ECE 638
Topics on Image Analysis

Module 64.0 Binary Segmentation

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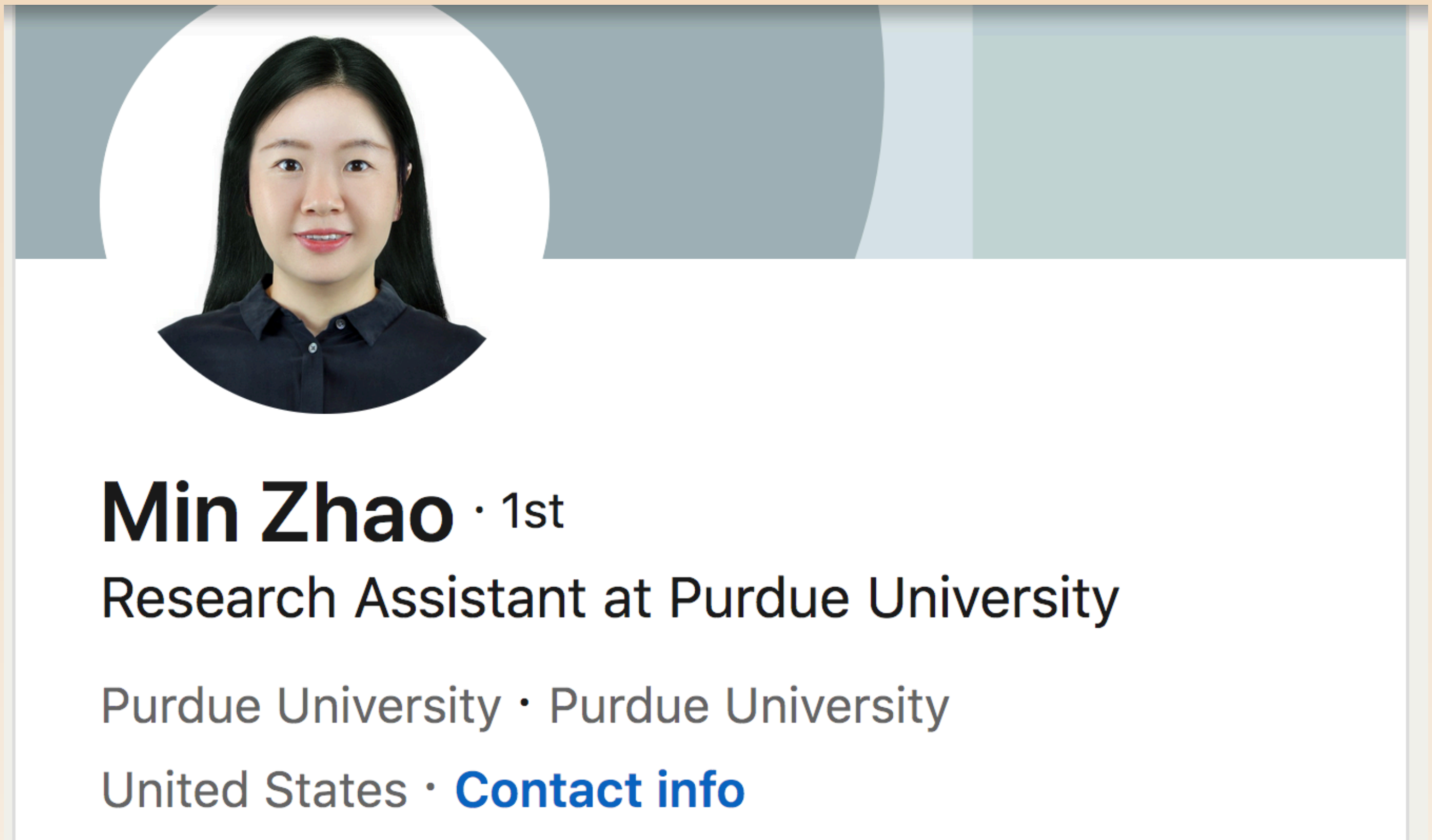
Synopsis (1/2)

- Binary segmentation
  - Otsu’s method
  - Twice Otsu method
  - Valley emphasis method
- Clustering methods
  - K-means algorithm (unsupervised)
  - Choosing the number of clusters
    - Elbow method
    - Other approaches
  - Spatiochromatic feature space
  - K-nearest neighbors (supervised)

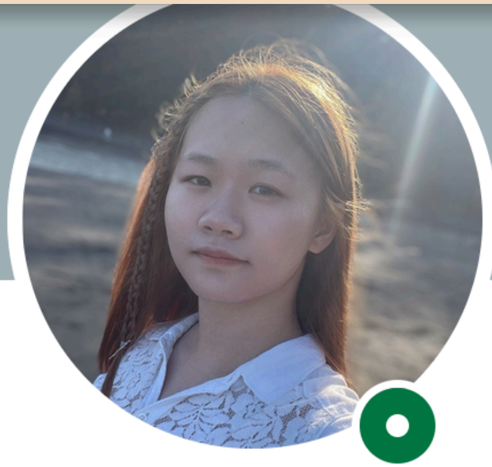
Synopsis (2/2)

- Edge-based methods
  - Sobel operator
  - Color-aware Sobel operator
- Adaptive bilateral filter
- Connected components
- Edge-thinning
- Morphological operations

About the authors (1/3)



About the authors (2/3)



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Detection, Imaging, and Quantification of DNA-Based Pathogen Based on Inkjet-Printed Test Strips

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Abstract

It is known that the foodborne pathogen E.coli O157:H7 from contaminated food can cause severe disease in our bodies and even death. Therefore, the detection of foodborne pathogens in our daily diet is crucial for global public health. We have previously reported an affordable, rapid, and simple method for detect-

the pattern for the capture of *E.coli* O157:H7. The readouts of test results can be interpreted either by our image analysis system or by the naked human eye. We prove that our PTSs can successfully detect the presence of *E.coli* O157:H7 down to 10^2 CFU/ml [3].

