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A smartphone-based bacterial colony phenotyping instrument based on the reflective elastic light-scatter pattern

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ABSTRACT

A portable bacterial colony classification tool based on colonies' reflective elastic light-scatter (ELS) patterns has been developed using a smartphone, a green laser, and a projection screen material. As the collimated beam from the laser illuminates the bacterial colony, backscattered photons interfere and generate a unique pattern on the screen material determined by the unique morphology of the colony. The phone camera, which is located behind the screen, captures the pattern. The collected patterns are utilized to extract the distinctive scatter-related features across different organisms for the classification process. Unlike other tools that use transmitted ELS patterns, the novel device measures the reflective signal, and therefore this ELS technique can be applied to organisms that are grown on opaque media such as blood agar, chocolate agar, which normally prohibits the transmission of the light and generation of forward ELS patterns. The adaptation of the smartphone camera as an imaging device dramatically reduced the system to a palm-size instrument. This made it wholly portable and easy to carry. For validation of the instrument, two different bacteria species, *E. coli* and *L. innocua* were grown on opaque agar media and tested. The results showed over 90% of overall accuracy in differentiating the organisms.

Keywords: Smartphone, portable instrument, bacterial colony, laser scattering, reflection, pathogens

1. INTRODUCTION

In the area of food security, clinical studies and bio-surveillance, identifying useful solutions for classification of microbiological sample in a rapid and accurate manner has been constantly sought after[1–3]. Recently, a single diode-laser based elastic light scatter (ELS) technology was reported with label-free phenotyping of bacterial species based on their morphological differences of colonies. The effectiveness of the technology was published with promising classification results for several bacterial genera, including *Salmonella*[4], *Vibrio*[5], *Listeria*[6], *Campylobacter*[7], and *E. coli*[8]. In addition, interaction of the colony shape and the incoming laser were investigated based on ESL phenomena for a single wavelength[9], multi wavelengths[10], and speckle analysis[11].

The abovementioned technology was applied to particular set of growth media which typically allows laser light to pass through the bacterial colony to generate a unique ELS patterns for discrimination on a sensor below the agar. This includes most of the transparent growth media such as brain heart infusion (BHI) agar, trypticase soy agar (TSA), nutrient agar, and several chromogenic media which utilize transparent agar. On the other hand, there are some opaque growth media such as blood agar, chocolate agar (CHA), and buffered charcoal yeast extract (BCYE) agar to name a few which are important for pathogen detection. One of the common elements of these opaque agar is that either the nutrient or agar completely blocks the incoming laser such that no visible patterns can be generated in transmission mode. Therefore, an alternative is to generate the ELS patterns for these types of colonies using a reflection mode to evaluate scatter patterns generated

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Initial effort to provide proof-of-principle has been reported in a prototype design of reflection scatterometer[12] and provided a scatter pattern classification from four different genera. However, one of the drawbacks of the proposed system was the large dimensions of that system. As shown in Figure 1 (A)-(B), the overall system was 300 mm (W) × 400 mm (L) × 600 mm (H), which required large bench space and also height clearance of 50 cm from culture dish to the imaging camera which limited the system's application into well-defined laboratory conditions.

