Chapter 2
Laser Scanning Confocal Imaging of Forensic Samples and Their 3D Visualization

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ABSTRACT

This chapter describes the application of confocal imaging in fluorescence and reflection modes and the analysis of the three-dimensional (3D) data sets of samples relevant to forensic medical investigations. In the last three decades, confocal microscopy has become a widely used technique in the fields of biological and medical sciences. Gradually, its use is becoming more widespread in forensic sciences as it offers numerous advantages over conventional wide-field microscopy. One of the key advantages is the generation of sharply focused 3D data stacks of imaged material, without out-of-focus blur. The technique generates digital optical sections from sample surface down to a depth of 100-300 μm from which a multitude of structural, sculptural and optical parameters in 3D and 4D can be obtained and analysed. This chapter discusses several examples of confocal imaging for medical forensic applications, including the 3D analysis of finger prints, hair, skin abrasions and grass pollen exine morphology to provide new diagnostic and prognostic information. The chapter also covers practical applications of a powerful 3D visualization and analyses software.

INTRODUCTION TO CONFOCAL MICROSCOPY TECHNIQUE

Over the past three decades, confocal microscopy has revolutionized biological sciences and has become a common technique in many scientific disciplines as it rapidly overcame the popularity of conventional optical microscopes. Its development involved a timely convergence of a multitude of disciplines – biology, optical physics, computer sciences, chemistry, mathematics, engineering, etc. The result was a dramatic transformation of

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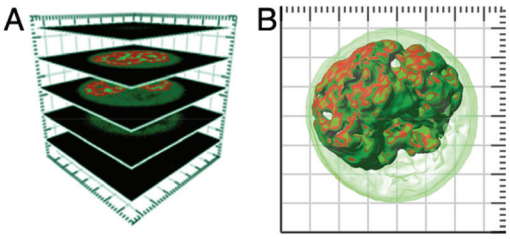
the study of cells and tissues in biological and biomedical sciences. The pace of the development of confocal imaging in the forensic sciences is accelerating, although its use is still relatively uncommon. It is beginning to be employed in instances when detailed inorganic sample, histological and cellular studies can provide forensic pathologists with the diagnostic techniques that add a wealth of 3D digital data and help in resolving difficult cases. The technique appears to be especially powerful when 3D microstructural information is required or when screening for molecular or pathological abnormalities occurring as a result of injuries or poisoning (Turillazzi et al., 2008).

The superiority of confocal imaging is largely due to the ease of obtaining high-resolution subsurface images of specimens with minimal preparation, without the need for chemical fixation or embedding. This is achieved by the elimination of image degrading out-of-focus light and by controllable the depth of field. Light detection is limited to a confocal volume within a sample by positioning a pinhole in front of the detector. The pinhole focuses the light emitted or scattered from the specimen before it reaches the detector. A confocal microscope, thus, provides an almost perfectly focused plane (the z-plane) without contribution of out-of-focus light from above or below it, although generally some out-of-focus light is usually detected. Acquiring the best objectives for the system is still important because the degree of z-plane optical resolution depends almost entirely on the aperture of the objective used, called the Numeric Aperture (N.A.). The higher N.A., the less out-of-focus light and the greater the z plane resolution.

A sequence of optical sections (i.e., sequential images) collected at incremental steps perpendicular to the optical axis of a specimen makes up a z-series (Figure 1). The series of steps are driven by a computer-controlled stepping motor that changes the focus by predetermined increments, set up

Figure 1. Confocal imaging of a live cell by optical sectioning (A) and 3D reconstruction of the sections using image analysis software (Imaris, Bitplane). Image by Christopher Hammang



by the microscope operator, that be as low as 0.1 micrometer or 10s of micrometers. The specimen is thus imaged in 3D (xyz) by performing serial optical sections without the need to physically cut through it, providing a non-invasive capability. The optical sectioning capability of confocal microscopes enables imaging and analysis of cellular, tissue or sample architecture, as well as any pathological alteration, without having to physically cut the specimen and in an environment with minimal alteration to biological reality. Even thick and relatively opaque specimen can be visualized by confocal imaging.

Specimen may also be viewed in a four-dimensional mode (4D microscopy, xyzt) – a z-series data is collected at time intervals producing a 4-dimensional data set with three spatial dimensions (x, y, and z), the time being the fourth dimension. The 3D and 4D data sets can be viewed as stereo pairs imaged at each time point, can be played back as a movie, or, imaged as xyz 3D reconstructions over a time series or as a 2D montage. Optical slice series through cells or tissues and 3D representation contains a great deal more information about the structure, morphology, sub-cellular location of fluorescence, localization of various cellular constituents and their relationship and special distribution (Figure 1 B).

Performance Evaluation and Scheme Selection of Person Re-Identification Algorithms in Video Surveillance
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