

## SHORT COMMUNICATION

# GTG banding pattern on human metaphase chromosomes revealed by high resolution atomic-force microscopy

S. THALHAMMER\*, U. KOEHLER†, R. W. STARK\* &amp; W. M. HECKL\*

\*University München, Institute for Crystallography, Theresienstr. 41, 80333 München, Germany

†MGZ Medizinisch Genetisches Zentrum München, Bayerstr. 53, 80335 München, Germany

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## Summary

Surface topography of human metaphase chromosomes following GTG banding was examined using high resolution atomic force microscopy (AFM). Although using a completely different imaging mechanism, which is based on the mechanical interaction of a probe tip with the chromosome, the observed banding pattern is comparable to results from light microscopy and a karyotype of the AFM imaged metaphase spread can be generated. The AFM imaging process was performed on a normal  $2n = 46$ , XX karyotype and on a  $2n = 46$ , XY, t(2;15)(q23;q15) karyotype as an example of a translocation of chromosomal bands.

## Introduction

Chromosome banding techniques have facilitated the precise identification of individual chromosomes. The GTG banding obtained by digesting the chromosomes with proteolytic trypsin followed by Giemsa staining is the most widely used in routine chromosome analysis. The interpretation of the GTG bands is still in progress. A direct role of Giemsa stain in producing the GTG bands was suggested (McKay, 1973). Several authors implied that chromosomes contain a pre-existing structure and this is subject of enhancement after GTG banding. But it is still unclear how this enhancement occurs (Comings, 1978; Ambros & Sumner, 1987). It is hypothesized that the differences between positive and negative GTG bands may be induced by the spatial organization of chromosomal protein and DNA.

Since the invention of atomic force microscopy (AFM)

(Binnig *et al.*, 1986), high resolution imaging has been performed on various biological applications including chromosomes (Putman *et al.*, 1992). Musio and colleagues worked on imaging the longitudinal patterns in untreated human chromosomes and imaged the chromosome structure after GTG banding (Musio *et al.*, 1997). The detection of numerical chromosomal abnormalities of untreated chromosomes with AFM was done by Ergün *et al.* (1999).

In our experiments we performed AFM on human metaphase chromosomes after GTG-banding on a normal  $2n = 46$ , XX karyotype and a  $2n = 46$ , XY, t(2;15)(q23;q15) karyotype. We present data that show that reliable GTG karyotypes can be generated from AFM data.

