Short Communication

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Advanced Microbiology 2018: Grayscale measurements of microbial colonies- Enric Maroto Fernandez- University of Barcelona

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This work presents a facile technique that employs flatbed scanners for the measurement of colony grayscale values. Use of grayscale conversion of sRGB-based color images simplifies initially complex three dimensional color space attributes into a single dimension, allowing for a simplified approach to the detection and monitoring of colony chromogenesis. The performance of 4 often-used grayscale conversions is assessed using Letheen agar in combination with two chromogenic dyes, triphenyl tetrazolium chloride (TTC), and tetrazolium violet (TV) in cultures of three model microorganisms (Escherichia coli, Pseudomonas aeruginosa and Staphylococcus aureus). The effects of different concentrations of the chromogens and the differences in color development over time are evaluated. Affordable approaches to interpret derived data are suggested and insights related to analysis of color development are supplied. Metrological aspects of the technique are duly addressed. Thus, particular care is devoted to characterize the measurement technique employed, to highlight its limitations, and to assess cross-device reproducibility of obtained results. The suggested method is simple and resorts to affordable and readily available devices and software. This technique can be applied in culture media enhancement, phenotypic characterization of microorganisms, especially in the detection of colony color development.

Grayscale images are distinct from one-bit two-color black-and-white images, which are the only two colors: black and white (also known as two-level images or binary). Grayscale images have many shades of gray in between. Grayscale images can be the result of measuring the intensity of light at each pixel at a given weighted combination of frequencies (or wavelengths), and in such cases, they are a single frequency. (in practice, a narrow band of frequencies) is captured. Frequencies can be derived from the origin of the electromagnetic spectrum (eg infrared, visible light, ultraviolet, etc.). A colorimetric (or more likely photometric) grayscale image is an image that has a defined grayscale color space,

In biology, a colony is made up of two or more conspecific individuals living in close association or connected to each other. This association is usually for mutual benefit such as enhanced defense or ability to attack larger prey. It is a solid medium on the surface (or inside) of identical cells (clones), usually derived from a single parent cell, such as a bacterial colony. In contrast, solitary organisms are those in which all individuals live independently and have all the functions necessary to survive and reproduce.

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Colonies, in the context of development, may be composed of two or more unitary (or solitary) organisms or modular organisms. Unit organisms have a defined development (defined life stages) from zygote to adult form, and individuals or groups of individuals (colonies) are visually distinct. Modular organisms have indeterminate growth forms (undefined life stages) followed by genetically identical modules (or individuals), and it can be difficult to contain. In the latter case, the modules can have specific functions within the colony. Some organisms are essentially independent and form facultative colonies in response to environmental conditions while others must live in a colony. For example, some carpenter bees will form shared colonies.

For most applications the colonies are lighter than the bottom plate; For the latter, the Iris applies grayscale image thresholding algorithms such as the Otsu algorithm. Typically, such thresholding algorithms operate on the histogram of the image brightness and effort to select a threshold that best separates the background pixels from the foreground. For applications such as biofilm and morphology readings (CR plates), where the brightness of a colony is relative to its background (may be a lighter or darker than a background), or may vary in the test, Iris uses the Marr-Hildreth algorithm , also known as the Gaussian Laplacian algorithm. This algorithm first applies a Gaussian smoothing filter to the grayscale image. Next, a second order derivative of the Gaussian is calculated, Brightness in zero values denoting abrupt changes. These pixel locations are then used as colony boundaries. To initially estimate colony centers, Iris calculates the image thresholding algorithm (Otsu) of the colony's ultimate erosion point after applying gray levels. This approach is also used in the case of manually cropped single colony images. Colony size is measured by all available software such as growth capital.

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