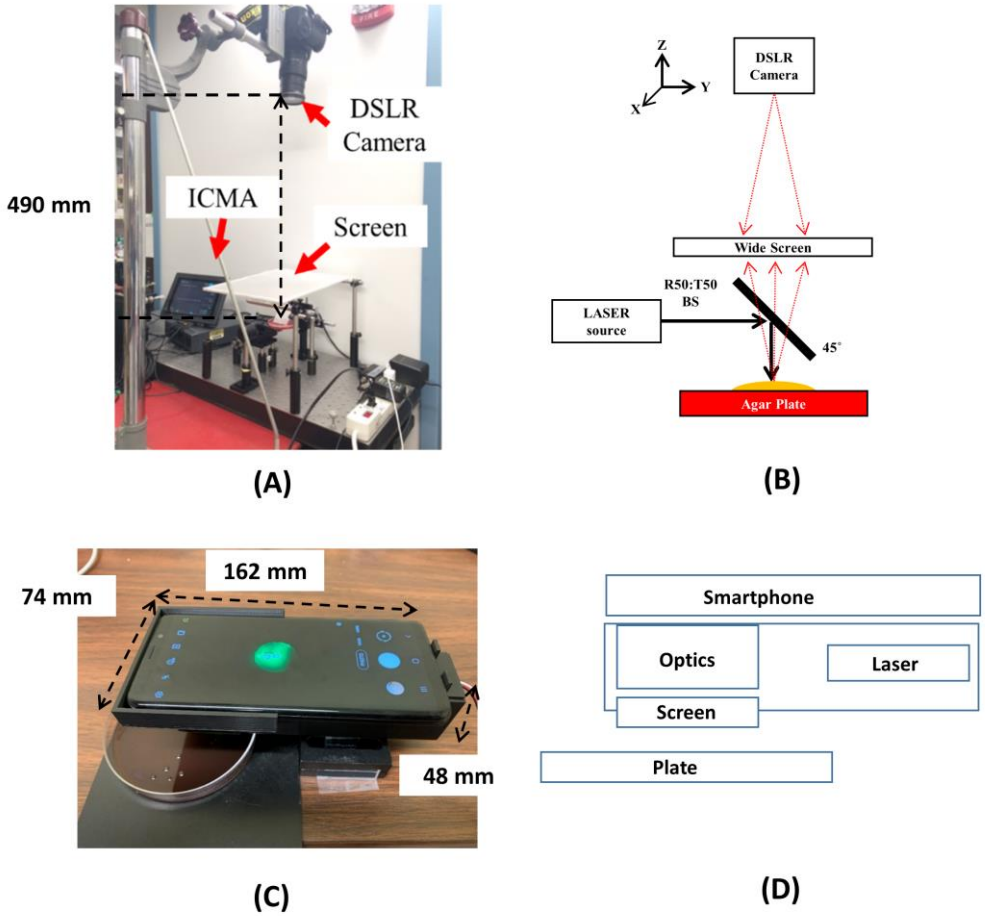

Figure 1. Photo of previous and current design of the reflection scatterometer. (A) previously reported reflection scatterometer which employs a DSLR camera and a bench top system. The imaging distance required was around 490 mm from the camera to the bacterial colony. (B) shows the schematic diagram of the previously reported device. (C) displays the photo of the prototype reflection scatterometer using a smartphone which dramatically reduced the size of the instrument. (D) displays the core components including the diode laser, optics and transparent screen material. Figure 1 (A-B) is reproduced from previous report[12] with the permission from the publisher.

Smartphone handsets have become widely available and become a ubiquitous device that can be easily usable and contain many useful features make the smartphone more than a simple communication device[13,14]. Characteristics such as large random-access memory (RAM), high-speed central processing unit (CPU), high-resolution complementary metal oxide semiconductor (CMOS) sensor, and wi-fi network are similar to the modern-day computers needed for laboratory-based testing. These features now enable the smartphone to be the basis for a field-deployable instrument. Many authors have explored this idea and introduced diverse attachments that transduce the signal of interest[15–19]. Smartphones have been transformed into microscopes[20,21], colorimetric devices[18,19,22–24], luminometers[25–27], and spectrometers[28–31]. Here we report the first design and initial validation of a smartphone-based ELS instrument that can

provide reflective scatter patterns from colonies grown on an opaque media. This report will describe the major design consideration, sample preparations, and data analysis method. Validation data will be provided for two types of different genera of bacterial samples.

2. MATERIALS AND METHODS

2.1 Instrument design

Figure 1 (C)-(D) shows the picture and schematic diagram of the smartphone-based portable scatterometer. The main body of the device was 3-D printed using black PLA material to minimize the stray light inside the optical chamber. In addition, non-reflective material was used to cover the inner part of the optical train starting from the laser and all the way to the screen. A 5-mW, 532 nm diode-pumped solid-state point laser was utilized as an interrogating light source which was powered by 5-volt voltage adaptor. The smartphone used as a Galaxy S9 model with 12-megapixel image sensor which was used for imaging the scatter patterns that was formed on the screen material. This phone provided manual mode which enabled users to control the camera setting (i.e. exposure time, aperture, focus, etc.) so that repeatable imaging conditions could be maintained throughout all the experimental procedures.

