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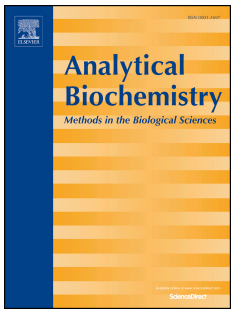

ImageJ-based semiautomatic method to analyze senescence in cell culture

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Technical Note **Javier Lozano Gerona**

Title

ImageJ-based semiautomatic method to analyze senescence in cell culture.

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Abstract

β-Galactosidase accumulates in the lysosomes of senescent cells of certain tissues. Cell staining with X-gal is a common procedure to detect senescent cells in culture. However, the organelle nature of the staining makes automatic count impossible, requiring time-consuming manual counting or expensive alternative techniques such as flow cytometry to effectively determine the amount of stained cells. Here we present an analysis strategy for images of X-gal stained cells which can be implemented into a macro for the ImageJ software overcoming some of the drawbacks of computational analysis of organelle staining.

Keywords

ECFC; microscopy; β-Galactosidase; senescence; ImageJ.

Abbreviations

X-gal	5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside
UV	Ultraviolet
SA-β-Gal	lysosomal β-Galactosidase
DAPI	4',6-diamidino-2-phenylindole
ECFC	Endothelial Colony Forming Cell

1. Introduction

Counting cells on images has become usual procedure in cellular biology. A number of software applications has been developed (ImageJ (1), CellProfiler (2)) that fulfil this task. However, there are still cases where these applications fail to correctly distinguish individual cells or events that the researcher wants to measure. The only way is then to analyze those images by eye, one by one, which is time consuming and often subjective.

In particular, this problem comes up when analyzing SA-β-Gal activity in cell culture using the X-gal cytochemical staining. Since the discovery in 1995 that this enzyme accumulates in the senescent cells (3), this method is widely accepted as a convenient way to detect senescent cells, and broadly applied to many cell types in culture or tissue sections. Senescence detection by X-gal staining is accurate when the cells are not in a confluent state since SA-β-Gal expression is, among other factors, increased with cell confluence (4), although recent studies (5) indicate that this effect may be avoidable by working at the optimal pH for the enzyme activity. The usual protocols work at a suboptimal pH of 6 in order to not stain non-senescent cells (6).

Actually, X-gal staining labels lysosomal organelles, not the entire cells. As a consequence, cell counting algorithms could consider each organelle as a single event, which gives rise to too-high estimates of the number of senescent cells.

Several approaches have been undertaken to make the quantitation less subjective and more precise (7). Some of them make use of flow cytometry to distinguish the signal emitted from each single cell. However, flow cytometry is expensive in terms of equipment and reagents. Others use SA-β-Gal activity measurement in total protein extracts (8), which is appropriate for global comparisons but can not determine the actual number of senescent cells. Considering cell counting by eye, Shlush et al. (5) proposed that the subjective error could be fixed by measuring the amount of green and blue pixels of images. However, for this one needs to manually select cells, which may be even more time consuming.

Here, we describe an alternative approach that is based on the simultaneous analysis of pairs of images from the same microscopic field: One to locate the cell nuclei by counterstaining with DAPI, and the other to detect X-gal staining in organelles according to previously described protocols. We programmed a macro, named *Senescence Counter*, implemented into ImageJ, which computes numeric results and saves a considerable amount of time.