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Step-and-scan maskless lithography for ultra large scale DNA chips

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Abstract

A maskless photolithography test bed was constructed to examine the requirements for stepper-based synthesis of Ultra Large Scale DNA chips (ULS-DNA chips). The test bed is based on a microscope optical layout with a 5 × reduction imaging lens and micro/nano controlled staging at the image plane. Spatial light modulation is enabled by a Digital Micromirror Device (Texas Instruments DMD 0.7XGA) and the positioning system is composed of a piezoelectric nano-positioner (nPoint Inc., Madison, WI) mounted on a high precision linear-motor stage (Newport Corp., Irvine, CA). With this test bed we examined the requirements of overlay and alignment in a stepper-based DNA microarray synthesis system. We demonstrated multi-field chip synthesis with a spot size of 3.15 μm at the 5 × reduction. All tests were verified by standard hybridization, and fluorescence microscopy. In addition to our demonstration of step-and-scan lithography for DNA chip synthesis, we drafted and modeled an imaging optic for a production scale tool capable of synthesizing DNA chips containing up to 20 million pixels.

Keywords: DNA synthesis; DNA microarrays; Maskless photolithography; Overlay; Oligonucleotide synthesis

1. Introduction

"DNA chips" are genetic sensors designed to measure the amount of genomic DNA in a sample by hybridization to a set of single-stranded DNA probes known as oligonucleotides, immobilized on a glass surface. These probes detect the existence of the sequences of interest in the sample because of the high sequence selectivity of hybridization. Fluorescence tags are used to reveal the location of the hybridization, and hence the existence of a sequence complementary to the programmed sequences on the chip. Since each probe only reveals the existence of its complementary sequence, it is often necessary to have a large number of probes in order to obtain useful results. The increase in density for DNA chips is driven by the need for affordable gene expression assays in the form of "DNA Microarray" [1], or "GeneChips" [2]. The parallel synthesis of short oligonucleotides (10–70 nucleotides (nt)) is based on a solid-phase photo-chemistry and is the basis for commercial DNA chips made by Affymetrix Inc., [3,4]. A "Moore's Law-like" trend can be observed in the historical data (Fig. 1) describing the density evolution of Affymetrix chips [5]; this trend shows clearly the evolution to higher density chips. The highest density DNA microarrays are synthesized by photolithographic methods analogous to the techniques used in the semiconductor industry. We note that in addition to scaling up the density, researchers are also finding new ways to get more information from microarray chips by improved probe design and by making chips re-usable [1]. The original method is based on a proximity exposure, i.e. on a mask aligner type system, that requires chrome masks for spatial light modulation [3]. In this method the need for many photolithographic masks (typically $4N$ masks, where N is the number of nt in the sequence – i.e., up to 280 masks for 70 nt) can quickly become a limiting factor for highly complex chips.

The need for photolithographic masks was eliminated with the invention of the Maskless Array Synthesizer (MAS) [6]. This maskless tool combines a Texas Instrument Digital Light Processor (DLP, often called "DMD" for Digital Micromirror Device in earlier papers) [7], a

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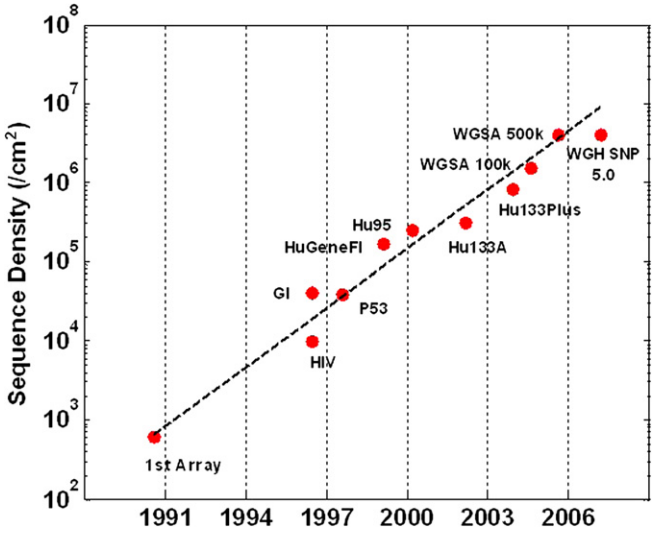

Fig. 1. DNA spot density per generation of Affymetrix chips vs. year of commercial release [5]. Notice the exponential growth, similar to Moore's law.