2.2 Imaging optics

This innovative portable scatterometer was made possible by using the smartphone camera as the imaging device along with our custom designed imaging optics for miniaturization of the overall device dimension. First, to utilize the smartphone camera, an f = 50 mm plano-convex lens was mounted in front of the smartphone camera to provide the correct focus for the bacterial colony samples. Second, a combination of both specular light and scattered light provided a challenging imaging condition. The smartphone image sensor's dynamic range does not meet the standard of typical scientific cameras so once exposure time was minimized, detail patterns from outer rim of the colony pattern were not recorded. Additionally, when increasing the exposure time of the smartphone camera, the overall image was oversaturated and failed to show any details of the patterns.

This was solved by utilizing a translucent screen that balanced the scattered light so that the smartphone camera was able acquire a quality image. In addition, several different attempts were made to provide a compact imaging module to facilitate both quality and small size device. The first attempt for minimization was to utilize the polarization beam splitter (PBS) where polarization and beam splitter function were integrated into a cube format as shown in Figure 2(A). However, initial testing by imaging a white color paper revealed some issues with transmission capabilities over the whole beam splitter band wavelength region. Figures 2(B) shows the camera screen from a white color paper for evaluation of color change/transmission issues. When an actual bacterial colony was imaged, this phenomenon affected the transmission of the scatter light particularly on the outer region of the ELS pattern. This could be potentially impact the quality of the classification due to the utilization of the Zernike moments in the analysis.

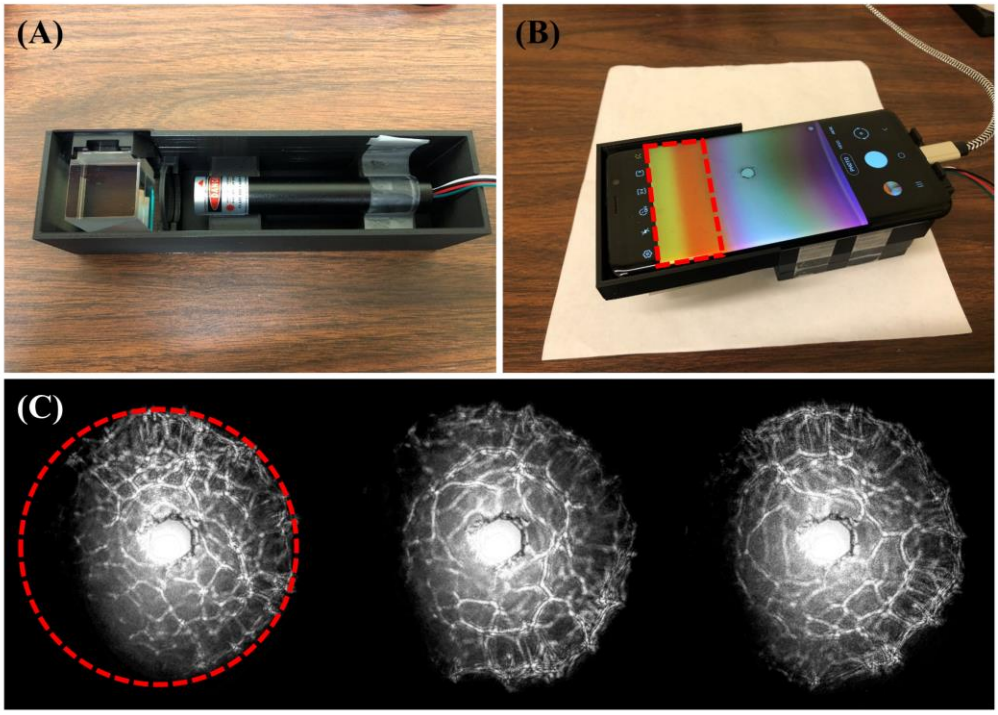

Figure 2. Design of the imaging module for the smartphone-based portable scatterometer. (A) displays the polarization beam splitter (PBS) based optical module. (B) testing result with solid white color sheet shows color distortion and transmission issue on the red-dotted line area. (C) shows the effect of the PBS on the scattering patterns. The left-most region of the light propagation was blocked and patterns were not completely generated.

