

## Microbialites at Gusev Crater, Mars.

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### Abstract

The Mars Exploration Rover Spirit investigated plains at Gusev crater, where sedimentary rocks are present. The Spirit rover's Athena morphological investigation shows microstructures organized in intertwined filaments of microspherules: a texture we have also found on samples of terrestrial stromatolites and other microbialites. We performed a quantitative image analysis to compare 45 microbialites samplings with 50 rover's ones (approximately 25,000/20,000 microstructures). Contours were extracted and morphometric indexes obtained: geometric and algorithmic complexities, entropy, tortuosity, minimum and maximum diameters. Terrestrial and Martian textures resulted multifractals, while terrestrial abiogenic minerals showed a simple fractal structure. Mean values and confidence intervals from the Martian images overlapped perfectly with those from terrestrial samples. The probability of this occurring by chance was less than  $1/2^8$ ,  $p < 0.004$ . Our work shows the presumptive evidence of microbialites in the Martian outcroppings explored by "Spirit", confirming our previous results concerning the Martian outcroppings explored by Opportunity at Meridiani Planum: unicellular life was widespread on the ancient Mars.

**Keywords:** Spirit mars exploration rover; Stromatolites; Microbialites; Fractal analysis; Life on Mars

### Introduction

Rover Spirit touched down the volcanic plains of Gusev Crater on 4 January 2004, for almost ten years it has runned on the Martian surface. From the landing site to "Pot of Gold" site (on sol 200 about), extensive/destroyed volcanic outcroppings, often covered by weathering coatings and/or floating on eolic sands, as crater ejecta were found. The Rover then reached the inner basin of Columbia Hills, the images showed here layered sequences, containing sulfate and other soluble elements, due to ancient large process of aqueous alteration [1-3]. At Home Plate, where are the most evident geologic feature investigated in the Inner Basin, these layered sequences appeared formed by erosion of basaltic rocks and pyroclastic rocks, deposited as alluvial fans and/or as eolian sand sheets [4-7]. In effect, ancient volcanic ash, known as "tephra", and hydrothermal deposits created in a nearby volcanic eruption, initially covered much of the Columbia Hills and the surrounding area [8], and then was covered by deposit of a cold lake [9]. The wind and weathering processes eroded most of that deposit, also carrying away much of the evidence for the ancient lake: an environment where to search for life could be interesting.

Stromatolites/microbialites are a frequently named target of life-detection missions on Mars [10-13]. Stromatolites/microbialites are an organization of primitive cyanobacteria, analogous to coral reefs that grow/grew in vast colonies. Fossil stromatolites, as well other microbialites, can be identified through their macro-meso-micro-

characteristic structures that result from the growth patterns of their constituent bacteria [14-17].

Rizzo observed by a qualitative visual inspection that images of Martian outcroppings (selected frames from images obtained by Opportunity and Spirit rovers) presented features resembling terrestrial stromatolites/microbialites [18-20]. Recently, we performed a fractal morphometric quantitative analysis of the Martian outcroppings explored by the Rover Opportunity revealing evidences of the presence of microbialites at Meridiani Planum [21]. In order to search for possible signature of microbialites in other Martian regions, we have performed a morphometric analysis of the Martian outcroppings shot by the Athena facility aboard of the Rover Spirit, comparing fractal morphometric indexes of the microtextures evidenced in the Martian outcropping with the ones of terrestrial microbialites.

### Materials and Methods

All the Martian images elaborated in this work are referred to float or weathered rocks, somewhere ejected by meteoric impact.

### Samples

This study undertakes a systematic analysis of black and white Microscopic Images (MI) obtained by Athena (Figure 1), a camera mounted on the NASA Mars Exploration Rovers (MER) "Spirit", selecting all the images that presented microspherules and filaments of microspherules (V.R.). The field of view of Athena is  $1024 \times 1024$  pixels in size and its optics provide a square frame of 32 mm of-field sampling at the working distance of about 63 mm from the front of the

lens barrel to the object plane, consequently having a resolution of about 30 micrometers [22]. In particular, 20 selected MI images obtained by the rover Spirit have been chosen and 50 samplings obtained (Figures 1 and 2), approximately corresponding to the presence of 20,000 microspherules/intertwined filaments of microspherules.

These cuttings having dimensions of about  $\frac{1}{4}$  of the original images (8-10 mm) were subjected to a slight contrast increase (Microsoft Publisher software) for an optimal vision of the microstructures. The studied cuttings were related to polished surfaces by MER's Rock Abrasion Tool (RAT) or to exposed surfaces of the Martian outcroppings.