## Material and methods

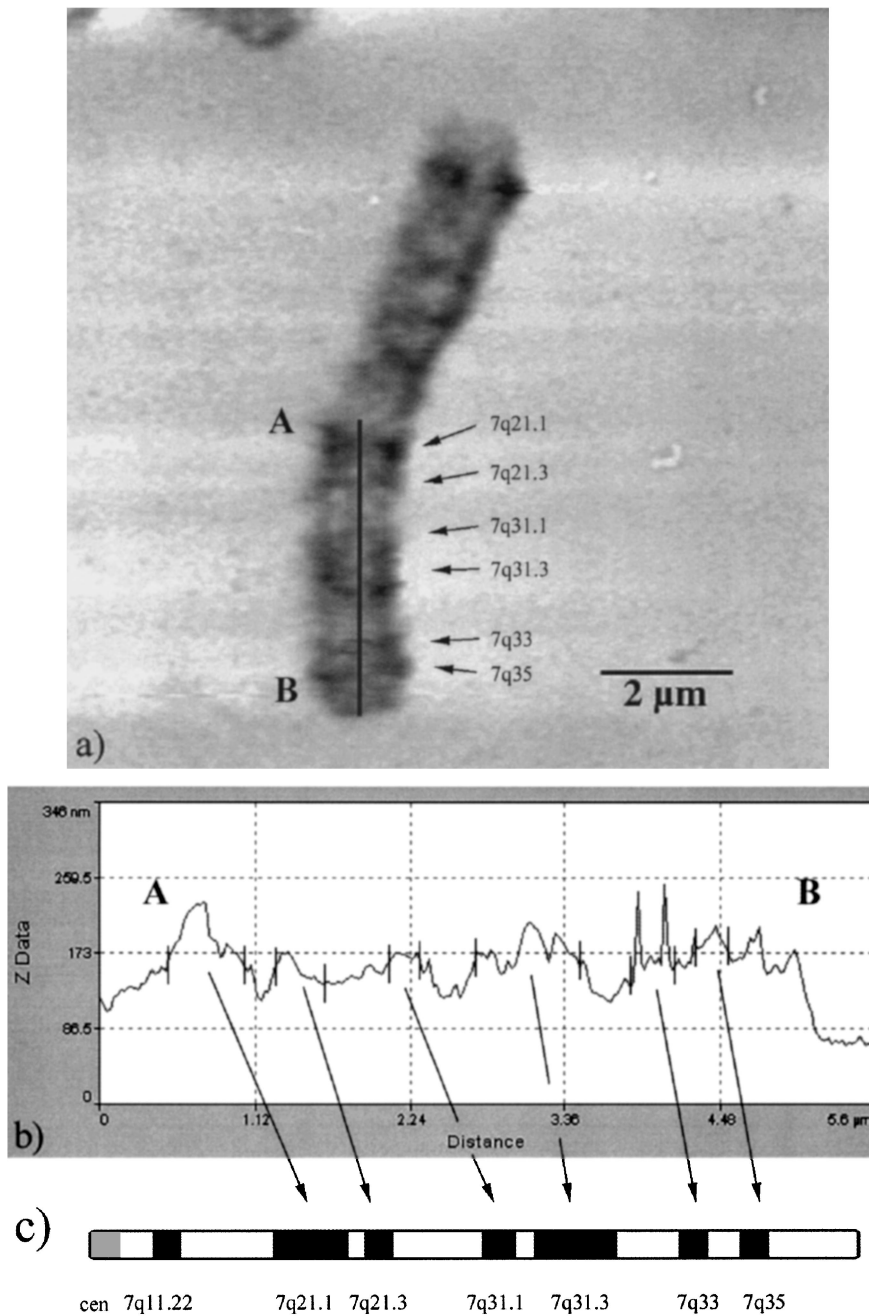
### *Chromosome preparation and GTG banding*

Heparinized human whole blood (0.4 mL) was cultured at 37 °C for 72 h in 10 mL Gibco Chromosome Medium 1A. Cells were arrested with colchicine ( $10 \mu\text{g mL}^{-1}$ ) for 30 min. Chromosome preparations were made by incubating the cell suspension in 0.075 M KCl at 37 °C for 13 min, followed by a fixation step in a freshly prepared mixture of 3 : 1 methanol : acetic acid at  $-20$  °C. GTG banding was performed by incubating the glass slides in a 0.05% trypsin solution (Difco) at 37 °C for 15 s, followed by rinsing the slides in phosphate-buffered saline buffer and staining in a 5% Giemsa stain for 8 min. The slides were rinsed with water and air dried.

### *Atomic-force microscope*

An atomic-force microscope (AFM, Topometrix Explorer) with 130  $\mu\text{m}$  xy-scan range and 10  $\mu\text{m}$  z-scanner was used. It was mounted on top of an inverted microscope in order to select the metaphase spreads. For imaging the GTG

Correspondence: Professor W. M. Heckl, Institut für Mineralogie und Angewandte Kristallographie, Ludwig-Maximilians-Universität, Theresienstr. 41, 80333 München, Germany. Tel.: +49 89 2394 4331; fax: 0049 89 2394 4331; e-mail: w.heckl@lrz.uni-muenchen.de



**Fig. 1.** (a) AFM image of a GTG banded chromosome 7 homologue, topographic AFM image, grey scale inverted: the bright and dark banding pattern is detectable; (b) line measurement through points A and B of the q-arm of chromosome 7; (c) idiogram of the q-arm of chromosome 7.

banded metaphase chromosomes in contact-mode stiff cantilevers were used (pointprobe CONT, spring constant  $c = 0.3 \text{ N m}^{-1}$ , nominal tip radius  $r < 10 \text{ nm}$ ). The loading forces during AFM measurements were 10–20 nN.

#### Image acquisition

The scanning procedure of the microscope is controlled by the software SPMlab 3.06. The topographic and error signal image were recorded. The representation of the topographic image was done in grey-scale with subsequent inversion of

the image for easy comparison with known optical microscopy karyotypes.