1:1 Offner imaging system, and associated micro-fluidics [6]. The MAS is the versatile platform for rapid high-density array synthesis commercialized by Roche-NimbleGen Inc. Currently, it can provide up to 4.2 million pixels by step-and-repeating three times a 1.4 million pixels SXGA chip [8]. The chip is still limited to a spot size of $13.6 \times 13.6\ \mu m^2$, determined by the individual mirror size on the DMD, and the $1\times$ optical system. A next-generation MAS will require reduction optics coupled to a reaction cell capable of step-and-repeat motions. To examine the feasibility of such a system we constructed a test bed based on a microscope design. Experience gained from these test bed experiments supports the design of a production scale system that could enable the synthesis of Ultra Large Scale DNA chips (ULS-DNA chips), potentially containing upwards of 20 million probes per chip, far exceeding the capacity of commercially available DNA chips. Most importantly, reagent consumption is kept essentially the same as those made by current $1\times$ MAS (non-reduction based system) so that the cost is essentially the same of current state-of-the-art chips. As in silicon technology, we increase the number of features per chip while keeping the fabrication costs constant.

2. Maskless microscope photolithographic test bed

The central component in our system is the DMD. The DMD offers high-contrast, high-resolution and rapid spatial light modulation [7] making it an attractive device for photolithographic applications. Several groups have attempted to take advantage of DMD technology to develop small cost-efficient photolithography tools for the purposes of rapid prototyping in photoresist [9–11]. Naiser et al. [11] demonstrated the use of a maskless Microscope Projection Lithography System (MPLS) for the synthesis of DNA microarrays and photoresist patterning, but without step-and-repeat functionality. A maskless stepper-based photolithographic system using a liquid crystal spatial light modulator for photo-patterning on photoresist was reported by Kessels et al. [12]. Our approach merges both DMD technology with motorized staging to achieve step-and-repeat synthesis of DNA microarrays that incorporates image-reduction. With this test bed we can fully realize the advantages in downscaling the projected image as well as test the overlay requirements for DNA microarray synthesis.

Our maskless microscope photolithographic test bed consisted of DMD Discovery Kit 1100 (DMD coated for UV) mounted in the image plane with respect to the UV microscope objective (OFR Inc., LMU-5 ×). Highly uniform illumination is provided from a 200 W HgXe arc-lamp filtered for I-line, relayed by a rectangular light-pipe, and then imaged on to the surface of the DMD. A functionalized glass microscope slide [13] is positioned at the focal plane within an in-house designed reaction cell, structurally adapted for our staging system. Staging consisted of a nano-positioner (nPoint Inc., NPXY100Z25A/ C300 controller) mounted on top of a single-axis linear-motor stage (Newport, UTM25PP.1/ESP300 controller). A camera built from a 20 × Mitutoyo 0.28 NA microscope objective, CCD camera (Watec American Corp. LCL-902 K), and InfiniTube relay assembly, is used to observe the surface of the substrate and image plane simultaneously through the reaction cell. Reagents are delivered to the reaction cell by a PE Biosystems Nucleic Acid Synthesizer Expedite.

To provide references for positioning and focus, chromium fiducial targets were produced on the surface of the glass substrates by standard metal/photoresist "lift-off". These chromium targets enabled the implementation of an "Image Lock" program, similar to the method reported by Kim et al. [14]. Thorough removal of residual photoresist from our lift-off process proved to be a critical factor in the preparation of chip substrates. Any remaining resist contaminated our silanization process and as a result either reduced or eliminated the presence of synthesized product on our chips. In addition, the use of these fiducials with our image lock algorithm was prone to error. Noise generated by mechanical vibrations, fluctuations in temperature, and over-saturation of the CCD resulted in an unstable Image Lock process.

3. Results

In our implementation we expose three adjacent fields sequentially for each cycle (or sequence layer) until the complete sequence is synthesized for all three fields. For example, for a given cycle (i.e. for the addition of a single base) a fine positioning alignment step is performed, exposure is delivered with the appropriate DMD mask for the first field. The coarse stage then moves the substrate to the second field position where the alignment and exposure

