Phytomorphology **56**(1&2) pp. 1-4, 2006

VISUALIZING CYANOBACTERIA IN SOIL CRUSTS FROM A SEMI-ARID ENVIRONMENT USING CONFOCAL FLUORESCENCE IMAGING[1]

VASSILIOS SARAFIS[3], RAINER HEINTZMANN[4] & ARNON KARNIELI[2]
[3]School of Information Technology and Electrical Engineering and School of Life Sciences, University of Queensland, Queensland, Australia
[4]Max Planck Institute for Biophysical Chemistry, Göttingen, Germany
[2]The Remote Sensing Laboratory, Jacob Blaustein Institute for Desert Research, Ben Gurion University of the Negev, Sede Boker Campus, Israel
e-mail : karnieli@~~bgumail.~~bgu.ac.il Fax : 972-8-~~6596704~~

Abstract

Confocal fluorescence imaging of samples of biological soil crusts from an arid zone yields a three dimensional view of the distribution of cyanobacteria within the crusts. The permeation of the crusts by oil immersion enables a clear view of their interior. Results show clusters of coccoid and filamentous cells within the crusts.

Keywords : Israel, microscope, Negev, 3D image

Abbreviations : BSC – biological soil crusts, He – Helium, Ne - Neon, 3D – three dimensions

Introduction

The study of the interior structure of biological soil crusts (BSC) is fraught with difficulties for a visual or structural analysis since it is impossible to look into the soil due to the severe scattering of soil particles. Belnap (2001) gives an account of BSC structures in various environments. Belnap et al. (2001) displays a diagrammatic view of BSC showing a three dimensional structure of a crust. However, the described structure is not the result of imaging through a crust. The imaging difficulties are caused by the presence of soil mineral particles that cause a strong scattering of light due to the jump in refractive index at the interface between air and crust particles.

Artifact-free sectioning of soil is possible only in the absence of hard particles such as sand grains. Filling the crust's air spaces with immersion oil greatly reduces the refractive index differences. Scattering is thus substantially reduced. This effect suffices to permit imaging the cyanobacterial distribution in three dimensions (3D) within a thickness of crust approaching 1mm using confocal fluorescence imaging methods (Wilson & Sheppard 1984).

In arid or semi-arid soils, the BSC are stabilized by the inclusion of cyanobacteria within the surface layer of soil, which help to bind soil particles together by mucilaginous coating.

Lange et al. (1992) describe Western Negev BSC with moss protonema and rhizoids together with *Microcoleus sociatus* cyanobacteria mainly holding the crusts together with some coccoid cyanobacteria and chlorophyte eukaryotes.

The aim of the work was to assess the spatial distribution of cyanobacteria inside the superficial crust of a soil from an arid environment.

Material and Methods

BSC samples were picked up in the sand field of the northwestern Negev, Israel (Karnieli & Tsoar, 1995). Here, the BSC mostly contain cyanobacteria, where *Microcoleus vaginatus* is the dominant species accompanied by *Scytonema, Schizothrix, Calothrix, Chroococcidiopsis, Nostoc*, and *Phormidium* (Danin et al. 1989, Dor & Danin, 1996).

---

1. MS received : 26 May 2005; revised :
2. Author for correspondence
We thank Thomas M. Jovin and Donna Arndt-Jovin from the Max Planck Institute for Biophysical Chemistry for supplying the experimental equipment. R. Heintzmann acknowledges the financial support through a fellowship by the Max Planck Institute. Eli Zaady is thanked for helpful discussions on the biological composition of the crusts and Stefan Höppner is thanked for improving this manuscript.

We chose dry crusts as a starting point, due to the fact that they were easier to sample as compared to wetted crusts. The crusts were sufficiently stable, enabling undisturbed physical removal from the site of collection. Changes in distribution of cyanobacteria due to rain and other hydration regimes were not included in the present study. No shaking of the crusts was allowed prior to imaging in order to stabilize the specimen against changes in structure due to possible movements of crust particles or cyanobacteria.

