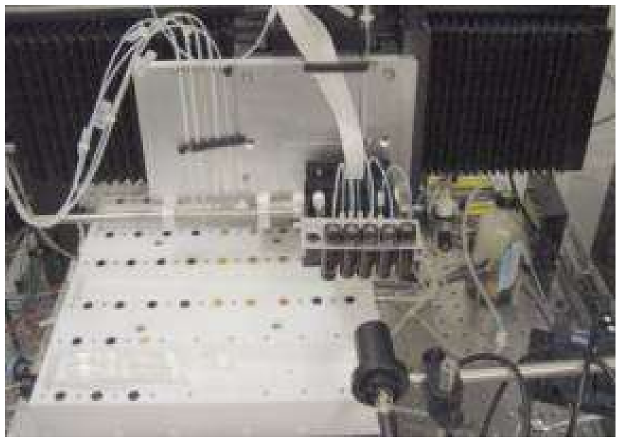
Assembly manual for the POSaM:

THE ISB Piezoelelctric Oligonucleotide Synthesizer and Microarrayer



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INTRODUCTION

We all know that, despite their inherent problems, microarrays have become an important tool in any lab that wants to do cutting edge molecular biology. If you didn't think that the technology was important, you wouldn't be reading this, so we'll will try to keep the rhetoric to a minimum and just tell you what you need to know to build and run the instrument we call POSaM, the Piezoelectric Oligonucleotide Synthesizer and Microarrayer. We will try to give you a bit of the history of how the instrument came to be, and how it turned out the way it is at this time. Hopefully, those descriptions can be helpful and maybe even amusing. We also hope that those of you who actually build one of these will take part in the development of a better instrument and new methods to take advantage of its speed and flexibility. In this respect, we are following in the steps of Brown, DeRisi, and their co-workers at Stanford, who made their pin-spotting device available to the scientific public back in the 90s. We feel that the early release of these plans helped speed the development the instruments and methods that are now used, and that de novo synthesized array development has suffered by not having an open source model to build on.

Currently microarrays come in several flavors: cDNA arrays, PCR DNA arrays, BAC arrays, and oligonucleotide arrays that are constructed using pre-existing libraries of characterized DNA molecules. These are usually pin spotted onto glass slides or membranes, although some non-contact printing systems are available. One of the problems with these arrays is that you have to collect all those clones, amplify them, and store them in freezers where they can be collected by a dedicated grad student, technician, or post-doc before they can be printed onto the glass slides. That means that you have to have a complete library for every organism you want to study stored in a freezer somewhere in your lab area, and then you have to keep track of where every clone is in that library. If you want to expand the array to include new genes, you first have to acquire physical copies of those genes. Any of you involved in the Human Genome Project know that this is really a nightmare of book keeping and lots of mistakes can happen as you are getting that well-characterized clone onto a microarray in the spots you want it in. (SPOTS, not singular, you want replicates. You need replicates. The more replicates the better, both of the specific gene and the experiment itself.) There is also a fair amount of cost, both initial and continuing, that goes along with acquiring, replicating, storing and retrieving these libraries.

Wouldn't it be nice to be able to do without them? Of course. That's one of the reasons that Afffymetrix started making in situ synthesized oligonucleotide arrays. They could store all their sequences in a computer file, and we all know that computer storage has come down in cost faster than the processors have gone up in speed. It costs essentially nothing to store and recall a virtual library of sequences from the RW memory of a computer. Of course this has some problems as well, particularly in designing the appropriate reporter to unambiguously identify each of the genes you want to measure, but we'll get to those. Affymetrix has now had more than 10 years to develop their product and they haven't been sitting still on their technology. There have been lots of reviews and comparisons of Affy arrays and others [Islam et al., 2002; Kuo et al. 2002; Li et al., 2002; Relogio et al., 2002; Rogina et al., 2003;] and you should read some of them. But one of the problems, in our eyes anyway, is that the chemistry used by Affy (photocleavage of the protecting groups on the dNTP monomers), is not all that efficient, so the oligos that can be synthesized are relatively short–20 to 25 bases at the maximum. It turns out that it takes several to many 25 mers to unambiguously identify any specific gene, which means that instead of 30K -40K reporters to cover the genome, it takes 10 to 20 times more reporters or 300 to 600 thousand different reporters to identify all the genes expressed in a genome. Until recently, even the photolitographic technology used by Affy could not put that many features on a single slide. Their new masking technologies may be able to put a whole genome on a slide now, however, since you have to make 4 new masks for every position in an oligonucleotide, it gets very expensive to change an array, and it also takes time to design and manufacture these new masks. That really takes this technology out of the reach of most academic labs (Xeotron and Nimblegen are now using dynamic micromirror displays for maskless photolithographic oligoarray synthesis, but these are also unavailable to most labs).

