

# Returning to Europa: can traces of surficial life be detected?

J. Chela-Flores<sup>1,2</sup> and N. Kumar<sup>3</sup>

<sup>1</sup>The Abdus Salam ICTP, Strada Costiera 11, 34014 Trieste, Italy

<sup>2</sup>Instituto de Estudios Avanzados, IDEA, Caracas 1015A, Venezuela

e-mail: chelaf@ictp.it

<sup>3</sup>Raman Research Institute Bangalore-560080, India

**Abstract:** There is at present a possibility for returning to Europa with LAPLACE, a mission to Europa and the Jupiter System for European Space Agency's Cosmic Vision Programme. The question of habitability by the identification of reliable bio-indicators is a major priority. We explain the options for approaching the question of selecting the right instrumentation for measuring the more abundant sulphur isotope, in spite of the fact that <sup>32</sup>S is isobaric (same *m/z*) with <sup>16</sup>O<sub>2</sub>. Two technologies are available for investigating the possible biogenicity of the surficial sulphur on the icy patches discovered by the Galileo mission. We argue that there is a need to use higher-order statistics in the data that are to be gathered with the instruments chosen for the payload (ion-traps for orbital measurements, or penetrators for surficial measurements). In particular, we argue in favour of data analysis taken from an orbital spacecraft that addresses fluctuations of the data retrieved, rather than the mean. For this purpose, we reconsider the significance of deviations of sulphur abundances relative to normal (meteoritic) values. In the present work, we consider the experimentally testable possibility of biogenically driven isotopic anomalies in the light of statistical data analysis. The fluctuation test that is being proposed in the context of future missions to Europa may well be appropriate to a laboratory experiment with sulphur-reducing bacteria with the corresponding isotopic fractionation.

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## Introduction: the European icy surface and its surrounding cloud

### *Habitability of Europa and instrumentation for its search*

The arrival of the Galileo mission in the Jovian system gave rise to a National Aeronautics and Space Administration (NASA) meeting at San Juan Capistrano and related publications, where the habitability of Europa was discussed, both in terms of microbiology (Chela-Flores 1996) and novel instrumentation for missions to the Jovian system. Early discussions, long before the proposed LAPLACE mission, also considered the possibility of exploring Europa's habitability in the future with a submersible called a hydrobot (Horvath *et al.* 1997). This question is still relevant a decade later, in terms of new NASA autonomous underwater vehicle (AUV) called ENDURANCE for the Astrobiology Science and Technology for Exploring Planets (ASTEP) programme (Doran *et al.* 2007). Significant papers have more recently addressed the conditions for establishing a stable ecosystem. They include discussions of the biochemistry (Chyba 2000; Schulze-Makuch & Irwin 2002), as well as the relevance of sulphur in the biogeochemistry of Europa (Zolotov & Shock 2003; Chela-Flores 2006).

There is a new generation of instruments that would be suitable for the exploration of Europa (Gowen *et al.* 2008). In

addition, further missions have been envisaged beyond LAPLACE, namely NASA's Europa Explorer (NASA 2008) and the intriguing EUROX proposal (de Vera *et al.* 2008). The present paper focuses on new instrumentation for future missions to Europa in the coming decade.

### *Assumptions*

The main point of the present paper is that sampling a large number of particles from many locations on the surface of Europa is perfectly compatible with the LAPLACE mission that the Consortium is now planning for activity in the coming decade. The impact-generated dust clouds surrounding the Galilean moons discovered at the end of the Galileo mission consist of particles that have been kicked up by hypervelocity impacts of micrometeoroids on to the satellites' entire surfaces (Kruger *et al.* 2003).

The main assumptions of this work are as follows.

- (i) The non-water-ice materials, including sulphur, are endogenous.
- (ii) Of the two options for an endogenous source of surficial sulphur, we consider a biogenic source, rather than cryovolcanism.
- (iii) Of all of the chemical elements, sulphur is assumed to have isotopic fractionation that may be clearly linked to biogenic activity.

- (iv) The sulphur fractionation on the cloud surrounding Europa should to a large extent reflect the same sulphur fractionation that is taking place on the surface, since the origin of the cloud is, as we said above, due to particles that have been expelled by hypervelocity impacts of micrometeoroids on the entire surface. The instrumentation, already successfully completed for ion-trap mass spectrometry on a comet nucleus (the Ptolemy instrument and the Rosetta space mission), is already in the planning stages with the view of its eventual applications for LAPLACE (Taylor *et al.* 2007; Todd *et al.* 2007).

