Microarray scanner for flexible, parallel and spectral fluorescence detection

Carsten Glasenapp, Holger Krause, Wolfgang Mönch, Hans Zappe

Institute of Microsystem Technology, University of Freiburg
Laboratory for Micro-optics

mailto:glasenap@imtek.de

To realize a new type of biochip-reader, a liquid-crystal display for the generation of dynamic holograms for individual illumination of microarrays was employed. In combination with a CCD-based spectral detection-unit, the resulting demonstrator was used to evaluate this new approach.

1 Introduction

The main task of a microarray scanner is the spectral detection of an array of fluorescent spots. Generally the array is spotted on a plastic or glass substrate. To stimulate the fluorescence, these spots have to be illuminated, typically by flood illumination. In this case, high background fluorescence reduces the sensitivity. To improve performance, holographic excitation setups may be used to illuminate only the spots and not the background [1]. Holographic excitation by a static diffractive element is only useful for microarray biochips with a fixed spot-grid. For enhanced flexibility, holographic excitation by using liquid crystal displays as dynamic diffractive optical elements (DDOEs) was demonstrated here. With this new technology, we are able to excite an arbitrary spot grid with two laser wavelengths.

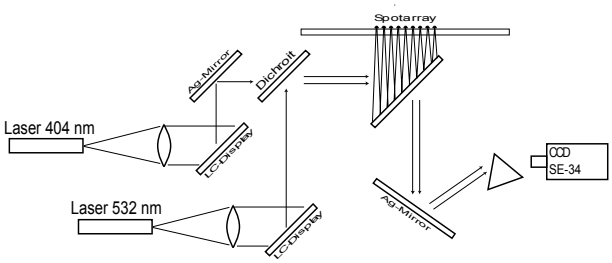

Fig. 1 *Experimental setup of holographic excitation with two laser sources and two DDOEs*

2 Holographic array excitation

Holographic array excitation is performed using two reflective liquid crystal displays (LCD) for two-dimensional modulation of the phase of an incident wavefront. The required spatial phase modulation for obtaining a desired intensity distribution is realized with computer generated holograms (CGH) which are loaded into the LCDs. The DDOEs are able to generate an arbitrary spatial phase modulation of an incoming light wave. The appropriate phase modulation can generate a defined three dimensional modulation of the light intensity behind the DDOEs [2]. This could be a single light spot, an array of spots or other arbitrary intensity patterns. Since the DDOEs are able to vary their diffractive properties in real time, it is possible to move a spot, to change the period of a spot array or to generate other types of time-varying intensity distributions. Colored holograms can be generated by the use of two or more DDOEs and two excitation wavelengths.

Fig. 1 shows the principle setup of our microarray-reader. Two DDOEs are illuminated by two expanded laser beams (λ= 404 nm @ 10 mW; λ= 532 nm @ 15 mW). A dichroic mirror couples the two separate light paths together to illuminate the microarray. With this arrangement, we are capable of exciting an arbitrary biochip array with two different light sources simultaneously. In Fig. 2 we see the beam profile of a 500 µm spot grid. The profile is homogeneous and the contrast is greater than 95%. The high contrast is important to detect small dye concentrations by reducing the background fluorescence.

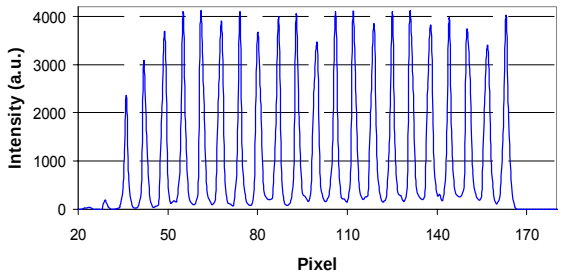

Fig. 2 *Line of 21 focused beams generated with a DDOE; Intensity per spot: 10 µW @ 404 nm; Beam diameter 160 µm; Beam period: 500 µm*

3 The spectral fluorescence detection-unit

To detect the fluorescence light of the stimulated spots, a spectral detection unit was designed. The goal, simultaneous spatial and spectral analysis of the fluorescent light, was attained by using a 2D-CCD camera. One dimension is used for spatial information, the other for the spectral distribution. The picture of a line of spots is imaged by a high-NA objective onto the camera chip. To obtain a continuous spectrum of the fluorescent light, a

glass prism is positioned in the optical path. The spectral resolution was set to 2 nm, enough to separate two or three different typical fluorochromes. A higher resolution would reduce the sensitivity.

The fluorescent emission is red-shifted with respect to the excitation wavelength, typically by about 20 nm. To detect weak fluorescence light, this effect thus requires filters which block the stimulating laser wavelength. In the setup used here, a special filter combination is used to measure the fluorescence of a set of fluorochromes. A long-pass-filter blocks the shortest laser wavelength (404 nm) and a notch-filter blocks the second laser line at 532 nm.

