The con-focal method on verifying focal plane of MAS machine

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Abstract: The Maskless DNA Array Synthesizer (MAS) is very efficient and flexible device on custom designed DNA array fabricating. To make the MAS's focal plane keep stable is important during the chip synthesizing process. In this paper we bring a creative idea using con-focal method to verify focal plane of MAS machine. That method is very sensitive on focal plane verification and could be used on other optical lithography system or the projecting system, which need real time focal plane precision control.

OCIS codes: (220.1440) Alignment; (220.3740) Lithography

References and links

1. Sangeet Singh-Gasson &Roland D. Green, "Maskless fabrication of light-directed oligonucleotide microarrays using a digital micromirror array," Nature Biotechnol. **17,** 974 (1999).
2. T.Wilson, C. R. J. Sheppard, *Theory and Practice of Scanning Optical Microscopy,* (Academic Press, London, 1984), Chap. 3.
3. C.J.R. Sheppard and D.M. Shotton, *Confocal Laser Scanning Microscopy*, (Springer, New York, 1997), Chap. 3.

1. Introduction of Maskless DNA Array Synthesizer

There is a growing interest in the development of the so-called DNA chips for a variety of applications in medicine, biology and genetics. These "chips" are created by forming a regular array of short strands of DNA (oligomers) on a glass substrate. In order to increase the number of different sequences on a chip, the size of each individual "pixel" is of the order of 13-16 microns. Typically the pitch is around 13.7-17 microns, with a separation of 0.7-1 microns. Ideally one would like to program in excess of several hundred thousands of different sequences on the chip. Interestingly enough, this number is similar to that of video display systems, with XGA being 700,000 and SXGA 1.3 millions. Existing processes use either "spotted arrays", whereby each site is created using a servo-controlled micropipette (up to 20,000 sites) or using an inkjet system (up to 50-60,000 sites), or an approach based on photolithography. This process is the one yielding the highest density chips. Until now, the photolithographic process was based on the use of standard contact printing with quartz masks. This approach is efficient in fabrication, but hard to modify since there is always a lag time in fabricating the masks. A maskless approach would be highly preferred, because it would cut down the cycle time for modifications to the layout to hours, and reduce the cost of fabrication.

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The process of this approach is illustrated in Fig. 1. We have implemented this idea in the Maskless Array Synthesizer, where a virtual mask is defined by Texas Instrument Digital Light Porcessor chip (DLP). The general idea is presented in Fig. 2, and described in [1].

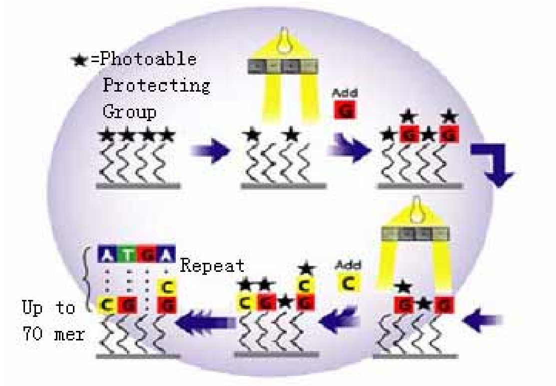

Fig. 1. The process of maskless array photolithography approach for DNA chip

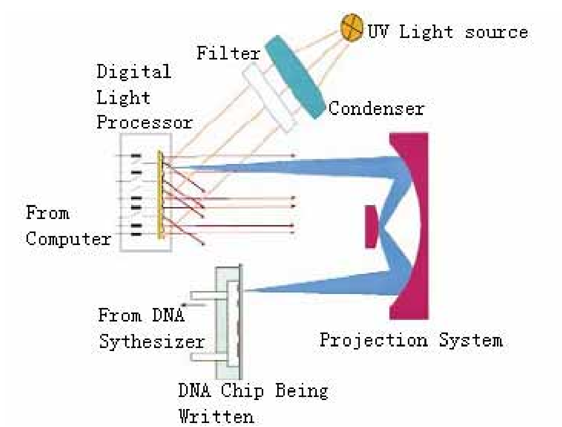

Fig. 2. The idea of the device with DLP Maskless array Synthesizer

2. The demand of verifying focal plane of MAS machine

In this paper we concentrate on a specific issue of the optical system. Because of the relatively long time required to form a DNA chip, the optical system must be very stable for several hours. For economy, the system should also be as simple as possible and thus we have been exploring various approaches for stabilizing the image. We note that in this system there is no overlay requirement, because the cell is never moved from its position, and all the chemical processing is done in-situ. However, focus must be maintained during the whole fabrication process, and lateral displacement minimized. We notice during the process, the defocus is a large term, and that in our optical system the depth of focus is of the order 57 microns. Hence, a simple and sensitive system for the detection of focal plane movement would be an important addition to the system. Various approaches have been proposed, relying on image processing or alignment marks but these are either cumbersome or expensive to implement. In our approach we use the programmability of the DLP to create a simple and effective confocal system. The enhanced sensitivity to the focal position of the confocal system allows us to

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quickly monitor focal displacements, and correct for them. In addition, this approach does not require marks on the DNA chip, and is thus very general. With the addition of marks, this system can also easily be used to detect lateral shifts.

