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An overview of emerging hyphenated SEM-EDX and Raman spectroscopy systems: Applications in life, environmental and materials sciences

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ABSTRACT

This review covers the potentials and limitations of novel hyphenated SEM-EDX/Raman spectrometer systems (different set-ups) to typify complex materials from disparate fields, as outlined through case studies in bio- and geomaterials, minerals, forensic science, pharmaceutical materials, and cultural heritage items. Emphasis is placed on analytical advantages, restrictions and challenges that must be faced to optimize analyses and achieve the full capabilities of this emergent analytical tool. Our aim is to promote its use and encourage users to explore new applications on challenging materials, by providing published analytical protocols and guidelines.

Contents

1. Introduction

The last two decades have demonstrated that there is great demand, seen to be crucial in various fields of science, for a particular type of physicochemical technique. Specifically, this should provide profound morphological, chemical and molecular characterization of complex nano- and micro-sized material specimens, whether of purely organic, inorganic or hybrid composition. It must preclude sample preparation -to preserve original structures-, and must be performed in the same region of interest (ROI) via a unique instrument. Since such a task is a substantial analytical challenge, this request has recently driven the hyphenation of scanning electron microscopy-energy dispersive X-ray spectroscopy (SEM-EDX) and Raman spectroscopy (RS) into a single system. The novel

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technique offers new insights to unravel the nature and structure of certain specimens not yet entirely characterized. Its development is the result of the collective efforts of innovative scientists searching for powerful and versatile analytical techniques to tackle holistic characterization of hybrid materials. SEM-EDX and RS are full-grown and robust techniques with numerous proven applications in diverse scientific fields [1,2]. A review of the basic principles of both techniques, and the technical aspects involved in coupling them can be found elsewhere [3].

Combining SEM-EDX and RS results in a potent analytical approach that merges morphological and elemental information provided by SEM-EDX, with molecular, structural and electronic data obtained with RS. The key aspects of the success of this hyphenated system are sample visualization at high spatial resolution and chemical information delivered by SEM-EDX, and identification of polymorphs, allotropes and organic/inorganic components supplied by RS. Most materials characterization requires visual examination of the sample, which usually is done with optical microscopy (OM) and/or SEM. The high spatial resolution and good depth of field typical of SEMs make them an ideal device for a material's first observation. Thus the benefit of performing analyses using a hyphenated SEM-EDX/Raman system in place of using RS furnished with an OM (micro-Raman spectroscopy, MRS hereafter) is that SEMs supply accuracy in visualizing sample features. SEMs overcome the restrictions of MRS, moreover using a field emission (high resolution) SEM (FESEM) concerning image spatial resolution (up to 4 orders of magnitude better than OM) and depth of field and contrast, such that the ability to recognize and analyze a ROI is simpler and more precise than using MRS (MRS resolution is ca. 1–2 μm, it is ca. 3 nm for conventional SEMs and ca. 1 nm for FESEM). The additional capability of SEMs to perform elemental analysis when connected to an X-ray spectrometer (SEM-EDX) increases the versatility of this hyphenated technique.

In spite of this, as we will show in the reviewed articles, new practical and technical challenges must be faced when performing coupled SEM-EDX and Raman analyses, since Raman bands are weaker than in MRS, sometimes to the point that Raman signals cannot be obtained. These difficulties and limitations arise from the mutual influence between SEM and RS when connected, as reported in several of the revised papers. The solutions require comprehensive knowledge of factors affecting Raman spectra, expert operators and optimization of analytical procedures -including sample substrate effects [4–7].

2. Hyphenated SEM-EDX/Raman spectrometer systems

Different technical set-ups can be implemented to produce unique hyphenated systems. In this section we show the present state of the art in coupling SEMs and Raman spectroscopic techniques. In 2000 the development of such hyphenated systems began with custom-built systems. Once the related technical difficulties in coupling the stand-alone techniques had been solved, hyphenated systems with diverse set-up configurations began to be marketed, although at different times. At present there are four commercial systems available on the market based on two different principles, i.e. correlative miscroscopy (CM) and *in-situ* SEM-Raman measures using an interface positioned below the SEM's pole piece (Fig. 1).

2.1. Correlative SEM -Raman microscopy systems

CM is used to depict the combined information obtained from the same ROI of a sample utilizing at least two different types of microscopy techiques. Correlative SEM-Raman microscopy systems mean that the two systems are "off-axis" indicating that the Raman optics are settled outside the (SEM) electron beam axis. Here the SEM-EDX and Raman data are acquired sequentially after a precisely calibrated movement of the SEM stage. Initially SEM-Raman CM involved time-consuming relocation of a ROI; however at present advanced CM supplies automated procedures to guarantee fast and accurate analysis of a target region. The original non-commercial home-built system of SEM-Raman CM was designed by Aksenov and co-workers in 2000 under the name CRM-SEM (confocal Raman microscope–SEM/EDX) [8,9], later renamed CRSEM (confocal Raman SEM) by Van Apeldoorn et al. [10]. In 2011 *HybriScan Technologies* BV (*HybriScan* company, The Netherlands), together with JEOL and FEI companies as partners, marketed a correlative Raman-SEM microscopy system under the name of *HybriSCan Molecular Microscope* (HSCMM), allowing correlated SEM-EDX and MRS analyses of the same object (http://www.nanounity.com/sem-raman.php). HSCMM consists of three main modules: a Raman spectrometer, an optical module to SEM and a *Hybriscan* pick-up module mounted in the SEM vacuum chamber. After SEM examination of a sample, the SEM stage moves that sample under the lens of the *Hybriscan* Raman microscope. A design of this "off-axis" system can be found in [3]. The HSCMM can be integrated with an EDX cryogenic module [11].

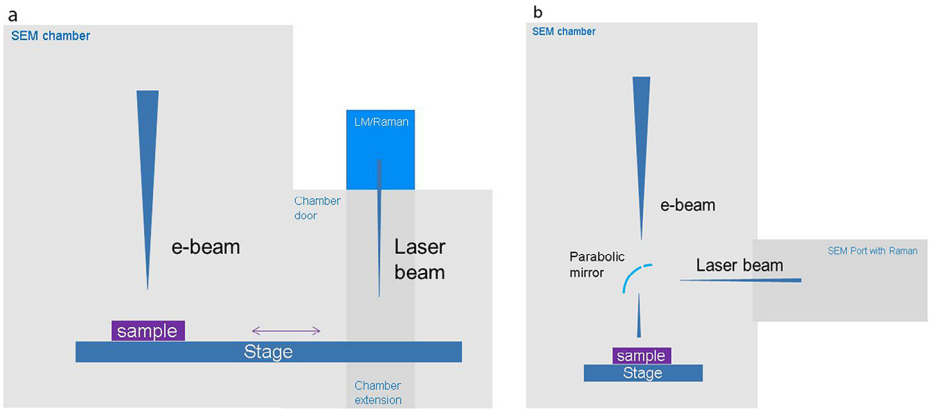

Fig. 1. Schematic of (a) correlative SEM-Raman microscopy systems showing the Raman optics settled outside the SEM electron beam axis; SEM and Raman data are sequentially acquired after a precisely calibrated movement of the SEM stage; and (b) In-SEM Raman systems based on an "on-axis" principle; here simultaneous SEM and Raman measurements are performed on a ROI (Courtesy of S. Freitag, Carl Zeiss Microscopy GmbH).