Confocal fluorescence imaging permits a high lateral (X,Y) resolution as well as optical sectioning along the optic axis (Z) (Wilson & Sheppard 1984, Brakenhoff et al. 1979). A Zeiss LSM 310, modified to be used with various lasers for fluorescence illumination, was used as a confocal microscope. The efficacy of the confocal mode of operation is improved in the fluorescence mode as demonstrated by Wilson and Sheppard (1984). We chose fluorescence objective of low magnification, a 2.5x Zeiss Fluar 0.25 NA (∞/ 0.17) as the imaging element. While a higher numerical aperture would have yielded better higher resolution, we would not have been able to scan a large area due to the objective's limited field of view. Another advantage of a low magnification is the large working distance of several millimeters, thus permitting observations deep into the crust. The minimization of scattering was achieved by permeating the dry crust by oil immersion. The oil easily penetrated the crust, making it transparent for the imaging distances used (up to a millimeter). A first series of images displaying the soil particles was made in the green emission mode with excitation by an argon laser at 488nm and detection using a 530/30nm band pass filter. The second series was collected by excitation by the 633nm line of a He : Ne laser, collecting the red fluorescence emitted by the cyanobacteria using a 665nm long pass filter. The two series of XY images were combined into a multicolor stack. This yielded a 3D set of color images. We imaged the maximum area possible by using the lowest magnification available. Three-dimensional data were collected by imaging a XY series in Z increments of equal size. The data were taken at sizes up to 5mm in XY and 1.2mm in the axial (Z) direction. A composite stereo image was then created from the data. This stereo composite image was generated with a lateral motion of half a pixel per slice of a series along the +X or -X direction for the images viewed with left and right eye respectively. Although the stereo view is not strictly a 3D reconstruction, the transparency of the object keeps the difference to true 3D images on a marginal level and these images thus closely resemble the 3D images desired. From the same acquired image stacks, we also selected a vertical view of a slice in XZ projecting a particular Y breadth

