Chapter 2

Microfluidic Technologies and Instrumentation for Printing DNA Microarrays

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Introduction

DNA microarray technology enables the simultaneous analysis of thousands of sequences of DNA for genomic research and diagnostics applications. The power of this technology was demonstrated primarily by the work of Affymetrix (17, 4, 21, 14, 31, 12, 11) and of the Stanford University groups of Patrick Brown and Ronald Davis (8, 9, 15, 18, 19, 25-29).

The Affymetrix approach involves the *in-situ* synthesis of oligonucleotides on a solid substrate using photolithographic techniques. The result is a microarray or "DNA chip", an array of tens of thousands of unique oligonucleotides in an area of several square centimeters. Biological samples are tagged with a fluorescent dye and incubated with the array. Fluorescent sequences in the probe mixture that are complimentary to array sequences will bind or hybridize. Interrogation of the array with fluorescent scanning device reveals the sequences that have a complimentary match.

The Stanford groups have used a variety of printing approaches, whereby tweezers, split pins, Micro Spotting pins, or ink-jets are use to deposit pre-synthesized oligonucleotides or PCR products onto solid substrates such as chemically-treated microscope slides. After printing, the microarrays are processed to remove unbound DNA and probed with a fluorescent sample. Similar to the Affymetrix approach, the location and intensity of the fluorescent signal provides quantitative information about the sequences present in a biological sample. Several recent reviews provide a good overview of the field (1-3, 5-7, 10, 13, 20, 23, 26, 30).

This chapter reviews the microfluidic technologies and instrumentation for printing or "spotting" microarrays as well as examines some of the practical considerations in using a

pin printing system. It should be noted that there has been some confusion about nomenclature in the microarray field, and this review uses the terminology established in the 1970s for filter-based methods out of deference to the pioneers in that field. The "target" is the DNA spotted on the surface to form the microarray and the "probe" is the labeled DNA that is hybridized to the surface-bound DNA of the microarray.

Microarray Printing Technologies

Making a microarray by printing involves delivering a small volume of DNA sample, the target DNA, onto the solid surface. The volume delivered is typically in the nanoliter (10^{-9} L) or picoliter (10^{-12} L) range. These volumes are below the range capable of being dispensed by typical liquid handling systems. Thus, new technologies have emerged to enable the production of microarrays.

The technologies for spotting DNA material onto a substrate fall into two distinct categories: Non-contact and contact dispensing. Non-contact dispensing involves the ejection of drops from a dispenser onto the surface. The most common type of non-contact dispensing uses ink-jet printing technologies, which are essentially modifications of devices used for printing ink onto paper. Contact printing involves direct contact between the printing mechanism and the solid support. Contact printing devices include solid pins, capillary tubes, tweezers, split pins and micro-spotting pins or "ink stamps", all of which deliver sample spots onto the solid surface. These two classes of technologies are described in the following sections.

Non-Contact Ink-Jet Printing

Ink-jet type printing involves the dispensing of the target DNA using a dispenser derived from the ink-jet printing industry. The DNA sample is withdrawn from the source plate up into the print head and then moved to a location above the slides. The sample is then forced through a small orifice causing the ejection of a droplet from the print head onto the surface. Two types on non-contact ink-jet technology, piezoelectric and syringe-solenoid, are currently being used to print DNA microarrays (Fig. 1).

Piezoelectric Printing Technology

Piezoelectric printing technology uses a piezoelectric crystal (e.g. ceramic material) closely apposed to the fluid reservoir. One configuration places the piezoelectric crystal in contact with a glass capillary which holds the sample fluid (Fig. 1a). The sample is drawn up into the reservoir and the crystal is biased with a voltage, which causes the crystal to deform, squeeze the capillary, and eject a small amount of fluid from the tip. An example of an array made with a piezoelectric dispensing system is shown in Figure 2.

The fast response time of the crystal permits fast dispensing rates, on the order of several thousand drops per second. Furthermore, the small deflection of the crystal results in drop volumes on the order of hundreds of picoliters. To date, piezoelectric dispensing technology has been shown to work for making small numbers of gene expression microarrays (26), but has been slow to develop into a commercially viable dispensing system. The main difficulties in implementing piezoelectric dispensing include air bubbles which reduce the reliability of the system, relatively large sample volumes, and difficulties with sample changing. Most of the commercial activity has been carried out by Packard Instrument Company and Incyte Pharmaceuticals.

Syringe-Solenoid Printing Technology

Syringe-solenoid technology combines a syringe pump with a micro-solenoid valve to provide quantitative dispensing in the low nanoliter range. Shown schematically in Figure 1b, a high-resolution syringe pump is connected to both a high-speed micro-solenoid valve and a reservoir through a switching valve. For printing microarrays, the system is filled with a system fluid, usually water, and the syringe is connected to the micro-solenoid valve. Withdrawing the syringe causes the sample to move upward into the tip. The syringe then pressurizes the system such that opening the micro-solenoid valve causes droplets to be ejected onto the surface. With this configuration, the minimum dispense volume is on the order of 4-8 nL. However, given the positive displacement nature of the dispensing mechanism, the reliability of the system is very high. An example of an array made with the syringe-solenoid technology is shown in Figure 3.

Pin Printing Technology

The second and more common means for dispense-based printing of microarrays involves the use of rigid pin tools and related technologies that were developed originally for use in making filter arrays. In surface contact printing, the pin tools are dipped into the sample solution, resulting in the transfer of a small volume of fluid onto the tip of the pins. Touching the pins or pin samples onto the slide surface leaves a spot, the diameter of which is determined by the surface energies of the pin, fluid, and slide. The typical spot volume is in the high picoliter to low nanoliter range.

Pin printing can be done using solid pins for transferring samples from microwell plates onto microscope slides (24). The tips of the solid pins are generally flat and the diameter of the pins determines the volume of fluid that is transferred to the substrate. A recent modification of solid pins involves the use solid pins tips with concave bottoms, which have been shown to print more efficiently than flat pins in certain cases. Because the loading volume of both types of solid pins is relatively small, only one or a few microarrays can be printing with a single sample loading, making the overall printing process rather slow.

