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Review Article

Shedding quantitative fluorescence light on novel regulatory mechanisms in skeletal biomedicine and biodentistry

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Summary Digitalized fluorescence images contain numerical information such as color (wavelength), fluorescence intensity and spatial position. However, quantitative analyses of acquired data and their validation remained to be established. Our research group has applied quantitative fluorescence imaging on tissue sections and uncovered novel findings in skeletal biomedicine and biodentistry. This review paper includes a brief background of quantitative fluorescence imaging and discusses practical applications by introducing our previous research. Finally, the future perspectives of quantitative fluorescence imaging are discussed.

Contents

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1. Introduction

Undoubtedly, biologists and dental and medical scientists have long benefitted from the microscope since the initial studies of biological structures reported by pioneers such as Robert Hook and Antoni Van Leeuwenhooek in the 17th century [1—3]. The imaging quality has much improved following advances in the development of technology and manufacturing. We are standing in a historical decade of microscopic development, with the awarding of the Nobel Prize in Chemistry to Osamu Shimomura, Martin Chalfie and Roger Tsien in 2008 for the discovery and application of green fluorescent protein (GFP), and the current awarding of the Nobel Prize in Chemistry to Eric Betzig, Stefan W. Hell and William E. Moerner in 2014 for the development of super-resolution fluorescence microscopy. In this review, the application of confocal imaging and image processing in hard tissue biology is discussed by introducing our research on skeletal biology.

2. Confocal microscope

Confocal laser scanning microscopy (CSML) is designed to allow only light originating from the nominal focus to pass through a detector, such as a photomultiplier tube (PMT). To scan the specimen, a tightly focused laser is used, and the emitted light is eliminated by a pinhole placed just before the PMT, so that the image is constructed by spatially mapping the detected (well-focused) signal in accordance with the position of the scanning spot (confocality). This confocality of the scanning spot and the detected light enables the exclusion of out-of-focus blurring, thus improving the signal-to-noise ratio and image resolution.

Principally, CSLM can obtain a better resolution image than widefield fluorescence microscopy. However, to obtain a significant advantage by CSLM, the pinhole has to be closed to an extent where most of the emitted light is discarded, thus only a faint signal is detected. Therefore, for the practical use of CSLM, the pinhole must be opened even wider than the theoretically optimized size to obtain suitable images. In this case, it is extremely important to notice that the obtained signal may include some degree of out-of-focus signals.

3. Image processing and quantitative analyses

In the fluorescence microscopy system, charged-coupled devices (CCD) or photomultipliers (PMT) are used as detection apparatuses that capture visual information in a numerical form. Digitalized fluorescence images are composed of a number of pixels (or voxels in three-dimensional images) that contain numerical information such as color (wavelength), fluorescence intensity and the spatial position. By taking advantage of this digital information, reliable quantitative data can be extracted from acquired fluorescence images. Therefore, the quantitative analysis of labeled molecules or cellular functions in a spatial manner is possible, which also facilitates the comparison analysis of numerical data sets and subsequent statistical analyses. These technical advantages can be beneficial to understand a given biomedical phenomena in a compressive manner from the tissue to subcellular levels.

4. Fluorescence morphometry

Our research group has applied quantitative fluorescence imaging on histological skeletal sections by measuring the three-dimensional distributions and intensities of fluorescence signals, which is referred to as ''surface rendering'' [4—8]. Surface rendering is a common image processing method that provides the surface area, volume, signal intensity, fluorescence wavelength and spatial information of a given signal that is three-dimensionally distributed. Another image processing technique we have applied is filament tracing, which enables us to trace a network of cellular process, such as neuronal dendrites and osteocytic cellular processes, in a three-dimensional manner [4]. Using this image processing method, the number, diameter and branching number of cellular processes can be numerically scored. Therefore, we can quantitatively estimate the cellular network pattern.

5. Visualization and quantification of the cellular input levels of TGF-β and BMP signaling in cartilage development

Transforming growth factor-β (TGF-β) and Bone morphogenetic proteins (BMP) have long been described as critical growth factors of cartilage development and chondrocyte

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