

Color Generation Using Coffee Rings Formed by Yeast Cells and Polystyrene Beads, and Its Application to Optical Elements

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The coffee ring effect is a phenomenon in which a ring-shaped pattern forms when droplets containing particles evaporate. This ring formation is caused by capillary flow during the drying process, and some particles remain irregularly distributed within the ring. When light is shone on these residual particles at an appropriate angle, this structure exhibits a unique color. In this study, we investigated the macroscopic and microscopic coloration phenomena of coffee rings formed by polystyrene beads (diameter 0.5–3 μm) and yeast cells. The macroscopic coloration was observed by spectral analysis, and the microscopic coloration was observed by optical microscopy. We also discussed the formation of speckle patterns by coffee rings formed by yeast cells. These results suggest that coffee rings formed by yeast cells function as optical elements, indicating the potential for utilizing microbial cells as optical elements in sensors, structural color devices, and other applications.

1. Introduction

The coffee ring effect is a well-known phenomenon in which a ring-shaped deposition pattern forms after the evaporation of a droplet containing fine particles, as illustrated in Fig. 1(a).^(1–3) This ring formation is driven by capillary flow (outward flow) generated during the drying process, which transports suspended particles toward the perimeter (outer edge) of the droplet.^(4–6) During this process, the residual particles become irregularly distributed within the ring. Figure 1(b) shows an observed image of a coffee ring formed on a glass slide by polystyrene (PS) beads with a diameter of 3 μm . The particles aggregate irregularly to form amorphous regions, but in certain areas, they locally assemble into two-dimensional and three-dimensional colloidal crystals. Such ring-shaped accumulation structures are observed when droplets containing dispersed microparticles evaporate. The macroscopic observation of such coffee rings demonstrates structural coloration originating from the residual microparticles.^(7–10) Employing non-spherical (e.g., elliptical) microparticles has been reported to effectively reduce color non-uniformity in applications such as inkjet printing.^(11,12) Recently, attempts have been made to extend this phenomenon to the biological field. In cases where microbial cells are

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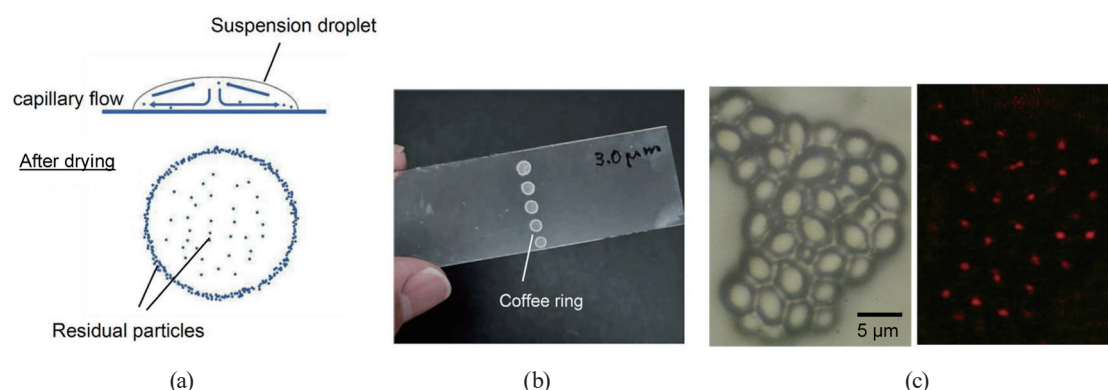


Fig. 1. (Color online) (a) Schematic of the coffee ring effect. (b) Coffee rings formed by PS beads with a diameter of 3 μm . (c) Optical microscopy image of cells remaining in a coffee ring formed from a yeast suspension (left) and focal spot image of transmitted red laser light (right).

physically treated as a type of microparticle, studies include the observation of evaporation-induced patterns in droplets containing motile and non-motile bacteria⁽¹³⁾ and the evaluation of the effect of changes in surface wettability caused by exposure to the laboratory environment on the evaporation-driven deposition patterns of bacteria.⁽¹⁴⁾ Yeast (*Saccharomyces cerevisiae*), widely used in breadmaking, is also a microorganism and is therefore expected to exhibit similar behavior to a microparticle. Figure 1(c) shows the optical microscopy image of cells remaining in a coffee ring formed from a yeast suspension, as well as the focal spots formed when laser light is transmitted through them. As shown in the figure, a typical yeast cell is ellipsoidal with a thick convex lens shape, having a major axis of approximately 4–5 μm , a minor axis of approximately 3 μm , and a height of approximately 3 μm . Because these yeast cells transmit light, they form a clear focal spot upon laser illumination. Thus, the individual yeast cells forming the coffee ring can function as microlenses.