#### Results and discussion

Figure 1(a) shows a topographic AFM image of a GTG-banded chromosome 7 homologue. The morphology of the chromosome is preserved; the banding pattern and the fibrous nature are detectable. Structural protrusions along the chromosome corresponding to the dark bands in Fig. 1(a) are detectable. A linescan of the q-arm, from A

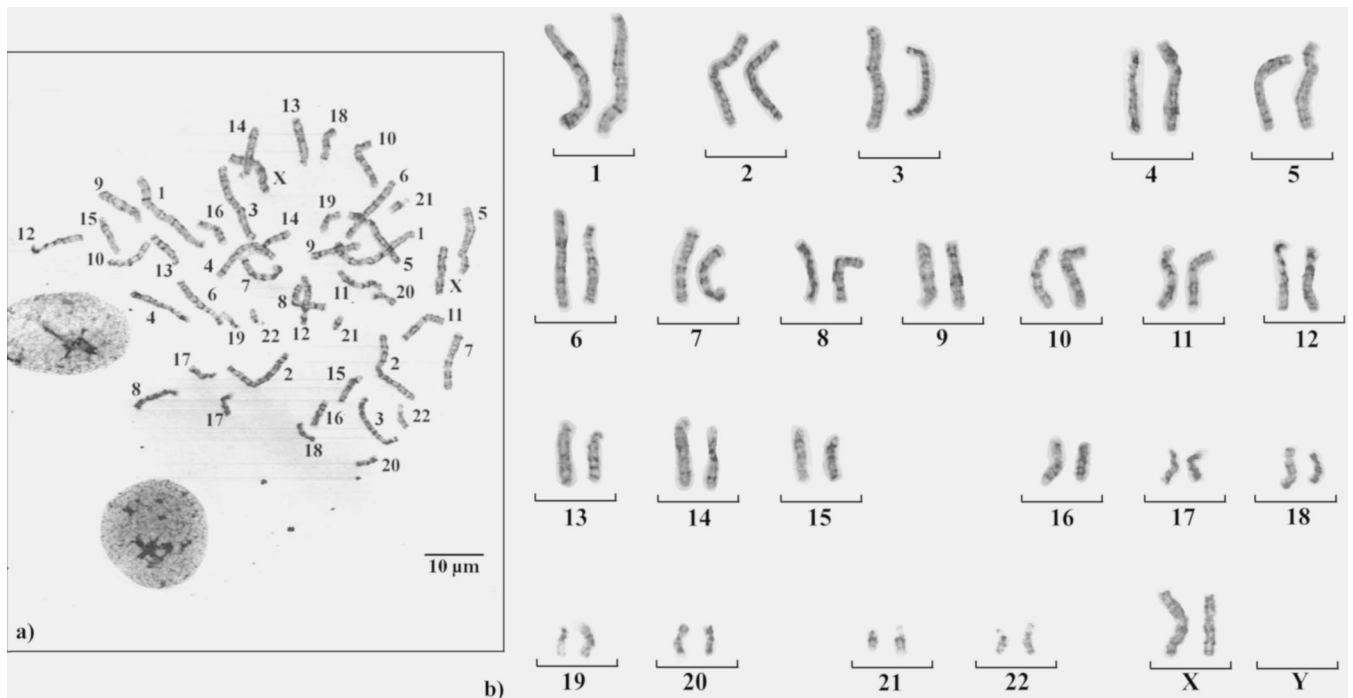


Fig. 2. (a) AFM image of a  $2n = 46$ , XX metaphase spread; (b) AFM-image of a  $2n = 46$ , XX karyotype.

to B (Fig. 1(b)), shows differences in height between dark and light bands of about 90 nm. The length of the rises is about 540 nm. The corresponding bands are marked in the idiogram (Fig. 1(c)). It is known from chromosomes

imaged by scanning electron microscopy that the Giesma light and dark bands differ in height (Harrison *et al.*, 1981). One must be aware that the AFM image not only represents the topology of the sample surface but also the