#### *The abundance of oxygen on the atmosphere of Europa*

At the beginning of the Galileo mission, Hall *et al.* (1995) published an article, independent of the mission itself, in which the oxygen atmosphere of Europa was discovered. The oxygen atmosphere accumulates around this satellite through reactions that break up the water molecules to form molecular hydrogen and oxygen. The lighter hydrogen molecules escape from Europa, leaving behind an oxygen atmosphere. Atomic oxygen emission was detected from Europa. This was interpreted as being produced by the simultaneous dissociation and excitation of atmospheric oxygen by electrons from Jupiter's magnetosphere. These results imply that the atmosphere is dominated by molecular, rather than atomic oxygen. In providing an answer to the difficulty of developing the appropriate instrumentation to distinguish molecular oxygen-16 from  $^{32}\text{S}$  (isobarism, see below), the presence of a tenuous oxygen atmosphere should be taken into account. Indeed, Europa's molecular oxygen atmosphere is very tenuous with a surface pressure about  $10 \times 10^{-11}$  that of the Earth's atmosphere at sea level.

#### *The abundance of sulphur on the surface of Europa*

Of all of the biogenic elements, sulphur has the most relevant isotopic fractionation for the detection of traces of biogenic activity (Chela-Flores 2006). Once a primordial satellite internal silicate nucleus (e.g. on Europa) had entered their corresponding geochemical cycles, their initial isotope mixtures began to be redistributed. The Earth's upper mantle and crust are believed to reflect broadly the isotopic distribution patterns of chondritic meteorites (Libby 1971). A similar mechanism would apply equally to the silicate core of Europa (Oro *et al.* 1992). The new point we make here requires the definitions of the sulphur and carbon fractionation parameters. For the isotopic fractionation of sulphur, we restrict our attention to  $^{32}\text{S}$  and  $^{34}\text{S}$ . We define

$$\text{delta } ^{34}\text{S} = [({}^{34}\text{S}/{}^{32}\text{S})_{\text{sa}} / ({}^{34}\text{S}/{}^{32}\text{S})_{\text{st}} - 1] \times 10^3 \text{ (‰)}. \quad (\text{CDT})$$

For simplicity, this function will be referred to as the delta  $^{34}\text{S}$  parameter, or simply as the delta 34-S parameter. Its value is close to zero when the sample coincides with the corresponding value of the Canyon Diablo meteorite that is a troilite (FeS), abbreviated as CDT. This parameter allows a comparison of a sample (sa) with the standard (st) CDT. The

relevant terms are the dominant sulphur isotope ( $^{32}\text{S}$ ) and the next in abundance ( $^{34}\text{S}$ ). In fact,  $({}^{34}\text{S}/{}^{32}\text{S})_{\text{st}}$  coincides with the average terrestrial fraction of the two most abundant isotopes of sulphur. We obtain positive values of the delta 34-S parameter when by comparison we have a larger quantity of the less-abundant isotope  $^{34}\text{S}$ .

For the fractionation of the carbon stable isotopes we require the delta  $^{13}\text{C}$  parameter that is defined as follows:

$$\text{delta } ^{13}\text{C} = [({}^{13}\text{C}/{}^{12}\text{C})_{\text{sa}} / ({}^{13}\text{C}/{}^{12}\text{C})_{\text{st}} - 1] \times 10^3 \text{ (‰)}. \quad (\text{PDB})$$

The value of the delta 13-C parameter is close to zero when the sample coincides with the PeeDee belemnite standard (PDB), in which  $({}^{13}\text{C}/{}^{12}\text{C}) = 88.99$ . (The delta 13-C parameter is defined as begin equal to 0.00‰.) This parameter can be used as a good biomarker. On the Earth biota, for instance, there is ample evidence that photosynthetic bacteria, algae and plants have typical significant deviations that yield values of up to  $-30$  and beyond, due to biological processes (Schidlowski *et al.* 1983). These results are analogous to the deviations shown by fractionation due to bacterial sulphate reduction. Yet, negative values of the delta 13-C parameter do not arise exclusively from biogenic sources: in lunar fines, we know that life is absent, significant negative deviations in the delta 13-C parameter do in fact occur, but are absent in the corresponding sulphur parameter (Kaplan 1975). Thus, without prior knowledge whether we are in the presence of life in a given environment, negative values of the delta 13-C parameter do not arise exclusively from biogenic sources. For this reason we have mentioned above that sulphur is more relevant for studying possible biosignatures.