Previous studies on the interaction between yeast cells and light have focused on the optical behavior of individual, single cells or suspension, such as the optical trapping of yeast cells using optical tweezers⁽¹⁵⁾ and the evaluation of their light-scattering properties.⁽¹⁶⁾ However, in these conventional approaches, yeast cells are treated as being optically transparent, isolated microparticles, leaving the collective optical phenomena—such as coloration arising from their aggregated structures—unexplored. To date, there are no examples of actively applying the optical properties exhibited by their aggregated structures to engineering applications. Although light propagation in composite two-dimensional spherical arrays of artificial PS latex particles has been investigated,⁽¹⁷⁾ experimental reports on evaluating the aggregate structures and optical properties of biological yeast cells from an engineering perspective remain absent. If yeast cells can be used as functional optical microparticles, they could potentially be developed into eco-friendly optical elements, such as safe, bio-derived sensors and color-generating devices.

In our microscopic observations, various individual colors were observed for yeast cells as well, demonstrating a phenomenon similar to that of PS beads. Therefore, in this study, the optical properties of coffee rings formed by both PS beads and yeast cells are investigated, and the experimental results regarding macroscopic coloration phenomena, spectral measurements,

microscopic coloration behavior, and speckle pattern formation in the coffee rings formed by both types of particles are reported in this paper.

Furthermore, because the fabrication method employed in this study is inexpensive and safe, it holds significant potential for educational and artistic applications. In Appendix, we discuss the prospects of utilizing these findings as Science, Technology, Engineering, Arts, and Mathematics (STEAM) educational materials.

2. Materials and Methods

PS beads and yeast cells were used to form coffee ring patterns. For PS-bead-based coffee rings, suspensions containing PS microparticles of various diameters (0.5, 1, 2, and 3 μm ; Polybead® 2.6% Solids-Latex) were dropped onto glass slides (Matsunami Glass Industry, S1126) using the tip of a toothpick and subsequently air-dried. Similarly, for the yeast-cell-based rings, a suspension of commercially available dry yeast (Oh'my instant dry yeast, NIPPON Corp.) dispersed in pure water was dropped onto the glass slides in the same manner. The typical experimental conditions for coffee ring formation were a temperature of approximately 22–24 $^{\circ}\text{C}$ and a relative humidity of 45–60%. The yeast suspension concentration was approximately 3% by weight. No additives were used in the preparation of the suspension. Before depositing the suspension, the surface of the glass slide was carefully wiped clean with a microfiber cloth. The macroscopic coloration of the coffee rings was captured using a smartphone camera (iPhone SE). For spectral observations of the scattered light, an optical setup consisting of a transmission diffraction grating, a knife-edge slit, and a camera (Nikon 1 J1 for visible light and modified Pentax Q for near-infrared light) was used; further details are described in Sect. 3.2. Microscopic coloration was observed using a metallurgical optical microscope (Meiji Techno Co., Ltd., ML7100) equipped with a 20 $\times$ objective lens (OLYMPUS, LMPLFLN20 $\times$, numerical aperture = 0.4).

3. Experimental Results and Discussion

3.1 Observation of coloration in coffee rings

Figure 2(a) shows a photograph of a coffee ring formed by PS beads, observed in backscattered sunlight with the sun behind the observer. In contrast, Fig. 2(b) shows the coffee ring observed via scattered sunlight transmitted through the sample; this observation was carried out at an appropriate angle to avoid direct sunlight and clarify the visibility of the coloration. In both cases, distinct coloration was observed, which showed a dependence on the diameter of the PS beads. Because the incident sunlight can be approximated as a plane wave—meaning it illuminates the coffee ring at a uniform angle—it is clear that the observed color variations are primarily dependent on the size of the microparticles. It is considered that this macroscopic coloration is a manifestation of diffracted light resulting from microparticle scattering. Figure 2(c) shows the relationship between the concentration and color of a suspension of 1- μm -diameter PS beads. It can be seen that the color of the coffee ring does not depend on the concentration of the fine-particle suspension.