To permit the printing of multiple arrays with a single sample loading, several groups have developed printing capillaries, tweezers, and split pins that hold larger sample volumes than solid pins and therefore allow more than one array to be printed from a single sample loading. Capillaries and tweezers have found use in research applications (16, 27, 29), though the open configuration of the sample channel leads to irregular loading volumes; moreover, the need to break the meniscus of the loaded sample by tapping the tweezers on the surface reduces printing durability in a production setting (Fig. 4). Split pins, developed by several groups, have a fine slot machined into the end of the pin to hold sample. When the split pin is dipped into the sample solution, sample is loaded into the slot. Tapping the pin onto a solid surface with sufficient force deposits a small volume of sample (Fig. 4).

The most widely used printing technology for microarray manufacture was developed by TeleChem International. TeleChem's Micro Spotting pins work like ink stamps, whereby sample solution is loaded into each pin, which can be customized to hold from 0.2-1.0 µl of sample solution (Fig. 4). The sample solution on the end of the pins is then brought in contact with the substrate. When the pins are moved away from the surface, the attractive force of the substrate on the liquid withdraws a small amount of sample from the sample

channel of each pin. TeleChem pins are available in a wide range of tip dimensions (http://arrayit.com), allowing the user to obtain spot sizes from 75-360 μm in diameter. An example array made with the TeleChem Chipmaker™ 3 pin is shown in Figure 5.

A variation of the pin printing process is the pin-and-ring technique developed by Genetic MicroSystems. This technique involves dipping a small ring into the sample well and removing it to capture liquid in the ring. A solid pin is then pushed through the sample in the ring and sample trapped on the flat end of the pin is deposited onto the surface. Complete details on this technique can be found in a later chapter of this book.

Summary

A comparison of the different printing technologies is shown in Table 1. Various parameters are shown relative to the different technologies. Many of the numbers are estimates for comparison purposes only and will depend on the specific application.

I. The Microarray Printing System

General Requirements

One or more of the above printing technologies can be used in an instrument for making microarrays. Putting a printing technology into a system requires both hardware and software infrastructures. There are several general requirements:

Robust: Must function for an extended period (>24h) without user intervention due to hardware or software errors.

Automated: Must process a large number of samples (3,000 to 10,000) with little or no user intervention.

High Precision: The microarray spots need to be highly regular with tight tolerances and duplicates slides need to have high reproducibility.

Hardware

Print head

The print head is the core of the system, being the device that transfers sample from the microplate to the slides. For non-contact, inkjet type printing, the dispense head consists of 4 to 16 channels or lines for dispensing. Each line is connected to a syringe pump for aspiration of the sample, prior to dispensing to the slide. For pin-based dispensing, the pins are held such that they can move freely up and down in the print head during contact with to the glass surface. Some configurations have the pin spring-loaded, though the preferable configuration allows the pins to move along the Z axis using gravity to return the pins to the resting position in the print head.

The spacing of pins or nozzles is determined by the source microplate configuration. Since most microplates contain either 96 or 384 wells, the center-to-center spacing is 9.0 and 4.5 mm, respectively. As will be discussed later, since the spacing of the nozzles or pins in the print head is much larger than the spacing of spots on the microarray, the spots typically do not directly map from the source plate to the array.

Plate and Substrate Handling

The microarraying system should have accommodations for both the source plates, 96 or 384 well plates, and the microarray substrate, typically 1" x 3" x 0.039" (25 mm x 76 mm x 1 mm) glass microscope slides. The source plate and slides are usually placed on a

platform that can both be addressed by the print head. Both the source plate and each slide must be held securely to permit accurate loading and dispensing of the sample.

The number of source plates will depend on the number of spots per slide. For example, to place 10,000 spots on a slide, the system will need to accommodate 104 x 96 well plates or 26 x 384 well plates. To accommodate this number of plates, three options exist: 1) manually place each plate on the printing platform 2), load each plate onto the platform using an automated plate handling system, or 3) arrange each plate on the platform that can be addressed by the print head. The first approach is simple but labor intensive, especially if the printing process takes more that 8 hours, the second approach is more automated but more expensive, and the last approach results in very large system (13.5 ft^2 for 100 microplates).

XYZ Positioning Stage

The XYZ positioning stage moves the print head relative to the source plate and the slides. All positioning stages have a certain level of positioning error. Generally, the smaller the error, the more expensive the positioning system. Several parameters of positioning stages are important when specifying the quality of a stage:

Repeatability. The error in moving from position A to a series of positions and returning to position A.

Accuracy. The error in moving from position A to position B, usually expressed in terms of +/- microns.

Resolution. The smallest step the stage is capable of moving.

Linear Velocity. The maximum speed (mm/sec) a stage can move in one dimension and maintain its position.

Positioning Feedback. The means for determining the current position of the stage.

In general, there is a trade-off between many of these parameters. For example, the maximum velocity is limited by the stage resolution; the greater the resolution, the slower the stage. The cost of the stage is related to many of these parameters. High resolution and high repeatability increase the price. Likewise, encoded stepper motors offer very high accuracy and positioning feedback, but are more expensive than non-encoded motors. Modification of the Biomek 2000 (22) provides an inexpensive solution for laboratories that need a functional microarray system.

Environmental Control

A variety of environmental conditions can affect microarray manufacturing. The two most important factors, humidity and dust, must be controlled to obtain the highest quality microarrays. Humidity must be controlled to prevent the sample evaporation from the source plates and from the sample channels of the pins during the arraying process. For plates configured in a stack, the plates act as lids for plate below to minimize sample evaporation. For the plate in use, if the printing time is long and the evaporation rate is high, then some sample evaporation will occur during the printing process. For example, if the cycle time for printing a set of spots (load sample, print on each slide, wash and dry pins) is 1 minute using 4 pins, then each 384-well microplate will require 1.6 hours to process. If evaporation is excessive during this time, the concentration of the DNA in the sample wells will increase

causing a gradual increase in the concentration of the arrayed DNA. Proper humidity control virtually eliminates sample evaporation.

Humidity control is usually achieved by use of a humidity chamber that encloses the arraying device. Humidity should be controllable over a wide range and have a feedback mechanism for maintaining the humidity at a pre-determined level. Relative humidity of 65%-75% is usually sufficient for most applications. Humidity >75% can be problematic because condensation can occur, leading to the wetting of metal parts of the positioning stage.

Dust contamination must be minimized or eliminated to create high quality microarrays. Dust from ceiling tiles, ventilation systems and the user can settle on the slide during the arraying process. This can lead to printing inaccuracies as well as to false readings during slide scanning (dust is highly fluorescent). A humidity chamber is usually sufficient to minimize contamination due to dust and particulate matter.