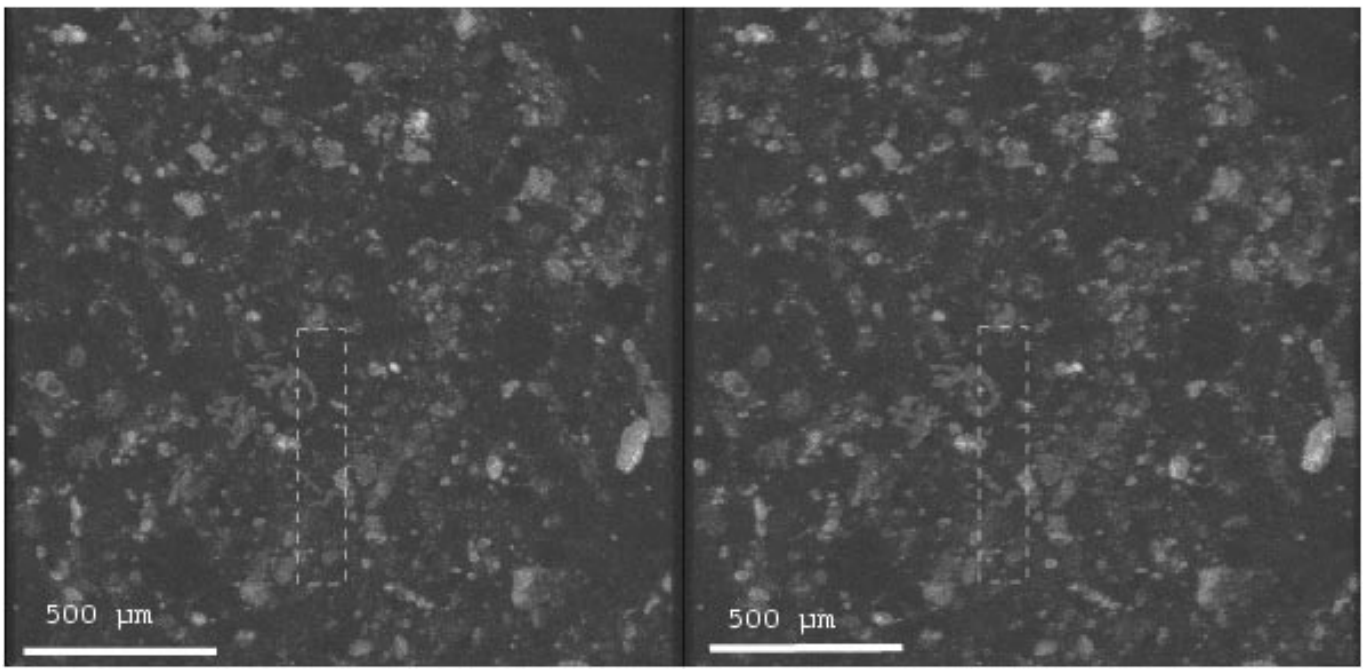

Fig. 1 — Using a stereoscope or amalgamating the images, one can view the 3D structure of biological material embedded in a matrix of crust particles.

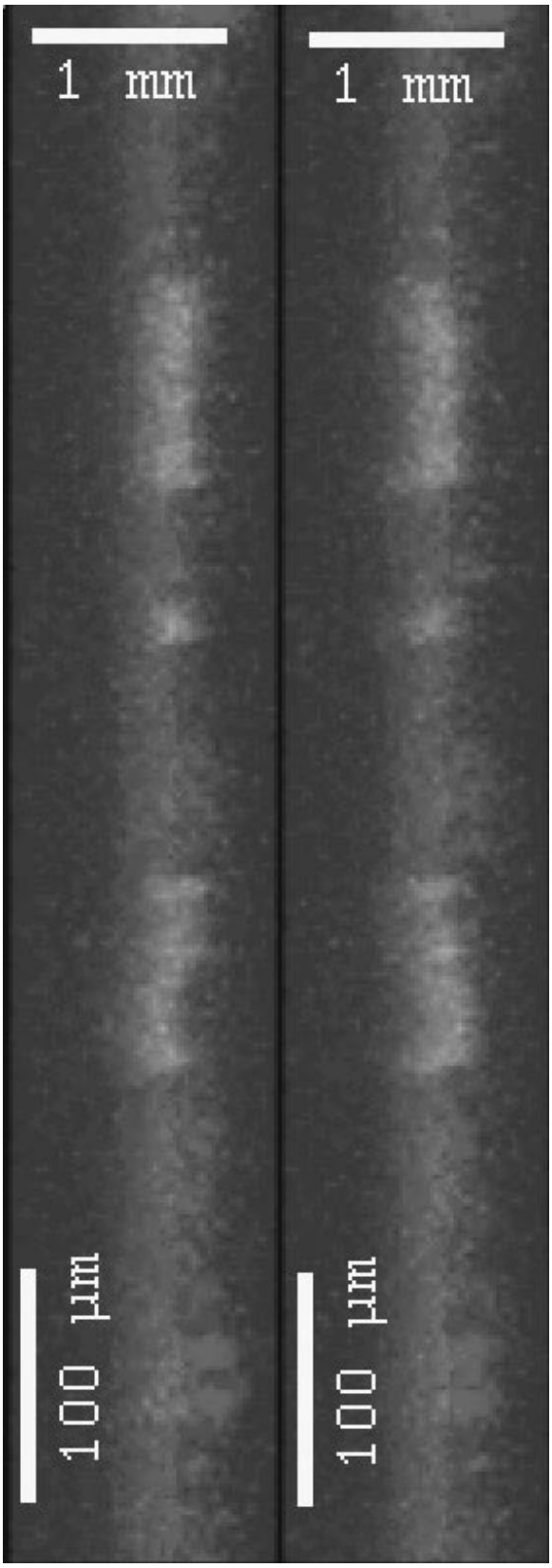

Fig. 2 — A stereo-projected thick XZ-section from the 3D region indicated by the dotted box in Fig. 1.

as shown by the dotted region in Fig. 1. In a similar manner, another stereo view was created from this region perpendicular to the previous one.