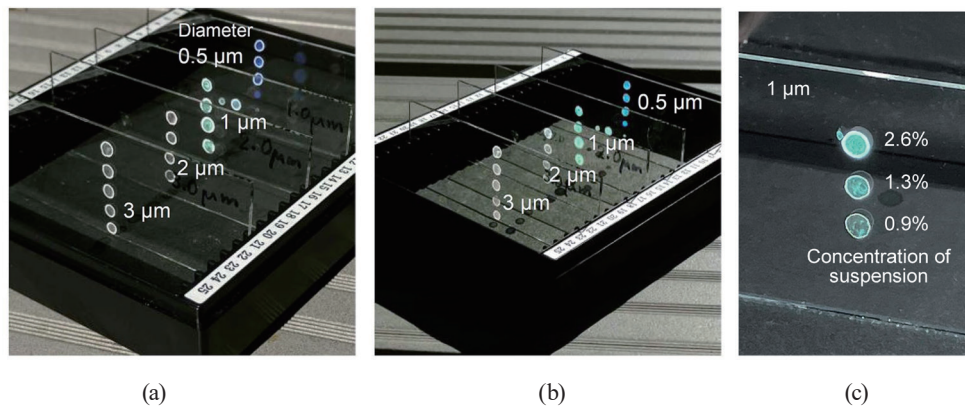


Fig. 2. (Color) Photograph of light diffracted from a coffee ring formed by PS beads, observed under sunlight. Because the sunlight used for illumination can be considered a plane wave (i.e., collimated light), it is incident on the coffee ring at a uniform angle. (a) Backscattered sunlight, with the sun behind the observer. (b) Scattered sunlight transmitted through the sample. (c) Relationship between concentration and color of a suspension of 1-μm-diameter PS beads.

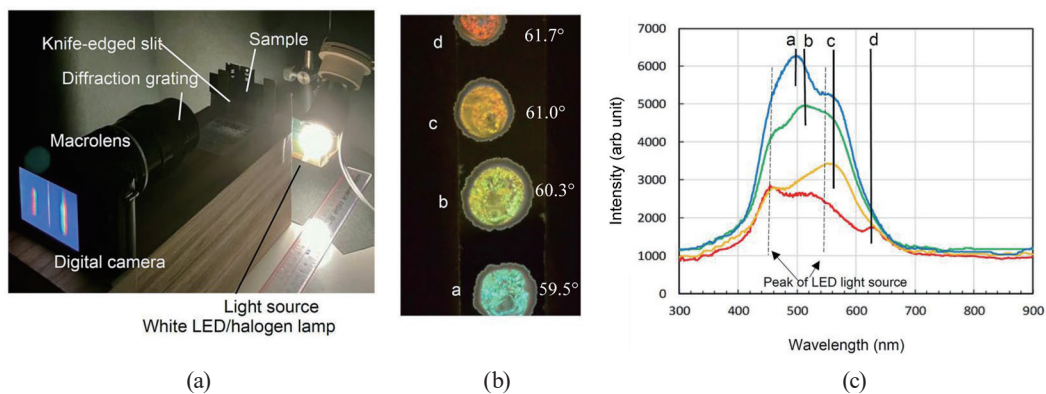


Fig. 3. (Color) (a) Experimental setup for measuring light diffracted from a coffee ring. (b) Light diffracted from coffee rings formed by PS beads with a diameter of 1 μm. (c) Spectra of light diffracted from coffee rings.

3.2 Observation of diffraction spectrum of coffee ring formed by PS beads

Next, the spectrum of the diffracted light from the coffee ring was observed. Figure 3(a) shows the measurement setup, which consists of a light source, the coffee ring, and a camera. In the experiment using PS beads, a white LED was used as the light source. To capture the spectrum, a knife-edge slit formed using razor blades was placed directly in front of the coffee ring on the camera side, and a transmission diffraction grating (STARANALYSER100, 100 lines/mm) was positioned in front of the camera lens (Micro Nikkor 55 mm F2.8). The wavelength spectrum was analyzed using a spectroscopic observation software kit (RSpec, GOTO TELESCOPE). The image displayed on the camera monitor shown in Fig. 3(a) is a spectrum of the light source captured through the slit. The wavelength resolution of the spectral analysis was approximately 3 nm, calculated from the slit width used in this experiment (40 μm), the imaging lens, the camera, and the optical magnification. Figure 3(b) shows a photograph of

light diffracted from a coffee ring formed with 1- μm -diameter PS beads. Here, the light source was positioned diagonally below the sample to prevent the direct light from entering the camera. The color of the diffracted light clearly shifted from blue to red depending on the incident angle of the light (from positions a to d, corresponding to 59.5 to 61.7°). By positioning a slit on the camera side of these coffee rings, the individual spectrum of each ring can be observed.