#### *The origin of sulphur on the European surface*

The presence of sulphur on Europa is widespread and patchy. For instance, sulphur is a prominent chemical element in the water-ice surface in the area north of the equatorial region, between  $0^\circ$  and  $30^\circ$  N and between the longitudes  $240^\circ$  and  $270^\circ$ . These patches were described with the Galileo measurements (McCord *et al.* 1998). Even much smaller patches were described in the same report.

External implantation would be expected to produce a more uniform surface distribution if the source was ions from the Jovian plasma. Based on combined data from the Galileo mission, it has been argued that the non-water-ice materials, including sulphur, are endogenous (Fanale *et al.* 1999), which is our assumption (i) above. The colouration is clearly due to endogenic sulphur in the frequent cases of lineae and mottled terrain since, as we have mentioned above, implantation from the Jovian plasma would be expected to produce a more uniform distribution (Carlson *et al.* 1999). The only remaining argument is the possible exogenous implantation in the dark trailing hemisphere. However, higher spatial resolution in the ultraviolet suggests that the main role of sulphur from Io and the Jovian magnetosphere is mainly an agent in sputtering erosion (Fanale *et al.* 1999, especially the section 'Conclusions'). Thus, all three terrains where the sulphur patches may occur are caused by endogenic material.

The main argument of this paper is that there are methods from biogeochemistry that can be refined to separate the biogenic sulphur from that of inorganic sources. Two alternative scenarios are possible.

First, effusive cryovolcanism is clearly one possible endogenous source of the non-water-ice constituents of the surface materials (Fagents 2003). It is possible to interpret the non-water elements on the icy surface as the product of eruptions on the seafloor that were subsequently raised to the icy surface (Thomson & Delaney 2001). In this view, cryovolcanism on Europa is more like the ‘black smokers’ that are found on the Earth seafloor, and possibly also on Mars: the Dao Vallis outflow channel on Mars is a possible site of hydrothermal springs in Mars’ past. The robotic explorer *Spirit* found deposits of pure silica, a form of silicon that occurs when hot water reacts with rocks (quartz is a pure silica) (Squyres *et al.* 2008).

Second, an alternative possibility is that sulphur may be biogenic. This conjecture is to be tested in future European missions. Certain aspects of sulphur as a biomarker, however, deserve further discussion. For instance, the harsh radiation field due to the Jovian magnetosphere enveloping Europa could possibly invalidate the biogenicity test that our paper is proposing for forthcoming Europa missions. However, the stopping depth for implantation by exogenous sulphur ions (provided by Io’s extreme volcanic activity) is expected not to exceed a thin layer of 1 cm of the surface (Greenberg 2005). Thus, organic molecules (carrying a biosignature through the negative values of the delta 34-S parameter) would not be disrupted beyond such a thin layer. Therefore, due to the preliminary results of the British Penetrator Consortium (Gowan *et al.* 2008), a modest penetration depth of their penetrators into the icy surface of Europa would be sufficient to correctly interpret results of mass spectrometry (the delta 34-S parameter with its possible signal of biogenicity) without any radiation interference.

### Fluctuations of the data about the mean for a number of measurements

#### *Instrumentation in orbit and on the surface of Europa*

We focus our attention on fluctuations of the data about the mean that should be taken on the icy European surface, or in orbit of the cloud that should bear the imprint of biogenicity as in assumption (iv) above. In other words, a large number of measurements ought to be performed in two different cases: first in orbit, measuring the dust cloud that surrounds Europa (Kruger *et al.* 2003; Taylor *et al.* 2007), and also at different locations *in situ*, where a set of penetrators may reach the surface. The latter approach would see its first steps on our own moon (Smith & Gao 2007). Such measurements with either technology would eventually have to face at least two problems: isobarism of  $^{32}\text{S}$  and oxygen and sensitivity.

