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On the use of Si-based nanohole arrays as near-field biochips

H.-G. Eberle, C. Dressler, H. Oertel, J. Beuthan, G. Müller

***Abstract*. Near-field biochips based on nanohole arrays produced in silicon wafers are described and the first studies of cells and DNA with the help of a laser scanning microscope are presented.**

Keywords: *nanoholes, biochips, biomedicine*.

1. Introduction

At the current stage of molecular genetic research, the methods of biomedical analysis are being combined more and more with nanotechnological methods. The biochip and microarray technologies used at present often suffer from fundamental limitations such as inefficient data processing, lack of flexibility, and high costs. We describe a two-dimensional array of light nanosources for high-resolution investigations of biological samples, which allows the user to overcome some of these problems. The light sources represent arrays of holes with a diameter less than 200 nm etched into a thin silicon film. These arrays containing up to 160000 single holes are irradiated by a scanning laser beam, for example, in a laser scanning microscope (LSM).

An advantage of this setup is that it allows the samples to be kept in a physiological environment (liquid medium). Another advantage is that a signal is accumulated considerably faster than in the case of conventional near-field techniques. This permits in fact real-time studies of the kinetics of specific biological reactions.

Now that the scientific community has mapped nearly the entire human genome, an even greater challenge is becoming evident: to find the reason for the large amount of information contained in the genome. This task involves two main areas: functional genomics, which comprises the further and accurate study of DNA sequence variety in order to fully understand its function, and proteomics, the study of how the genes control the production of proteins as well as deciphering the full repertoire of proteins encoded by a genome [1 – 2].

Biochips are broad technological platforms on which a variety of biological processes can be analysed using miniaturised high-throughput screening. Biochip technology promises to revolutionise life sciences research, diagnostic medicine, and drug synthesis [3 – 4]. Current biochip technologies can be divided into two basic categories. The first type comprises passive biochips on which the bioanalytical reactions are diffusion-dependent; examples of passive chips are DNA/protein microarrays and biomolecule-attached bead arrays [5]. Active biochips are the second category in which microfabricated electrodes or channels are used to facilitate bioanalytical reactions; examples of active biochips include bioelectronic chips for direct-current field-assisted DNA hybridisation and direct-current-based microfluidic capillary electrophoresis chips for the separation and characterisation of bioanalytes [6].

The major limitations of current sample processing and measuring are high costs, laborious procedures, and low signal-to-noise ratio (S/N). The last restriction results from the readout principle of the commonly used CCD chips [7 – 9]. The S/N ratio can be improved by cooling the CCD chip, but this may adversely affect the sample condition and handling.

A passive biochip proposed in this paper allows one to carry out high-throughput analysis featuring improved selectivity and sensitivity, providing solutions for the critical needs in pharmaceutical R&D, clinical diagnostics, and biomedical research. Its advantages are the improved S/N ratio and implementation in LSMs.

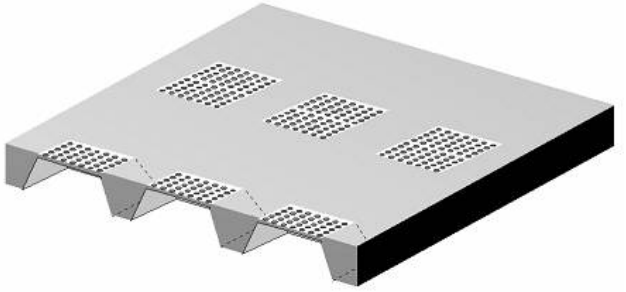

Figure 1. Nanoarrays in a silicon chip. Size: 12 × 12 mm, 9 membrane arrays (1 × 1 mm) 3 × 3 screens; membrane thickness: 1.5 – 2.5 μm; arrays: 200 × 200 or 400 × 400 holes each.

H.-G. Eberle, G. Müller Laser Medizin Technologie Berlin GmbH Fabeckstr. 60-62, 14195 Berlin, Germany. Tel: +49 (0) 30 8449 2332, Fax: +49 (0) 30 8449 2399; e-mail: h.eberle@lmtb.de;
C. Dressler, J. Beuthan, G. Müller Institut für Medizinische Physik/ Laser-medizin, Freie Universität Berlin, Universitaetsklinikum Benjamin Franklin, Fabeckstr. 60-62, 14195 Berlin, Germany
H. Oertel Fraunhofer-Institut für Siliziumtechnologie (ISiT), Fraunhoferstr. 1, 25524 Itzehoe, Germany; e-mail: oertel@isit.fhg.de

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2. Materials and method

Arrays of 200×200 or 400×400 conical holes were produced by a series of subsequent lithographic and etching procedures using SOI silicon wafers (630 μm thick) as the starting material. The wafer is than made thinner from the opposite side, to a thickness of approximately 2 μm (Fig. 1). The hole channels are conical in shape (Fig. 2) with an entrance diameter always larger than 500 nm (0.5–2.0 μm). Finally, a thin Al- or Ti-film (40 nm) was deposited to improve the S/N ratio for light propagation through the holes. The smallest exit aperture achieved in this way had a diameter of 190 nm.