Results

The results show the images of the soil biological crusts' structure in dual color. The green fluorescence (displayed in green) shows the embedding crust mineral particles. The BSC contain cyanobacteria, as demonstrated for a sample from the same geographical location by Karnieli and Sarafis (1996) and as described taxonomically by Danin et al. (1989) and Dor and Danin (1996). In Fig. 1 we see cyanobacteria in the red fluorescent channel (displayed in red) aggregated in small clusters as well as in small groups or singly. They are mostly filamentous but coccoid and rod shaped forms also occur. They range from mainly isolated pockets up to extensive mats extending into the crusts (Fig. 1). We also saw rod shaped and coccoid forms. These extend through various levels of the crusts. Filaments displayed in Fig. 1 are dispersed and twisted in all three dimensions. They may or may not be clustered. They may be intermixed in small numbers in the crusts in populations or occur singly. We know from the events during crust wetting (Karnieli & Sarafis 1996, Belnap 2001) that the cyanobacteria travel to the crust surface.

In the dry state (when infiltrated deeper into the crust structure) the bacteria's sheath secretions serve to bind the areas in contact with the organisms. In Fig. 2, 3D distributions of the cyanobacteria from the region described by the dotted box of Fig. 1 are shown. The curved shapes of some of the cyanobacteria inside the crust are particularly well depicted.

Discussion

Fluorescence is emitted only from the cyanobacteria, when excited at a wavelength of 633 nm. There is another advantage related to the lower scattering of longer wavelengths. Under these conditions, good penetration power of the excitation wavelength is achieved in a reasonably well-corrected system compared to other shorter excitation wavelengths. The shorter excitation wavelength allows us to see the crust mineral particles forming the mineral matrix embedding the cyanobacteria.

The images show filamentous rod-shaped and coccoid microorganisms with an inhomogeneous distribution throughout the crust. According to our earlier spectroscopic study (Karnieli & Sarafis 1996), they are probably cyanobacteria similar in appearance

to those described by Danin et al. (1989) and Dor and Danin (1996). The crust structure is known to vary during periods of rain. Cyanobacteria migrate towards the surface of the crust and wander along the surface when the crust is wetted. They retreat into the crust during dry periods (Karnieli & Sarafis 1996, Belnap 2001).

We propose the use of crossed circular polarizers in a future study, with the crust mounted between the polarizers so as to clearly show the quartz particles forming the inorganic matrix of the crust in whichever orientation. Confocal imaging between crossed circular polarizers should enhance the contrast and allow good discrimination imaging in 3D (Wilson & Juskaitis 1995). This method would reflect the pattern of distribution more accurately than any classical widefield microscope unable to perform optical sectioning. Combining 3D images of the cyanobacteria with the crystalline particle 3D images would give a more complete picture of the distribution of cyanobacteria in the crusts.

Magnetic resonance imaging of dry crusts at low field strengths of 0.5 Tesla (MacFall & van As 1996) could yield a more detailed isotropic structure of these biogenic crusts, as optical microscopy usually has a reduced Z-resolution in comparison to the in-plane resolution (Heintzmann & Sarafis 2001). Low field strength corrects for magnetic susceptibility, which generates artifacts of widening the air spaces in comparison to high field strength imaging (Rofe et al. 1995). However, oiled or wetted samples could be imaged at high field strength showing increases in resolution for the oil filled and water filled crusts and changes taking place during hydration.