#### *Isobarism of $^{32}\text{S}$ and oxygen*

$^{32}\text{S}$  is isobaric (same  $m/z$ ) with  $^{16}\text{O}_2$ . Since the surface of Europa is predominantly composed of water-ice (contaminated with

non-water-ice chemical elements), oxygen may be produced in the impact (or even in the ion trap of the detector), since it is well known that a low-density oxygen atmosphere surrounds Europa. Alternatively, the isobaric difficulty may be found with *in situ* measurements with a set of penetrators. Even if it is the surface that were chosen to be probed, the presence of a tenuous atmosphere surrounding it could, in principle, lead to isobaric challenges to the interpretation of the data.

Consequently, we can expect water, or even oxygen, to be components of the particles that the instrumentation will encounter either in orbit or on the surface in measurements that will be closely associated with the surficial sulphur. (This chemical element is known to be present on Europa since measurements by the Galileo mission discovered sulphur patches.) Sufficient resolution would be hard to achieve with the available design to identify the contributions from  $^{32}\text{S}$  and oxygen at  $m/z$  32 and 34. (At least this is true for the dust detectors that are being tested for the current Cassini mission.) So we either need to study how much oxygen might be seen in the ion trap at Europa (and hence its interference with the  $^{32}\text{S}$  measurement), or we need to find an alternative means to eliminate the  $^{32}\text{S}\text{--O}_2$  ambiguity (described below in the section on low-cost penetrators).

#### *Sensitivity*

Improving the current technology (of the dust detectors) would imply the need to count about  $1 \times 10^6$  sulphur ions to get a precision of  $\pm 5\%$  on the delta 34-S parameter. So the likely ion counts (namely, the sensitivity) require special attention.

### The Luria–Delbrück fluctuations

#### *The fluctuation test*

A pioneer in the field of molecular biology, Salvador Luria conducted a variety of experiments using bacteriophages (viruses that infect bacteria). He believed that bacterial resistance to bacteriophages results from mutations rather than adaptations on the part of individual bacterium, the leading theory in the early 1940s (Luria 1947). Luria developed the fluctuation test to confirm the Max Delbrück theory (Luria & Delbrück 1943).

The test consists of a method for demonstrating that bacterial mutations pre-exist in a population before they are selected; a large parent bacterial culture (population) is subdivided into small parts (samples) that are grown independently without mixing and the number of (resistant) mutants in each subculture is determined. Now, the number of mutants in the subculture will fluctuate from sample to sample because in some a mutant arises early (giving an exponentially large number of progeny through multiplication), while in others, the mutant arises late, giving exponentially fewer progeny. The resulting large fluctuation dominating the mean eventually became known as the ‘fluctuation test’.

A more lucid explanation of the fluctuations is worth recalling (Nanjundiah 1999): Luria had realized that if the mutations were acquired as a result of exposing a growing culture of bacteria to phage, the number of resistant individuals would vary very little from one experiment to the next. The observed number would fluctuate slightly about the average. On the other hand, if a mutation could occur even before the bacterium was confronted with phage, the number of resistant bacteria would depend on the time elapsed since the mutation had taken place. The number of resistant individuals would show large fluctuations, since the total number of bacteria in the culture, which is continuously growing, keeps increasing exponentially.

#### *Physical characteristics of biological specimens*

The overwhelming biological evidence is that bulk growth processes of living tissue proceed by multiplicative rather than additive increments and, therefore, measures of body size should follow a lognormal rather than normal distribution. Indeed, despite common claims of normality, the sizes of plants and animals are approximately lognormal. The same is true of bacterial cultures.

Differences in size due to sexual dimorphism or other polymorphisms such as the worker/soldier/queen division in social insects may, however, make the joint distribution of sizes deviate from lognormality. In addition, the assumption that the linear size of biological specimens is normal leads to a non-normal distribution of weight (since weight/volume is roughly the third power of length and Gaussian distributions are only preserved by linear transformations). Conversely, if we assume that it is the weight that is normal, then we are led to non-normal lengths. This is a problem because there is no *a priori* reason why one of length, and not the body weight, should be normally distributed. Lognormal distributions, on the other hand, are preserved by powers, so the ‘problem’ goes away if lognormality is assumed. Thus, lognormal seems more natural in this sense.

