



ELSEVIER

Ultramicroscopy 86 (2001) 241–246

ultramicroscopy

www.elsevier.nl/locate/ultramic

Scaling-index method as an image processing tool in scanning-probe microscopy

F. Jamitzky^{a,b,*}, R.W. Stark^a, W. Bunk^b, S. Thalhammer^a, C. R ath^b,
T. Aschenbrenner^b, G.E. Morfill^b, W.M. Heckl^a

^a*Institut f ur Kristallographie und Angewandte Mineralogie, Universit t M nchen, Theresienstr. 41, 80333 M nchen, Germany*

^b*Max-Planck-Institut f ur extraterrestrische Physik, Giessenbachstr. 1, 85749 Garching, Germany*

Received 14 July 2000

Abstract

The scaling-index method (SIM) is a novel tool for image processing in scanning-probe microscopy. Originating from the theory of complex systems, the SIM can be used in order to extract structural information from arbitrary data sets. This method can readily be applied to the analysis of digital atomic-force microscopy (AFM) images. Especially for biomedical diagnostics, where genetic material is investigated by various microscopic methods, a reliable image segmentation based on the SIM algorithm is helpful. As a first application, AFM-images of GTG-banded human metaphase chromosomes (with G bands obtained by Trypsin using Giemsa) are compared with micrographs from conventional light microscopy by means of a scaling-index analysis. While the grey-level distributions of the optical and the AFM-images are largely different from each other, the scaling-index images are remarkably similar. Using this method, a fingerprint of an image can be produced which helps in the classification and interpretation of the measured data.   2001 Elsevier Science B.V. All rights reserved.

PACS: 07.05.Pj; 61.16.Ch; 87.16.Sr

Keywords: Atomic force microscopy; Image processing

1. Introduction

From an abstract point of view, an image obtained by means of scanning-probe microscopy can be thought of as a point distribution. A point distribution may be the representation of a given data set, e.g. a time series in state space following

the technique proposed by Packard and co-workers [1]. Also, images can be thought of as a number of points in an appropriate state space: A black and white image (see Fig. 1), for example, can be interpreted as a number of single ‘states’ in an appropriate state space: each pixel has three state variables — the spatial coordinates (x, y) and a grey scale z . Similarly, a pixel of a colour image is defined by a set of five state variables, (x, y) and the (r, g, b) components, expressing the colour content of the pixel.

The basic idea of using local scaling properties for image analysis is closely related to the analysis

*Corresponding author. Max-Planck-Inst. f. extr. Physik, Giessenbachstr.1, 85749 Garching, Germany. Tel: +49-89-23944317; fax: +49-89-23944331.

E-mail address: f.jamitzky@mpe-garching.mpg.de (F. Jamitzky).

of the fractal geometry of an object. Each point in the state space will be characterised by its structural surrounding. In addition to the canonical properties of an image pixel, i.e. spatial coordinates and colour quantities, one obtains a structural measure. This can be used for a number of basic tasks in image analysis, for example structural decomposition, texture detection, noise reduction, and pattern recognition.

This leads to the idea that the scaling-index method (SIM) [2] can be used for structural decomposition and for noise reduction of atomic-force microscopic (AFM) images of biological specimen [3]. Another potential application of the SIM is the analysis of microscopic data obtained on chromosomal material. For example, the light microscopic investigation of G-banded metaphase chromosomes is the most widely used technique in routine chromosome analysis for biomedical diagnostics [4].

Beyond the diffraction limit of light microscopy, AFM [5] offers a direct approach for high-resolution imaging of biological samples without the requirement of a special specimen preparation. In the past, various biological applications were developed for AFM including the investigation of chromosomes [6–10]. Musio and colleagues worked on imaging the longitudinal patterns of untreated human chromosomes and imaged the chromosome structure during G-banding treatments [11,12].

In this study, we show that the scaling-index method provides a useful tool for the analysis of AFM images of human metaphase chromosomes. AFM data are compared to data obtained by conventional light microscopy using this method.