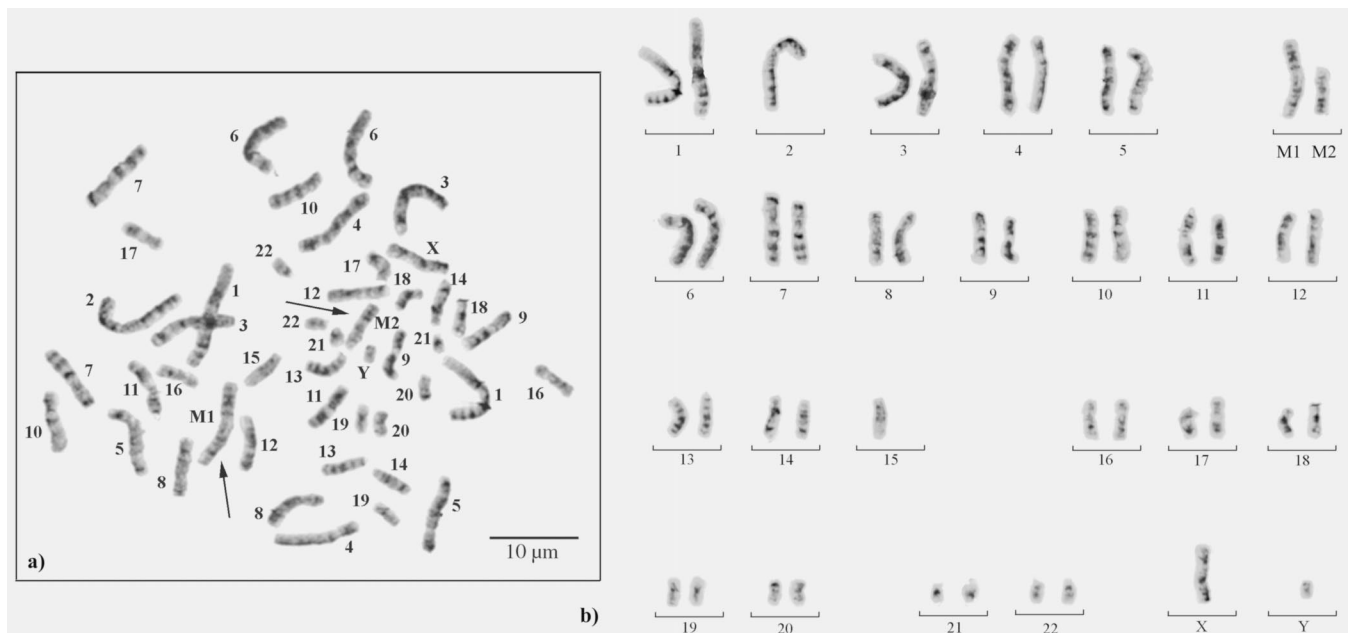


Fig. 3. (a) AFM image of a  $2n = 46$ , XY,  $t(2;15)(q23;q15)$  metaphase spread, the derivative chromosomes are indicated by an arrow; (b) AFM image of the  $2n = 46$ , XY,  $t(2;15)(q23;q15)$  karyotype.

compressibility of the sample, therefore height is partially expressed as topography.

Figure 2(a) shows a human  $2n = 46, XX$  metaphase spread. The light and dark bands are clearly detectable and all chromosomes could be identified. Figure 2(b) shows the corresponding karyotype. In order to prove the potential to characterize individual GTG banded subchromosomal changes, a  $2n = 46, XY, t(2;15)(q23;q15)$  karyotype was investigated. Figure 3(a) shows the metaphase spread of a male carrier at this translocation, Fig. 3(b) the corresponding karyotype. The derivative chromosomes are marked with M1 and M2 in the metaphase spread and karyotype.

To summarize, AFM is capable of imaging structural chromosomal abnormalities on the nanometre scale. The presented technique requires no additional preparation steps for high resolution imaging and thus minimizes the risk of incorporating artefacts in the specimen. When compared with the state of the art standard optical microscopy the AFM method leads to the same reliable results, but additionally allows the mechanical manipulation of the related chromosomal area. For example, we have shown that AFM nanoextraction of chromosomal regions with additional degenerated polymerase chain reaction can create chromosomal painting probes (Thalhammer *et al.*, 1997).

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