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OPTIMIZATION OF X-RAY MICROTOMOGRAPHY TO INVESTIGATE SAMPLES OF ERECT CALCAREOUS ALGAE TARGET OF CLIMATE CHANGE STUDIES

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Summary: X-ray transmission microtomography of the fronds of the calcareous algae *Ellissolandia elongata* to study the structure of the reef. *Ad hoc* tomographic set-up has been developed for investigating the complexity of reef structure, for optimizing X-ray spectra and fluxes adapted to frond transparency, particularly in non-calcified portions.

1. INTRODUCTION

Through a multidisciplinary approach, this work aims at developing *ad hoc* methodology to investigate the structure of the reef-forming calcareous algae *Ellissolandia elongata* widely distributed in the Mediterranean Sea (0-1m deep). This species is able to form ecosystems, thus creating spaces for other organisms to live and feed on. Due to its skeletal structures and metabolic activities, it plays an important role by mitigating the effects of climate changes on its associated fauna [1, 2].
E. elongata reefs are formed by encrusting bases from which develops pinnate and erect fronds (height: 1.5-5.0 cm), composed of calcified portions alternating with non-calcified ones which allow the fronds to be flexible when subject to flow. Within the reef, the fronds are highly dense especially close to the base of the reef (Fig. 1). In order to study the reef complexity and fronds structure of this habitat providers, an accurate optimization of transmission X-ray microtomography is required. In more detail, our goal is a quantitative assessment of the frond density of the reef, by counting, slice per slice along the growth axis (vertical), the area occupied by the fronds normalized to the area of the sample-container. An experimental setup is developed on purpose in order to obtain microtomography of these biological samples.

2. EXPERIMENTAL METHOD

The tomography set-up, developed and assembled at the ENEA Laboratories in Frascati (Italy), is composed of a microfocus X-ray source (about 30 μm spot size), a rotator and a two-dimensional hybrid detector called PIXIRAD, located next to the sample (contact radiography) in a fixed position. The X-ray tube provides a spectrum from 15 to 35 keV and a beam current of 300 μA, optimized to maximize the imaging contrast of the sample. PIXIRAD detector is an INFN-Pisa Spin-off, it works in photon counting in the range 2-100 keV and it is noise free [3]. PIXIRAD is realized by coupling an X-ray sensor made of a thin layer of crystalline CdTe (650

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μm) to a large area VLSI CMOS pixel ASIC. The CMOS VLSI chip has an active area of 30.7×24.8 mm², organized on a matrix of 512×476 pixels each one of 55×55 μm². The sample is rotated with angular steps of 1° and the radiographies (476 x 512 pixels) are acquired in 3 sec per position.
The filtered back-projection (FBP) is routinely used because it is fast and provides a good quality of sample reconstruction. The projections have to be uniformly distributed over the range of 180°. Alternatively, we use the iterative algorithm of Lucy-Richardson, based on the Maximum Likelihood. The algorithm converges at the maximum of the objective function and it uses a multiplicative correction applied in a matricial form at any iteration. However, the limit is the duration, because it results two orders of magnitude slower when compared to the FBP.

3. RESULTS AND CONCLUSIONS

Our study revealed the potential of the technique in biological studies. The high quality of the obtained 3-D image reconstructions allows good qualitative and quantitative data (fronds density, volume, available spaces, etc.) and poses the base for the use and development of this tool for biological applications. In fig 1(b) 141 particles (sections of the branches) have been found, whose areas are listed in the array aside the image. The green line represent the area of the sample-container, used as normalization. For the different samples we found a density ranging from 8 to 35 % at the base of the algae going to 0 at the top, at about 20 mm height

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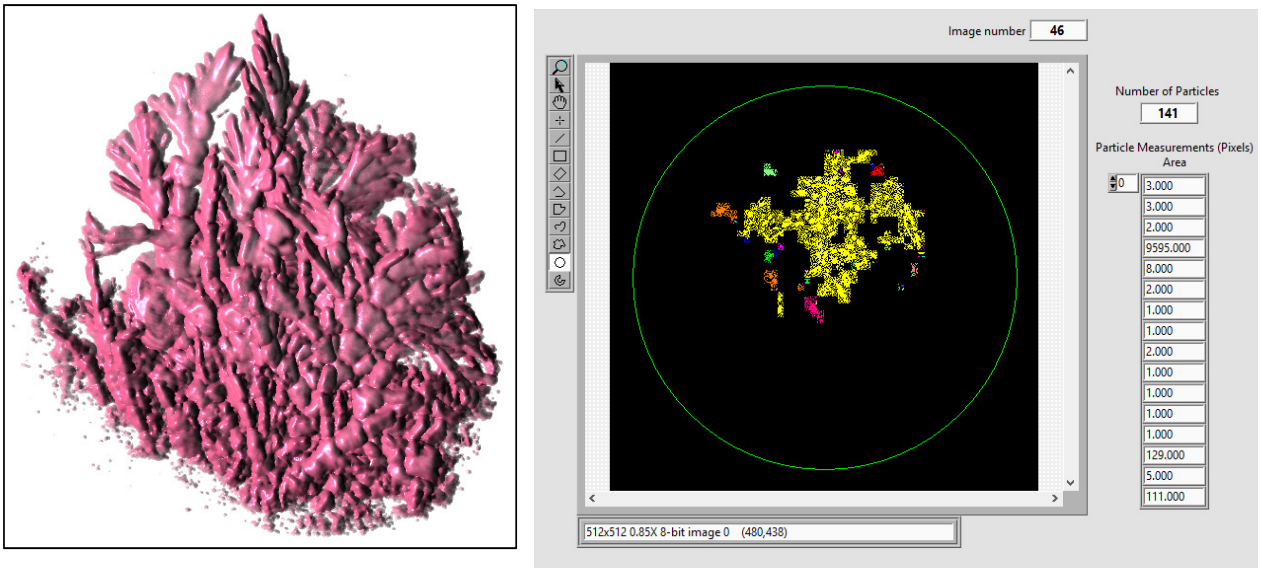

(a) (b)

Fig. 1 3-D reconstructions of the sample (a) and measurement of the areas of the reef (141 particles) at the slice 46 (b)