3. The confocal focus shift testing on MAS system

3.1 The theory of confocal optical system [2][3]

For conventional optical system the image intensity can be describe as:

$$I\ (v)=[2J_1(v)/v]^{2} \tag{1}$$

That is well known as Airy disk, here v is a dimensionless radial optical coordinate

$$v = 2\pi r_1 \sin\alpha/\lambda \tag{2}$$

In the confocal optical system, the image intensity is given by:

$$I\ (v)=[2J_1(v)/v]^{4} \tag{3}$$

From the function of above, the point spread function of a confocal system is sharper than that of an equivalent optical system, and is actually equal to the square of the conventional optical system's PSF.

The confocal system can be simply set up as what is showed on Fig. 3. The detector is set up on the con-focal plane of the source point, a pin hole pupil can be set in front of the detector to make it work as a pin detector. If the object is right on the focal plane, the beam focus by the lens will be reflected back to the lens in the same path just with the up-ray and down-ray swap the position, but the beam will focus on the detector as a point. When the object defocus, as dashed line showing on Fig. 3, the beam reflected back through lens but focus in front or behind of the detector, on the detector plane, the image change to be a larger dish, it will be larger than a light point when the object on-focus

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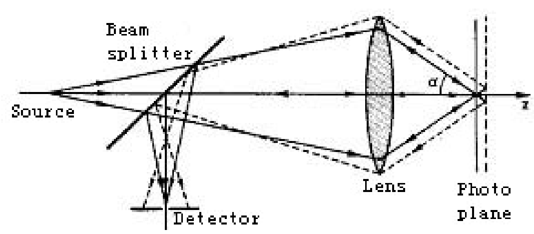

Fig. 3. Con-focal system in on focus and defocus condition

In a confocal system, the image of a pinhole is transmitted through the same pinhole after proceeding through the optical system, as typically done in confocal microscopy. The reference pinhole does not need to be the actual pinhole itself, but an equivalent aperture. The PSF is very sensitive to focal plane movements, and indeed confocal microscopes are often used as "sectioning" tools for transparent systems.

3.2. Implementation of a confocal system on MAS

The implementation described here forms the confocal system using the DLP itself. A small number of isolated micro-mirrors of DLP are turned "on" to define the virtual pinhole. Some amount of light is reflected by the interface glass-slide, and traces back to its origin on the DLP. When the system is perfectly aligned the returned light has maximum intensity. The virtual pin-hole before the detector can only let the light which is focus on focal plane go through and hit the detector, the total amount of power received by the detector will reduce when the focal plane shift and the light dish is larger than the on-focal spot. How much power received by the detector will be related to how the focus is. Any displacement in this system that causes a shift in the focal plane will be immediately detected as a drop in intensity. We note that the system can operate continuously providing real-time feedback to a compensating system.

3.3. The setup on MAS system focusing verification

The system is build as Fig. 4 illustrated. We put a glass slide with aluminum coating on the back as a feedback mirror and set it on the focal plane of MAS projecting system, the beam focus on the back surface of the aluminum coated slide will be reflected back through the same path to the projecting system and focus on the DLP, and then the beam will be reflected back to the same path to illuminator. Thinking each micro-mirror on DLP as a pin-hole, That is a con-focal system. A beam splitter is inserted between the illuminator and the DLP, so the feedback beam reflected back from the DLP can be collected by a power collection lens and then be received by the detector.