2.3 Sample preparation

As a proof-of-concept, classification of phenotyping two bacterial species was conducted using *Escherichia coli* and *Listeria innocua*. Samples were prepared on opaque agar media and the organism identities were confirmed by PCR using the primers for ATCC strains *E. coli* ATCC 25922, and *L. innocua* ATCC 33090. All organisms were subcultured from -80 °C freezer stored stock cultures and streaked on trypticase soy agar (TSA) (Bacto™, BD Diagnostics, Franklin Lakes, NJ, USA). The streak plates were incubated at 37 °C until the colonies were visible. One colony from each organism culture was picked and serially diluted in 4 ml buffer solution (PBS) three times by a dilution factor of 1:40. A 50-µl aliquot of the last dilution tube was spread on the opaque media plate using an L-shaped sterile spreader (Globe Scientific, Mahwah, NJ, US) and the dish was incubated at 37 °C until the diameter of the colony reached 500 – 1000 µm (typically 12-18 hours). The diameter of the colony was controlled to have a comparable size of the reflection pattern across the species. For opaque media, brain heart infusion (BHI) agar with 5% horse blood was prepared to investigate the reflective ELS patterns of the sample bacteria and explore the change of the patterns with respect to the nutrition media.

2.4 Image processing and classification

Opaque media was placed under the instrument and incident laser was manually placed closer to the target bacterial colony. The smartphone camera app was started and scatter patterns were captured by manually centering the image. Captured images were exported to a computer and images and subsequently preprocessed by Matlab. The patterns were first saved as grayscale, cropped to a square image with 1024 × 1024 pixels, and adjusted for contrast. Next, the noise reduction process followed in order to remove speckle. Images were then imported into Baclan software which is an in-

house developed software suite which provides training and analysis of scatter patterns. Following the procedures described in our previous studies, two groups of features were extracted: pseudo-Zernike moments and Haralick texture features. Pseudo-Zernike moments are invariant orthogonal moments computed using pseudo-Zernike polynomials. They capture the characteristics of circular and uniform patterns. Haralick texture features were included to increase the feature space, contributing to a more accurate recognition of disordered patterns. A classification model was constructed using a sup-port vector machine (SVM) algorithm. 10-fold cross-validation (CV) with a 20-times repetition was performed to calculate the accuracy, sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV).

3. RESULTS

3.1 Imaging chamber

A core design concept of the imaging chamber is shown in Figure 3(A)-(B). The reduction of the scatter pattern intensity was caused by the structure of PBS where two of triangular beam splitter were integrated together with polarizers. Therefore, the integrated module was separated into three parts: plate beam splitter with 50:50 transmission to reflection ratio, and two plate polarizers. Figure 3(C) shows the construction of the modified imaging module with the first plate polarizer on the top of the imaging cube and the second plate polarizer placed immediately adjacent to the laser diode. To provide both imaging capability and interrogating laser to the colony, the plate beam splitter was placed beneath the first plate polarizer (Figure 3(D)). This structure utilized the same amount of space while removing the lower transmission phenomena observed in Figure 2 with our previous design.