As regards terrestrial stromatolites, unambiguously determined as biogenic, 20 quoted images and 45 samplings were obtained (Figure 3) from the WEB (from which one sample of terrestrial stromatolite photographed by Athena and one from cultured cyanobacteria, see Figure 2 or photographed at the Regional Museum of Natural Sciences, Turin, approximately corresponding to 25,000 microspherules/intertwined filaments of microspherules (Figure 2). They have been acquired and magnified in order to obtain the same dimensional scale ( $\pm 10\%$ ), resolution and acutance of the Athena imagery (V. R.). They were analyzed using the same procedures of the Martian images.

### Image analysis

The contours present in the terrestrial and Martian images (biogenic stromatolites and selected images shot by Spirit) were automatically extracted from the images and converted to single pixel outlines by a canny-edge filter (Digital Image Magnifier software by Strikos Nikolaos: <http://www.softoxi.com/digital-image-magnifier.html>), (fixed sigma and low threshold values, equals to 0.9 and 12, respectively) (Figure 4).

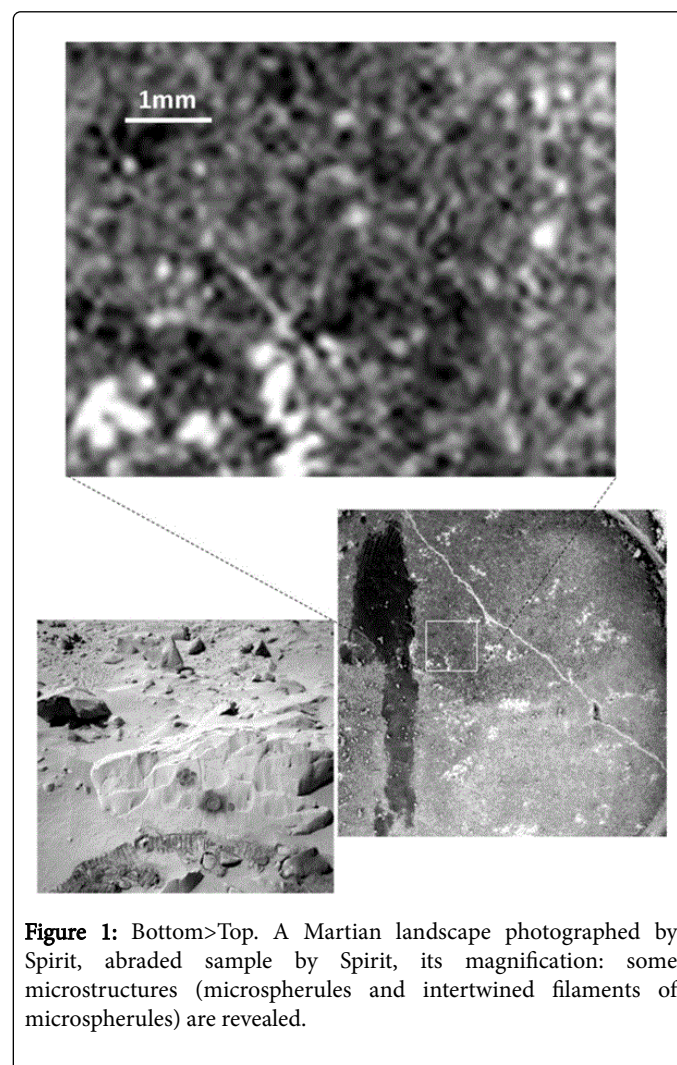
The obtained textures were characterized by analyzing their geometrical complexity, entropy (Information Dimension), algorithmic complexity (L-Z, randomness) and tortuosity (Dmin). Minimum and maximum diameters of the microspherules were also measured.

### Geometrical Complexity, D0

To evaluate the geometrical complexity of the patterns, the local fractal dimension was measured using the box-counting algorithm. Resulting our texture multifractals, as identified by the two straight lines on the log-log plot (Figure 5), the algorithm was applied for the two regions: 200-10 pixels=2 mm - 0.1 mm and 10-5 pixels=0.1 mm-0.05 mm. Briefly, each image was covered by a net of L square boxes and the number of boxes containing any part of the outline Nb(L) was counted. The slope of the log-log plot of Nb(L) vs. 1/L represented the fractal dimension of the distribution (Figure 5) [23]. The existence of log-log straight lines ( $p<0.001$ ) justified the use of the fractal analysis, applied here as a tool to obtain the morphometric indexes. The method was validated by measuring computer-generated Euclidean and fractal shapes of known fractal dimensions (Circumference=-0.7%; Square=+0.4%; Triadic Koch island=-0.9%; Sierpinski's Triangle=-1.5%).

