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Review Article

Study of early events during herpes simplex virus type 1 infection by confocal microscopy

Roger D. Everett *

MRC-University of Glasgow, Centre for Virus Research, 8, Church Street, Glasgow G11 5JR, UK

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ABSTRACT

Laser scanning confocal microscopy is a powerful technique that can be applied to study the localisation and behaviour of proteins and nucleic acids in many experimental situations. It is a particularly useful technique for the study of virus infections because of the changes that occur in the distribution and amounts of both viral and cellular proteins as infection develops. These changes reflect key stages and important regulatory events that govern the efficiency of infection. Using herpes simplex virus type 1 infected cells as an experimental model, this article provides guidance for users new to confocal microscopy on basic principles and techniques. The emphasis is on recognising, diagnosing and avoiding potential artifacts, and the workflow of the production of high quality, technically correct images.

1. Introduction

Laser scanning confocal microscopy is a very powerful technique that can be used, quite literally, to illuminate the events that take place in virus infected cells. The insights gained from observing the behaviour of viral and cellular proteins as infection progresses have propelled many aspects of virus research and have led to the discovery of several important aspects of virus-cell interactions. This article arises from the author's studies on the early events that occur during herpes simplex virus type 1 (HSV-1) infection, but rather than reiterate a review of the scientific background to the work (which has been presented in detail elsewhere [1,2]), the intention is to provide guidance which should be more generally applicable. HSV-1 proteins are expressed in a temporal cascade, with Immediate-Early (IE) proteins being expressed first, followed by Early proteins (which enable viral DNA replication) and finally the Late proteins, which are mainly structural components of the virus particle (reviewed in [3]). The IE proteins, particularly ICP0, ICP4 and ICP27, have regulatory properties that are necessary or essential for efficient infection. Their expression can be observed by microscopy soon after infection has initiated, and they interact with cellular and/or viral components in manners that change both rapidly and dramatically as infection progresses. These changes in viral protein localisation and accumulation reflect crucial aspects of virus/cell interactions and the development of infection. Therefore the observation of these changes, coupled with other approaches, contributes essential insight into the understanding of HSV-1 biology.

While in some quarters it may be fashionable to downplay the significance of 'observation-based research' it remains true that observation can be of immense value in the initial steps of scientific discovery. The detection of novel or unexpected phenomena through microscopy has led to the development of many hypotheses and paradigms that would be unlikely to have been discovered were it not for the simple art of observation.

Modern microscopes are becoming increasingly complex, with many technical advances that allow hugely sophisticated analysis. The details of these technologies will vary according to the instrumentation that is available, and they are in continuous further development. Therefore this article does not provide a guide to the state-of-the-art techniques or hardware. Instead, the aim is to provide a primer for users who are new to confocal microscopy, with the intention of providing practical advice to aid the production of high quality images while avoiding the pitfalls that can render the results meaningless. It is assumed that the reader has access to a basic confocal microscope with a selection of lasers, and that the aim is simply to acquire images of infected cells. Further information on the principles of confocal microscopy, image preparation, basic theoretical background and references to more technical aspects of microscopy can be found in an excellent guideline article published in the Journal of Cell Biology [4].

2. The theoretical framework of laser scanning confocal microscopy

The principles of fluorescence microscopy are straightforward. Proteins or nucleic acids within a cell are labelled fluorescently,

Abbreviations: FITC, fluorescein isothiocyanate; ICP0, infected cell protein 0; ND10, Nuclear Domain 10; PBS, phosphate buffered saline; PML, promyelocytic leukaemia (protein).