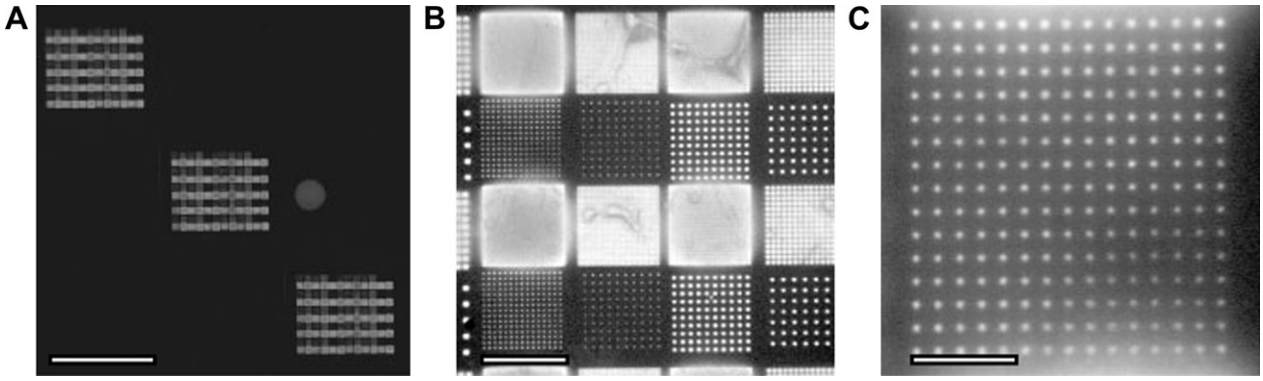

Fig. 2. A. Three adjacent fields of our resolution test pattern. Fluorescent image captured by ArrayWoRx bio-chip scanner for CY3 fluorescence. Scale bar represents 3 mm. B. Resolution test pattern showing the various DNA spot sizes and spacings determined by the DMD projected mask. CY3 Fluorescent image captured at 10X on a Nikon E800. Scale bar represents 165 μm. C. Single-pixel exposure with 2 pixel/mirrow spacing between each spot. Single-pixel area in fluorescent image measured to 3.15 μm. CY3 Fluorescent image at 40 × Nikon E800. Scale bar represents 50 μm.

steps are repeated; the process is then repeated for a third field position. At this point the substrate has been exposed in all the desired locations, the phosphoramidite reagent is admitted into the reaction chamber, and other chemical operations are performed in situ [6]. The cycle is repeated for the addition of the next-base, until the overall layer has been completed, and all of the ACTG have been attached. Typically, power measured at the image plane was around 60–100 mW/cm^2 depending on the lamp age. A target of 7–10 J/cm^2 dose provided adequate irradiation for hybridization signals of comparable intensity to those made in a 1 × MAS. We note that an image-reduction M should increase the intensity delivered by a factor of the magnification squared M^2; however, the refractive optics used in our test bed only allowed for an increase of intensity to a factor of approximately $\frac{1}{3}M^2$. We repeatedly synthesized microarray patterns with accurate alignment with and without image-locking functionality. Attempts at more complex chips produced relatively lower-quality results because of scattered light (flare) which, may cause erroneous deprotection and errors in the Image Lock process.

In our resolution tests we synthesized oligonucleotide chips patterned with a layout containing different spot sizes at several different spacings, where each spot contained the same sequence (i.e, 3′-CTGGTCCCACCACCAAGTACTAC-TACTG-5′). Each field exposed contained this resolution test pattern generated by the DMD and it provided information about the resolution limitations in our test bed. Hybridization with complementary sequence labeled with Cy-3 fluorescent molecule revealed that single mirror exposures spaced one to two mirrors apart suffered in resolution because of scattered light and aberrations within the system (Fig. 2).

Using our resolution test chips we then tested the ability to recover the fluorescent data from chip scanners and fluorescent microscopes. An ArrayWoRx Bio-Chip Scanner whose highest resolution was 2 μm, was able to capture fluorescent data but the edges of the spots were blurred, as expected. Improved resolution was obtained with a confocal microscope system (Leica TCS SP2 AOBS) but the amount of signal decreased with the improved resolution. The best compromise we found in imaging ULS-DNA chips was in the E800 Nikon Epi-fluorescent microscope. DNA microrray spots with diameters on the order of 1–3 μm should be a suitable spot size for this type of synthesis and detection.

4. Production scale tool design and discussion

Our demonstration brought insight to the many design considerations for a production scale tool, such as the need for automated focal position monitoring of the synthesis cell, increased mechanical stability for longer and more complex synthesis, and also higher resolution from our projection optic. Overall, we were successful in proving the concept at a 5 × reduction with step-and-repeat synthesis, but clearly a system with better optics and staging is needed to create more complex chips.

We developed a projection imaging system with the goal of increased resolution and reduced flare at the 5 × image-reduction. Our design for an imager quickly converged to resemble a system designed for EUV optical lithography, albeit with much reduced performance requirements. In particular we refer to a design, originally developed at Lawrence Livermore National Labs for a EUV stepper [15]. Our design is relaxed to work at I-line and is a two asphere mirror design. While this design has strict tolerances both in surface roughness of the mirrors as well as in positional control, the requirements are considerably easier to meet than for an EUV tool. To image the full object field (area of DMD) with moderate distortions the optics themselves become quite large in order to satisfy the joint requirements of the NA and of small field angles. In conclusion, a bench top test bed was used to validate the concept of applying a stepper tool approach to synthesizing DNA microarrays. These initial tests have led to a more ambitious design for a production scale tool capable of generating 20 M pixel DNA chips.

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