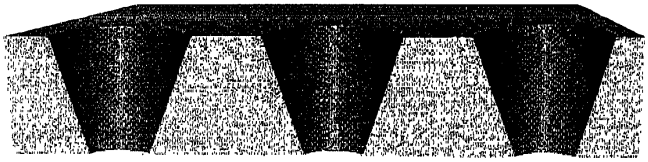

Figure 2. Approximate shape of the apertures etched in the silicon wafer.

To reduce the exit diameter further, a thin Si_3N_4 layer was deposited at high temperature. The arrays were coated additionally with an extra 80-nm thick metal film to increase the optical damping between the holes. The smallest apertures were 60 nm in diameter (Fig. 3), but no far-field transmission through these holes could be observed. Light transmission into the far-field (without the interaction with a sample!) was observed down only to ~160 nm (Fig. 4).

The nanoarrays were designed in order to provide platforms for a variety of biological processes in the course

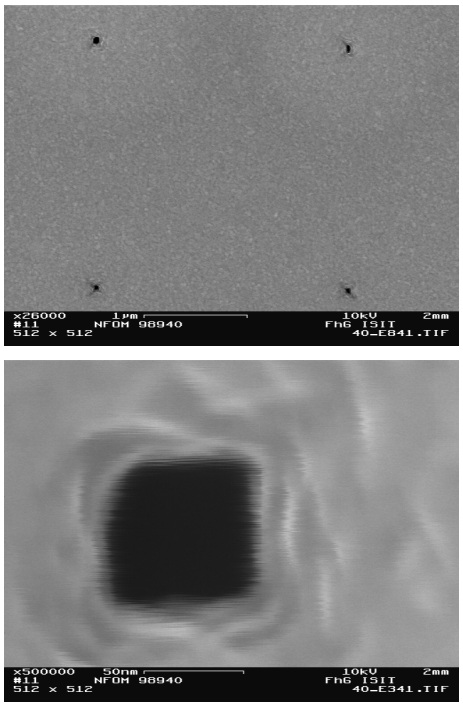

Figure 3. (a) Detail of a 400×400 array with 60-μm apertures, (b) single 60-μm hole.

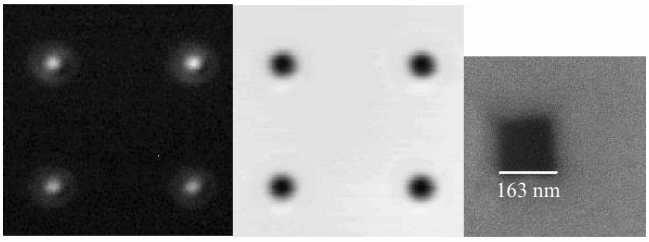

Figure 4. Transmission and reflection image recorded with the laser scanning microscope (LSM) (Fig. 5); excitation wavelength 543 nm, $20 \times /0.75$ and SEM image (from left to right) of an array with 163-nm apertures; distance between the apertures is 5 μm.

of a miniaturised optical high-throughput screening. These arrays then can be analysed by a commercial LSM using transmission and fluorescence microscopy simultaneously. This would ensure flexible sample preparation, handling, and a very fast digital data acquisition. The experimental setup is shown in Fig. 5.

The spacing between the individual holes is large enough to prevent more than one aperture being excited at one time. Using an LSM 410 (Carl Zeiss, Jena, Germany), the fluorescence is detected at the excitation side, which means that the fluorescence light has to penetrate the nanohole before it is detected in the far-field. This limits the usable exit aperture diameter to approximately 200 nm because of