### Entropy (Information Dimension, D1)

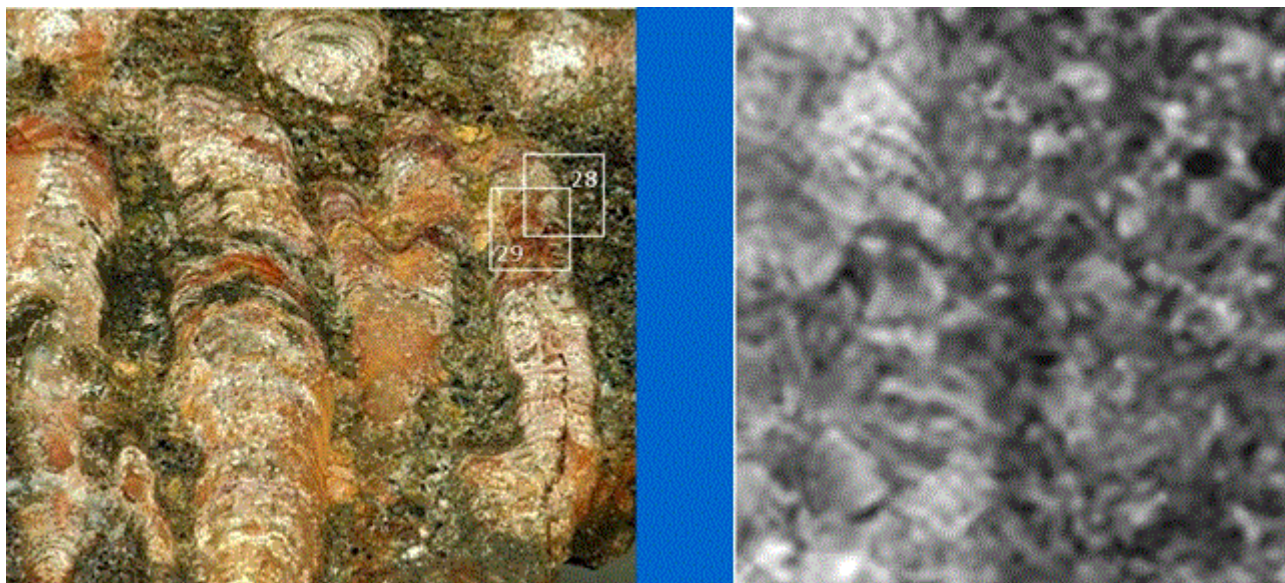
To evaluate the information (entropy) present in the patterns, information dimension, D1, a robust estimate from a finite amount of data that gives the probability of finding a point in the image, was calculated. The set was covered with boxes of linear size, d, from 200 to 10 pixels and from 10 to 5 pixels as above, keeping track of the mass,  $m_i$  (the amount of pixels) in each box, and calculated the information entropy  $I(d)$  from the summation of the number of points in the  $i$ -th box divided by the total number of points in the set multiplied for its logarithm [24]. The slope of the log-log plot of Information entropy vs.  $1/\text{box side length}$  represented the information dimension of the distribution. The method was validated by measuring computer generated Euclidean and fractal shapes of known information dimensions. The existence of log-log straight lines ( $p<0.001$ ) justified the use of the fractal analysis, applied here as a tool to obtain the morphometric indexes.



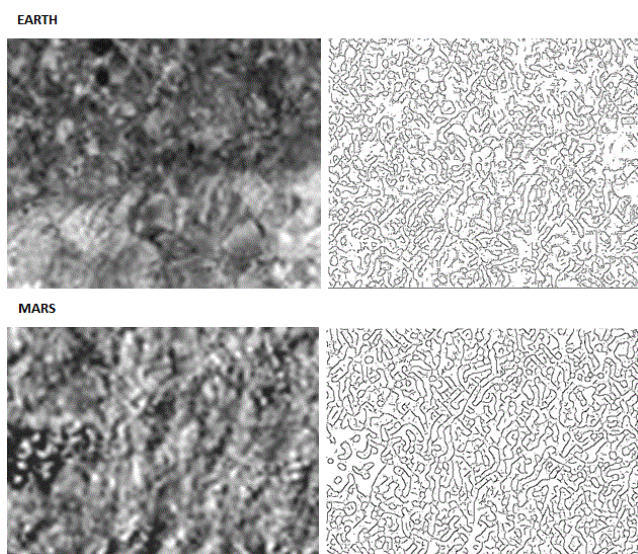
**Figure 1:** Bottom>Top. A Martian landscape photographed by Spirit, abraded sample by Spirit, its magnification: some microstructures (microspherules and intertwined filaments of microspherules) are revealed.