One of the conditions that a successful biosensor must be meet is that the detection results show a low variation between

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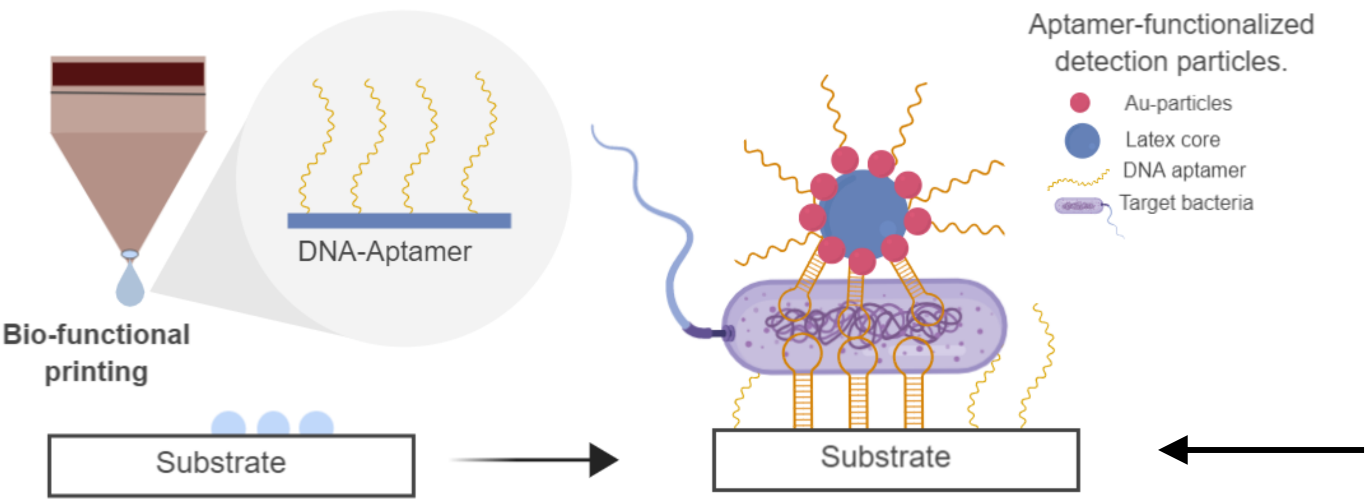
2. Program Objectives

- ❑ Develop processes for inkjet patterning of nano-functionalized biocompatible and biodegradable cellulose test strips;
- ❑ Develop image analysis systems for high-speed inspection and process control.



3. Detection platform

Our approach successfully combines three technologies:

1. Aptamer-based detection
2. Reproducible mass production by Inkjet printing
3. Image analysis with mobile phone camera to quantitatively correlate color intensity to pathogen concentration.

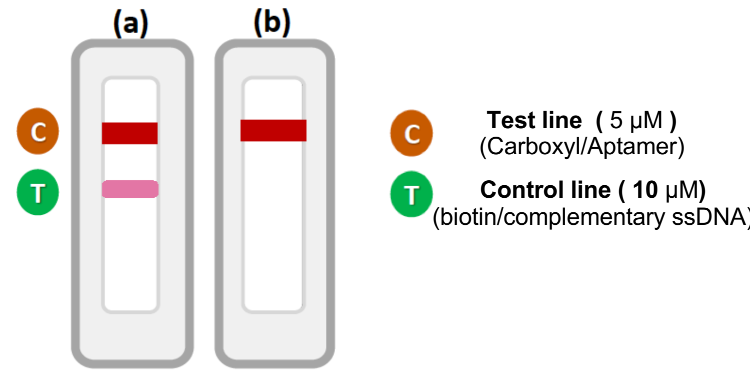


Fig. 2: Test Interpretation: (a) positive and (b) negative.

