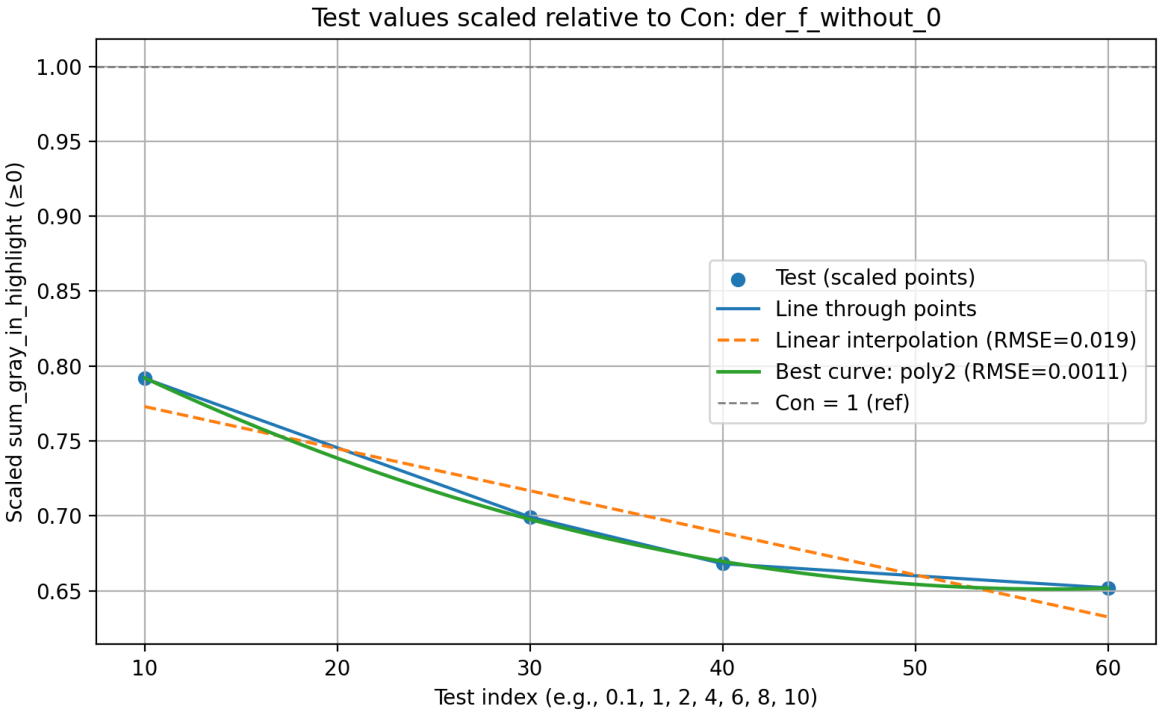

Figure 5: Scaled test-line intensities relative to the control exclude concentration points below 1 (Der F assay).

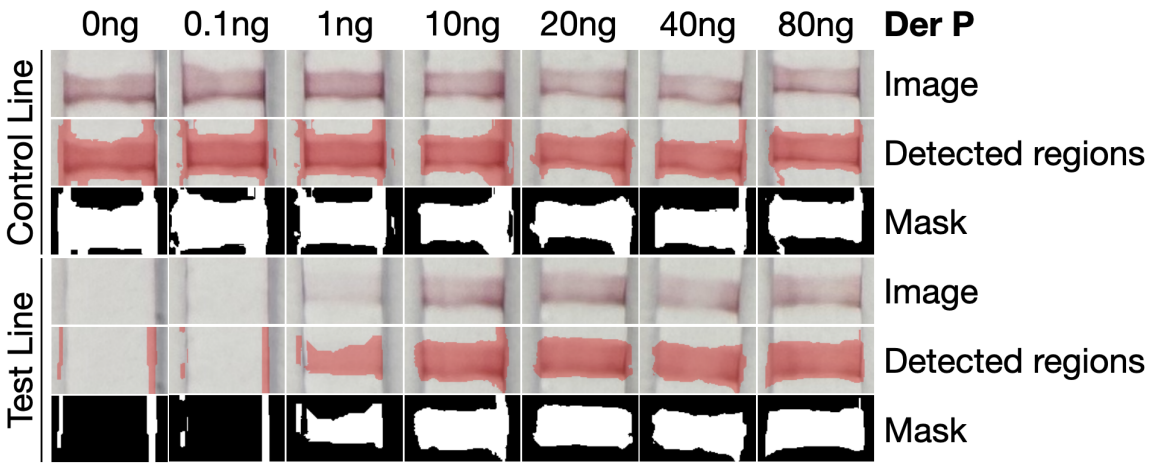

Figure 6: The mask for Der P assay strips.

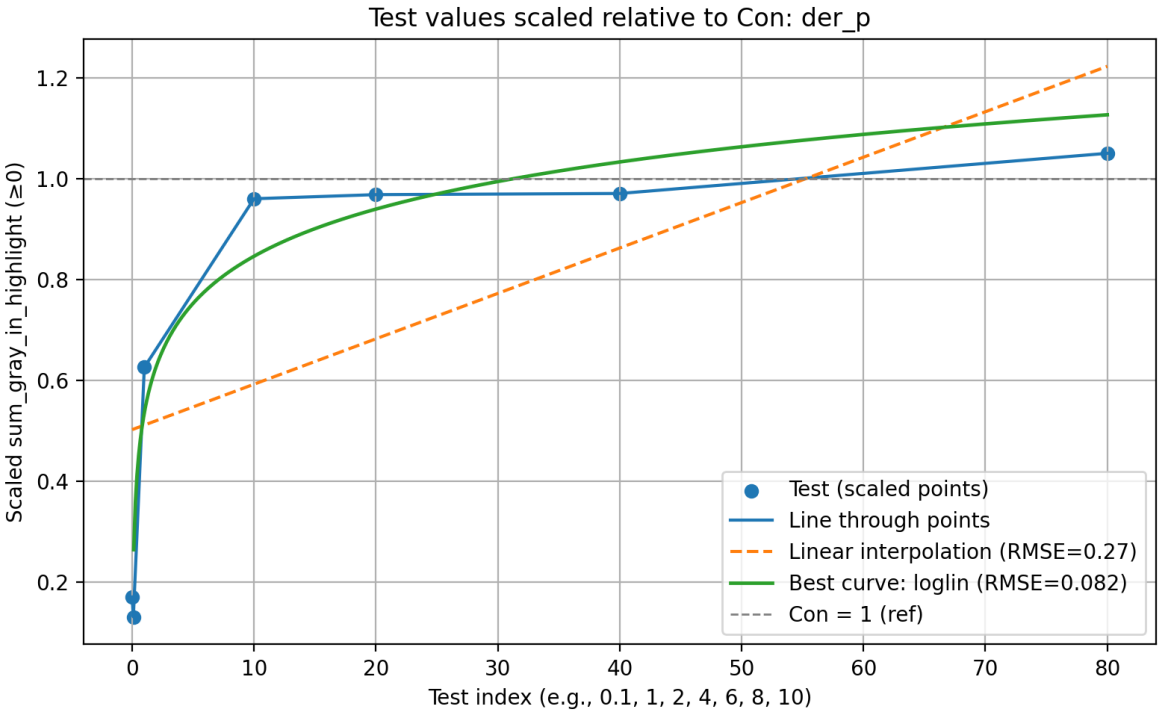

Figure 7: Scaled test-line intensities relative to the control (Der P assay).