Instrument Control Software

The microarray printing process involves moving the print head to the sample plate, loading sample, making spots on the substrate, and washing the dispense head prior to the next sample. The control of this operation is accomplished by software that communicates with the arraying instrument. Software of this type can span the spectrum of easy-to-use (but inflexible), to difficult-to-use but extremely flexible. For most applications and end-users, a certain amount of software flexibility is required until microarray experimentation becomes routine and stable.

Sample Tracking Software

The microarray process involves moving a sample from a source plate to the microarray, hybridizing the microarray with probes, scanning the slide, and evaluating the spots. Sample tracking software is required to track the sample through this process such that spots on the array can be readily identified.

Practical Considerations

This section outlines the practical considerations of using Micro Spotting pins for printing microarrays onto glass slides. Although broadly applicable, these considerations apply primarily to Telechem ChipMaker™ pins used on a Cartesian PixSys PA Series Workstation.

Array Substrate

The substrate for printing the array must be rigid, amenable to surface chemistry modifications, and have low background fluorescence in the region of fluorescent dye excitation wavelengths. The most commonly used substrate is the 25 mm x 76 mm glass microscope slide, although a number of groups have begun exploring porous polymeric surfaces (polymers coated onto glass substrates) or plastic substrates for producing microarrays.

Surface Chemistry

The surface of the microscope slide must be treated prior to use for several reasons. First, a suitable functional group must exist on the surface for attaching the target DNA to the

glass. Target DNA will not attach to naked glass. Attachment prevents the target DNA from being washed away during slide processing and hybridization. Second, a hydrophobic surface will allow a higher printing density since the spotted sample (hydrophilic) will spread less on a hydrophobic surface than an untreated (hydrophilic) surface. Currently, two chemical functionalities are commonly available on glass slides. An amine or lysine coated slide is used for adsorption of DNA onto the glass slide (ionic interaction between the negatively charge phosphodiester backbone of DNA and the positively charged slide surface). An aldehyde functionalized surface is used to covalently attach amino-modified DNA onto the surface via reaction with free aldeyhyde groups using Schiff's base chemistry. In addition to chemistries that provide a two-dimensional attachment surface, DNA attachment can also be achieved by coating the slide with polymeric reagents such as thin layers of acrylamide.

Substrate Effects

The substrate can have large effects on the overall microarray experiment. Substrate materials with elevated intrinsic fluorescence will decrease the sensitivity of the assay. Poor surface treatment can lead to poor attachment of DNA to the slide. Non-homogeneous surface treatment will result in variations in the amount of attached DNA. Finally, residual material from the slide treatment step can lead to background fluorescence.

Pin Characteristics

The ChipMaker^{TM} and Stealth^{TM} pins from TeleChem contain a stainless steel shaft with a fine point. Machined into the point is a narrow gap that acts as the reservoir for

sample loading and spotting. The pins are mounted in a print head such that the pins float under their own weight when touched onto the substrate. Up to 64 pins, on 4.5 mm centers, can be mounted in the print head.

The pins have a loading volume of 0.2-0.6 µL and can produce spots ranging from 100 to 360 µm, depending on sample and surface properties. Given the fine structure of the pin, care must be used in handling the pins. Although they are robust when touched onto the surface in the Z-direction, movement of the pin across the surface in the X or Y directions may cause the tip to bend. Also, dragging the tip of the pin across a surface can result in clogging of the pin sample channel. In the event that a pin becomes clogged, it can be cleaned with an ultrasonic bath. It should be noted that extensive exposure to ultrasonic waves is not recommended since this may weaken the pin tips and compromise durability in a production setting.

Array Layout Options

Print Area

DNA samples must be spotted or printed in an area defined by the area detectable by a fluorescent detection device. For example, the ScanArray 4000 and 5000 confocal scanners (GSI Lumonics) can detect an area 22 mm x 72 mm whereas the ScanArray 3000 has a 22 mm x 60 mm scan area.

Print Head

The print head contains an array of holes, each holding a single ChipMaker™ or Stealth™ pin. The standard ChipMaker™ 2 print head holds up to 32 pins in a 4 x 8 array, wherease the ChipMaker™ 3 print head holds up to 48 pins in a 4 x12 array. The Stealth™

print heads hold either 32, 48 or 64 pins. Both the ChipMaker™ and Stealth™ print heads holds pin on 4.5 mm centers.

The printhead is oriented relative to the plate such that pin A1 corresponds to well A1 of a microwell plate. For a 384 well plate, pins may be placed in any of the positions for printing since the 4.5 mm pin spacing matches the 4.5 mm well spacing. For a 96-well plate (9 mm well spacing), every other position can be used (A1, A3, C1, C3, …).

Single Pin Printing

Printing arrays with single pins is the most straightforward type of printing, although it is the most time consuming. With single pin printing, a source plate can be directly mapped into an array. In other words, 384 samples from a source plate can be spotted as a 16 x 24 array such that the spot A1, B1, C1, etc. in the array corresponds to well A1,B1, C1, etc. of the source plate. This makes post-hybridization analysis trivial. Using a single pin and 0.25 mm spot-to-spot spacing, 21,120 spots (55 x 384-well plates) can be placed on a slide.

Multiple Pin Printing

Printing with more than one pin is faster than a single pin but requires more planning for array layout more sophisticated sample tracking. To spot with multiple pins, the pins are dipped into the sample wells to load the pins and then touched in unison onto the surface to create separate spots. If the pins are on 4.5 mm spacing, the first round of spotting produces spots on 4.5 mm spacing. The next rounds of printing are done by spotting with a small offset (100-400 µm) from the previous location. This permits maximum spot density but

requires de-convolution at the analysis stage to identify the sample spot. The following are possible configurations:

1 x 8 Pin Configuration (ChipMaker™ 2). If 8 pins are placed in row one of the ChipMaker™ 2 print head (4.5 mm centers) and used to sample column-wise from a 384 well plate, then an array containing 8 subgrids will be generated. As the arraying process proceeds, spots from Pin A1 will approach spots printed by Pin A2 (sample B1). With the appropriate choice of center-to-center spacing, the desired number of spots can be printed between pins. For example, if spots are placed on 0.28 mm centers, then 16 spots can be placed between adjacent pins (16 x 0.28 = 4.5). Sixteen is desirable since it is an even multiple of a 96- or 384-well plate.

