

Method for direct confocal acquisition of fluorescence from X-gal staining on thick tissue sections

A research group of the Andalusian Public Health System and FISEVI, in collaboration with the University of Seville, has developed a new simple technique based on X-gal fluorescence emission and mathematically-based optical correction, to obtain high quality fluorescence confocal images.



Description

Among the different reporter systems, X-gal staining has emerged as an optimal choice based on its simplicity and high sensitivity. However, this procedure does not permit the direct confocal acquisition of images, thus preventing the identification of labelled cells on the depth (Z) axis of tissue sections. This makes it difficult to precisely define whether or not a reporter gene is expressed in a particular cell within a complex tissue, despite combining the detection of the X-gal with immunolabelling of other cell markers, producing a significant amount of incorrect co-localization on thick tissue sections. Previous attempts to solve this major limitation have been based on the use of specific antibodies against β gal. However, X-gal staining is considerably less costly and offers a lower detection threshold and a better signal to noise ratio than immunofluorescence.

This new technology provides a simple method to obtain high quality fluorescence confocal images directly from X-gal-stained tissue sections. This technique is based on the fact that the X-gal precipitate is able to absorb light in 570–700 nm range, and emit fluorescence in the 650–770 nm wavelength range. Moreover, the procedure is totally compatible with classical immunofluorescence, allowing a combination of the LacZ reporter system with multiple fluorescent labels.

As part of the applied methodology, a mathematically-based optical correction protocol has been designed, which is a reliable method to significantly increase the signal to noise ratio for weak fluorescence.

1. Allows high quality confocal images obtained from X-gal-stained sections with a regular confocal microscope without photoactivation, being totally compatible with the use of most common fluorescent dyes or fluorophores for immunolabelling.
2. Valuable tool in gene expression and cell tracing studies. It could be also applied to evaluate cell senescence in tissue or tumor sections.
3. The fluorescent property of X-gal makes the reporter a good candidate for new applications such as flow cytometry or dynamic fluorometry.



Intellectual Property

This technology is covered by an International Patent Application.



Aims

We are looking for a partner interested in a license or/and collaboration agreement to further develop and exploit this innovative technology.



Classification

Area: ICT

Technology: Digital pathology



Advantages