The growth of black mold (Aspergillus brasiliensis) in black-colored samples such as hair color and mascara was measured with an automatic count system based on time-lapse shadow image analysis (TSIA). A. brasiliensis suspended in a lecithin and polysorbate (LP) solution of each sample (hair color or mascara) was spread on a potato dextrose agar medium plate containing LP. The background image darkness of the agar plate could be adjusted to attain accurate colony counts. 95 colonies in hair color and 22 colonies in mascara could be automatically determined at 48 h. The accuracy of the colony counts could be confirmed from the time-lapse image data. In contrast, conventional visual counting at a specified time could not determine the number of colonies or led to false colony counts.

Key words: Time-lapse shadow image analysis / Aspergillus brasiliensis / Hair color / Mascara.
Three-dimensional shadow images of fungal colonies at concentrations other than the selected one. In this test mold, spores of the plate could hardly be counted by visual observation.

According to the TSIA algorithm arrangement of TSIA, the adverse effect of hyphae to darken the image and cause false counts was avoided. Consequently, TSIA recognized the center of mold properly and colonies were counted accurately.

In the case of the test liquid of hair color, N became 1 at 28 h and then increased up to 95 at 48 h. The image data captured during this growth period are shown in Fig. 3a. Black spots that were greater than the threshold size and continued to grow were converted to white spots. However, non-growing black spots were not dark enough to be decided as colonies. Rather they seemed to be hair color stains. Another difficulty was whether a large colony should be counted as 1 or as multiple colonies. Such multiple colonies could not be determined without comparing the image data captured at previous times. In contrast, TSIA results showed 67 colonies (Fig. 2 and Fig. 3a, 39 h, right panel). After this time, only TSIA could follow the increase in colony numbers.

In the case of mascara, TSIA could determine the number of colonies although the images of plate were much more complicated. The number of colonies determined by TSIA was 22 at 48 h (Fig. 4). In comparison with the hair color samples, there were many more black spots such as non-biological particles and dust that gave noise signals (Fig. 5a). The number of colonies visually counted at 42 h was 21 (Fig. 5b). However,
it was difficult to visually distinguish colonies from mascara stains at 39 h. On the other hand, it was also difficult to visually decide count to decide whether a large spot was a single colony or multiple colonies. In contrast, TSIA could distinguish single colonies from multiple colonies.

In conclusion, we could find the optimum conditions to control for the darkness of the background images in TSIA. Thus, the improved system could be successfully applied to the accurate counting of fungal black colonies on PDA containing black hair color and black mascara. At the present, however, it cannot be validated by the present method by using the conventional

![Image](image.png)

**FIG. 3.** Image data of *A. brasiliensis* in a hair color sample. (a) Images captured at the culture time indicated (left panel) and colonies determined by TSIA (right panel). Black areas encircled by white broken lines (0 h) indicate noise signals. (b) Example of a visual colony count. The left panel at 39 h in (a) was enlarged. The number of colonies is 62+4?, where ? indicates 1 or multiple colonies.

![Image](image.png)

**FIG. 4.** Colony enumeration of *A. brasiliensis* in a mascara sample.

![Image](image.png)

**FIG. 5.** Image data of *A. brasiliensis* in a mascara sample. (a) Images captured at culture time indicated (left panel) and colonies recognized by TSIA (right panel). (b) Example of a visual colony count. The left panel at 42 h in (a) was enlarged. The number of colonies is 21.
visual count method, because conventional methods can hardly provide reliable results as described above. Then it should be necessary to use standard materials of viable microbial cells that may be prepared by the same procedure reported by Matsuoka et al., (2014). The validity of the TSIA results might be verified by conducting time-lapse visual inspection, after all.

After the validation, the present method could be applied to the evaluation of antifungal agents in such materials and should be of practical importance. Such a performance may be attained by other photometric methods using staining dyes.

REFERENCES