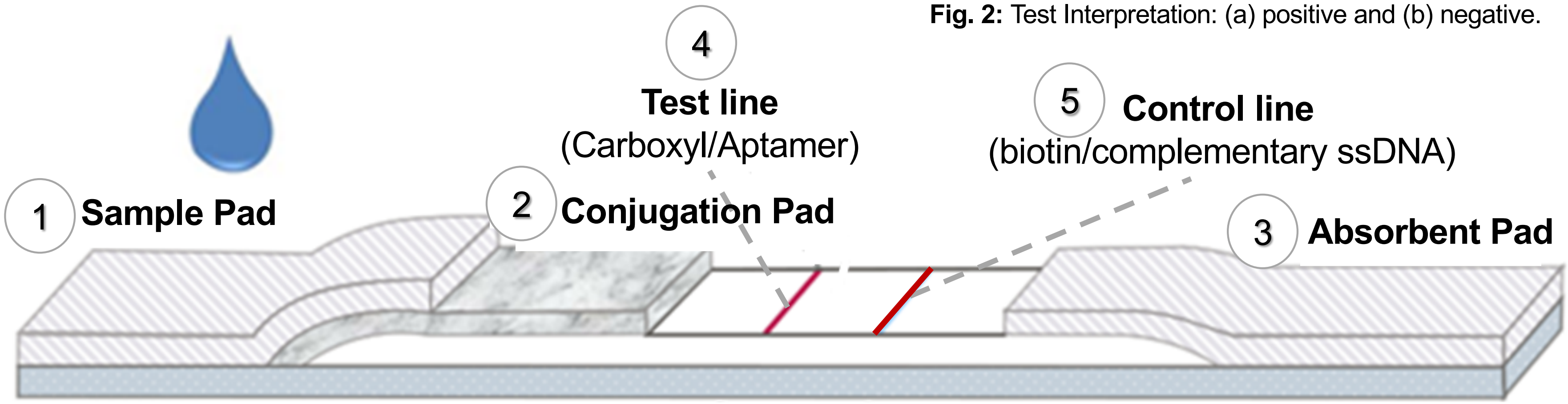


Fig. 1: Configuration of a lateral flow test strip

- S. Diaz Amaya, **M. Zhao**, L.K. Lin, C. Ostos, J.P Allebach, G.T.C. Chiu, A.J. Deering, and L.A. Stanciu. Inkjet Printed Nanopatterned Aptamer-Based Sensors for Improved Optical Detection of Foodborne Pathogens. Small, p.1805342. (2019).

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4.1 The printing systems

- Thermal Inkjet PicoJet System: (HP TIPS)
  Drop volume: 2 - 200 picoliters

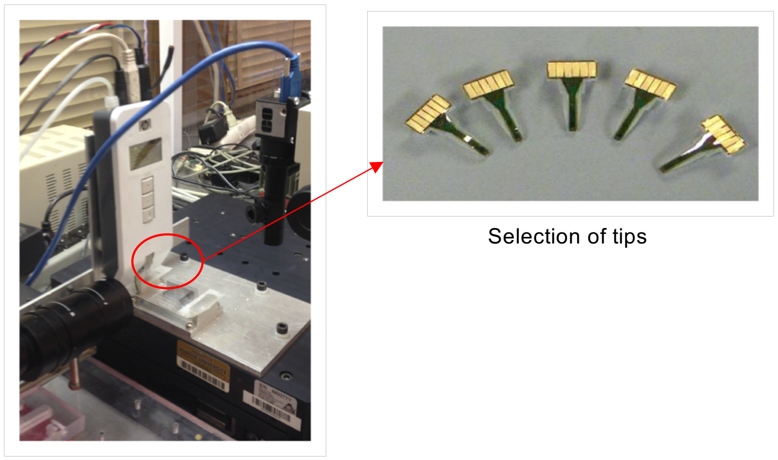

Fig. 1: The components of the TIPS
(TIPS: Thermal Inkjet Picojet System)

- PipeJet System: (BioFluidiX, Freiburg, Germany)
  Drop volume: 1 - 70 nanoliters

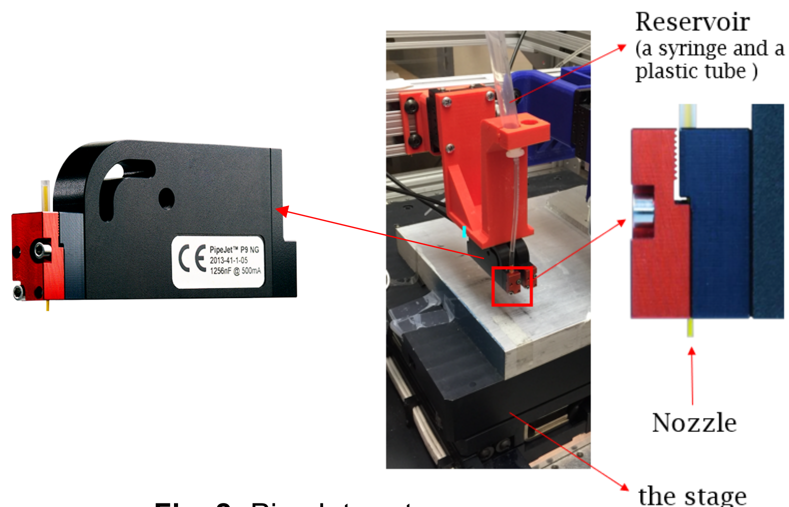

Fig. 2: PipeJet system

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5.1 Image analysis | Pipeline and result

RGB Color Space → CIE L*a*b* Space → Grayscale images (ΔE from background) → Segmentation (Otsu's method) → Binary image (8-point connected component)