2. Experimental

2.1. Chromosome preparation and GTG banding

Human whole blood (0.4 ml) was cultivated at 37°C for 72 h with RPMI-medium, fetal calf serum (10%) and phytohemagglutinin (0.5%) as mitotic agent. Cells were arrested with colcemid (10 µg/ml) for 30 min. Chromosome preparations were made by incubating the cell suspension in 0.075 M KCl

at 37°C for 13 min and fixing in a freshly prepared mixture of methanol and acetic acid (3:1) at 0°C and stored at room temperature for several days. GTG-banding was performed, incubating the glass slides in a 10% trypsin-solution at 37°C for 25–60 s. After a washing step in PBS buffer, the slides were stained in a 5% Giemsa-solution for 8 min. The slides were rinsed with water and air dried.

2.2. Atomic-force microscopy

A commercial AFM (topometrix explorer) with 130 µm xy scan-range and 10 µm z -scanner was used. The AFM was mounted on top of an inverted light microscope (Zeiss Axiovert 135) in order to select the metaphase spreads. For imaging the GTG-banded metaphase chromosomes in contact mode siliconcantilevers were used (point probe CONT, spring constant $c=0.3$ N/m, nominal tip radius $r<10$ nm). The loading forces during AFM measurements were 10–20 nN. The scanning procedure of the AFM is controlled by the software SPMLab 3.06. In order to realise the deflection mode imaging [13], the topographic as well as the error signal image were recorded. The representation of the deflection (error) image was done in 8-bit grey-scale.

3. Scaling-index method

The scaling-index method is a realisation of computing the distribution of pointwise dimensions and is briefly explained as follows. For each point of the distribution the number N of points is counted, which are located within an n -dimensional sphere of radius r , centred at \mathbf{x}_i :

$$N(\mathbf{x}_i, r) = \sum_j \Theta(r - |\mathbf{x}_j - \mathbf{x}_i|). \quad (1)$$

In this equation, Θ is the Heaviside function with $\Theta(x) = 1$ if $x \geq 0$ and zero if $x < 0$. The distribution function $N(\mathbf{x}_i, r)$ is computed within a specified range $r \in [r_1, r_2]$, the so-called scaling range.

For many cases and wide scaling regions, one finds that as r increases, the number of points in the sphere is approximately given by a power law:

$N(\mathbf{x}_i, r) \propto r^\alpha$. The scaling-index α is then determined by approximation of the power-law index. The first-order approximation is given by the difference ratio:

$$\alpha_i = (\log N(\mathbf{x}_i, r_2) - \log N(\mathbf{x}_i, r_1)) / (\log r_2 - \log r_1) \quad (2)$$

r_1 and r_2 are specified by the lower and upper limit of the scaling range. This scaling-index α describes the structural surrounding of the point at location \mathbf{x}_i and is a property of that point and of the location \mathbf{x}_i in phase space, as well. It is comprehensible that α_i is characterising the structural component the point P_i belongs to. By using an appropriate scaling range it comes out, that points which are part of clumpy structures will be represented by α -values close to 0. Points which are embedded in straight lines are identified by values around 1, such points in a flat distribution by values around 2, and so on. One of the most interesting properties is that for point distributions filling the n -dimensional space homogeneously α -values close to the embedding dimension $n = d_E$ are assigned.

The normalised frequency distribution $p(\alpha)$ of α -indices to a given point distribution is therefore in some sense a statistical measure of the distribution of elementary structural components. In particular, the fraction $p(\alpha)$ with $\alpha < n$ indicates the part of structural ordered points which can be extracted from the entire distribution.

After these remarks on the correlation between α and structural nature, it is obvious that the setting of the scaling region is a crucial point in employing this technique in practice. From a theoretical point of view, the specification of the scaling region is insignificant for the results as far as the distribution function $N(\mathbf{x}_i, r)$ is scaling free.

In practice, a pre-analysis of the scaling behaviour may be helpful because the choice of the scaling range is a non-trivial task and depends on the size of the objects under investigation.