The spectra obtained by this method are presented in Fig. 3(c). The peaks of both the white LED light source (approximately 450 and 540 nm) and the diffracted light from the PS bead coffee rings (approximately 490 to 630 nm) are clearly observed. This result demonstrates the angular dependence of the diffracted light with respect to the light source. The experimental results in Fig. 3 were observed under an oblique-illumination and normal-observation configuration (non-specular diffraction). Although a simple Bragg reflection alone cannot account for this angular dependence, by assuming a two-dimensional hexagonal close-packed colloidal crystal, the colloidal layer acts as an optical thin film with an effective refractive index.⁽¹⁸⁾ Under this model, the angular dependence of the wavelength shift would be explained by diffraction from the $\{11\}$ planes, taking into account an angle-dependent correction term.

3.3 Observation of coloration and spectrum of coffee rings formed by yeast cells

Figure 4(a) shows an image of light diffracted from a coffee ring formed by yeast cells. Depending on the relative positions of the coffee ring and the halogen lamp light source, whitish and slightly pinkish colors were observed. Figure 4(b) displays the optical spectra corresponding to the light source and yeast-cell coffee ring. Although the measurement system was the same as that shown in Fig. 3(a), a halogen lamp was employed as the light source instead of the white LED, as the latter lacks emission in the NIR region. Note that this halogen lamp features a surface coating, resulting in complex spectral characteristics. In addition, a camera with sensitivity extending into the IR region was utilized for this measurement as mentioned in Sect. 2.

To evaluate the differences in characteristics between the light source and the yeast-cell-based coffee ring, the spectra of both the light source and the coffee ring were normalized to their respective intensities at 580 nm in the visible light region. Consequently, the diffracted light from the coffee ring showed a distinct increase in peak intensity within the wavelength range of approximately 660 to 850 nm, clearly differentiating its spectrum from that of the halogen lamp light source. It is considered that this measured increase in light intensity in the NIR region is due to the residual yeast cells within the coffee ring.

3.4 Observation of color transmitted through coffee rings

Next, we observed the color transmitted through coffee rings formed by PS beads and yeast cells. Figure 5(a) shows a photograph of sunlight transmitted through a coffee ring formed by PS beads (0.5–3 μm in diameter) and projected onto white paper, captured using a smartphone camera. The color of the transmitted light clearly varies depending on the bead diameter. The light transmitted from the coffee ring with 0.5- μm -diameter beads tends to be red, indicating a

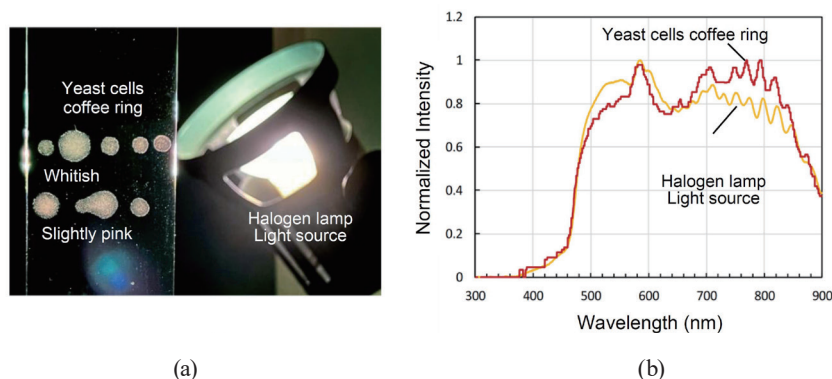


Fig. 4. (Color) (a) Color of light scattered from a coffee ring formed by yeast cells. (b) Spectrum of light from the coffee ring.