MARS (Rover Microscopic Imagery selection)				EARTH (fossil and living microbialites)			
Original image	Sampling	SOL	Source	Original image	Sampling	Stromatolites	Source
1	1	60	ZM131690279EFF1155P2939M2M1	1	1_5	Recent stromat. (a), Lagoa Salgada, Brazil (Srivastava, 1999; fig. 5)	<a href="http://sisep.cprm.gov.br/sitio041/sitio041.htm">http://sisep.cprm.gov.br/sitio041/sitio041.htm</a>
2	2_4	82	ZM133648332EFF2232P2979M2M1	2	6_8	Living microbialite, La Brava, Salar de Atacama, Chile	provided by EM Farias, Consejo Nacional de Investigaciones Cientificas y Técnicas (CONICET)
3	5	85	ZM133914740EFF2232P2979M2M1	3	9_12	Proterozoic columnar stromatolite, Mauritania (Borque collection)	<a href="http://www2.gel.ulaval.ca/personnel/borque/s4/stromatolites.aaciens">http://www2.gel.ulaval.ca/personnel/borque/s4/stromatolites.aaciens</a>
4	6	214	ZM145365403EFF8500P2959M2M1	4	13	Proterozoic stromatolite, South Africa (Schopf, 2006; fig. 1, c)	<a href="http://www.keyobs.be/fr/ebonino/html/stromatoliti.html">www.keyobs.be/fr/ebonino/html/stromatoliti.html</a>
5	9	228	ZM146609673EFF8600P2976M2M1	5	14	Devonian columnar stromatolite, Australia (Borque coll.)	<a href="http://www2.gel.ulaval.ca/personnel/borque/s4/stromatolites.aaciens">http://www2.gel.ulaval.ca/personnel/borque/s4/stromatolites.aaciens</a>
6	10	259	ZM149359510EFF8800P2960M2M1	6	15	Stromatolite shots on Earth by Athena	<a href="http://marsrover.nasa.gov">http://marsrover.nasa.gov</a>
7	11	299	ZM152912555EFF89AIP2957M2M1	7	16	Proterozoic stromat., Collenia type, Michigan, USA (University museum)	<a href="http://www.fossilmuseum.net/FossilGalleries/Stromatolites/str54">http://www.fossilmuseum.net/FossilGalleries/Stromatolites/str54</a>
8	12_13	333	ZM155931811EFF9946P2936M2M1	8	17	Silurian stromat., Gotland, Sweden (Nose et al., 2006; fig. 12)	<a href="http://www.sciencedirect.com/science/article">http://www.sciencedirect.com/science/article</a>
9	14_15	334	ZM156024338EFF9946P2957M2M1	9	18	Proterozoic stromat., Earraheedia type, Wiluna, Austr. (Bonino coll.)	<a href="http://www.keyobs.be/fr/ebonino/html/stromatoliti.html">www.keyobs.be/fr/ebonino/html/stromatoliti.html</a>
10	16_17	354	ZM157793588EFA269P2977M2M1	10	19	Proterozoic stromat., BIF type, Pilbara, Austr. (Bonino coll.)	<a href="http://www.keyobs.be/fr/ebonino/html/stromatoliti.html">www.keyobs.be/fr/ebonino/html/stromatoliti.html</a>
11	18	358	ZM158147821EFA269P2977M2M1	11	20	Paleoproterozoic stromat., Cochabamba, Bolivia (Bonino coll.)	<a href="http://www.keyobs.be/fr/ebonino/html/stromatoliti.html">www.keyobs.be/fr/ebonino/html/stromatoliti.html</a>
12	19_20	372	ZM159395697EFA2HPP2957M2M1	12	21	Paleoproterozoic stromat., Collenia type, Minnesota, USA (Bonino coll.)	<a href="http://www.keyobs.be/fr/ebonino/html/stromatoliti.html">www.keyobs.be/fr/ebonino/html/stromatoliti.html</a>
13	21	373	ZM159478547EFA2HPP2957M2M1	13	22	Paleoproterozoic stromat., Cochabamba, Bolivia (Bonino coll.)	<a href="http://www.keyobs.be/fr/ebonino/html/stromatoliti.html">www.keyobs.be/fr/ebonino/html/stromatoliti.html</a>
10	22_26	374	ZM159567193EFA2HPP2957M2M1	14	23	Cretaceous stromat., Pocalithus type, Potosi, Bolivia.	<a href="http://www.lowestone.com/acatalog/Fossils-for-sale.html">http://www.lowestone.com/acatalog/Fossils-for-sale.html</a>
11	27_31	386	ZM160631672EFA2K1P2957M2M1	15	24_26	Middle Devonian stromat., Orcadian Lake, Scotland (University Museum)	<a href="http://www.landforms.eu/orkney/Fossils/Stromatolite.htm">http://www.landforms.eu/orkney/Fossils/Stromatolite.htm</a>
12	32	511	ZM171727638EFAAJCP2977M2M1	16	27	Archean stromat., Wyoming, USA (EDCOPE)	<a href="http://www.fossilmall.com/EDCOPE_Enterprises/stromatolite">http://www.fossilmall.com/EDCOPE_Enterprises/stromatolite</a>
13	33	675	ZM186289394EFAJQVP2957M2M1	17	28_30	Precambrian stromat., BIF type, Minnesota, USA (James St. John coll.)	<a href="http://www.newark.osu.edu/faculty_staff/personal/istjohn/Documents">http://www.newark.osu.edu/faculty_staff/personal/istjohn/Documents</a>
14	37	688	ZM187443970EFAJXYP2956M2M1	18	31_35	Precambrian stromat., Chocabamba, Bolivia. Sample R2162.	Photos shot at Regional Museum of Natural Sciences, Turin.
15	38-40	754	ZM193315571EFAOA0P2956M2M1	19	36_40	Precambrian stromat., dolomitic, South Africa. Sample 3713.	Photos shot at Regional Museum of Natural Sciences, Turin.
16	41	770	ZM194720101EFFAPBCP2956M2M1	20	41_45	Middle Cambrian Stromat., Qiniamiao Formation, Xinshan, China. Sample GE05216.	Photos shot at Regional Museum of Natural Sciences, Turin.
17	44	1368	ZM247814647EFAWCCP2976M2M1				
18	45_48	1434	ZM253668740EFFAX05P2956M2M1				
19	49	1452	ZM255266694EFAAX16P2976M2M1				
20	50	1967	ZM300990702EFFB1E5P2936M2M3				