Literature Cited

Belnap J 2001 Comparative structure of physical and biological soil crusts, In *Biological Soil Crusts : Structure, Function and Management*, pp. 177–191 eds. J Belnap & OL Lange (Springer-Verlag : Berlin, Germany)

Belnap J, Büdel B & Lange OL 2001 Biological soil crusts : characteristics and distribution, In *Biological Soil Crusts : Structure, Function and Management*, pp. 4–30 eds. J Belnap & OL Lange (Springer-Verlag : Berlin, Germany)

Brakenhoff GJ, Blom P & Barends P 1979 Confocal scanning light microscopy with high aperture immersion lenses, *J. Microsc.* **117** 219-232

Danin A, Bar-Or Y, Dor I & Yisraeli T 1989 The role of cyanobacteria in stabilization of sand dunes in southern Israel, *Ecol. Mediterranea* **15** 55-64

Dor I & Danin A 1996 Cyanobacterial desert crusts in the Dead Sea Valley, Israel, *Algol. Stud.* **83** 197-206

Heintzmann R & Sarafis V 2001 Two point resolution in incoherent imaging, *Optik* **112** 114–118

Karnieli K & Sarafis V 1996 Reflectance spectrometry of cyanobacteria within soil crusts — a diagnostic tool, *Int. J. Remote Sens.* **8** 1609-1615

Karnieli A & Tsoar H 1995 Satellite spectral reflectance of biogenic crust developed on desert dune sand along the Israel-Egypt border, *Int. J. Remote Sens.* **16** 369-374

Lange OL, Kidron GJ, Budel B, Meyer A, Killian E & Abelovich A 1992 Taxonomic composition and photosynthetic characteristics of the 'biological soil crusts' covering sand dunes in the western Negev, *Funct. Ecol.* **6** 519-527

MacFall J & van As H 1996 Magnetic Resonance Imaging of Plants, In *Plant Biology. Current Topics in Plant Physiology* Vol. 16 pp. 33–76 eds. Y Shachar-Hill & PE Pfeffer (Nuclear Magnetic Resonance in ~~ASPP~~ : Rockville, Maryland, USA)

Rofe CJ, van Noort J, Back PJ & Callaghan PT 1995 NMR microscopy using large, pulsed magnetic-field gradients, *J. Mag. Res.* **108**B 125–136

write full form

Wilson T & Juskaitis R 1995 On the extinction coefficient in confocal polarization microscopy, *J. Microsc.* **179** 238-240

Wilson T & Sheppard C 1984 *Theory and Practice of Scanning Optical Microscopy* (Academic Press Inc. Ltd. : London, U.K.)

Author Please write the complete ref. with complete title, page no.'s and address according to phytomorphology format

Author please check the citations for original and also crosscheck the referee given in text with those in literated cited

