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Use of confocal laser scanning microscopy (CLSM) for the characterization of porosity in marble

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ABSTRACT

Confocal microscopy offers several advantages over conventional light optical microscopy, including the ability to control depth of field, elimination or reduction of background information away from the focal plane and the capability to collect serial optical sections and three dimensional imaging from thick specimens. Although the technique is widely used in many scientific fields, especially in biology and medicine, the use of confocal laser scanning microscopy is relatively unknown in the field of building materials. In the following article, an example of application of this technique to geomaterials is given, together with a description of sample preparation and a practical example of investigation of the porosity of bowing marbles.

1. Introduction

Although the basic concept of confocal microscopy was introduced in the mid-1950s by Marvin Minsky, the broad usage of confocal microscopy did not begin until the end of the 1980s, when the first commercial instruments appeared [1]. Due to further development of optics and electronics in the 1990s, which afforded more stable and powerful lasers, high efficiency scanning mirrors, fiber optics, better dielectric coatings and detectors, confocal laser scanning microscopy (CLSM) has become an essential and invaluable tool in biology and medicine (see for example [2–4]). Characterization of materials was partly covered by researches in paper technology [5], [6], while characterization of building materials remained relatively unexplored, with exceptions of some researches in the field of wood science [7], cement-based materials [8–10] and polymer coatings [11].

The basic principle of CLSM is the detection of excited light from the point location in the specimen, which is confocal with two pinhole apertures of the laser system and detector. Spatial filtering techniques eliminate out of focus light or glare, which gives high-quality images with good resolution. A schematic diagram of the optical pathway in a CLSM system is illustrated in Fig. 1. Emitted light from a laser is projected into the specimen by scanning mirrors. Secondary fluorescence emitted from points on the specimen scanned by a laser passes back through the objective to a beam splitter (dichromatic mirror) and is focused as a confocal point at the detector pinhole aperture. The same aperture discards rays that are reflected or fluoresced by planes that are not in focus.

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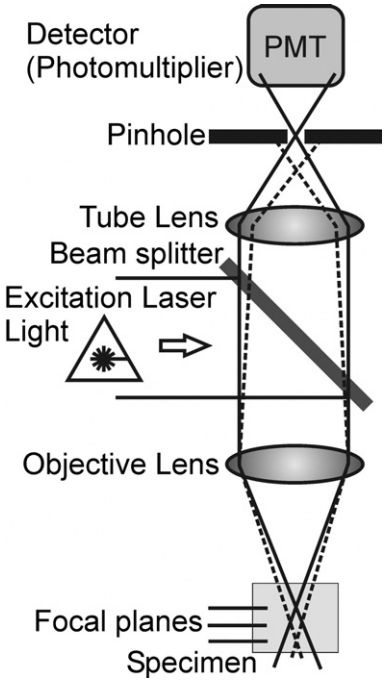

Fig. 1 – Schematic diagram of the optical pathway in a laser scanning confocal microscope.

Refocusing of light shifts scanned points on the specimen to a new plane that becomes confocal. This forms well-contrasted images that are sequentially deeper optical slices of the sample. As a result, a three dimensional representation of material can be assembled with corresponding software. The confocal image never exists as a real image and cannot be visualized through the microscope eyepieces; rather it is reconstructed point by point from emission photon signals by the photomultiplier and accompanying electronics.

A review of recent literature shows that confocal microscopy in the area of geomaterials is relatively unknown despite its huge potential. Confocal microscopy has been used for quantification of surface roughness of cement-based materials [8,9]. Kurtis et al. [9] demonstrated the utility of confocal microscopy for two additional applications:

1) imaging through glass aggregate, which provides a view into the material of the aggregate-cement surface, and facilitates a study of reactions that occur at aggregate/cement interface e.g. formation of alkali-silica reaction products (also see [10]), and
2) wet-chemistry CLSM, where contrast between solids and voids or solution is enhanced, which also leads to generation of a volumetric presentation.

One of the major obstacles in the study of stone materials using conventional microscope techniques is the absence of 3D visualization of microstructure. In order to characterise 3D properties, stereological tools are used to predict 3D porosity, grain-size distribution and texture on the base of IA of 2D graphs. The main aim of this study was to evaluate whether CLSM could be applied to imaging 3D pore system in bowed marble, as well as to its quantification with image analysis of obtained optical slices.

2. Materials and Methods

Three samples taken from two calcitic marbles, which are known as materials prone to hydro-thermal deterioration such as bowing of facade panels, were prepared for examination with a CLSM system. Sample A and sample B were samples of the same calcitic marble of known origin, before (sample A), and after artificial ageing (sample B), while sample C was taken from a detached cladding panel from a building in Ljubljana, Slovenia. After approximately 15 years of exposure to natural environment almost all panels in the cladding exhibit severe bowing [12]. Specimens with denotation "B" were taken from the panels artificially aged in the laboratory according to the method for determination of bowing potential. A description of the basic procedure for determination of bowing potential is given in NT Build 499 [13], while further procedures were developed in the frame of the European project TEAM — Testing and assessment of marble and limestone [14]. After 50 cycles of heating and cooling in the range 20–80 °C, panels exhibited bowing to a maximum of 4.482 mm/m.

From each sample, three subsamples were prepared. Three specimens of dimension $30\times30\times30$ mm^3 were cut from each sample in order to determine open porosity according to standardised method [15]. At the same time two polished sections of each sample were prepared for CLSM and scanning electron microscope (SEM) examinations. The list of samples and research methods used is presented in Table 1.

Sections for confocal laser scanning microscopy and scanning electron microscope analysis were first impregnated in a vacuum impregnation unit with epoxy resin which was mixed with fluorescent dye[1] in order to increase the resin's fluorescence. Fluoroscein is one of the most popular fluorochromes used in confocal microscopy because of its absorption maximum at 495 nm, which coincides well with 488 nm spectral line produced by Ar-ion laser. Impregnated sections were than dried and polished with a Ni-diamond pad (70 μm) and an oil-based suspension and then successively with combination of different pads and oil-based diamond suspensions of 15, 6 and 3 μm.

Polished sections were examined with a confocal microscope. Fluorescent images were acquired with plan-apochromatic objective (10× and 20× magnification with 0.45 and 0.75 NA respectively) using 488-nm Ar-ion laser excitation. Optical slices ($651.5\times651.5\times2$ μm^3) captured with instrument software and exported in BMP format were then ready for porosity and orientation measurements with freely available image analyses (IA) software Image J [16]. BMP files were transformed in 8 bit images. Thresholding was set in two ways: a) with automatic set threshold value and b) with a manual threshold value. For manual thresholding an SEM image (Fig. 2) was used as support for more precise definition of crack thickness in a single CLSM optical slice, (e.g. for determination of suitable threshold range). With this procedure some subjectivity is introduced into the IA procedure; never-theless, once the threshold range was manually set, the same threshold value (30–36) was then used for all measurements of

[1] Fluorescein (Sigma-Aldrich, St. Louis, MO, USA).