1 x 12 Configuration (ChipMaker™ 3). If 12 pins are placed in row 1 of a ChipMaker™ 3 print head and used to sample row-wise from a 384 well plate, then more of the slide can be printed with spots. Using the same spot spacing as 1 x 8 configuration, the number of spots increases to 14,976 (78 x 192 array or 39 x 384-well plates).

4 x 1 Pin Configuration for 384 Well Plates. Another alternative is to place 4 pins in column A of a ChipMaker™ 2 or 3 print head. With this configuration, 64 spots can be placed in a column along the short edge of the slide. Continuing to spot in a left to right fashion with 0.28 mm offsets, a total of 240 columns can be printed to fill the entire print area. The resulting 64 x 240 array contains 15,360 spots (40 x 384 well plates).

4 x 8 or 4 x 12 Configuration. For maximum throughput, the ChipMaker™ 2 or 3 print head can be used with the maximum number of pins, 32 and 48 respectively. In doing so, the spots from one pin approach the other pins two dimensions. For 250 µm spacing, each pin can print a 16 x 16 array for 8,064 spots for the 4 x 8 configuration and 12,288 spots for the 4 x 12 configuration. If the spacing is decreased to 150 µm spacing, then the total features increase to 28,800 for the 4 x 8 configuration and 43,200 for the 4 x 12 configuration.

Pin Configurations for 96 Well Plates. For 96 well plates, a similar strategy is used but the pin density in the print head is reduced by two since these plates have 9 mm well spacing.

Printing the Microarray

Sample Loading

The DNA sample to be printed on the slides is usually placed in a 96- or 384-well plate. For best results, a flat bottom plate is used with enough sample to provide a 1 mm layer (4-6 µl) on the plate bottom of the microplate. This technique uses minimum of sample volume in each well. Dipping the pin into a larger volume of sample results in the absorption of sample onto the outside of the pin causing printing irregularities early in the printing process and requiring pre-printing to enable consistent printing.

Pre-Printing

If the sample volume exceeds >6 µl per well for 384-well plates, the pins must be spotted a number of times on a slide to create consistent spots. This "pre-printing" is necessary to drain excess sample solution from the exterior of the pin. In the example shown in Figure 6a, three pins were loaded from wells containing 10 µL of sample per well (384

plate) and spotted a number times to show the change in spot size as a function of spot number. As the data show, after 10 to 20 pre-print spots, the spotting becomes more consistent (Fig. 6a).

Loading the pin with a larger volume of sample (i.e. dipping the pin deeply into the sample solution) results in a larger number of pre-prints required to achieve consistent spotting. As shown in the Figure 6b, pin loaded from wells containing 35 µL of sample require a larger number of pre-prints to achieve uniform printing.

Reproducibility

Spot reproducibility is a measure of spot variation during the printing process. Spot variations can be due to mechanical differences between pins, slight variations in slide surface properties, and changes in the pin during the printing process (e.g. a pin becomes clogged with particulate matter). This variation was measured using four pins and printing a Cy3-labeled 31-mer printing onto five slides. The results shown in Figure 7 reveal slight variations from pin-to-pin and from slide to slide. As can be seen, each pin has a consistent volume but there is a slight difference between pins, presumably due to slight mechanical differences in the pins or different surface properties of the pin. For example, the coefficient of variation of spots among each pin across the five slides ranges from 9.3 (pin 2) to 12.1 % (pin 4). The variation of spots across each slide ranges from 7.1 (slide 2) to 14.4% CV (slide 5). The overall variation for the entire data set is 12.6 % CV (Fig. 7).

Spot Size and Density

The size of the spot deposited on the glass slide determines the number of spots that can be printed on a slide. Spot size is related directly to the volume of sample deposited on the surface. The volume is determined by a number of factors:

Surface and Solution Properties. The properties (surface energy, viscosity) of the slide surface, pin surface, and sample determine the how much sample will be deposited and how much the sample will spread once deposited. For example, an aqueous sample deposited onto a hydrophobic surface (e.g. aldehyde slide) will result in a much smaller volume and spot size than the same sample printed on a more hydrophilic surface (e.g. untreated slide).

Pin Contact Surface Area. The surface area of the pin determines the initial contact between sample or pin and slide. The larger the area, the larger the spot size. For example, the two different Chipmaker™ pins (CM2 and CM3) which contain approximately 100 μm and 75 μm tips, respectively, were tested and shown to produce spot sizes proportional to the sample/pin tip surface area (Figure 8).

Pin Velocity. Though direct contact between the pin and the substrate is not necessary for printing with Micro Spotting pins, most users choose to lightly touch the slide surface to correct for the unevenness of the printing surface. If pin surface contact is chosen as means of calibration, the speed at which the pin strikes the surface can have an effect on spot size if excessive speeds are used. If the pins tap the surface at high velocity (>20 mm/sec), fluidic inertia may force a large volume of sample out of the pin, resulting in a large spot. Tapping the pins on the surface may also lead to mechanical damage of the pin tips. Unlike tweezers and split pins, TeleChem Micro Spotting pins **do not** require a tapping force for printing. Lightly touching the pin to the surface like an ink stamp

produces small spots and extends the durability of the pins. An estimate of spot density, given a spot size and center-to-center (CTC) spacing, is shown in table 2.

Pin Washing and Sample Carryover

Efficient cleaning of the pins during the printing process is necessary to prevent sample carryover which would complicate the hybridization results. ChipMaker™ and Stealth™ pins are cleaned by dipping the pins into distilled water and then removing the wash water from the pins with a vacuum. Repeating this procedure three times reduces sample carryover to <1 part per 10,000. To measure the effect of pin washing on sample carryover, two 31-mer oligonucleotides were spotted onto aldehyde-containing microscope slides. One of the oligonucleotides (target-positive) was perfectly complementary to the fluorescent probe, whereas the second oligonucleotide (target negative) was non-complementary (Fig. 9A). After spotting and drying, the oligos were attached to the slide using Schiff-base chemistry, hybridized overnight with Cy3-labeled positive probe and scanned. The resulting array is shown in Figure 9b. As can be seen, no carryover of the target-negative oligonucleotide can be detected.

Carryover can also been measured by direct spotting of Cy3 or Cy3-labeled oligonucleotide and a blank solution onto slides and comparing the results. Although a small but measurable amount of carryover is seen at the highest sensitivity settings of the scanner, this level is not directly applicable to DNA because the oily Cyanine dyes are more difficult to clean from the pins than unlabeled oligonucleotides or cDNAs.