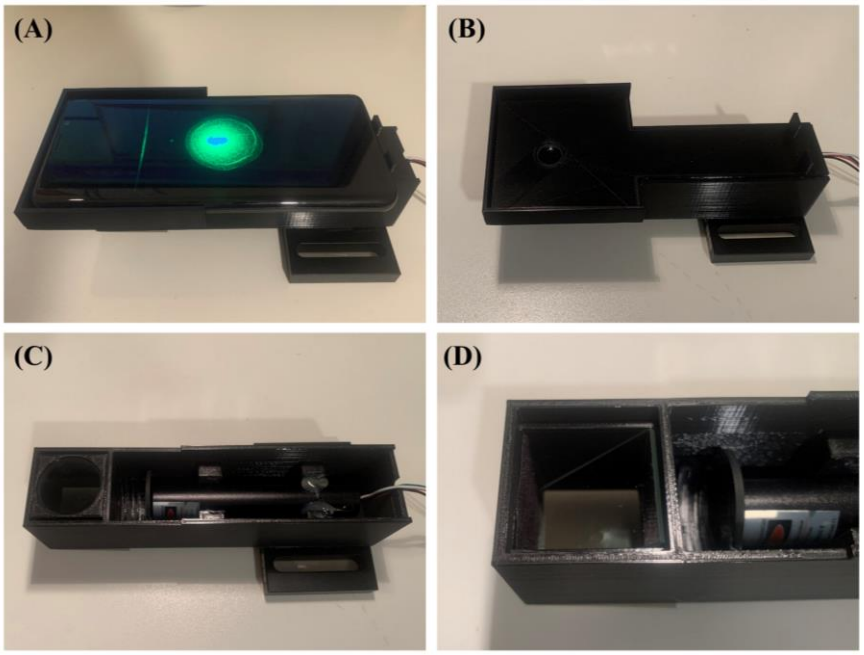

Figure 3. Final assembly of the proposed imaging chamber. (A) Assembly of the full system (B) assembly without a smartphone (C) Smartphone holder is removed to show the internal structure. Left-end shows the imaging optics which include the screen, plate beam splitter, and plate polarizer. (D) Close-up photo of the imaging module without the plate polarizer. Addition plate polarizer was shown right next to the laser diode.

3.2 Classification results

The representative pattern images of the two bacterial species are presented for the qualitative comparison in Figure 4. Based on a visual inspection, each species showed unique and distinguishable characteristics in their reflective pattern images. The colonies of *E. coli* produced patterns with non-uniform structures in the inner area and noncircular outer edges. *L. innocua* patterns also showed an irregular shape, but the peripheral area of its patterns differed from the patterns from *E. coli*; the patterns were not enclosed by borderlines, and the boundary was unclear. Compared to the previously reported images[12], *E.coli* and *L.innocua* didn't generated concentric ring patterns which was related to the opaqueness of the individual bacterial colony. Certain species provided more reflection than others and highly reflective colonies generated more ring-like structure due to the constructive/destructive interference of outgoing waves.

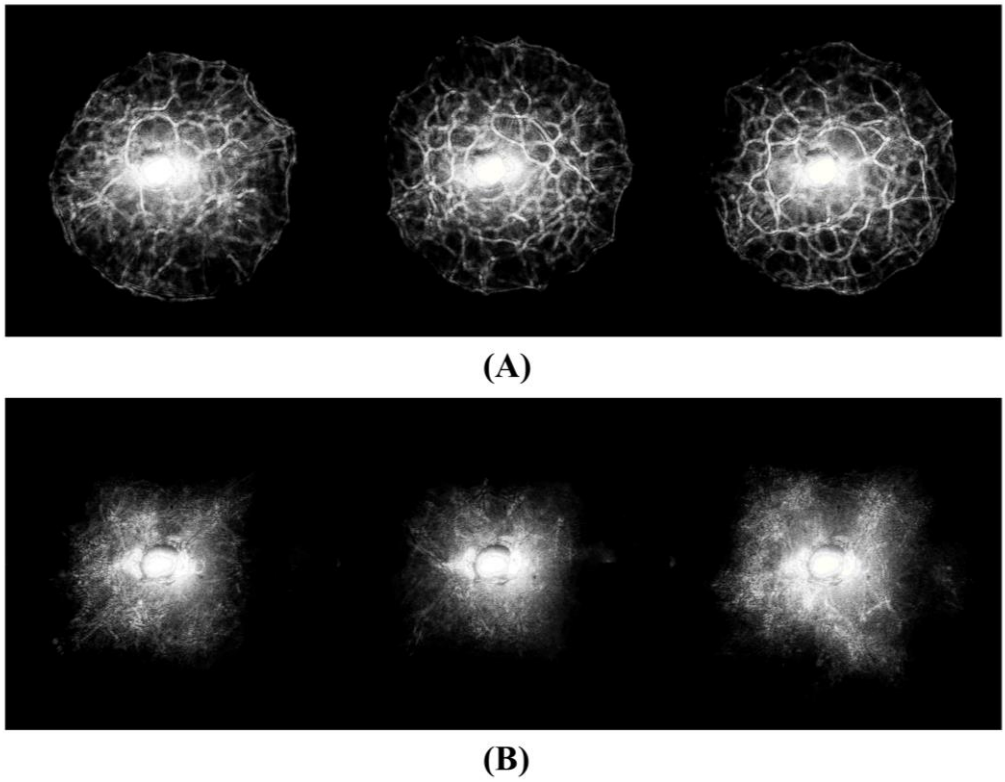

Figure 4. Representative reflective scatter patterns captured from the proposed system. (A) *E.coli* (B) *L. innocua.*