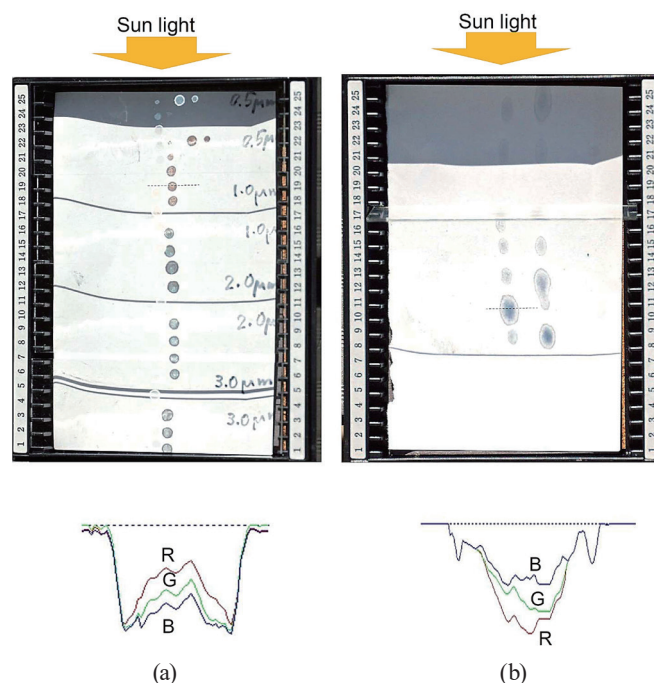


Fig. 5. (Color) Photograph of sunlight transmitted through a coffee ring and projected onto white paper. (a) PS bead coffee ring (0.5–3 μm in diameter). (b) Yeast cell coffee ring.

correlation between bead size and transmitted color across all tested sizes. The graph below Fig. 5(a) shows the RGB intensity ratio of the transmitted light, obtained using image processing software (StellaImage5, AstroArts Inc.). This graph demonstrates that the intensity of the R (red) band is higher than those of the G (green) and B (blue) bands. Similarly, Fig. 5(b) shows a photograph of sunlight transmitted through a coffee ring formed by yeast cells and projected onto white paper. Here, the transmitted light tends to be blue. The lower graph in Fig. 5(b) shows the RGB intensity ratio for the yeast-cell coffee ring, and it is clear that the intensity of the B band is higher than those of the R and G bands. These results demonstrate that the size dependence of microparticles in a coffee ring can be utilized to prepare a color filter.

3.5 Observation of microscopic coloration of coffee rings formed by yeast cells

The spectral characteristics of the coffee ring described above correspond to the observation of macroscopic diffracted light. Next, we microscopically observed the residual yeast cells within the coffee ring using a halogen lamp as the light source. Figure 6(a) shows the residual yeast cells within the coffee ring observed under epi-illumination. The residual cells within the ring are densely packed in some areas and sparsely distributed in others; notably, their arrangement remains irregular even within the densely packed regions. To observe the microscopic coloration of these cells, a dark-field illumination setup was used, where light from the halogen lamp was incident from the back (glass slide side) of the sample at an angle of approximately 45° . Figure 6(b) shows an optical microscopy image of the residual yeast cells captured under this dark-field illumination within the same field of view as Fig. 6(a). Under dark-field conditions, the yeast cells exhibited various colors ranging from blue to red. For comparison, Figure 6(c) shows a dark-field optical microscopy image of residual particles from a coffee ring formed by PS beads with a diameter of $3\ \mu\text{m}$. Similarly, to yeast cells, PS beads were observed to exhibit colors from blue to red. This coloration is attributed to Mie resonance, where specific wavelengths are selectively enhanced depending on the size parameter $\pi D/\lambda$ (the ratio of the particle diameter D to the wavelength λ). Consequently, particles exhibiting diverse colors appear within the field of view. For the $3\text{-}\mu\text{m}$ -diameter PS beads, the size parameter is approximately 17 for green light (e.g., at a wavelength of 550 nm), placing the system well within the resonance regime of Mie scattering theory. When the size parameter is large, the maxima of the scattering intensity appear with a fine wavelength dependence.⁽¹⁹⁾ Under these conditions, even a slight variation in diameter shifts the scattering to a resonance peak of a different order, leading to a significant change in perceived color.⁽²⁰⁾ Since yeast cells have a diameter of approximately $4\ \mu\text{m}$, their size parameter at 550 nm is approximately 23, which corresponds to Mie resonance. Therefore, similarly to PS beads, yeast cells are also observed as particles of various colors within the field of view of a microscope.