Author please locate the ref. in lit. cited and vise-versa

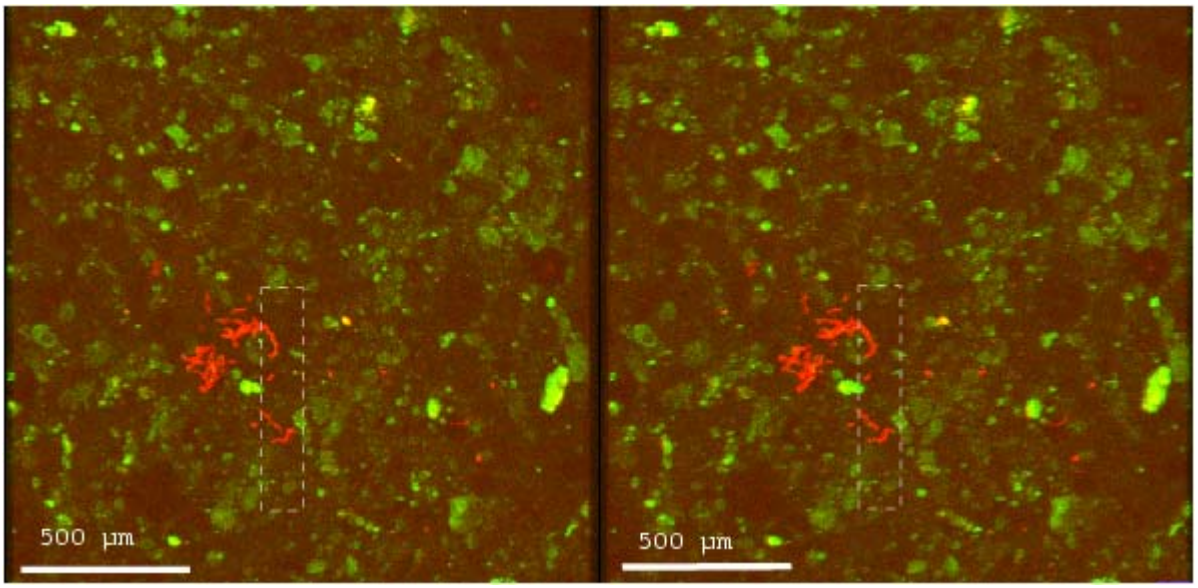

Figure 1

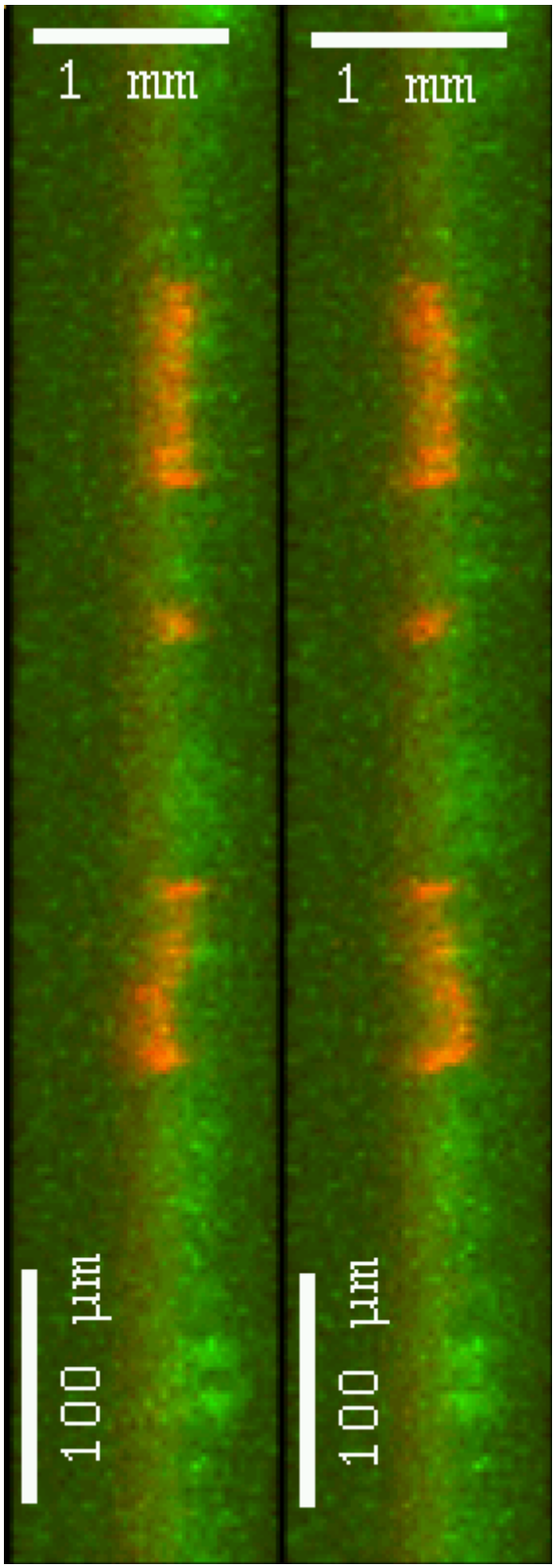

Figure 2