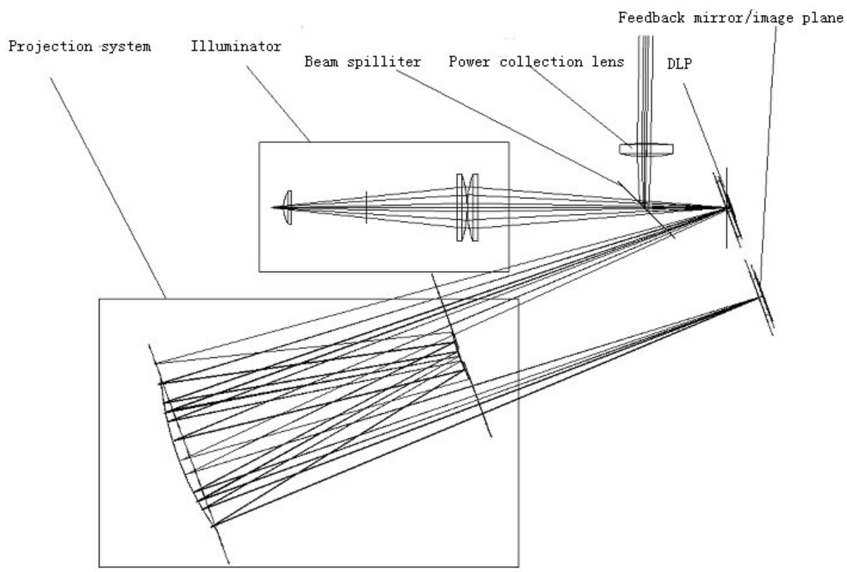

Fig. 4. The setup of DLP focus verification system

Each micro-mirror on DLP can be thought as a 16μm by 16μm pin-hole, when there is only 1 micro-mirror on, the beam illuminate on that single Micro-mirror pixel of DLP will be reflected into the projection system and focus on the feedback mirror, if the feedback mirror set right on the focal plane without defocus, the beam will be focus to a point by the projection system. Then, the beam will be reflected back on the same path to projection system and focus on the single micro-mirror pixel of DLP and being reflected by this micro-mirror to the feedback power collection system totally.

When there is defocus of feedback mirror the beam will focus before or behind the feedback surface, in this case, the spot formed on DLP by the feedback optical path will be bigger than it was when the feedback mirror set right on focus, some amount of power will lose, only the light hit the Micro-mirror pixel of DLP can be collected by the power collection system. That confocal setup is very sensitive to the focal shift on image plane, and could achieve a real-time control of focal plane verification system efficiently.

There are two issues need to be concerned when we setup and use this system. The first issue is because the feedback mirror is in the same plane of image plane (DNA chip). The misalignment of this mirror, such as the tilt, will have a direct effect on the performance since the projection system is telecentric. The second issue is the feedback mirror has to be within the field of the projection system, which will reduce usable field of view significantly.

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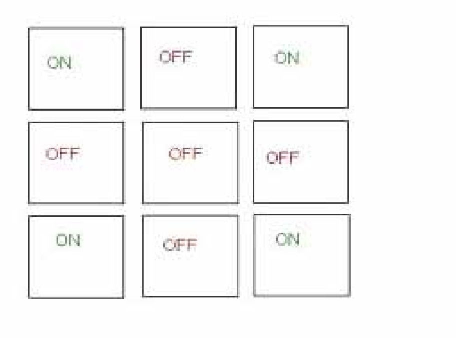
4. The test result

4.1 Test setup

On the real test set up, we use a tele-centric lens to be the feedback beam receiver, a Hamamstu CCD camera is attached to the lens work as a detector. Because when only one micro-mirror is on the power received by the digital camera is too weak, so on the real test we use 4 micro-mirrors, and that is "four in nine pattern", which is showed on (a) of Fig. 5. The pattern project to the CCD image plane is showed as (b) of Fig. 5.



(a) 4 in 9 pattern

(b) The image of 4 in 9 pattern on feedback CCD

Fig. 5. The 4 in 9 pattern and its image

The intensity falls off with the defocus can be detected with the setup mentioned before. A motorized actuator and high performance motion controller is being used to scan a 2mm area near the focal plane, the CCD camera record intensity of each defocus position. Figure. 6 shows the intensity change in different focal shift.