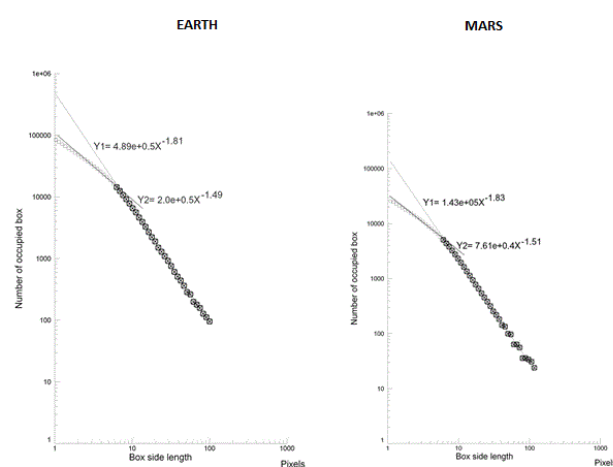
Figure 2: List of sampled images: Martian samples photographed by Spirit and terrestrial (biogenic) stromatolites selected by web sites or photographed at the Regional Museum of Natural Sciences, Turin.



**Figure 3:** Sample #28 (left) from a Precambrian stromatolite (Banded Iron Formation type, Minnesota, USA, James St. John Collection), enlarged (right) in order to obtain the same magnification resolution and acutance than the Athena enlarged images (Figure 1). Microspherules and intertwined filaments of microspherules are revealed. This stromatolite lived billion years ago in an anoxic Earth (as Mars today).



**Figure 4:** Terrestrial sample of a (biogenic) stromatolite (sample 28, the same of figure 3, top, left), its automatically extracted contours (top, right); Martian sample photographed by Spirit Rover (sol 228, figure 2) and its automatically extracted contours (below, right). Magnification is the same ( $\pm 10\%$ ).



**Figure 5:** Left, log-log plot of a terrestrial sample of a (biogenic) stromatolite, and, right, of a Martian sample photographed by the Spirit Rover. Magnification is the same. Both the samples are multifractals, as evidenced by the presence of two straight lines (from 200 to 10 pixels, from 10 to 5 pixels) with different slopes. Please, note also the remarkable similarity of their critical exponents (geometrical complexity or local fractal dimensions by box-counting).