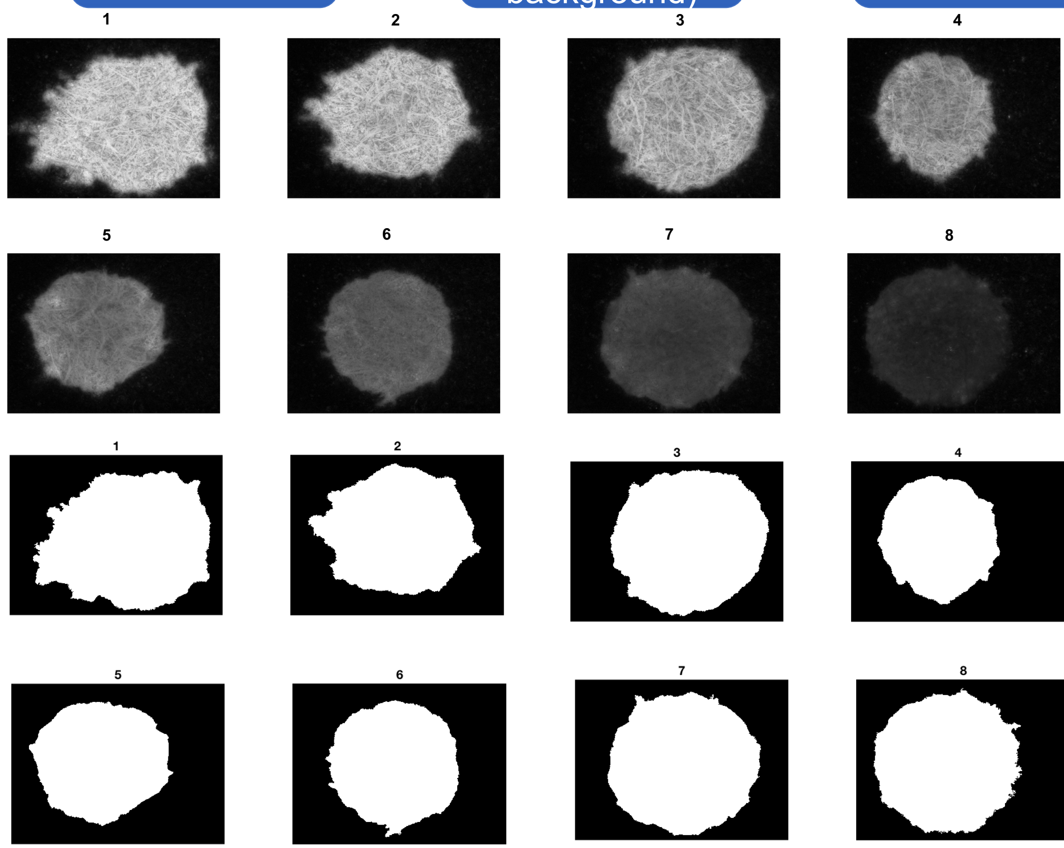

Fig. : The grayscale images and binary images of 8 dots

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5.2 Image analysis | Printed test strips

- ❑ Develop an image analysis pipeline that can effectively detect *E. coli* O157:H7.
- ❑ Correlate color change of response area with the concentration of target.
- ❑ Determine the limitation of detection of our biosensor.
- ❑ Assess the variation among the responses in test lines of test strips by correlating the color intensity under a fixed concentration.

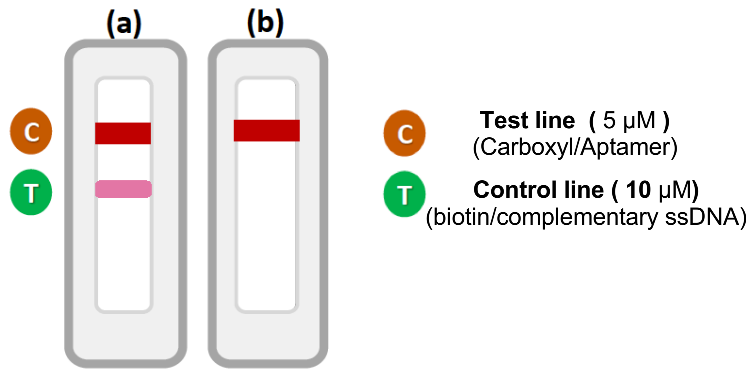

Fig. :Test Interpretation: (a) positive and (b) negative.

5.2 Image analysis | Samples of printed test strips

Goal: Correlate the color intensity with bacteria concentration on the printed test strips. Background substracted image analysis.

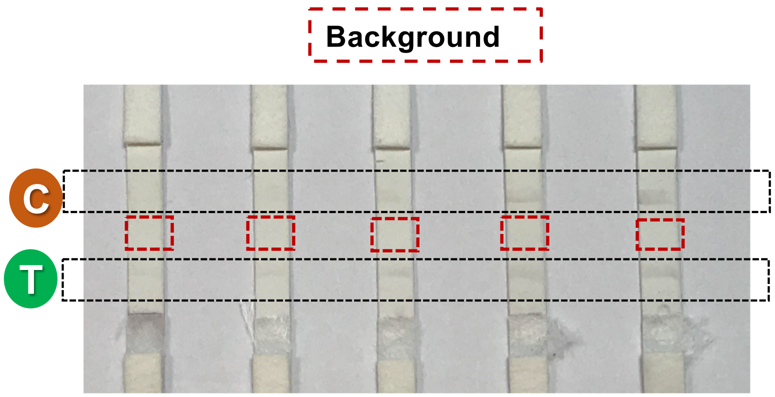

Fig. 1: Image captured by mobile phone camera (iPhone 7 plus)