### **Are there fluctuations detectable with low-cost penetrators on Europa?**

#### *Reliable biomarkers with isotopes and data fluctuations*

Sulphur isotope analysis is a valuable tool to be used in conjunction with other approaches for planetary exploration. Geological and biogeochemical data from many sources of the Precambrian demonstrate that pyrites and evaporates were formed biologically by dissimilatory sulphate reduction (Schildowski *et al.* 1983; Konhauser 2007). Rocks of Archaean age (older than 2.5 Ga) provide the best evidence of early metabolic processes. Their study allows reconstruction of the biogeochemical cycle for sulphur since the origin of life on Earth. The remarkable sulphur icy patches on the European surface will inevitably be targets for future space missions that are expected to return to Europa in the next decade. With landers or low-cost penetrators that could first of all be tried out on the lunar surface (Smith & Gao 2007), we would be in a position to test whether there are

fluctuations on various samples of the surficial ice, as we proceed to explain in the remainder of this section, where we show that Luria–Delbrück fluctuations, if present in the data that could be sampled by a set of penetrators, would be associated with biogenicity.

#### *A possible analogy of the Luria–Delbrück fluctuations*

We assume that the biota autochthonous to Europa consists of sulphur bacteria. Inasmuch as the sulphur-reducing bacteria metabolize the lighter isotope of sulphur ( $^{32}\text{S}$ ) in preference to its heavier isotope ( $^{34}\text{S}$ ), an isotope fractionation that produces an excess of  $^{32}\text{S}$  over its natural (abiogenic or meteoritic) abundance should be expected in general. In principle, this could serve as a biomarker that may be picked up by mass spectrometry.

At the simplest level, one would look for this excess (i.e. enhanced relative abundance) in the mean (namely, averaged over all of the samples collected). Given, however, that a biogenic source of this isotope excess would generate large fluctuations that should dominate the mean (unlike the usual Gaussian case expected for abiogenic origin), one is tempted to propose a statistical analysis of these fluctuations, rather than just the mean, somewhat analogous to the celebrated ‘fluctuation test’ that Luria and Delbrück introduced in a different context (as described above).

The basic idea is as follows: we consider a large number of samples analysed with the instrumentation that some penetrators would use on the surface of Europa.

#### *The generalization of the Luria–Delbrück test*

The generalization of the Luria–Delbrück fluctuation test to the case of a European biota would consist of a method of demonstrating that bacterial markers on the ice pre-exist from a population at various times in the geological past, say  $t_1, t_2, t_3, \dots$ , before a space probe is able to test the biogenic effect that is represented by the large isotopic anomalies with a significantly negative delta 34-S parameter.

The effect of a large parent population is divided into small parts on the Europa surface where the penetrators would carry out measurements with the appropriate instrumentation (mass spectrometry). The separate measurements would independently identify the biogenic isotopic anomalies in each of the various sections of the ice where the penetrators have landed. Measurements will fluctuate because in some biogenic effects would arise early ( $t_1 < t_2$ ), while in other sections of the ice the isotopic anomalies would arise late.

#### *Are non-Gaussian fluctuations characteristic biogenicity?*

Inasmuch as bacteria grow multiplicatively (exponentially), and not additively (linearly), a product, such as the  $^{32}\text{S}$  isotope, metabolized by a growing bacterial culture is expected to amplify any relevant parametric fluctuation exponentially and give a correspondingly broad distribution of the processed product  $^{32}\text{S}$ , where the mean is dominated by the fluctuation (variances), for example, a lognormal or Levy distribution rather than a normal (Gaussian) distribution.

This statistical feature was essentially the basis of the classic ‘fluctuation test’ of Luria and Delbrück, proposed in the context of the testing of a hypothesis on bacterial resistance by adaptation versus that of mutation.

In this work, we invoke basically an analogous idea, namely that of proposing the statistical fluctuations rather than the mean of the  $^{32}\text{S}$  isotope excess as a possible sensible biomarker for the sulphate-reducing bacterial population on Europa. (Note that the sulphide-reducing bacteria are known to metabolize the higher sulphur isotope  $^{32}\text{S}$  in preference to the heavier isotope  $^{34}\text{S}$ .) More explicitly, consider a culture of sulphate-reducing bacteria sampled from Europa. Now, the total (integrated) bacterial mass contained in the sample will clearly depend on the ‘age’ of the sample, measured in terms of the number of generations past. In fact, one would expect it to be an exponentially increasing function of ‘age’.