#### Algorithmic complexity (“randomness”, L-Z)

To determine the algorithmic complexity (“randomness”) of the patterns, relative Lempel-Ziv, L-Z, values were calculated according to

the Kaspar and Schuster algorithm [25] using the Chaos Data Analyzer version 2.1 software package (CDA; Pro, Academic Software Library, North Carolina State University, USA). Briefly, patterns of the original image were transformed into 16.732 points containing one dimensional vector, where each datum point was converted into a single binary digit according to whether the design is touched (=1) or not (=0). Relative L-Z values are close to 0 for a deterministic equation, close to 1 for totally destructured random phenomena.

### Tortuosity (Dmin)

Tortuosity, or the fractal dimension of the minimum path, Dmin, was computed for each cluster present in the image from the power law  $Ic=r^{Dmin}$ , where Dmin is the exponent that governs the dependence of the minimum path length between two points (Ic) on the Pythagorean distance r between them in a fractal random material. To obtain Dmin, the maximum diameter and the half perimeter of the microstructures present in the textures were measured using an automated procedure (Image Pro Plus software, Media Cybernetics, USA). For each image 100-500 microstructures were measured. The slope of the log-log plot (maximum diameter vs. perimeter) represented Dmin. The existence of a log-log straight line ( $p<0.001$ ) justified the use of the fractal analysis in order to obtain the morphometric index. The method was validated with the original one by Hermann and Stanley [26] with a maximum shift of  $\pm 3\%$ .

Geometrical Complexity and Information Dimension were calculated using the Benoit 1.3 software, (TruSoft Int'l Inc: <http://trusoft-international.com/benoit.html>). Algorithmic complexity and Dmin were calculated using a software written by us. All this four methods are routinely performed by one of us in biomedical works [26-31]. Minimum and maximum diameters of the microspherules (Earth and Mars) were automatically measured by Image Pro Plus software (Media Cybernetics, USA).

### Statistical analysis

Mean intra-and inter-observer coefficients varied  $<2.0\%$  and  $<3\%$ , respectively. Comparisons between the groups were analyzed by the Mann-Whitney U test and chi-square test; t-test was applied in order to verify the linearity of the log-log plots.

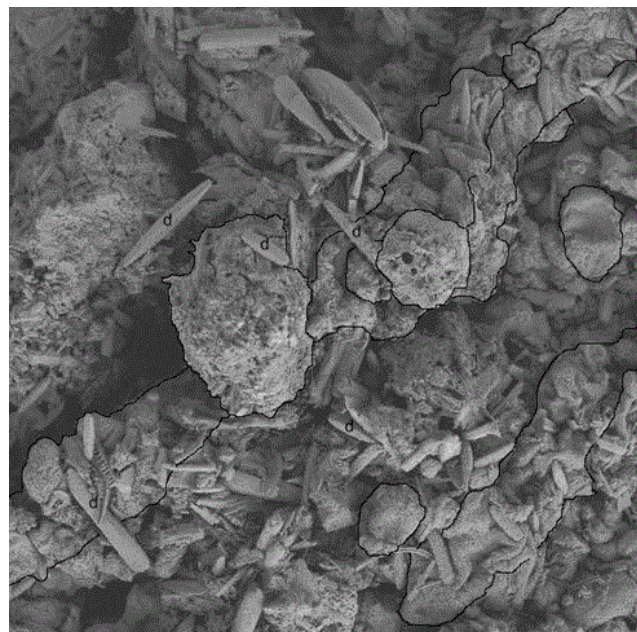
### Results

In the images photographed by Spirit as well from the images obtained from terrestrial microbialites a continuum pattern of microspherules and intertwined filaments of microspherules, dimensions of about 0.1 mm–0.3 mm, are present. Figure 6 show the same microstructures observed at high magnification after Scanning Electron Microscopy.

On Earth, these reports are prevalently referred to the microbialitic environment, where the microspherule, somewhere aggregate in linear arrays, is known as peloids [32-34].

The morphometric analysis reveals that both textures, from microbialites (Earth) and from selected MI images (Mars), present a multifractal aspect, as revealed by the two straight lines in the log-log plot (Figure 5). Two textures are present: the texture as a whole (200-10 pixels, corresponding to 2 mm-0.1 mm) and the microstructure inside the microspherules and the intertwined filaments of microspherules (10-5 pixels, corresponding to 0.1 mm-0.05 mm).