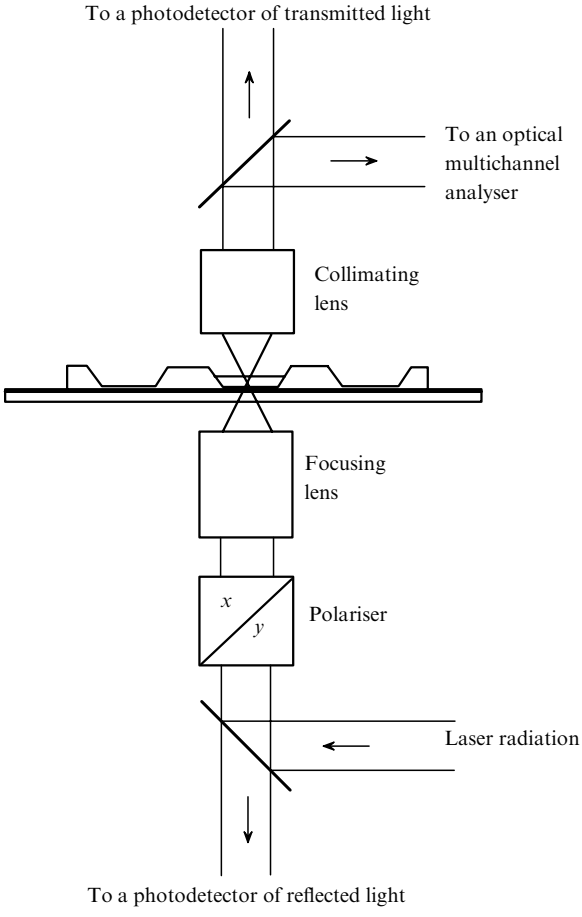

Figure 5. Scheme of excitation and detection with a confocal LSM (LSM 410 Carl Zeiss); transmission upwards, fluorescence downwards.

the strong attenuation of light passing through the conical nanochannel. The fluorescence signal intensity could be increased by enlarging the cone angle or by detecting the fluorescence in the sample half space. This improvement is planned at the next stage of the investigation.

The main advantage of using a 2-dimensional array of nanoholes is the separation of the laser beam excitation site and the sample location. This means that only a part of the sample is excited and gives a signal which is located in a near-field distance to the laser excited nanoaperture. Moreover, the separation between the holes is larger than the laser beam diameter, which limits the excitation to a single hole at a time. This leads to a great improvement in the S/N ratio due to the extremely low background level.

The nanoarray surfaces had to be conditioned appropriately before carrying out the bioanalytical applications. To achieve optimal near-field conditions, biocompatible and adhesive substrates for cells or analytes should be prepared. For this purpose the Langmuir – Blodgett film technique [10] was used. The arrays were coated with monolayers of thickness < 20 nm and roughness < 5 nm. The nanoarray coated with an adhesion layer and a sample and ready for a study is shown in Fig. 6.

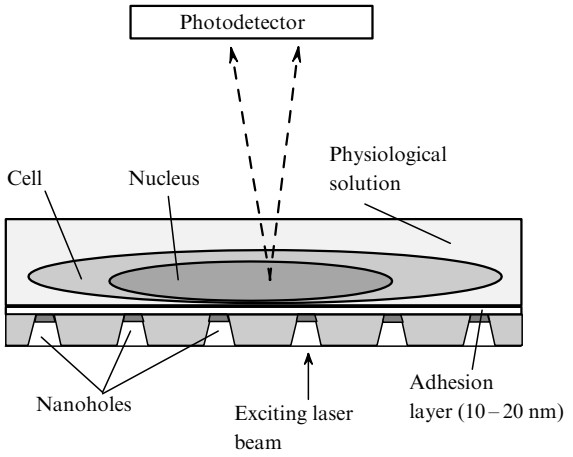

Figure 6. Schematic view of the laser excited nanoarray.

As fluorescence markers, Alexa or Cy3 and Cy5 dyes are widely used today because they provide a spectrally selective excitation using the laser wavelengths used in LSMs. These dyes are also available as reactive modifications suitable for specific applications. In addition, the detected fluorescence signals can be spectrally separated.

3. Results and discussion

Fibroblasts were grown on substrates prepared in the way described above. In these fibroblasts, the RNA rich regions of nuclei (nucleoli) were selectively detected after labeling with a nucleic acid stain. Fig. 7 shows the overlapped fluorescence and transmission images, the fluorescence signals mainly originating from the nucleoli.

The DNA fragments of sizes between 200 and 9300 nm were detected after staining with ethidium bromide (Fig. 8). The fluorescence and transmission signal intensities show the opposite behaviour indicating near-field optical conditions since in conventional far-field light microscopy single DNA fragments are not visible by absorption.

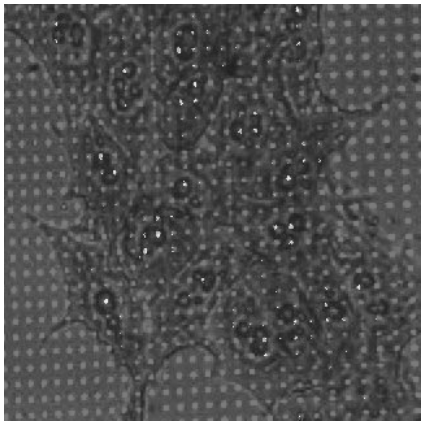

Figure 7. Fibroblasts grown on a nanoarray. Cells were stained with ethidium bromide. Confocal LSM imaging: overlay of fluorescence (white spots) and transmission (gray spots); the image size is $(112\ \mu m)^2$.