For each class, 50 reflective ELS pattern images were prepared for feature extraction, and the combination of pseudo-Zernike moments and Haralick texture features were employed to describe the patterns. The order of the pseudo-Zernike moments was set to 20, while the distance and the number of levels were set to 1 and 64, respectively, for Haralick texture. The total number of features extracted from each pattern image was 243, where 13 of them were from Haralick texture, and the others were from pseudo-Zernike moments. As a result of the classification, the CV matrix was obtained from an SVM-based classifier. These statistical parameters were calculated based on the CV matrix and provided Figure 5. The CV result was promising as every class demonstrated accuracy measures greater than 96%.

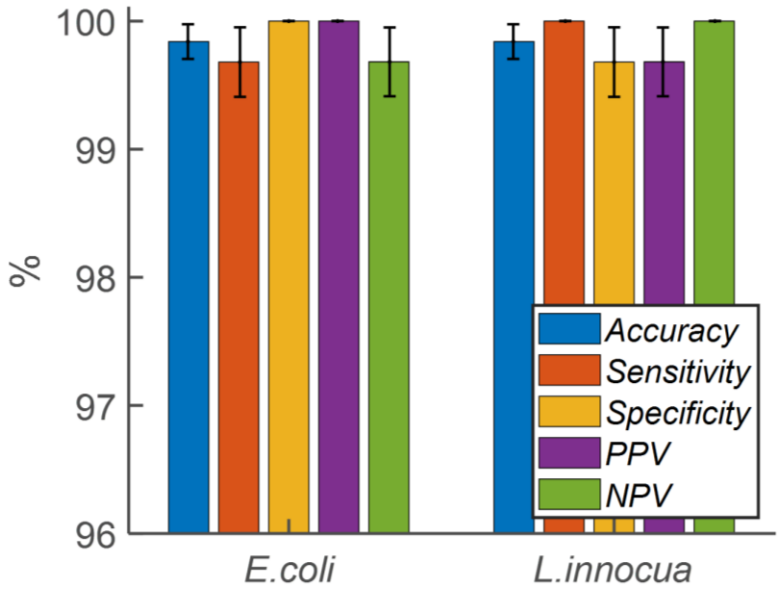

Figure 5. Statistical result from the cross-validation (CV) matrix. Five statistical categories were calculated for *E.coli* and *Listeria innocua* patterns where training and test set of images were randomly mixed for multiple times. Each occasion resulted in slightly different CV matrix. Overall results were above 95% accuracy with standard deviation of 0.27%

4. DISCUSSION

Preliminary results from current report indicate that our proposed system can capture consistent reflection type scatter patterns from bacterial colonies grown on opaque media. In many clinical settings, swabbing a patient sample and culturing on agar is the standard practice to first obtain the bacterial colony of interest. This is subsequently used for other bio-chemical testing or methods such as PCR analysis. With the significant reduction of size and readily available of smartphone devices, portable smartphone-based readers can be utilized in any clinical setting to obtain a first and rapid assessment of bacterial colony samples. The current data only shows the efficacy of result with respect to BHI with horse blood agar, but future work will expand this task to other widely used opaque media such as chocolate agar and buffered charcoal yeast extract agar. One of the key points required for evaluation is the wavelength compatibility with certain types of agar. As previously shown, growth media with blood cells failed to generate clear reflective patterns when a 632 nm laser diode was used[12]. This was understood to be related to the diffusive scattering interaction with the incoming laser and the bacterial colony and medium. Our current classification demonstration shows a promising result while the true test will be whether the overall system is capable of providing similar accuracy with large number of classes. Future work will expand this into 6-8 genera and species lever classification of the same genera.

5. CONLUSION

We present a portable, smartphone-based instrument that provides reflective ELS patterns from bacterial colony grown on an opaque media. An optical chamber design was reported that provided high quality images from two bacterial genera. Classification results were reported in five statistical categories which resulted in an accuracy of 96%. Future work will expand the number of opaque media and the number of species tested. Further development can allow clinical laboratories to quickly assess and identify bacterial samples collected from patients.

6. ACKNOWLEDGEMENT

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