DETECTION OF BIOSIGNATURES IN MILLION YEARS OLD FOSSILS WITH THE “STANDOFF BIO-FINDER”.

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Introduction: The “Standard Biofinder” has been developed at the University of Hawai‘i with the intent of quickly locating biological materials in wide geological contexts in planetary exploration [1]. The Standoff Biofinder locates bio-fluorescent materials by taking live images that highlight the short lifetime fluorescence emitting objects [2]. Since its creation [3-5], the instrument has been proven to distinguish between mineral and biogenic fluorescence, work from standoff distances (1-10 m) in daylight conditions with short measurement times (0.1s), and to differentiate between different biogenic materials by taking color images. These capabilities could help an exploration rover identify objects of interest for the ‘search for life’ beyond Earth and then dedicate other characterization techniques, such as Raman or LIBS, to determine the molecular and elemental composition of the selected targets. For this work, we used the “Color Biofinder” version of the instrument [5] in combination with time-resolved fluorescence measurements to assess its capabilities to detect and characterize fossils as biosignatures. Fossils are preserved remains from biological entities from past geological ages. It is possible that any biosignatures encountered in planetary exploration belong to ancient, extant life and are in fossil form.

Methodology: The key elements in the Biofinder are a nano-second pulsed laser to excite the fluorophores in the samples and a gated imaging detector to record the fluorescence images. The timing capability of both the laser and the detector allow for recording time-resolved fluorescence images. The laser beam was diffused to illuminate a 20 cm wide area and a compact CMOS color camera with a 1” lens imaged the scene. A notch filter kept the light at laser wavelength from reaching the detector and both the camera and the laser pulses were synchronized. Thus, images show short lived fluorescence while long lived or non-fluorescent areas appear dark [1]. The images reported here were obtained from a distance of 50 cm with simultaneous 355 and 532 nm excitation pulses from a Nd-YAG laser. Samples were excited with total pulse energy of 5 mJ at 355 nm plus 4 mJ at 532 nm. Time resolved fluorescence spectra were obtained with a combined Raman-LIBS-fluorescence remote system developed at the University of Hawai‘i [6].

Samples: The fossils used for this study were obtained from both commercial study fossil sets and the collection of the Geology and Geophysics department at the University of Hawai‘i at Mānoa. The specimens range in ages from the Precambrian eon (more than 543 million years old, Ma) to the Pliocene epoch of the Cenozoic era (1.8-5.3 Ma).

Results and discussion: Figure 1 shows the white light and short lived fluorescence images of shrimp fossils in their containing matrix. The fluorescence image was excited with both 532 and 355 nm laser and clearly shows the shrimps and shrimp pieces emitting strong fluorescence and being positively highlighted as biogenic. Small shrimp pieces not clearly visible in the white light image are clearly revealed in the fluorescence image, illustrating that the use of time resolved fluorescence greatly enhances fossil detection capabilities using laser stimulation techniques in paleontology.

Figure 1: Shrimp fossils from the Green River formation, Colorado, Wyoming and Utah, USA. Top: white light image; down: short lived fluorescence image.
The background soil matrix also shows biofluorescence. The stronger fluorescence of the shrimps is likely due to a higher amount of biogenic traces in the fossils.

We also tested the Biofinder with fossils of different ages. Figure 2 shows the white light and fluorescence images of 9 fossils from different geologic time periods. The Biofinder image shows positive detection of all the fossils as biomaterials. The oldest fossil, the Collenia stromatolites, between 2.2 and 2.4 billion years old (Ga) (Precambrian) shows clear fluorescence and the internal structure of the columnar colonies. We observed that younger fossils tend to show stronger fluorescence while older fossils show weaker fluorescence. We observed this trend over 40+ different fossils. This can be explained through the fact that younger fossils containing larger amounts of biogenic remains as decay of organic matter is time dependent. The time resolved spectral measurements confirmed the fluorescence of all the fossils fully decays within 50 ns, indicating short fluorescence lifetimes of <25 ns. Figure 3 shows a time-series measurement of the fluorescence emitted by the Green River shrimps (Fig.1). The strongest fluorescence arrives to the detector at 130 ns time delay. Subsequent spectra show weak or no fluorescence, indicating that the fluorescence is indeed short lived. For the shrimps, the fluorescence is broad and centered around 550 nm, perfectly correlating with the yellowish-white color shown by the color fluorescence image in Fig. 1.

Conclusions: The Standoff Biofinder developed at the University of Hawai‘i is able to identify biogenic fossils as old as 2.2 Ga, based on the fluorescence emitted by minute amounts of biogenic fluorophores. The Color Biofinder would be a powerful addition to the instrument suite dedicated to “search for life” as able to detect biological materials at >50 cm distance, with <1 ms measurement times, during daylight, without sample preparation and non-destructively.

Acknowledgments: This work was supported by NASA PICASSO, and NSF grant ‘Ike Wai.