Throughput

Printing throughput should be measured as total cycle time, a cycle being loading the pins, pre-printing, printing arrays, and washing. Table 3 lists some printing time estimates.

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Tables and Table Legends

Table 1. Comparison of printing technologies.

Parameter	**Printing Technology**		
	Piezoelectric	**Syringe-Solenoid**	**Micro Spotting Pin**
Minimum sample volume[a] (µl)	20-50	20-50	5
Loading volume[b] (µl)	5-10	5-10	0.2-1.0
Print volume (nL)	0.05-10	4-100	0.5-2.5
Spot size (µm)	125-175	250-500	75-360
Spot density (spots/cm^2)	500-2500	200-400	400-10,000
Programmable volume	yes	yes	no
Number of nozzles or pins	4-8	8-16	1-64
Delivery speed (spots/sec)	100-500	10-50	64
Simplicity	X	X	XXX
Robustness	X	XX	XXX
Cost per Spot	$$$	$$	$

a Volume of sample in the 384-well source microplate

b Sample volume of the dispensing device

Table 2. Microarray density.

Spot Size (µm)	CTC Spacing (µm)	Spots/cm^2	Total Spots (18 mm x 72 mm)
75	113	7,901	104,296
100	150	4,444	58,667
150	225	1,975	26,074
200	300	1,111	14,667
250	375	711	9,387
300	450	494	6,519
400	600	278	3,667
500	750	178	2,347

Shown are the densities (spots/cm^2) and total number of spots possible on a single microscope slide with an 18 mm x 72 mm print area, given a spot size and center-to-center (CTC) spacing given in microns (µm).

Table 3. Printing time estimates.

Step		**Time**	
	1 pin	**8 pins**	**32 pins**
Load pins with sample	2 sec	2 sec	2 sec
Pre-print 10 spots	4 sec	4 sec	4 sec
Print 48 slides	48 sec	48 sec	48 sec
Wash pins	6 sec	6 sec	6 sec
Total time for 1 cycle	1 min	1 min	1 min
Total time for 1 x 384-well microplate	6.4 hrs	0.8 hrs	0.2 hrs
Total time for 40 x 384-well microplates (15,360 spots)	256 hrs	32 hrs	8 hrs

Shown are microarray manufacturing specifications for the PixSys 5500 (Cartesian) using ArrayIt™ ChipMaker™ 2 Micro Spotting technology (TeleChem).

Figures and Figure Legends

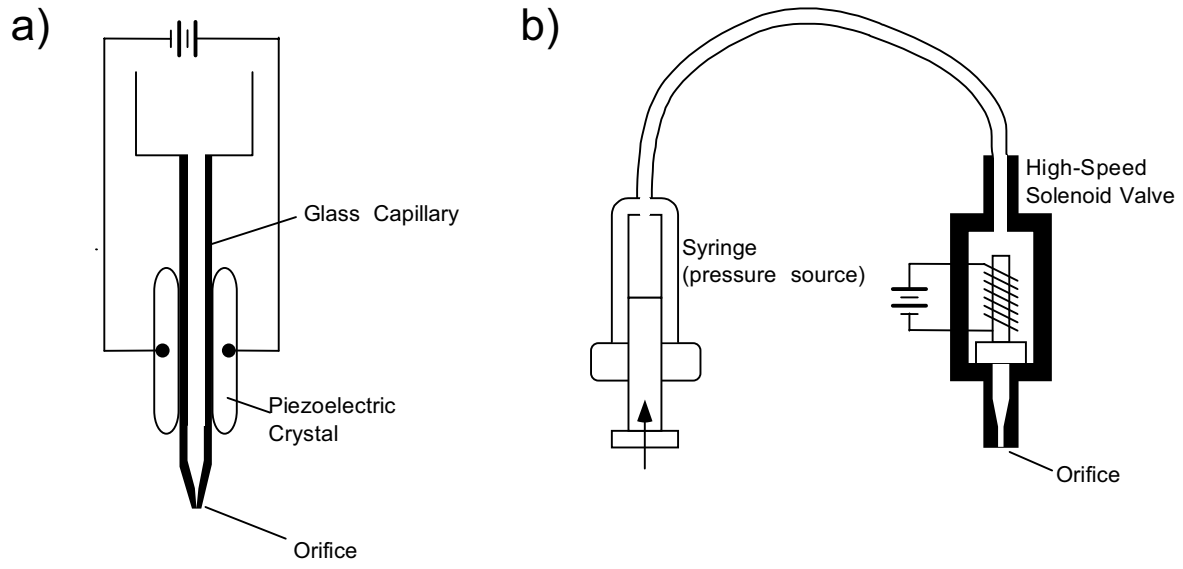


Figure 1. Types of non-contact inkjet dispensers a) Piezoelectric dispenser shown with piezoelectric crystal surrounding a glass capillary, b) syringe-solenoid inkjet-type dispenser shown with a high-resolution syringe pump coupled to a high-speed solenoid valve.

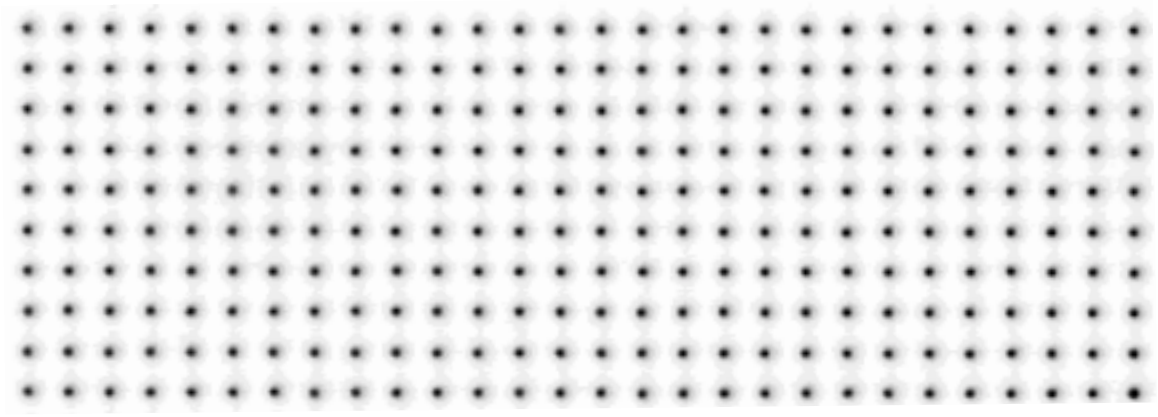


Figure 2 . Array of ^{33}P-labeled material made with a single channel piezoelectric dispenser. Spot spacing is 750 µm, spot size approximately 145 µm (Courtesy of Packard Instrument Company).