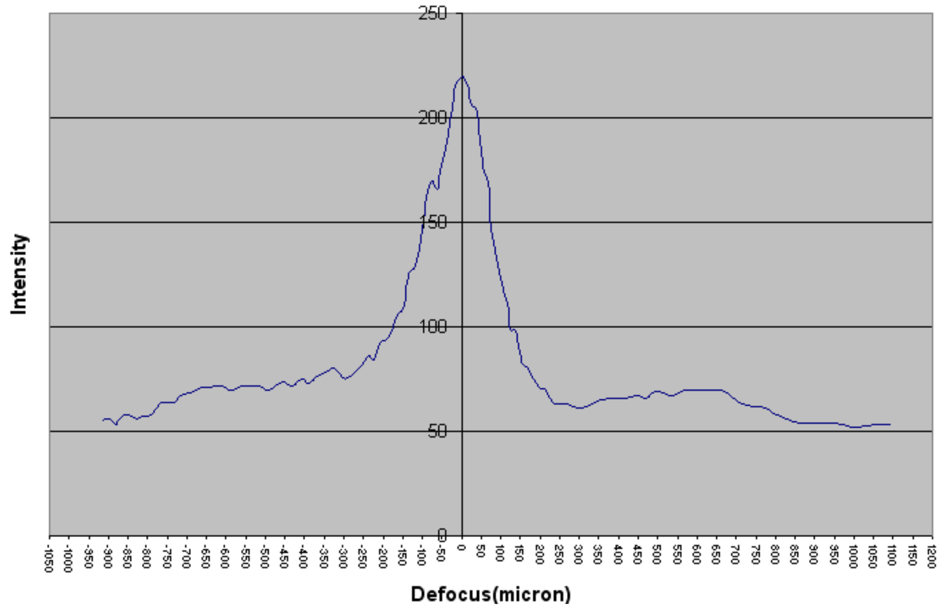
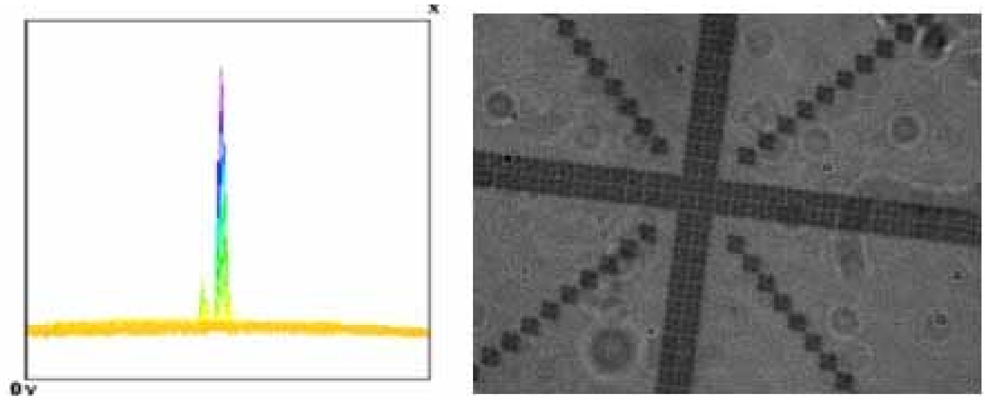


Fig. 6. The intensity change in different focal shift

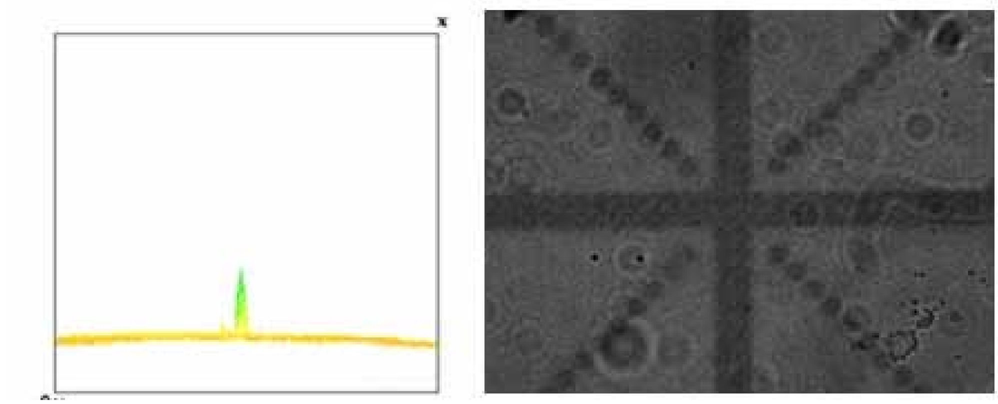
4.2 Test result

The test result is showed on Fig. 7 and Fig. 8. The illustration (a) of Fig. 7 and Fig. 8 are the signal value reading from the CCD camera, the illustration (b) is the cross pattern on DLP being projected to imaging plane and exposures in a radiachromic film, the cross features have 2 micro-mirrors line crosses with another 2 line located in 45° to this 2 main lines. Figure 7 shows the on-focus status, we can distinguish each DLP micro-mirror very clearly and the edge of each small Micro-mirror of DLP is sharp and clear. Figure 8 shows the defocus status, we can see when the feedback signal drop down to around 40% compare to the signal of on focus status, the image of Micro-mirror of DLP change to be much blurred.



(a) feedback signal (signal level 221) *(b) the cross pattern of DLP image on focal plane*

Fig.7. On focus position



(a) feedback signal (signal level 88) *(b) the cross pattern of DLP image on focal plane*

Fig. 8. Defocus position

5. Conclusion

From the test as we described, use the confocal method to check the focal plane of MAS is very sensitive, even small defocus can make feedback signal has a significant change, that can be a very useful way for focal verification on MAS machine and achieve a real time focal control. Also, this method can be widely used on focal plane verification for other optical lithography system or the projecting system which need real time focal plane precision control.

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