Although structural colors of fine particles driven by Mie resonance are often evaluated through electric field analysis,⁽²¹⁾ the present evaluation utilizes the size parameter to characterize the coloration. In addition, despite their non-spherical shape, elliptical yeast cells can be optically and structurally treated as thick convex lenses.⁽²²⁾ Indeed, taking into account their three-dimensional size and light-focusing capability shown in Fig. 1(c), the experimental results for yeast cells in Fig. 4 are remarkably similar to those of PS beads in Fig. 3. Furthermore, their Mie resonance behavior shown in Fig. 6 is comparable to that of spherical PS beads. Therefore, within the scope of this experiment, yeast cells can be optically regarded as equivalent to PS beads—functioning as transparent spherical optical elements. Consequently, the coloration induced by residual particles within the coffee ring could be explained by the superposition of diffraction effects from the two-dimensional colloidal crystal and Mie resonance from individual particles.

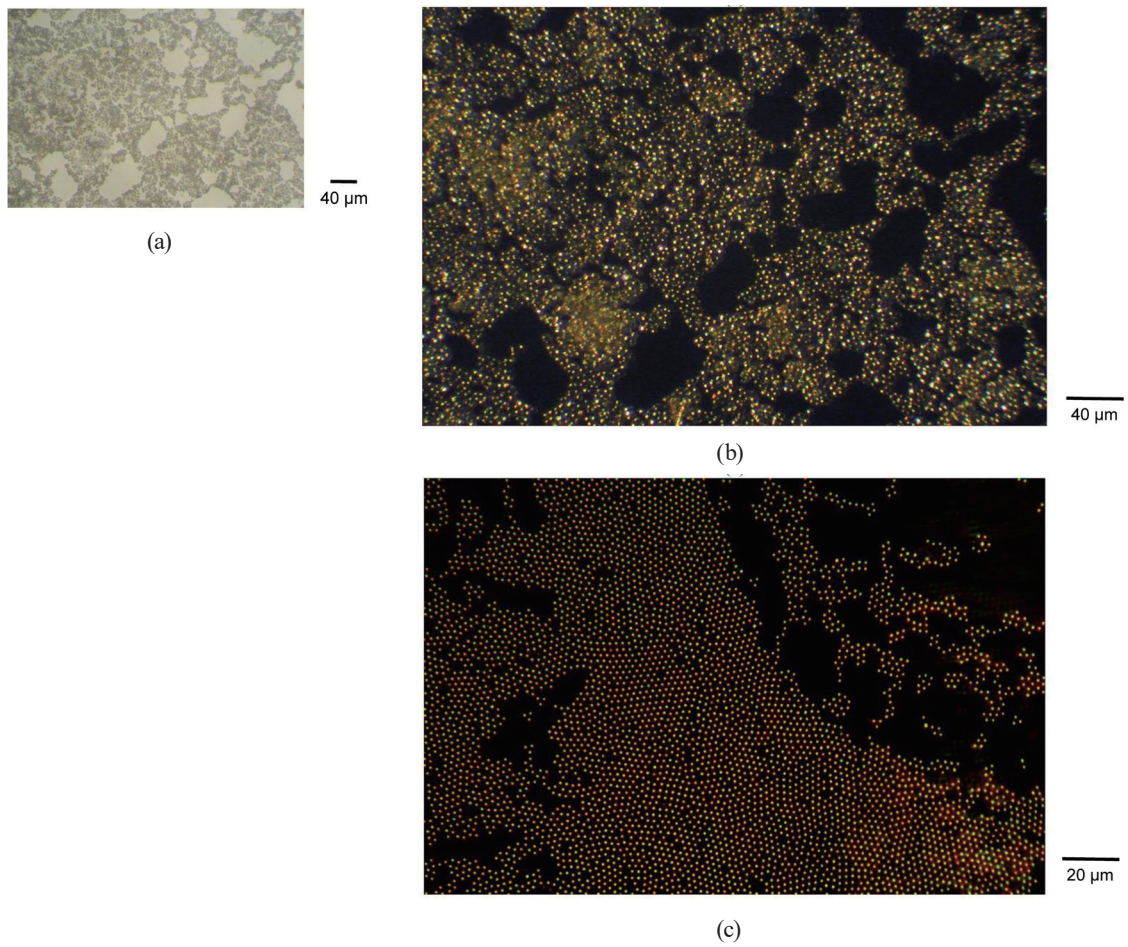


Fig. 6. (Color) Optical microscopy images of residual yeast cells within a coffee ring under (a) epi-illumination and (b) dark-field illumination. (c) Residual 3- μm -diameter PS beads within a coffee ring under epi-illumination.