However, the age must vary randomly from sample to sample. This randomness of the generational age of the sample fluctuates in the total bacterial mass generated. This exponentially large fluctuation of the bacterial mass will in turn translate into a corresponding exponentially large sample-to-sample fluctuation in the amount of  $^{32}\text{S}$  processed (‘fixed’) by the bacteria. This exponentially large fluctuation of the  $^{32}\text{S}$  isotope will give away its biogenic origin and thus constate the biomarker in question. It is, of course, assumed here that the samples have had no mixing in the past and are spatially well separated. Any homogenization by mixing will clearly kill the fluctuations.

#### *A simple model to illustrate our idea*

In the glossary and appendix, we provide some general remarks and definitions on higher-order statistics. Consider a culture of bacteria (the sample) that grows multiplicatively through  $n$  generations giving a population  $N(n) = 2n = e^{(\ln 2)n}$ . In the time-continuum limit (and hence in the large  $N$  limit), this is equivalent to the exponential growth  $N(t) = e^{t/T}$ , where  $e$  is the  $e$ -folding time and  $t$  is the culture age. Now the age ‘ $t$ ’ has a sample-to-sample random fluctuation with a distribution  $p(t)$  that we may take as

$$p(t) = (1/T)e^{-t/T}$$

an exponential for simplicity, with  $T$  the mean age. The two expressions combine to give the probability distribution for the bacterial population  $P(N)$  (and, therefore, of the product  $^{32}\text{S}$  metabolized):

$$P(N) = (t/T) (1/N^a),$$

which is a power law with exponent  $a = 1 + t/T$ . This is a fat-tailed (broad) Levy distribution in general with a Levy exponent ‘ $a$ ’. Now it is known that for  $1 < a < 2$ , the mean population is finite, but its variance is infinite ( $a > 2$  gives the usual Gaussian distribution).

This simple model is an extreme case of the dominance of fluctuations over the mean for a biogenically generated product.

## Discussion

Although the statistical method described in this paper dates back to 1943, Luria’s collaboration with Delbrück in the early 1940s was an event that in most people’s mind marks the formal beginning of molecular biology (Friedberg 2002). However, the statistical method itself is now fully integrated, not only in biological issues, but also in current physical sciences. For example, higher-order statistics is now widely used in electronic engineering, for example, in power-quality events in delicate electronic devices (Gerek & Ece 2006), and in nuclear physics, for example, in heavy-ion collisions (Borghini *et al.* 2001).

On the other hand, the next decade should see an attempt at probing a sulphur patch on the icy surface of Europa with mass spectrometry on a LAPLACE penetrator, or on a Europa lander platform from Phobos Heritage, namely a Roscosmos lander (Blanc *et al.* 2008). If such measurements were to reveal an isotope shift toward lighter fractionation ratios (without an obvious inorganic explanation), this would likely be a most successful candidate as an argument for life.

The samples to be collected in the future Europa mission would presumably be analysed by mass spectrometry for the isotopic excess of  $^{32}\text{S}$  over the natural abundance. For normalization, we can assume that the samples to be tested are all of equal mass. Clearly, the isotope  $^{32}\text{S}$  processed by the bacterial population in the sample will have increased over the time (generational age) of bacterial activity. However, bacteria grow multiplicatively and, consequently, the population will grow exponentially with time elapsed (i.e. the sample age).

Hence, any dispersion of this timescale (the primarily random variable here) will translate into an exponential dispersion in the bacterial population sampled. The latter will in turn translate itself into an exponential dispersion of the amount of processed isotope  $^{32}\text{S}$  over the samples. That would then dominate the mean. These non-Gaussian fluctuations that are characteristic of the biogenic origin of the  $^{32}\text{S}$  excess will be large and more sensitive, thus, a qualitatively distinct biomarker for the integrated bacterial activity on Europa.