* Fax: +44 0141 330 3520.
E-mail address: roger.everett@glasgow.ac.uk

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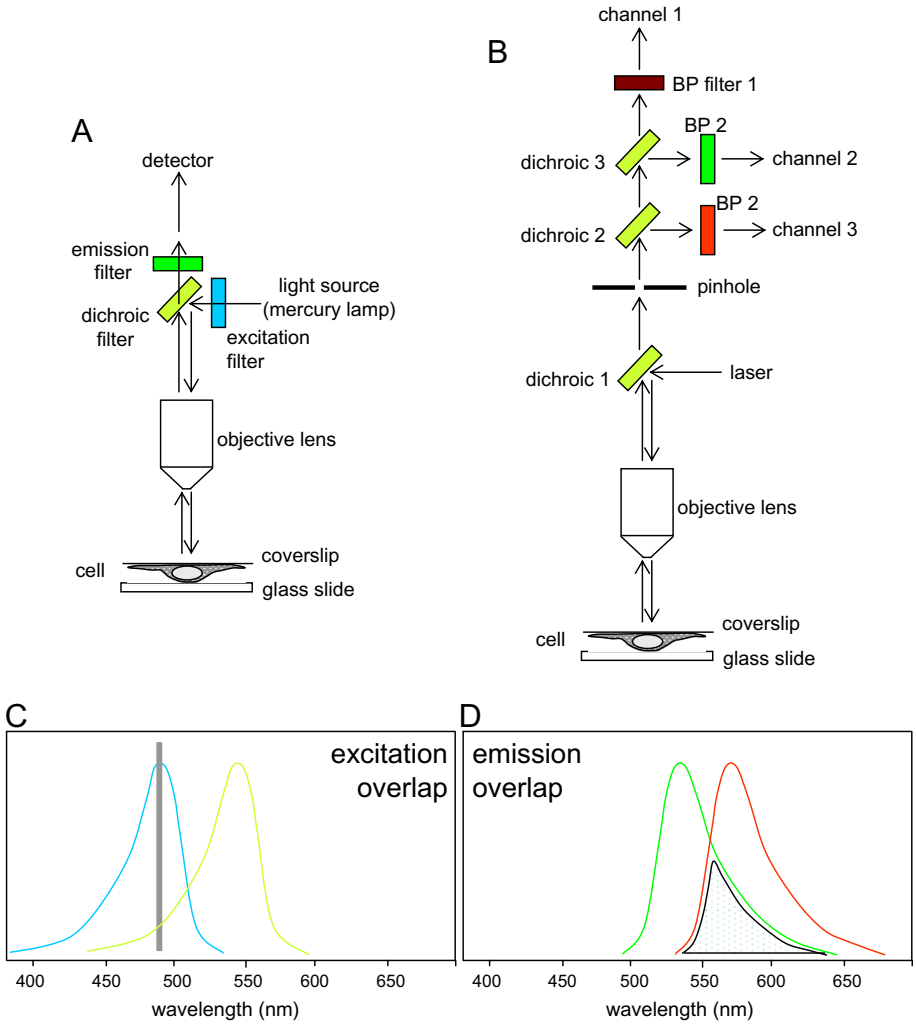

Fig. 1. Representation of simplified standard and confocal microscopy light paths, and of the principles of excitation and emission overlap. (A) The basic epifluorescence light path, with excitation, dichroic and emission filters. (B) The basic highly simplified confocal microscopy light path, with laser illumination and separation of the emitted light into different channels using a sequence of dichroic and band pass (BP) filters. (C) Illustration of the principles of excitation overlap. The blue line represents an idealised excitation spectrum of a green dye such as FITC, and the light green line that of a red dye such as Alexa 546. The vertical bar depicts excitation with a 488 nm laser, illustrating maximal excitation of the green dye and less efficient, but nonetheless significant, excitation of the red dye. Note that actual excitation spectra of different dyes and autofluorescent proteins are likely to be more complex than the simplified examples presented. D. Illustration of the principles of emission overlap. The green line represents an idealised emission spectrum of a green dye such as FITC, and the rd line that of a red dye such as Alexa 546. The shaded area depicts the wavelength range at which the emission spectra overlap. Note that red to green overlap can occur (depending on the band pass filter used for the green channel), but that green to red overlap is potentially much more substantial. The actual emission spectra of different dyes and autofluorescent proteins are likely to be more complex than the simplified examples presented.

then subjected to light at specific wavelengths that excite the fluorescent molecules, whose subsequent decay to a lower energy state is accompanied by the emission of photons of a lower energy (longer wavelength). There is a wide variety of fluorescent labels available, with different characteristics, thereby enabling simultaneous detection of two or more labelled species. The number of different labels that can be detected in a sample is limited by the range of lasers in the microscope system, the sophistication of the image acquisition hardware and software, and the availability of reagents that are specific to the macromolecules of interest. But however sophisticated the hardware and software, there are a number of simple and common technical pitfalls that can result in images that are, at best, misleading. It is important to understand the basics of the microscopy system so that these pitfalls can be avoided.

A highly simplified representation of the light path of a standard epifluorescence microscope is presented in Fig. 1A. A mercury lamp is most commonly used to provide wide spectrum illumination, then passage through an excitation filter selects the required excitation wavelength band. This usually covers a range of about 20 nm around the stated maximum. The light then hits a dichroic filter, set at an angle of 45°, that reflects light of that wavelength so that it is directed through the objective lens onto the sample. The emitted light returns through the lens and, being of a longer wavelength, is allowed to pass through the dichroic. These photons are then selected by a band pass emission filter (usually covering 30–50 nm) before being detected by eye or camera. The different channels can be visualised by inserting the appropriate filter sets (see http://www.aic-imagecentral.com/products/pdfs/hdbk4.pdf for an excellent description of filters and their characteristics). The core components of a typical standard confocal microscopy system are similar except that lasers provide excitation at defined wavelengths, avoiding the need for excitation filters. The emitted light passes through a pinhole that excludes all photons except those derived from the focal plane, hence the term confocal, and is detected by a scanning system that builds the image pixel by pixel. The emitted light is separated by dichroic and emission filters into wavelength bands or channels that are specific for the