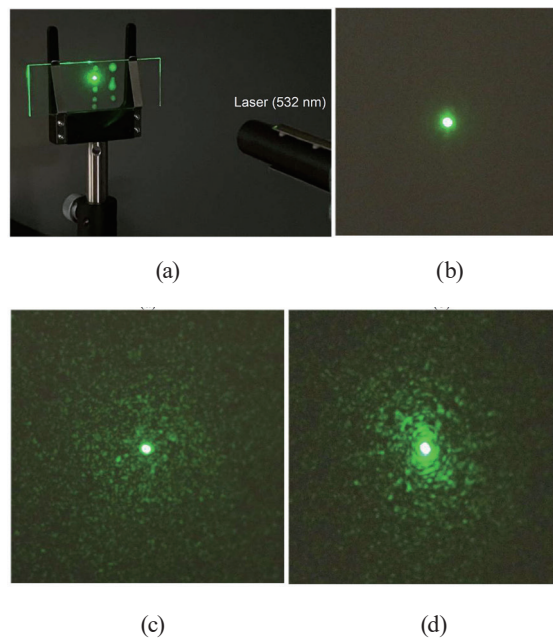


Fig. 7. (Color) (a) Experimental setup, (b) laser spot projected onto a wall, (c) speckle patterns formed by yeast cells, and (d) speckle patterns formed by PS beads (diameter: 3 μm).

3.6 Speckle pattern formation using coffee rings formed by yeast cells

Speckle patterns are granular, random intensity distributions that arise when coherent light, such as a laser, scatters off a rough surface or medium. This phenomenon occurs as a result of the interference of numerous scattered waves with various phase differences caused by surface irregularities. Using the experimental setup shown in Fig. 7(a), we investigated the speckle patterns generated by residual yeast cells within a coffee ring under illumination from a green laser (λ : 532 nm). Figure 7(b) shows the laser spot projected onto a wall at a distance of approximately 3 m, captured with a shutter speed of 1/1000 s. Figures 7(c) and 7(d) show the speckle patterns formed by yeast cells and PS beads (diameter: 3 μ m) in their respective coffee rings. These results demonstrate that distinct speckle patterns can be successfully generated using biological yeast cells. The speckle pattern is considered to change depending on the concentration and irradiation position. However, in principle, the pattern should not change if the irradiation position and the alignment of the light source do not change. Although it is difficult to precisely reproduce the alignment in this experimental setup, we have visually confirmed that the pattern rotates when the coffee ring is rotated without changing the optical axis. In other words, because it is controllable, it is also reproducible. Furthermore, a closer observation of Fig. 7(a) reveals that the laser light propagates to the edges of the glass slide and to non-irradiated areas of the yeast coffee rings. The incident laser light is captured by the yeast coffee ring, guided in the glass substrate via total internal reflection, and observed at the periphery of the glass slide's edge. This phenomenon indicates that the coffee ring formed by yeast cells functions as an efficient optical coupler. Together with previous findings regarding the microlens function of yeast cells,⁽²⁰⁾ these results suggest that microbial cells functioning as optical elements hold potential for bio-photonics applications.

4. Conclusions

In this paper, the experimental observation of the optical characteristics of coffee rings formed by PS beads and yeast cells was presented. The optical phenomena were characterized by the observation of macroscopic and microscopic coloration phenomena, spectral analyses, and speckle pattern formation. Although yeast cells were used as a microorganism in this study, other readily available and smaller species, such as lactic acid bacteria (0.5–2 μ m), are equally applicable. In the future, two-dimensional arrays of coffee rings composed of microorganisms of various sizes are expected to have applications in displays. By integrating the functions of photocouplers and filters, microbial cells may also function as optical elements such as sensors and color generators. Therefore, microorganisms can potentially be used as optical devices.

Acknowledgments

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Appendix1

The drying process of a yeast-cell suspension droplet was photographed from the side. The images are shown in Fig. A1. It can be seen that the droplet radius is fixed in the initial stages of drying, while the height of the droplet gradually decreases. Although it is difficult to measure the contact angle as the droplet shrinks (receding contact angle) from these photographs, a simple scale is provided in the figure.

Appendix2

The method presented in this paper is inexpensive and safe, and easy to use, and is implement in educational settings. In this appendix, considering the above results, we examine the potential of applying this method as a teaching tool in STEAM education, particularly in science and arts education.