Abiogenic pseudostromatolites, as well other abiogenic minerals, reveal always a simple fractal (and not multifractal) textures (Figure 7) [20,21].



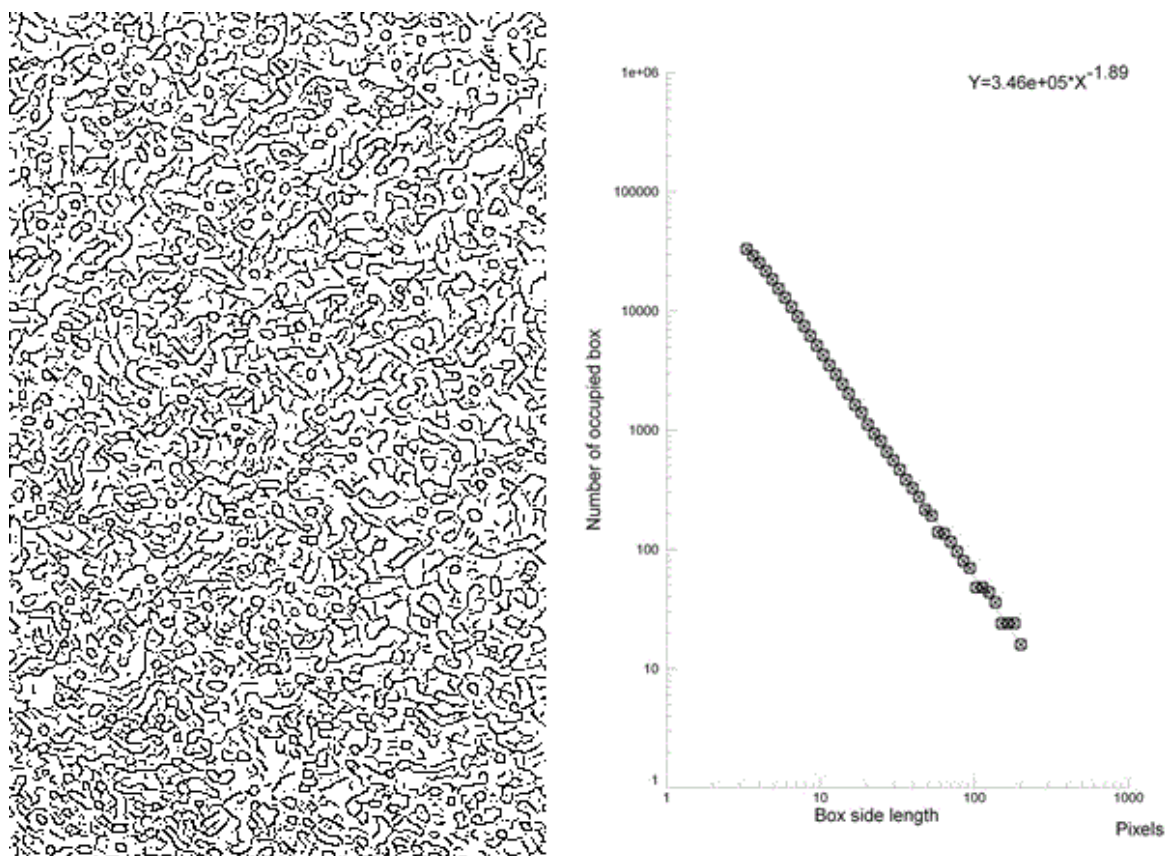
**Figure 6:** Scanning Electron Images of a living microbialite at La Brava (M.E.F.). The picture highlights occurrence of microspherules and filaments, minerals built by Cyanobacteria; other living forms, diatoms, are present, d, over the microfilaments. X 100.

The morphometric analysis also shows that the Martian and terrestrial textures were extremely similar to each other; the average values and confidence intervals of the eight independent morphometric parameters of the terrestrial and Martian images perfectly overlapped with each other (table 1). In these eight separate comparisons, the probability of this occurring by chance is  $1/2^8$  ( $p<0.004$ ).

### Discussion

The search of life on Mars is the main motivation behind the different research programs on the Martian surface. Our knowledge on Mars has remarkably increased after the last NASA missions, especially those called Mars Explorer Rover (MER, Opportunity, Spirit and Curiosity rovers) held, from 2004 on the Mars landscape at Meridiani Planum and Gale Crater, respectively. Indeed, the photo reportages realized by the Martian rovers confirmed the presence of water deposits on the surface of Mars [35].