Summarising, the algorithm is defined as follows:

For a grey-scale image (see Fig. 1) the scaling-index is computed by representing the image as a set of points in a three-dimensional state space defined by the coordinates x and y and the grey

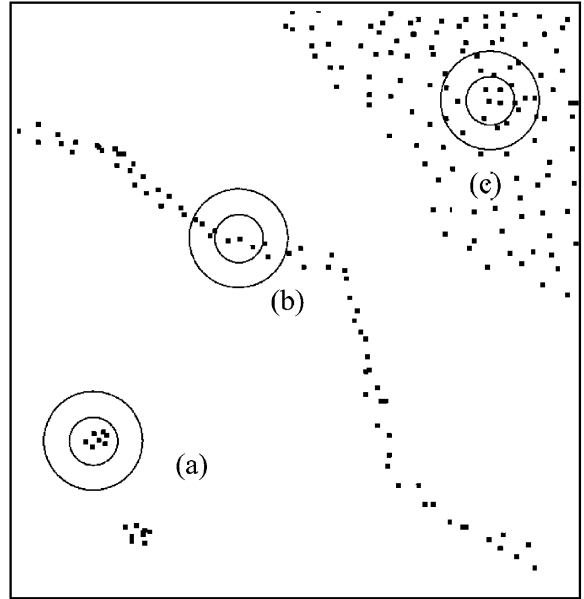


Fig. 1. Scheme illustrating the different dimensionality of point distributions. (a) A point-like structure. (b) A line-like structure. (c) An area-like structure.

value z . For every point P_i with coordinates (x_i, y_i, z_i) the number of points in a sphere with radius r_1 and a sphere with radius r_2 is determined and then the scaling-index is computed following Eq. (2). The obtained scaling-index is a measure for the local dimensionality of the region surrounding the point P_i .

4. Results and discussion

The scaling-index was computed for two images, the deflection signal AFM-image and the optical light-microscopic image of the same sample. In Figs. 2a and 3a, the optical and the AFM-image are shown. Obviously the optical image is fuzzier than the AFM-image due to resolution limitation of the optical imaging process. The two images differ in the grey-level distribution and are somewhat distorted with respect to each other which makes a quantitative comparison difficult using standard methods. Furthermore, the AFM-image was recorded in deflection mode where the AFM-

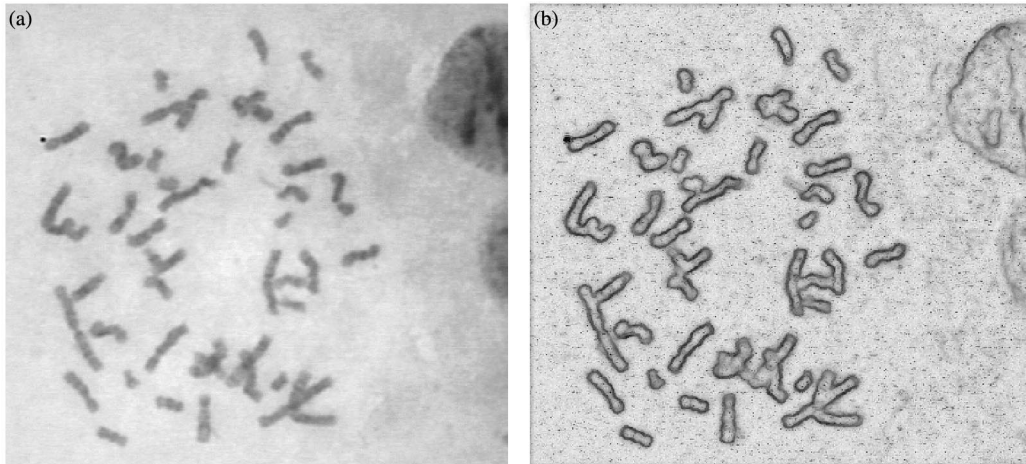


Fig. 2. (a) Light microscopic image of GTG-banded human metaphase chromosomes. (b) Respective scaling-index (image size approx.: $75 \times 75 \mu\text{m}$).