In addition, the usual Gaussianity is characterized by the disappearance of all cumulants of an order greater than two. The biogenically generated non-Gaussianity will show up as the non-disappearance of higher-order cumulants, say the fourth cumulant dominating the mean. In this context, an added advantage will be that the cumulants are semi-invariant, that is, that they are independent of any additive background bias, such as contamination in the mass spectrometry by the oxygen that is present on the external low-density atmosphere of Europa. The oxygen molecule is isobaric with  $^{32}\text{S}$  and, hence, would have been expected to create a disambiguation problem with the present technology from the point of view of resolution and sensitivity.

Due to the patchiness of the ice surface, the constantly replenished cloud around Europa that is generated by

micrometeorites would mirror the large S-isotope deviations that are caused locally by micro-organisms that are sulphur-reducing. Consequently, we would expect dust detectors in orbit around this satellite to record similar large fluctuations of the Luria–Delbrück type to the *in-situ* measurements on the surface itself. Again, no large-scale mixing has to be assumed.

Finally, while the fluctuation test is proposed here in the context of future missions to Europa, it may well be appropriate for a laboratory experiment with sulphur-reducing bacteria giving isotopic fractionation. Clearly, there are a number of technical challenges to be overcome before we can say with any certainty what we could measure regarding  $^{34}\text{S}/^{32}\text{S}$ , but the statistical fluctuation analysis as described here offers a possible approach to the interpretation of the data.

## Glossary

### Cumulant

Consider a scalar random variable of zero mean, say  $x$ , whose characteristic function is denoted by  $f(t)$ , where

$$f(t) = E\{\exp(itx)\}.$$

Expanding the logarithm of the characteristic function as a Taylor series, one obtains

$$\log f(t) = k_1(it) + k_2(it)^2/2 + \dots + k_r(it)r/r! + \dots,$$

where the  $k_r$  are constants. These constants are called the *cumulants* (of the distribution) of  $x$ . In particular, the first three cumulants (for zero-mean variables) have simple expressions.

### e-folding

*e-folding* is the time interval in which an exponentially growing quantity increases by a factor of  $e$ . The term *e-folding* time is also sometimes similarly used in the case of exponential decay to refer to the timescale for a quantity to decrease to  $1/e$  of its previous value.

### Kurtosis

*Kurtosis* is the fourth-order cumulant. This function can be considered as a measure of the non-Gaussianity of  $x$ . For a Gaussian random variable, kurtosis is zero; it is typically positive for distributions with heavy tails and a peak at zero, and negative for flatter densities with lighter tails. It can be expressed as

$$\text{Kurt}(x) = E(x^4) - 3(E\{x^2\})^2.$$

### Standard deviation

The standard deviation is the square root of the variance.

### Variance

Where the average (or mean) is a measure of the centre of a group of numbers, the *variance* is the measure of the

spread. The following two sets of numbers have the same mean, 10:

$$S_1 = \{10, 10, 10, 10, 10\}; \quad S_2 = \{0, 5, 10, 15, 20\}.$$

However, the first set has a variance of zero, as all of the numbers are the same; the second set has a variance of 50.

## Appendix. Higher-order statistics: Gaussian and non-Gaussian processes

A Gaussian process is completely identified by its first and second cumulants (mean and variance, cf. the glossary). If the process is non-Gaussian, the lower-order cumulants can only fit the best Gaussian model, while neglecting the non-Gaussian behaviour of the data that is under consideration (Gerek & Ece 2006). This is particularly significant in the topic of the present paper. As seen repeatedly since Luria and Delbrück completed their work, exponentially growing living systems drive the statistics away from the classical Gaussian distributions. Hence, the first- and second-order cumulants are not capable of yielding the differences between different non-Gaussian processes. The fourth-order cumulant (kurtosis, cf. the glossary) is essential.

The second-order parameters: mean and variance (cf. the glossary) and related functions are sufficient to completely characterize Gaussian processes, but in the biological context of our search for life on Europa (Chela-Flores 2007; Blanc *et al.* 2008), it is essential to go beyond this simple approach into higher-order statistics. A discriminating tool in the future ion-traps in orbit around Europa (Todd *et al.* 2007) will need to deal with parameters that contain higher-order cumulants. Alternatively, on the icy surface of Europa, if a few penetrators are capable of taking numerous samples from a wide distribution of sites where the Galileo mission detected well-defined patches, then once again higher-order statistics will prove to be a crucial discriminating tool for the data that are to be gathered.

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