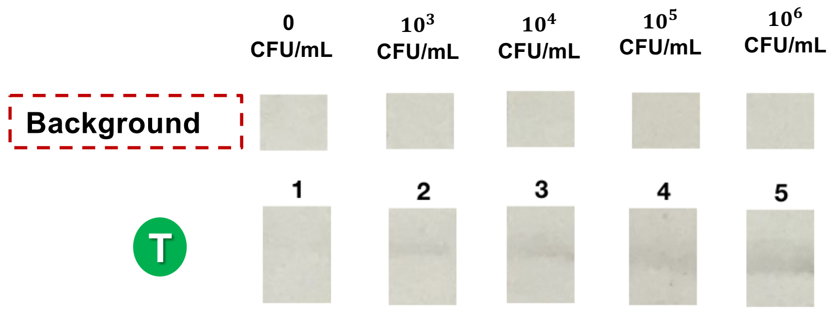

Fig. 2: Optical detection of decreasing bacteria concentration (*E. coli* O157:H7)

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5.2 Image analysis | Pipeline and result

RGB Color Space → CIE L*a*b* Space → Grayscale images (ΔE from background)

→ Segmentation (Otsu's method) → Binary image (8-point connected component) → Sum of ΔE of each test line

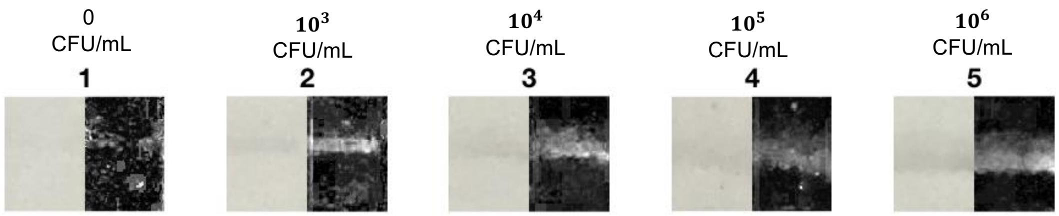


Fig. 1: Original images and gray level ΔE images images:

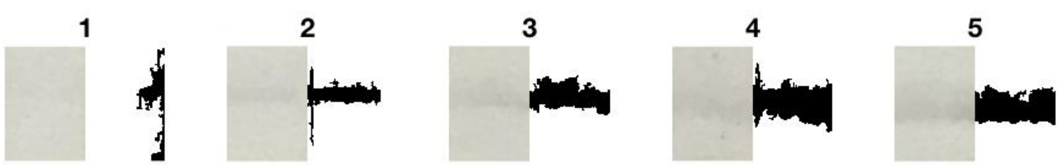
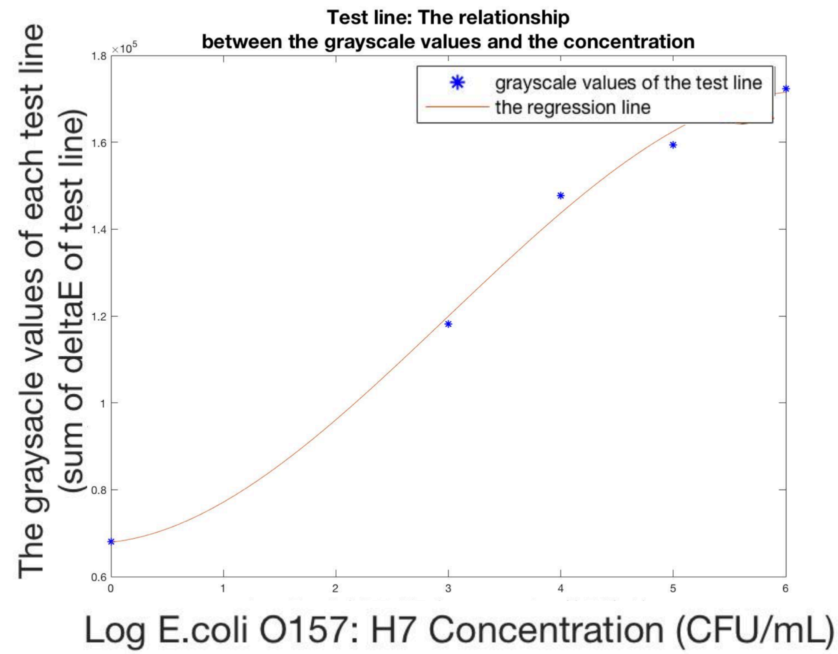