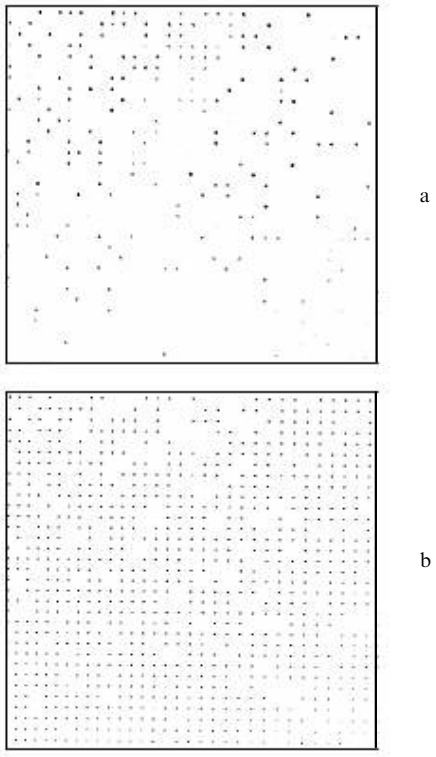

Figure 8. Fluorescence (a) and transmission (b) images of DNA fragments stained with ethidium bromide on a film-coated nanoarray. CLSM images are contrast inverted; the image size is $(96\ \mu m)^2$.

Biological interactions of samples and analytes may induce a spectral shift of the fluorescence signal. By using a wavelength discriminating detection mode, different interactions can be distinguished. The probability for different interaction processes can be evaluated by the method of scanning statistical microscopy (SSM) [11]. The transverse concentration (surface density) of molecular aggregates can be determined without knowing the dimensions of the particles by applying arrays with groups of holes with different diameters. The appropriate algorithm is described in detail in [11].

The procedure described so far used a statistical sample distribution on the array surfaces. At the next step, the regular nanoapertures can be loaded with molecular samples to be investigated in a well-defined manner using atomic force microscopy (AFM). In [12, 13], a method is described to deposit molecular samples with a high degree of order and nanometer accuracy. Using these AFM-based deposition techniques, versatile biochips with nanoscale resolution can be produced. The use of nanohole arrays, instead of the 'mechanical' AFM scanning [13], for measuring the modifications of the samples will provide a rapid data acquisition, with only a slightly inferior resolution.

4. Conclusions

The application of laser excited light nanosource arrays was tested with biomolecules and cells down to hole diameters of 280 nm. The arrays containing up to 1.6×10^5 holes are highly homogeneous relating to the hole diameter and distances. The smallest hole diameter achieved was 60 nm, and light transmission into the far field was observed for hole diameters exceeding 160 nm. The laser excited arrays offer a promising new tool for biosensors with the local near-field interaction. For this purpose, spectroscopic measurements must be carried out. The work of Palanker and Lewis [11] on scanning statistical microscopy may provide a useful support for the evaluation and interpretation of the results. By using an LSM for excitation, signal acquisition and processing, a versatile and fast high-throughput analysis could be carried out. Further, the method should be combined with the AFM procedure [13] to achieve a well-defined regular sample deposition, thereby producing very versatile biochips with near-field resolution and rapid data acquisition.

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References

1. Hebestreit H.F. *Current Opinions in Pharmacology*, **1**, 513 (2001).
2. Honore B. *Expert Rev. Mol. Diagn.*, **1** (3), 265 (2001).
3. Dhiman N., Bonilla R., O'Kane D.J., Poland G.A. *Vaccine*, **20**, 22 (2002).
4. *The San Diego Biotech. J.*, Oct/Nov., 18 (2001).
5. http://www.bioarrays.com/techpapers/spie.pdf
6. Cronin M.T., Boone T., Sassi A.P., Tan H., Xue Q., Williams S.J., Ricco A.J., Hooper H.H. *J. Assoc. Lab. Autom.*, **6** (1), 74 (2001).
7. Newton M.A., Kendziorski C.M., Richmond C.S., Blattner F.R., Tsui K.W. *J. Comput. Biol.*, **8**, 37 (2001).
8. Brown C.S., Goodwin P.C., Sorger P.K. *Proc. Natl. Acad. Sci.*, **98**, 8944 (2001).
9. Tseng G.C., Oh M.-K., Rohlin L., Liao J.C., Wong W.H. *Nucl. Acids Res.*, **29**, 2549 (2001).
10. Zasadinski J.A. *Science*, **263**, 1726 (1994).
11. Palanker D., Lewis A. *Biophys. J.*, **60**, 1147 (1991).
12. Piner R.D., Zhu J., Xu F., Hong S., Mirkin C.A. *Science*, **283**, 661 (1999).
13. Lee K.-B. *Science*, **295**, 1702 (2002).