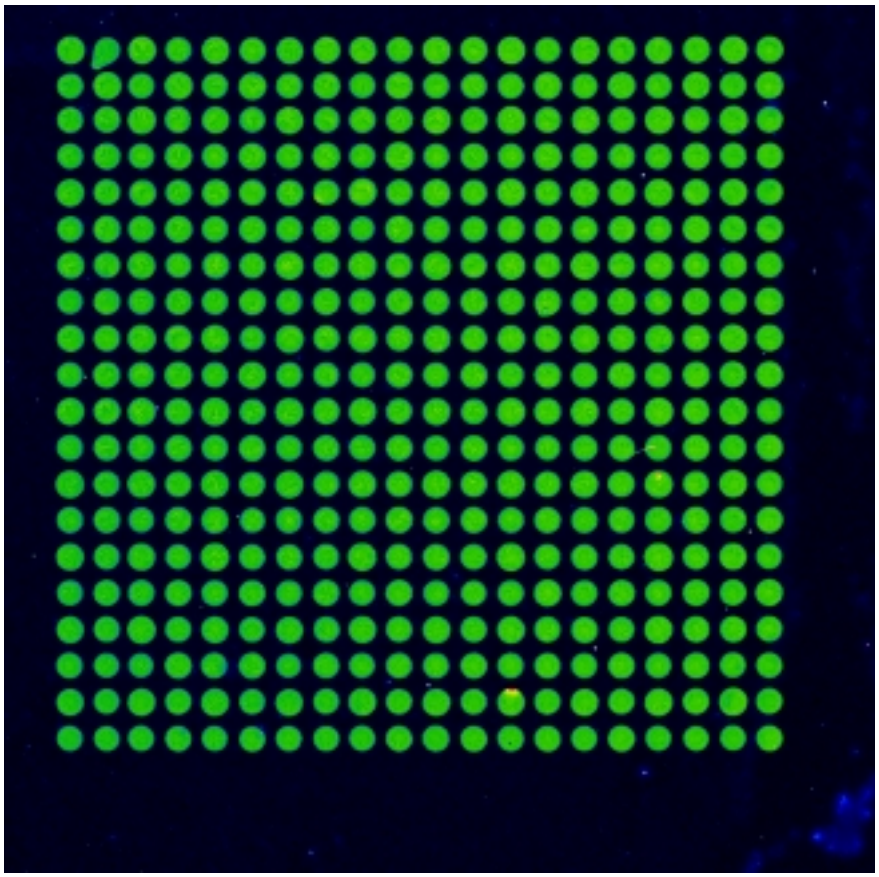


Figure 3. Array of 4.2 nL spots of Cy3 made using an syringe-solenoid type of dispenser. Spot spacing is 500 µm with spot size approximately 325 µm.

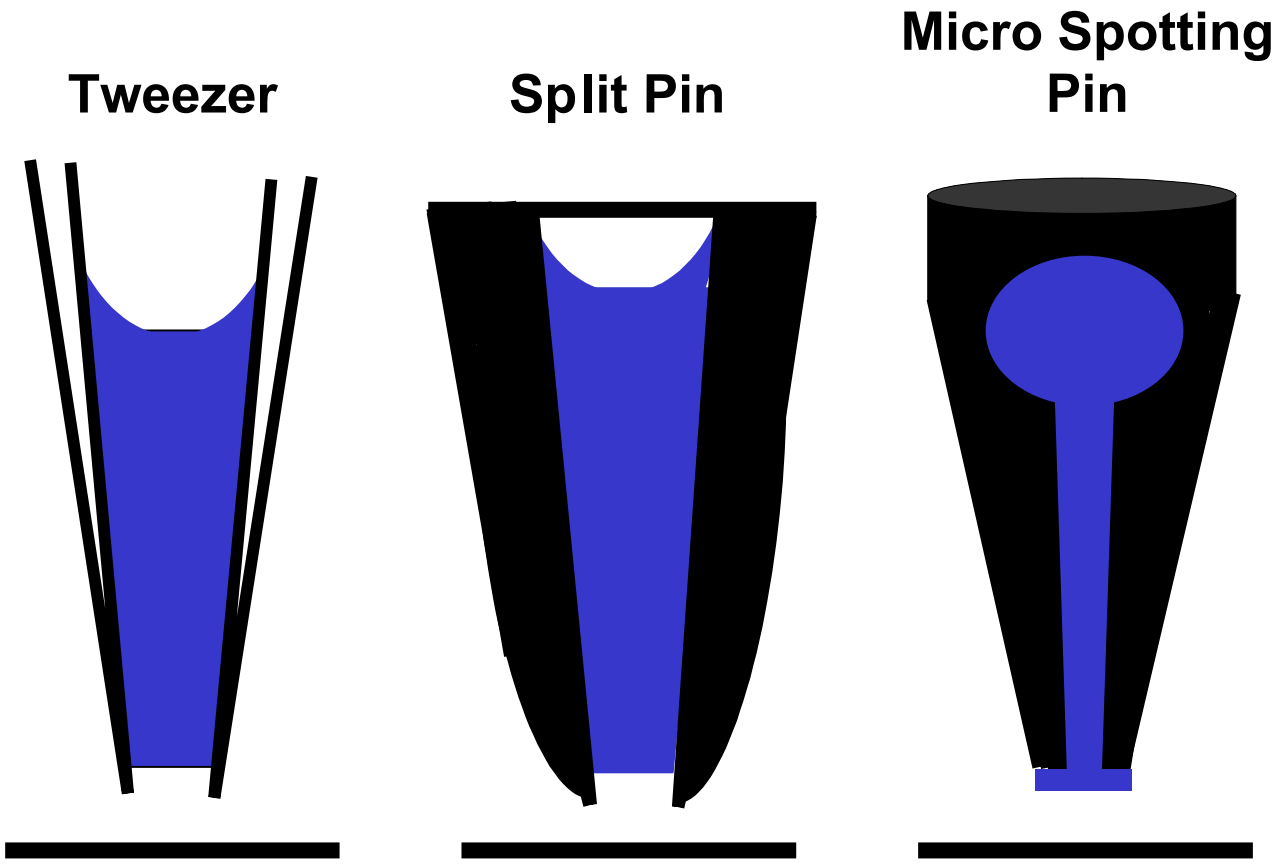


Figure 4. Schematic drawings of contact printing dispensers. The tweezer, split pin and TeleChem's ArrayIt™ brand Micro Spotting pin are shown loaded with sample solution (blue). Tweezers and split pins require a tapping force move sample down the channel onto the surface. TeleChem pins work more like ink stamping devices, whereby sample on the end of the pin is brought in contact with the surface and the substrate pulls a small amount of sample out of the fluid reservoir when the pin is moved away from the surface.