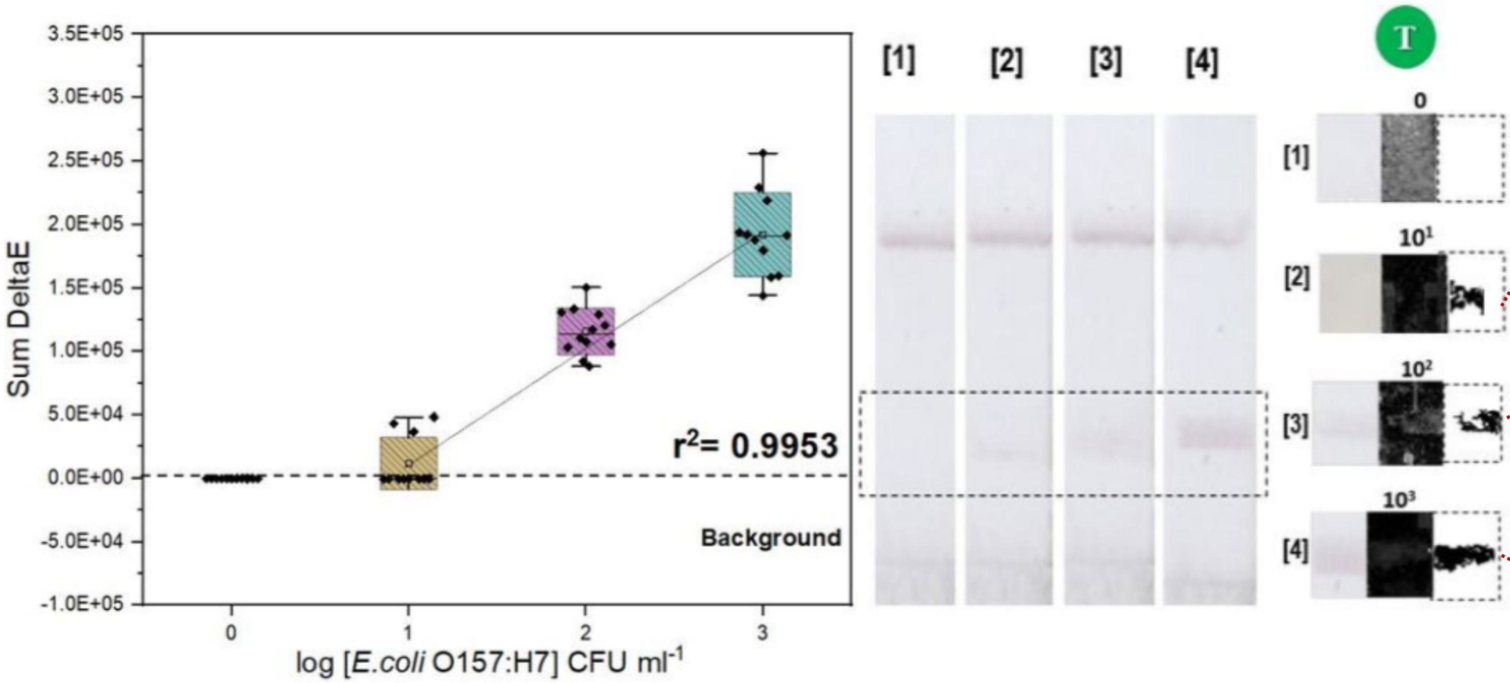
Fig. 2: Original images and binary images:

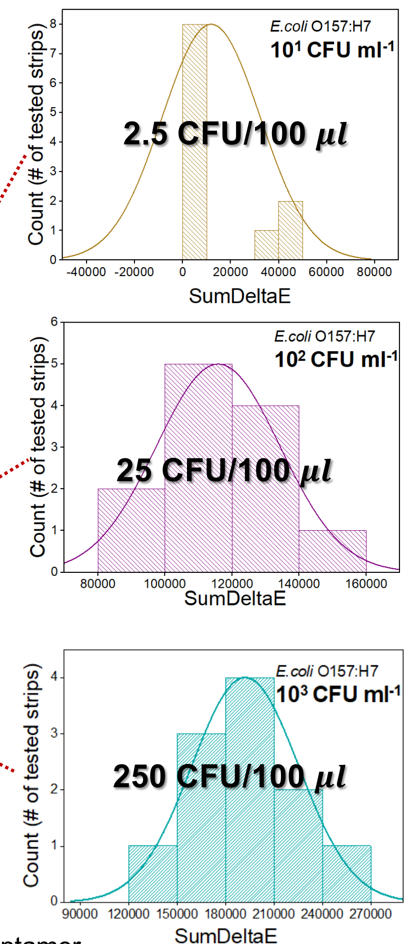


- Bio-inks were printed under the same conditions.
- The grayscale intensity is **directly proportional** to cell concentration.
- From low to intermediate concentration (0 to 10^2 CFU/mL) **an exponential correlation** is observed.
- At higher concentrations (10^4 to 10^6 CFU/mL) **a plateau effect** is predicted possibly caused by saturation.

5.2 Image analysis | Limitation of detection and the correlation

Goal: Detect the limitation of detection and find the correlation between the intensity and the concentration.





S. Diaz Amaya, **M. Zhao**, L.K. Lin, C. Ostos, J.P Allebach, G.T.C. Chiu, A.J. Deering, and L.A. Stanciu. Inkjet Printed Nanopatterned Aptamer-Based Sensors for Improved Optical Detection of Foodborne Pathogens. *Small*, p.1805342. (2019).

Binary Segmentation – Otsu's Method

Otsu's thresholding method is one of the best-known methods for automatic image segmentation [17]. Based on the histogram of a grayscale image, Otsu's method finds the optimal threshold t^* that maximizes the between-class variance $\sigma_B^2(t)$. The optimal threshold is expressed as follows:

$$t^* = ArgMax_{0 \leq t < L}\{\omega_1(t)\mu_1^2(t) + \omega_2(t)\mu_2^2(t)\} \quad (3.2)$$

where L is the number of distinct gray values ranging from 0 to L - 1, $\omega_1(t)$ and $\omega_2(t)$ are the probabilities of the two classes, and $\mu_1(t)$ $\mu_2(t)$ are the average gray values of the two classes. This method works well when the histogram has a strongly bimodal distribution. However,

[17] N. Otsu, "A threshold selection method from gray-level histograms," *IEEE Transactions on Systems, Man, and Cybernetics*, vol. 9, no. 1, pp. 62–66, 1979.