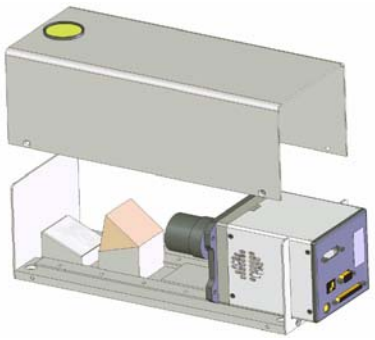

Fig. 3 *The spectral fluorescence-detection-unit showing the wavelength-dispersive prism and CCD camera with objective.*

The fluorescence detection unit is able to measure the spectrum of one line of fluorescence spots. In combination with a mechanical xy-scanner, the spectra of an entire microarray can then be read out line-by-line.

4 Detection of molecules-concentrations

The fluorescence intensity of a spot depends on the concentration of the molecules in the spot.

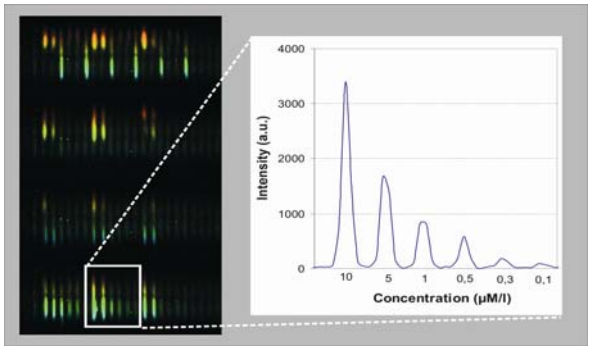

Fig. 4 *Detection of various molecule-concentrations*

We thus measured the fluorescence concentrations of a test-microarray to demonstrate the functionality of the system. Concentrations from 10 to 0.1 µM/l of the fluorochromes Dy 631, Dy 550 and Dy 415 (*Dyomics GmbH*) were spotted on a glass substrate. The drop volume of each spot was 1 nl. The resulting surface-concentrations ranged from $2 \cdot 10^5$ to $2 \cdot 10^3$ molecules per µm². In Fig. 4 we see four lines of the testchip measured with our demonstrator. In the bottom line, six concentrations of the dye Dy 415 from 10 to 0.1 µM/l were spotted. The plot on the right shows the dependence of the fluorescence intensity over the concentration. The detection dynamic range of three orders was limited by the 12-bit-CCD-camera.

5 Detection of emission spectra

The spectral fluorescence detection allows simultaneous use of multiple fluorescence dyes.

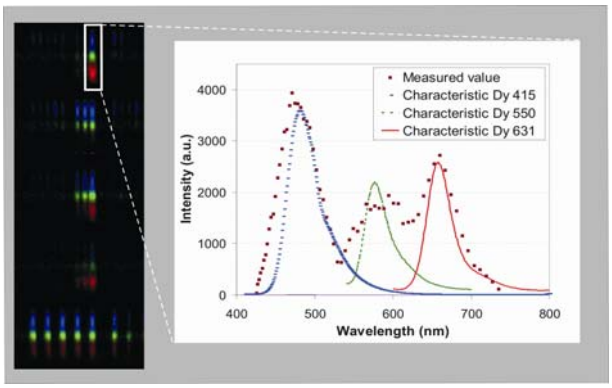

Fig. 5 *Detection of three different marker-molecules*

As may be seen in figure 5, it is possible to detect the fluorescence intensity of three different dyes in one spot of a microarray-biochip. The figure shows five lines of a test-biochip with different concentrations of the fluorochrome Dy 415 (blue), Dy 550 (green) and Dy 631 (red). The spectral intensity of the first line is plotted on the right hand of figure 5. We see three maxima at the wavelengths 470 nm, 580 nm and 660 nm. These maxima and curve-shapes are exactly the same as those of the dyes measured independently (colored lines in plot). The resolution of 2 nm is sufficient to separate each spectrum.

6 Acknowledgements

This work is part of the project *MOBA,* supported by the research program *Biophotonik,* funded by the German Ministry of Education and Research (BMBF). We would like to thank Sensovation AG and Thorsten Neumann of the chair of Chemistry & Physics of Interfaces (CPI) of the University of Freiburg for their cooperation and support.

References

[1] Blom H., Johansson M., Hedmann A.S., “Parallel fluorescence detection of single biomolecules in microarrays by a diffractive-optical-designed 2x2 fan-out element”, *Applied optics, Vol.41, No.16, 6/2002*

[2] Lesem L.B., Hirsch P.M., Jordan J.A.Jr.,. “The Kinoform: A new wavefront reconstruction device” , *IBM J. Res. Develop. 13 (1969) 150*