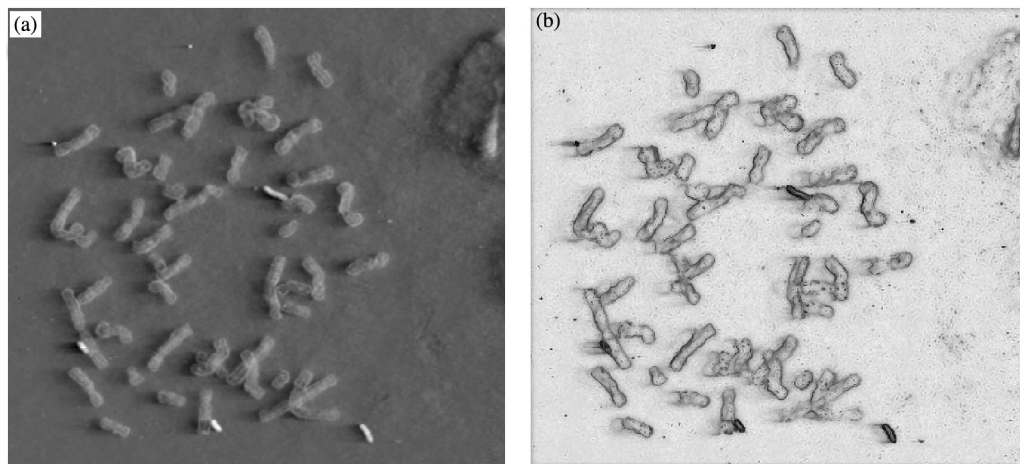


Fig. 3. (a) Atomic-force microscopic image of the GTG-banded human metaphase chromosomes (cf. Fig. 2a) obtained in deflection mode. (b) Respective scaling-index (image size: $75 \times 75 \mu\text{m}$).

data does not represent the topographic height, but the change of the topography.

In Figs. 2b and 3b, the respective scaling indices for the scaling range $r = 5$ to 10 pixel are shown. The scaling range is chosen in accordance with the predominating scale of structural components visible in the image. While the grey-level distribution of the original AFM and optical image are rather different, the scaling-indices of the two images are remarkably similar. This stems from

the fact that the scaling index is a measure of inherent information contained in the image, namely the local dimensionality near each pixel of the image.

In Fig. 4a, the frequency distributions of the grey levels of the original images and in Fig. 4b, the frequency distributions of the scaling indices, the so-called $N(\alpha)$ -spectra, are shown. While the grey-level spectra are vastly different, the $N(\alpha)$ -spectra are strikingly similar. The spectrum

of the AFM-image shows a strong peak at $\alpha = 2$. This is due to the flatness of the background of the chromosomes, which is mostly two-dimensional. The same peak also shows up in the spectrum of the optical image due to the same reasons. The distribution of the scaling indices of the optical image is slightly wider because the original optical image is a bit fuzzier than the AFM-image.

The $N(\alpha)$ -spectra can furthermore be used for the identification of corresponding regions of the images. In Fig. 5 the $N(\alpha)$ -spectra for two subimages of the optical and AFM-image are

shown. The scaling indices of the subimages are shown as inlays. Obviously, the spectra of corresponding objects are remarkably similar and thus can be used for an identification. A further investigation of this method will be presented elsewhere (Jamitzky and Bunk, in preparation).

5. Conclusions

In this paper an image processing method has been presented that can be used to extract topological information from multi-channel images. The results show that in future applications it will be possible to perform an image segmentation based on topological properties of the objects shown in the image. By comparing conventional optical images and images obtained by an atomic-force microscope, the independence of the proposed scaling index from the grey-level distribution of the particular images has been shown. While the grey-level distributions are largely different from each other, the distributions of the scaling indices are remarkably similar. Using this method, a fingerprint of an image can be produced which helps in the classification and interpretation of the measured data.

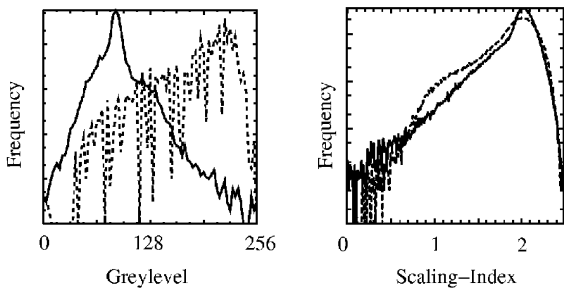


Fig. 4. (a) Grey-level distribution function of the optical (dashed) and the AFM-image (solid). (b) $N(\alpha)$ -spectrum for the optical image (dashed) and the AFM micrograph (solid). The structural similarity of both images is clearly reproduced despite the fundamental differences in the imaging process.