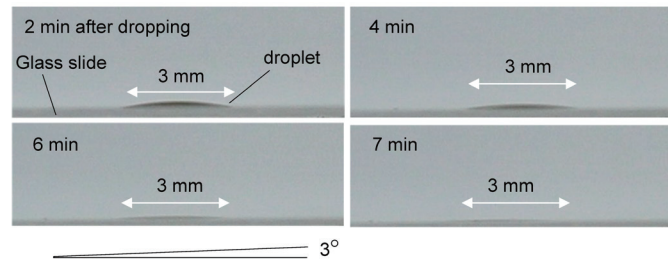


Fig. A1. Observation of process of drying a yeast-cell suspension droplet from the side. The scale bar is indicated in the figure.

STEAM education is an educational approach that aims to cultivate students' ability to independently identify and solve problems through interdisciplinary learning across the following five areas: Science (S) includes observing natural phenomena, conducting experiments, and testing hypotheses; Technology (T) is related to manufacturing processes and their underlying mechanisms; Engineering (E) is related to structural design and understanding; Arts (A) encompasses free thinking, aesthetic design, and expression; and Mathematics (M) provides fundamental analytical tools. In education, the ability to integrate knowledge across these fields to produce solutions to problems is increasingly in demand. Furthermore, curiosity, along with sensitivity and creativity, is essential for human beings. Integrating these STEAM areas will likely be effective in improving students' motivation to learn.

For example, creating visual designs utilizing the coffee ring effect described in this study can serve as an effective educational bridge. Figure A2 shows an artistic image created by arranging multiple coffee rings formed from PS beads with diameters of 0.5–3 μm .⁽²³⁾ Using such artistic images as an entry point, the science behind coffee ring formation can be utilized as an engaging STEAM educational tool for students and the general public. Specifically, the coffee ring formation process provides an intuitive platform for introducing fluid dynamics through concepts such as capillary forces and Marangoni convection. On a macroscopic scale, observing interacting light provides an opportunity to learn about optics, particularly the diffraction and interference of light.

By observing colors on a microscopic scale, students can gain practical experience with optical microscopes. While most students are only familiar with standard biological microscopes (using transmitted light), this situation introduces the techniques of reflected light and dark-field illumination. Through this experience, students will be motivated to modify standard biological microscopes to achieve dark-field capabilities. Furthermore, by using a laser pointer, students can learn the principles of quantum optics through the mechanism of laser emission. They can also deepen their understanding of the principles of technologies used in modern optical communications, such as speckle pattern formation, light wave guidance in glass, and grating couplers. Previous research has shown that PS beads can approximate spherical lenses and yeast cells can approximate thick convex lenses,⁽²²⁾ making this approach an ideal topic for optics education. In particular, the yeast cells used in this experiment are dried yeast, the same kind

used in everyday bread making. When students discover that familiar yeast cells are a “treasure trove of science,” they will be greatly surprised and gain a deeper understanding that the natural world is governed by systematic physical laws.

By using artistic imagery as an entry point, this approach can effectively impart the laws of nature not only to physics students but also to art students and the general public. In conclusion, the method proposed here functions as a highly effective STEAM education tool that guides diverse people towards scientific inquiry by showcasing the wonders of nature.

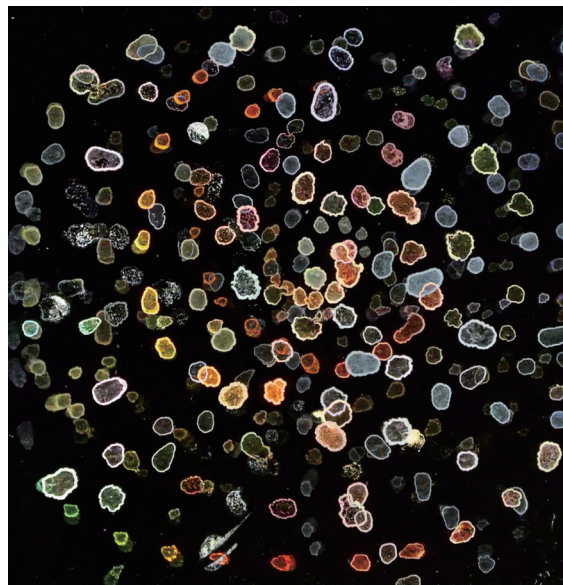


Fig. A2. (Color) Artistic image generated by arranging multiple coffee rings formed by PS beads.⁽²³⁾