Zoological Studies 34, Supplement I (1995)

Zoological Studies

Fluorescence *in Situ* Hybridization on Human Metaphase Chromosomes Detected by Near-field Microscopy

M.H.P. Moers, A.G.T. Ruiter and N.F. van Hulst
Applied Optics Group, Department of Applied Physics, University of Twente, P.O.Box 217, 7500 AE Enschede, The Netherlands

The high optical resolution and sensitivity of near field scanning optical microscopy (NSOM) promises important applications in biology and biochemistry. In particular fluorescence microscopy, with fluorescent labels as markers for biochemical processes, requires an improvement in resolution. *In situ* hybridization is an example of a technique that demands a higher resolution, especially when used for DNA mapping. With conventional optical microscopes DNA probes can be resolved at distances of about 500 nm. Modern techniques like electron microscopy and atomic force microscopy offer a much better resolution, but give problems with multiple 'color' ISH and with the preparation of the samples.

Near-field scanning optical microscopy (NSOM) might play an important role in the localization of genes on morphologically preserved inter- and metaphase chromosomes: the technique combines the high resolution of scanning probe microscopy with the optical selectivity and sensitivity of optical microscopy. A forthcoming, but important advantage is that a topographic (force) image is obtained simultaneously with the fluorescence image, such that the location of the fluorescence signals can be correlated to the morphology of the chromosome.

The near-field optical microscope used for the detection if *in situ* hybridization signals is integrated on a Zeiss Axiovert inverted microscope: chromosomes are inspected with a high quality epi-fluorescence microscope and preselected for measurements with the near field microscope. The sample is illuminated through a adiabatically tapered, aluminum coated optical fiber with a small aperture at the tip end. The size of the tip, approximately 100 nm, determines the illuminated area of the sample, and thus the resolution. A feedback on shear-force is used for distance regulation.

Fluorescence of the sample is collected by the microscope objective and detected by a photon counting avalanche photo-diode. The sample is raster scanned while both fluorescence and height are measured resulting in a simultaneously obtained fluorescence and topographic map of the chromosome.

The possibility to detect indirectly labeled DNA probes with a near-field scanning optical microscope is demonstrated. Both satellite and plasmid probes are accurately localized on human metaphase chromosomes. The fluorescence images show a lot of detail that is not seen with conventional fluorescence microscopy. Optical resolution in the fluographs is better than 100 nm. The fluorescent sites can be correlated to the topography of the chromosomes, which is recorded with a lateral resolution of about 50 nm. Furthermore, two color fluorescence *in situ* hybridization on a single chromosome is shown.

As an example of the results described above, a 7×7 μm image of a human metaphase chromosome 1 is shown. The chromosome is hybridized with dioxygenated p1-79 and immunologically detected with CY-3. We have combined the fluorescence signal and the topography in one image in order to emphasize the simultaneous recording of the signals. The topography of the chromosome is given in grey, and the chromosome structure is clearly recognized. The fluorescence intensity is given in orange. Several signals are observed at the telomer region, giving the location of the p1-79 probe. With conventional microscopy p1-79 usually is observed as a single spot at the telomer, although some sub-structure is recognized. Several other signals are found on the chromosome, which is probably due to non-specific hybridizations.

Concluding we clearly demonstrate the possibility and sensitivity to detect fluorescently labeled DNA fragments on human metaphase chromosomes with an optical resolution substantially better than possible with conventional microscopy. Furthermore, a force image of the chromosome morphology is afforded simultaneously.