The search on Mars of extraterrestrial microorganisms and, in particular, of cyanobacteria, the main building materials of terrestrial microbialites/stromatolites [36] has been underlined many times. A century of research on stromatolites has revealed diverse fabric and many structures together with a contentious history and various definitions.



**Figure 7:** Terrestrial abiogenic microspherules-containing granular sediment (a siltstone), showing microspherules in its automatically extracted contours (left), its log-log plots (right). The log-log plot is fractal (one single straight line from 200 to 3 pixels, corresponding to 2 mm -0.03 mm) but not multifractals like as the biogenic microbialites (Figure 5).

Besides, most of microbialites/stromatolites are carbonate in composition, but siliceous, phosphatic, iron, manganese and sulphate examples also occur: in recent view, stromatolites may be defined as microbialites, macroscopically layered authigenic microbial sediments, namely organosedimentary rocks [17]. In this frame, the outcroppings on Mars surface at Meridiani Planum (Opportunity Rover) or at Gusev Crater (Spirit Rover) represent an interesting field for microstructure/textures investigations [4,37].

In this work we analyzed enlarged photographic images, at the same magnifications, resolution and acutance, of stromatolites and of the Martian images obtained with the Athena system of the Spirit Martian Rover. At our range of magnification and resolution is possible to study microstructures in the 1-0.05 mm size range: a field characterized in the terrestrial microbialites/stromatolites by clotted fabric, typically formed by widespread micritic clots or lumps, agglutinated and set in an irregular sponge-like network structure or in intertwined turf-like communities [15-17], giving origin to the aspects of microspherules and intertwined filaments of microspherules (see, Figure 6, to look these microstructures at high magnification)

All these structures and aspects are very interesting for astrobiological purposes because they are difficult to explain as abiogenic artifacts. In fact, while the sediment structure follows universal and repetitive laws (revealing in the present work a simple

fractal structure, Figure 7, right), the biological structure, within the same environment, at the same meso-microscopic scales, appears to vary depending on the metabolic activity of the living organisms [38-43] (revealing in the present work a multifractal structure, Figure 5, left).

In this paper, a tangle of microspherule and intertwined filaments of microspherules has been evidenced on the Martian sediments photographed by the Spirit rover: a textural pattern that is also present in living microbialites as well in recent and fossil stromatolites [18-20]. Both the textures, from microbialite images (Earth) or from analyzed MI images (Mars), are multifractals, as revealed by the two straight lines present in the log-log plots (Figure 5). At a quantitative analysis, our objective morphometric approach reveals indexes of geometric and algorithmic complexity, entropy, and tortuosity, minimum and maximum diameters of the microtextures extremely similar among them: average values and confidence intervals of the eight independent fractal parameters between Earth (stromatolites and other microbialites) and Mars samples perfectly overlap. The probability of this occurring by chance is less than  $1/2^8$  ( $p < 0.004$ ).

We may note that at Spirit location we obtained the same results we have had analyzing sediments photographed by Opportunity Rover at Meridiani Planum [21], a region very far away from Gusev crater. On February 2015, Nora Nofke [44], in a visual inspection of images shot

by Curiosity Rover (landed in a further region of Mars, at Gale crater) Evidences of microbialites in the Martian outcroppings are noted signs of the presence of stromatolites in the Martian landscapes. multiplying: unicellular life was widespread on the ancient Mars.

	EARTH/ Mean (SD)	MARS (Spirit location)/ Mean (SD)
Geometric Complexity, High scale	1.82 (0.02)	1.81 (0.02)
Geometric Complexity, Low scale	1.48 (0.05)	1.51 (0.07)
Information Entropy, High scale	1.88 (0.01)	1.85 (0.02)
Information Entropy, Low scale	1.42 (0.05)	1.46 (0.05)
Randomness (Lempel-Ziv index)	0.46 (0.05)	0.48 (0.04)
Tortuosity (Dmin)	0.79 (0.03)	0.80 (0.03)
Minimum diameter (mm)	0.077 (0.001)	0.080 (0.002)
Maximum diameter (mm)	0.2066 (0.003)	0.2069 (0.004)

**Table 1:** Eight different morphometric indexes of microspherule/intertwined filaments of microspherules, mean and standard deviation, were obtained from 45 terrestrial microbialite images and 50 Martian images, corresponding to approximately 25,000/20,000 microstructures analyzed. Note the remarkable similarity of the Earth/Mars indexes: The probability of this occurring by chance is less than  $p < 0.004$ .

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