Binary Segmentation – Valley Emphasis Method

The valley-emphasis segmentation is a weighted Otsu's method. The idea of this method is to select the optimal threshold value corresponding to a grayscale value that has a small probability of occurrence and also maximizes the between-class variance, as in Otsu's method [17]. The objective function of this method is expressed as:

$$t^* = ArgMax_{0 \le t < L}\{(1 - p_t)(\omega_1(t)\mu_1^2(t) + \omega_2(t)\mu_2^2(t))\}, \tag{3.3}$$

where p_t is probability of occurrence of the grayscale value corresponding to the threshold t.

Binary Segmentation – Twice Otsu Method

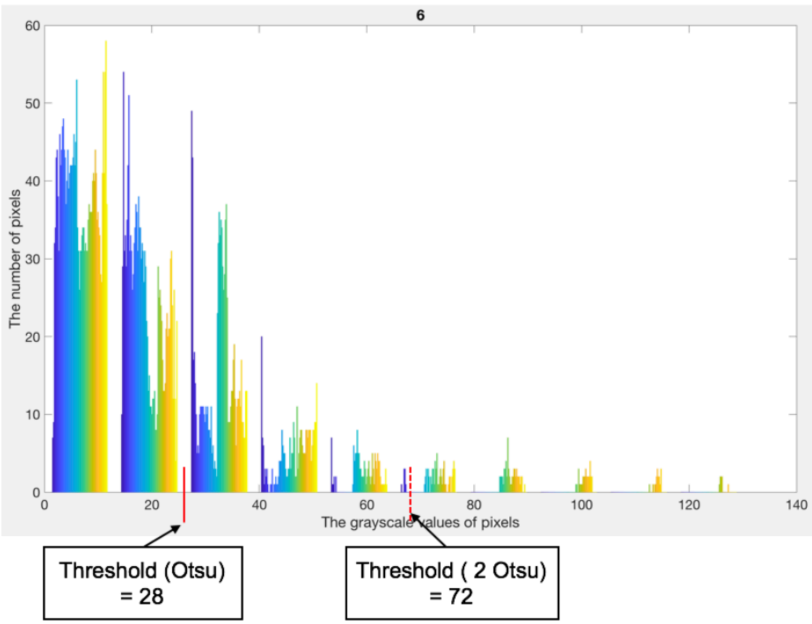
We can observe that the background variance is large for each image, so it is hard for a single threshold segmentation algorithm to effectively segment the target test zones from such kinds of background, as illustrated in Figure 3.3 O-7. It causes some of the background pixels to be classified as foreground pixels. To address this problem, we apply Otsu's method again to the segmented image after the first application of the single Otsu's threshold method. One of the examples that the segmented image after second application of single Otsu segmentation is shown in Figure 3.3 T-7, which is expected to remove extra background regions. This method is referred to as the twice Otsu method [32]. In our application, the criterion for an image to be treated with the twice Otsu method is that the ratio between the height and the width of the foreground should be higher than an empirically chosen threshold. We use 0.4 as the threshold in our application.

[32] D.-Y. Huang and C.-H. Wang, "Optimal multi-level thresholding using a two-stage Otsu optimization approach," *Pattern Recognition Letters*, vol. 30, no. 3, pp. 275–284, 2009.

Twice Otsu Method – Example

1. The basic idea:

   The criterion for an image applied to twice Otsu: $\frac{the\ height\ of\ foreground\ after\ single\ Otsu}{the\ width\ \ of\ foreground\ after\ single\ Otsu} > 0.4$ (empirical data)



a b c d

Fig. :Steps of Twice Otsu segmentation method: (a) Original image, (b) grayscale image, (c) segmented image after first application of single Otsu threshold method on (b), (d) segmented image after second application of single Otsu segmentation on object (dark) pixels of image (c).

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Segmentation – Histogram Thresholding

1. The basic idea: Initially, we regard that every non-empty gray level is a separate mode contained in a cluster. Then the distance between adjacent clusters are computed, and the smallest distance pair is merged. The estimated threshold for the usual two-level thresholding is obtained by iterating this operation until two groups of gray levels are obtained.

$$Dist(C_{k1}, C_{k2}) = \sigma_I^2(C_{k1} \cup C_{k2})\sigma_A^2(C_{k1} \cup C_{k2})$$

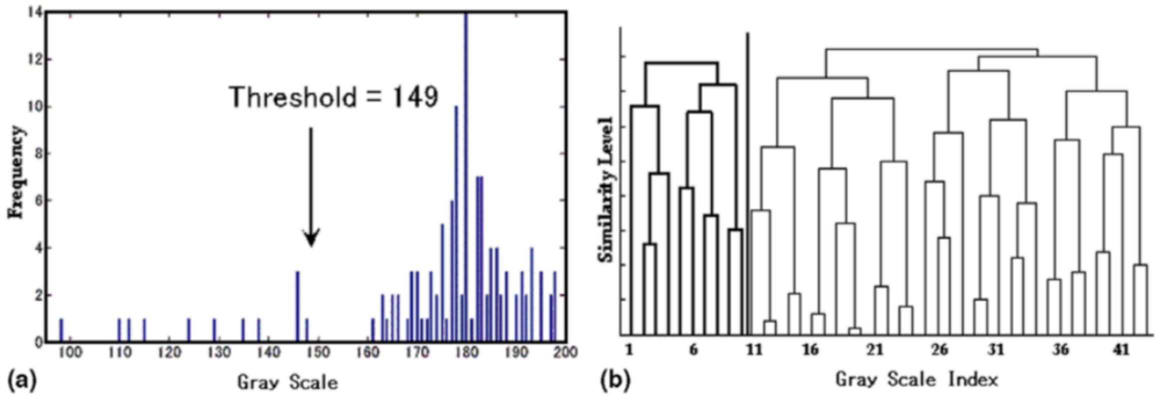

Fig. 1. (a) Histogram of the sample image and (b) the obtained dendrogram.