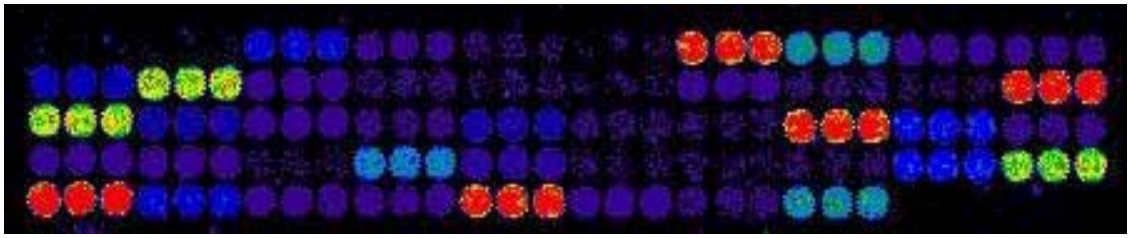
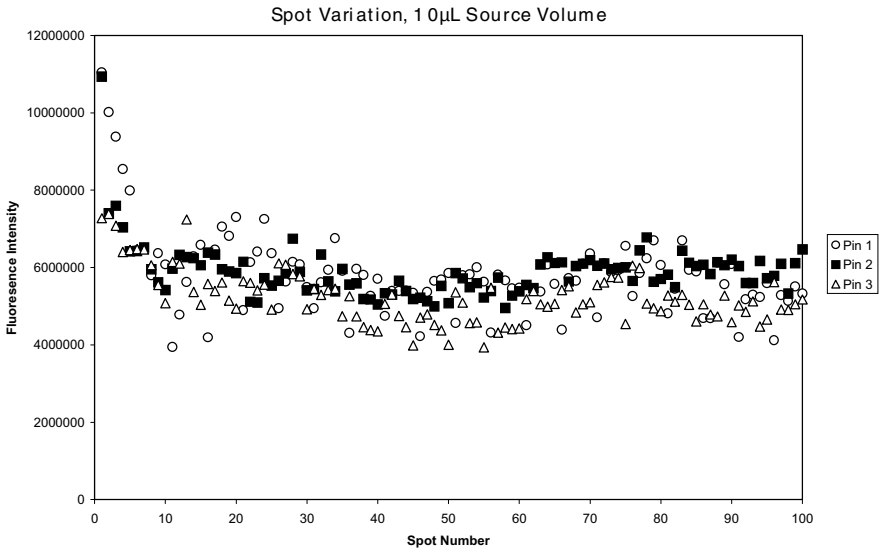


Figure 5. An example of a printed and hybridized microarray made with TeleChem's Micro Spotting pin technology (Chipmaker^{TM} 3 pins); spot spacing, 140 µm; spot size, approximately 125 µm, with targets spotted in triplicate. Fluorescent image generated with a ScanArray 3000 (GSI Lumonics).

a)



b)

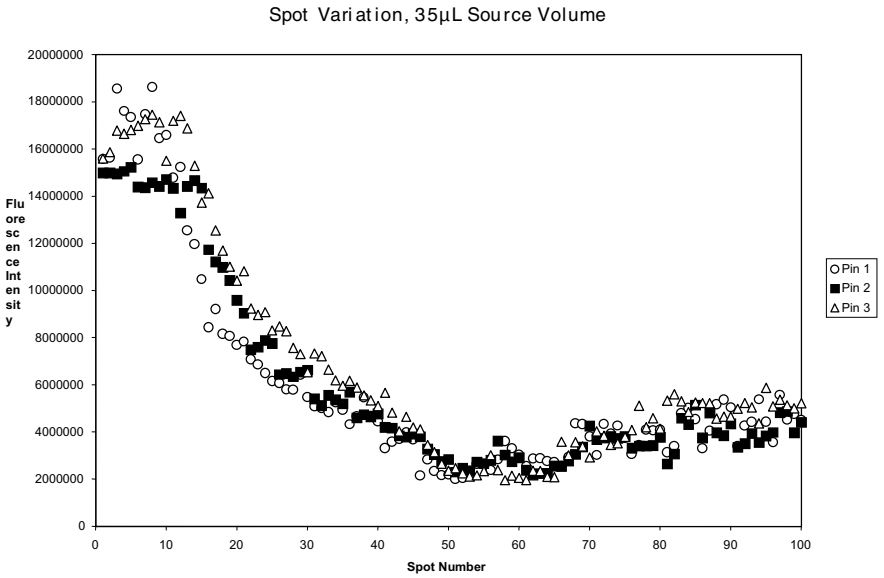


Figure 6. Spot variation as function of spot number for a) 10 µL of sample per microwell and b) 35 µL of sample per microwell. The sample was a Cy3-labeled 31-mer in 1X Micro Spotting solution (TeleChem) in square-well, flat-bottom 384 well plate. The procedure used a PixSys 5500 gridding robot (Cartesian) equipped with four Chipmaker 2 pins (TeleChem) printing on CSS aldehyde-modified glass slides (CEL Associates) with the following steps: 1) dip the pin into the sample such that the pin contacts the bottom of microplate, 2) print 100 spots on one slide (with no pre-prints), 3) wash the pin with three water dip and vacuum wash cycles, 4) scan the slide with a ScanArray 3000 (GSI Lumonics), 5) calculate fluorescent intensities by integrating each spot.