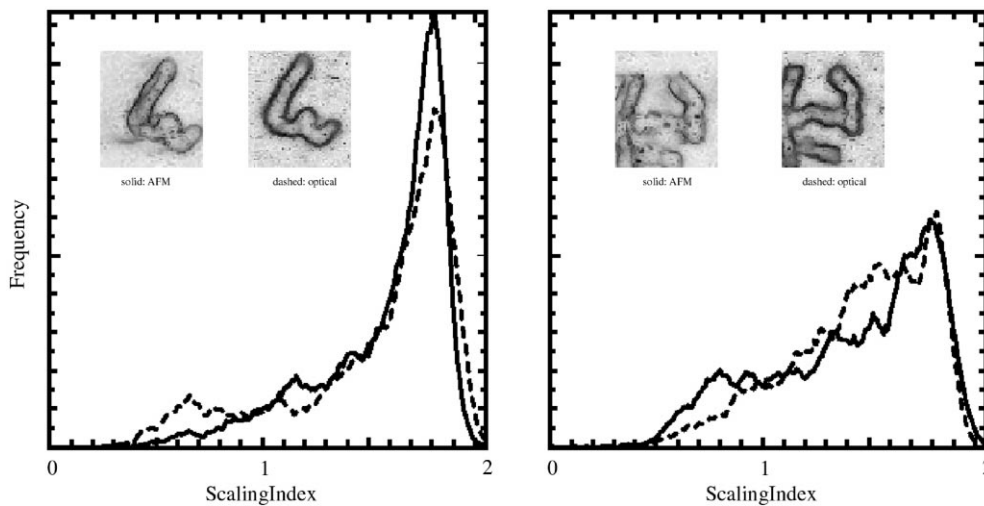


Fig. 5. $N(\alpha)$ -spectra for two different subimages of the optical and the AFM-image. The subimages are shown as inlays.

References

- [1] N.H. Packard, J.P. Crutchfield, J.D. Farmer, R.S. Shaw, *Phys. Rev. Lett.* 45 (1980) 712.
- [2] C. R ath, G. Morfill, *J. Opt. Soc. Am.* 12 (1997) 3208.
- [3] F. Jamitzky, R.W. Stark, G. Morfill, W.M. Heckl, *J. Comput.-Assisted Microsc.*, in press.
- [4] ISCN, An International System for Human Cytogenetics Nomenclature: Birth Defects, Original Article Series, Vol. 21, No. 1, March of Dimes Birth Defects Foundation, New York, 1995.
- [5] G. Binnig, C.F. Quate, C.H. Gerber, *Phys. Rev. Lett.* 56 (1986) 930.
- [6] C.A.J. Putman, K.O. van der Werf, B.G. de Grooth, N.F. van Hulst, F.B. Segerink, J. Greve, *Ultramicroscopy* 42/44 (1992) 1549.
- [7] P. Rasch, U. Wiedemann, J. Wienberg, W.M. Heckl, *Proc. Nat. Acad. Sci. USA* 90 (1993) 2509.
- [8] S. Thalhammer, R.W. Stark, S. M uller, J. Wienberg, W.M. Heckl, *J. Struct. Biol.* 119 (1997) 232.
- [9] R.W. Stark, S. Thalhammer, J. Wienberg, W.M. Heckl, *Appl. Phys. A: Mater. Sci. Proc.* 66 (1998) 579.
- [10] J. Tamayo, M. Miles, A. Thein, P. Sothill, *J. Struct. Biol.* 128 (1999) 200.
- [11] A. Musio, T. Mariani, C. Frediani, I. Sbrana, C. Ascoli, *Chromosoma* 103 (1994) 225.
- [12] A. Musio, T. Mariani, C. Frediani, C. Ascoli, I. Sbrana, *Genome* 40 (1997) 127.
- [13] C.A.J. Putman, K.O. van der Werf, B.G. de Grooth, N.F. van Hulst, J. Greve, P.K. Hansma, *Proc. SPIE* 1639 (1992) 198.