59 * Arifin, Agus Zainal & Asano, Akira. Image segmentation by histogram thresholding using hierarchical cluster analysis. *Pattern Recognition Letters.* pp. 1515-1521, (2006).

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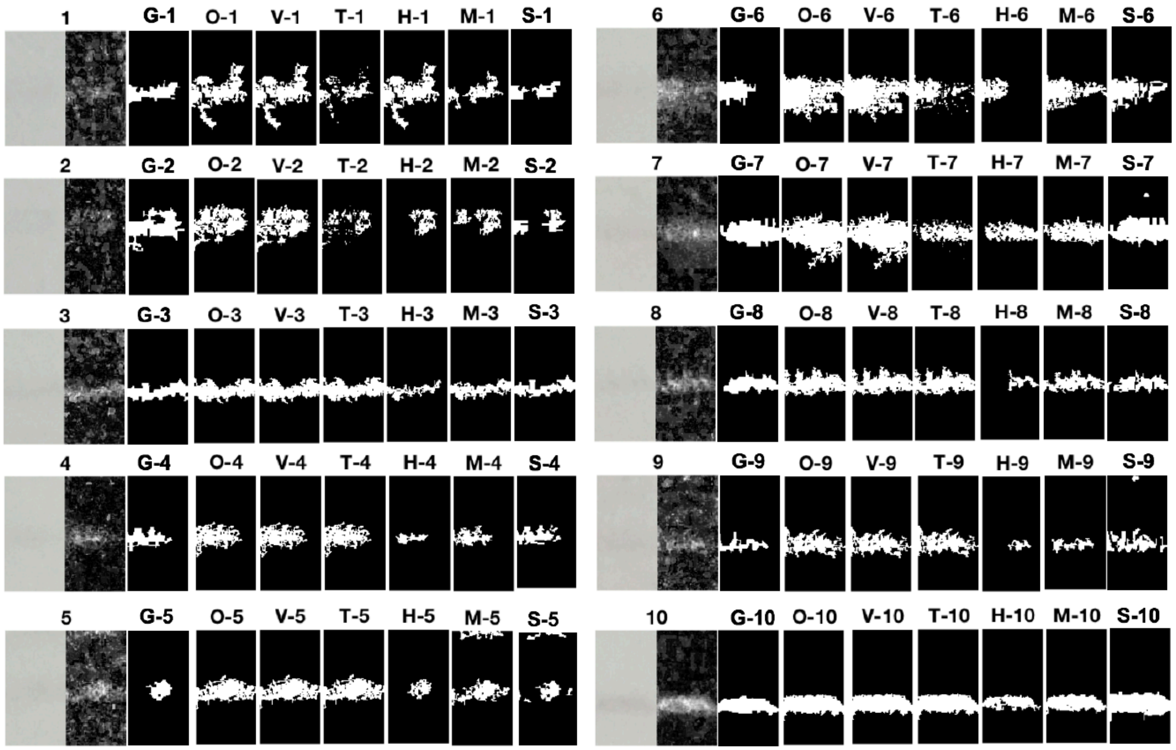
Segmentation – Multilevel Thresholding Method

There is another multilevel segmentation method which is an extension of Otsu's method, called the multilevel thresholding method [33]. Assume there are m thresholds, $(t_1, , t_m)$ dividing the image into m classes. Then, the optimal thresholds can be determined by the following equations:

$$
\begin{aligned}
Maximize\ J(t) &= \sigma_0 + \sigma_1 + \sigma_2 + \ldots + \sigma_m \\
where\ \sigma_0 &= \omega_0(\mu_0 - \mu_T)^2\ , \\
\sigma_1 &= \omega_1(\mu_1 - \mu_T)^2\ , \\
\sigma_2 &= \omega_2(\mu_2 - \mu_T)^2\ , \quad \ldots \\
\sigma_m &= \omega_m(\mu_m - \mu_T)^2\ .
\end{aligned}
\tag{3.5}
$$

where σ_0, ..., σ_m and μ_0, ..., μ_m, μ_T are the variance, the mean intensity of each class, and the mean intensity for the whole image, respectively. Each of the grayscale images in

[33] P. Sathya and R. Kayalvizhi, "Modified bacterial foraging algorithm based multilevel thresholding for image segmentation," *Engineering Applications of Artificial Intelligence*, vol. 24, no. 4, pp. 595–615, 2011.



(**G, O, V, T, H, M**, and **S** represent the abbreviation of the ground truth segmentation result, Otsu's method, valley-emphasis method, twice-Otsu method, histogram thresholding method, multi thresholding, and SVM, respectively.)

Observation:

- The segmentation results of **Otsu's** and the **valley-emphasis methods** are very close: poor signal-to-noise ratio, hard precisely segment the test zone (O-7 and V-7).
- **The twice Otsu method** can extract the refined test zone from the backgrounds.
- **The histogram thresholding method**: cause information loss (H-4).
- **The multilevel thresholding method** extracts the relatively bright region in the grayscale image.
- The segmentation results of **the SVM method** is mostly determined by the training data.

Fig. : The original images, grayscale images, and segmentation results for test images by the selected methods.