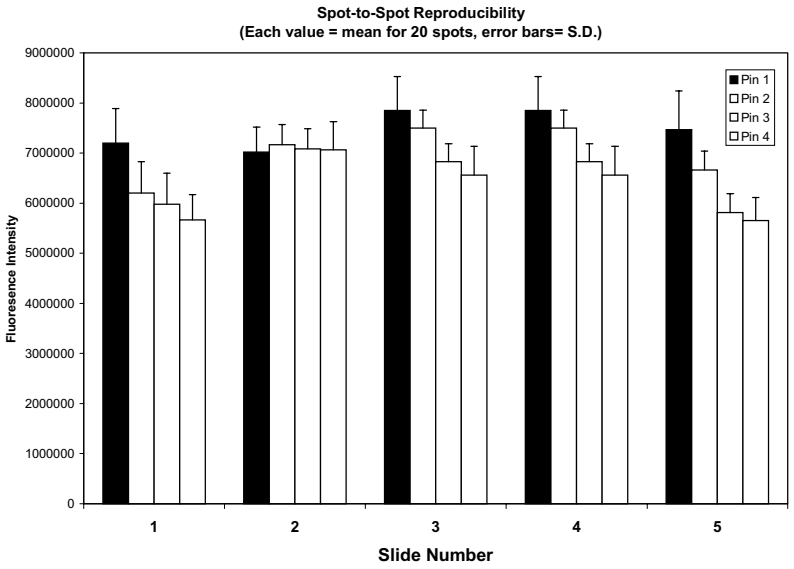


Figure 7. Variation of spot intensity as a function of four different pins and five slides (each bar represents an average of 20 spots). Overall CV (standard deviation divided by the mean, times 100) for data set is 12.6%. Experimental conditions: Sample, Cy3-labeled 31mer in 1X Micro Spotting solution (TeleChem); Procedure: 1) dip pin, 2) pre-print 20 spots, 3) print one spot per pin per slide for five slides, 4) dip and vacuum wash, 5) repeat with same sample to produce 20-spot array, 6) scan with ScanArray 3000, 7) integrate spots, 8) compute CV for 20 spots.

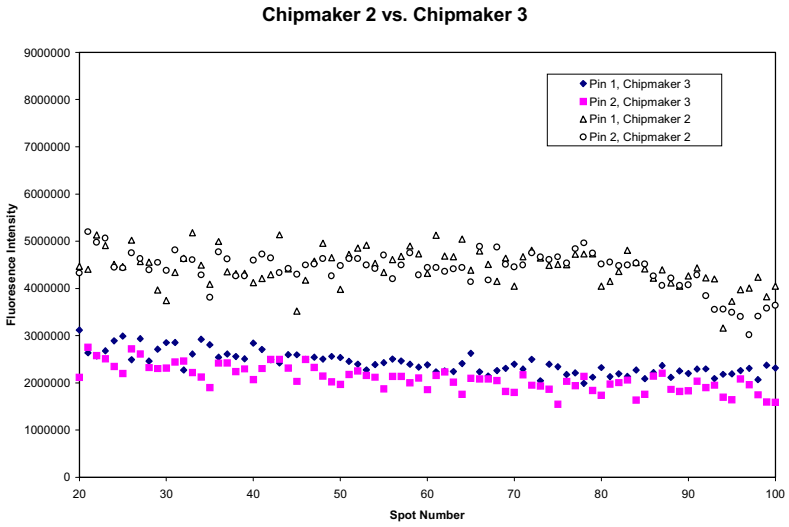
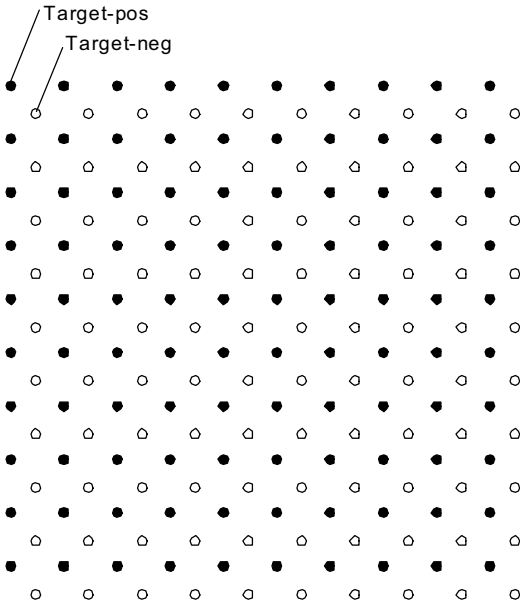


Figure 8. Spot size as a function of spot number for two different size TeleChem pins. Experimental conditions, same as Figure 7, except 30 pre-prints prior to printing.

a)



b)

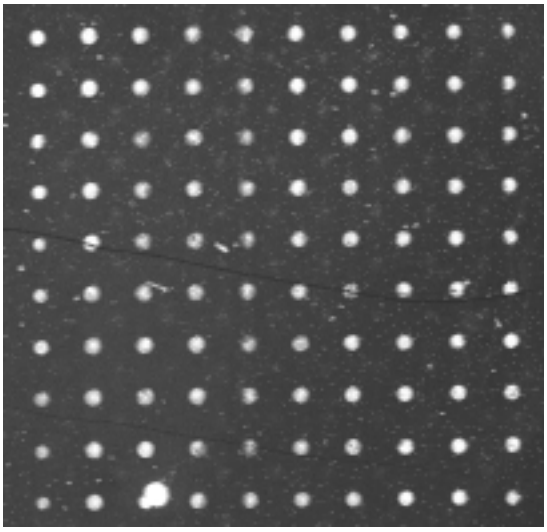


Figure 9. Effect of pin washing on sample carryover. a) schematic layout of two test 31-mers printed on aldehyde (silylated) slides. One oligonucleotide (target-pos) was 100% complementary to the fluorescent probe, whereas the second oligonucleotide (target-neg) was non-complementary. The microarray was printed as follows: print 10 spots of the target-pos oligonucleotide, wash pin twice (dip into water, vacuum dry, dip, dry, dry), print 10 spots of the target-neg oligonucleotide, wash pin twice (dip into water, vacuum dry, dip, dry, dry), repeat until a total of 400 spots are printed. After spotting and drying, the oligos were attached to the slide using Schiff's base chemistry, hybridized overnight with Cy3-labeled positive probe (complimentary to the target-pos oligo), washed, and scanned three times with a ScanArray 3000 (GSI Lumonics) set at 100% laser power and 80% PMT.