Back in the 1990's, Blanchard and Hood demonstrated at the University of Washington that off-the-shelf inkjet parts might be used to deliver standard phosphoramadite chemistries to the modified surface of a slide in order to synthesize new oligoarrays very rapidly and inexpensively. Blanchard and Hood actually put together a prototype inkjet arrayer that was used as an asset during the formation of Rosetta Inpharmatics. Rosetta (and then Agilent) went forward and developed an elegant inkjet array synthesizer based on these original plans, but, because this is a commercial instrument, it isn't simple enough to be constructed or maintained by a regular molecular biology lab. In addition, it has taken more than 5 years of development to get a catalog of inkjet arrrays to the end-user. This, we feel, has held up development of new methods that can take advantage of the speed and flexibility inherent in this design. So we (the Hood Laboratory, now at the ISB) decided to develop an inkjet arrayer that almost anybody can build and operate. That is where the POSaM platform came from. We were not trying to compete with the throughput of the industrial-size Agilent/Rosetta arrayer. It would cost too much to build that into a machine for the end users (at this stage at least). We just wanted to make an instrument that could be built and used by anybody interested so that new methods and designs could be investigated to make better in situ synthesis array–much as the release of the plans for the pin-spotter did for more traditional arrays.

We are not guaranteeing this instrument or the arrays it makes. It is an academic instrument that works fairly well. It is good for prototyping arrays and designing and testing reporters. You might want to start the design for your arrays on this machine and, when they work the way you want, have Agilent or Nimblegen or Xeotron or Affymetrix make them for you in large enough quantities and in high enough quality that you can do all your experiments. You could also use the arrays from the POSaM platform itself and not order commercially made arrays. That is up to you and how comfortable you feel with the results you have gotten. Presently, we feel most array results need to be confirmed using different methods anyway. The bottom line is that you (any good molecular biology lab with some organic chemistry experience and a little engineering help) can build and run this instrument. You can make arrays and test methods. And you can do it fast and inexpensively. We generally make 4 to 6 10K feature arrays of 40mer oligos overnight, deprotect them the next morning, hyb, wash and scan them during the day, and have some results within 24 hours of putting the slides on the machine. We figure the cost is less than $80 dollars per slide, including labor costs. That's quick and cheap, and quickness and cheapness are good when you are designing something new. You can test lots of different reporters and protocols and not bust your budget or have to wait weeks or months to do it. You need to keep in mind that the printing element used in the POSaM was developed for the low-cost commercial color printing market where 100% reliability is not expected, therefore the matter of quality control is very important.

This is the technical and operational manual for the POSaM inkjet oligonucleotide synthesizer. POSaM is open source and licensed under GNU type licenses. The idea is for the community to help make a better machine and better methods by working together using a design funded by the NIH, and therefore the public and we believe that instruments developed with public funds should be available to the public. If you have questions or problems, call us at 206-732-1276 or email us at posam@systemsbiology.org. We actually have some funds for user support and will be happy to try to answer your questions so we can make a better machine together. Note that this is the first edition of the user manuals and--while it makes perfect sense to us--we are familiar with the instrument. It is possible that some of the instructions are really as clear as mud to everybody else; we are counting on your input to make this a better document.

SYSTEM OVERVIEW

Here is a quick overview of how we built the arrayer. We tried to use off-the shelf components wherever possible and we tried to keep the size reasonably compact. As a very rough estimate, it can take one person three months to assemble this machine. This is based on the time spent by students here on our second machine and includes purchasing lead time.

Overview of the arrayer.

The arrayer is build around a three-axis servo positioning system mounted to an 89x119cm optical table and enclosed within a sealed acrylic cover. Array substrates are chucked into a Teflon slide holder mounted to the Y-axis stage. A piezoelectric print head and six valved wash nozzles are mounted to the X- and Z-axis stages which move above the slide holder. Reagent and waste bottles also remain inside the cover. Servo amplifiers, the servo controller (6K4), power supplies, the supervisory computer (PC), and most electronics are packaged in a 19" rack outside the cover. All processes of the arrayer are directly controlled by the PC except the basic motion control functions that are handled by the 6K4.

The Epson™ F057020 print head (available from Agson Electronics, Cherry Hill, NJ) print head is uniquely suited to inkjet microarraying. It contains six fluid channels that can hold the four standard monomers, the catalyst, and still accept a modified monomer, monomer mixture, or preformed linker. It contains 192 total nozzles. The droplet size quoted by the manufacturer is a mere 6 pL. Droplet size varies with viscosity and surface tension, and consequently temperature, but based on solvent consumption for the work described here, our droplet sizes are closer to 10 pL. The newer Epson print heads actually have more nozzles that squirt smaller volumes (4 pL). More nozzles can result in faster synthesis. These tiny droplet volumes (4-10 pL) enable high spot densities and make consumption of reagents hard to measure, but that keeps the costs down.

Most importantly, the F057020 is a piezoelectric print head so it can dispense a fairly wide range of solvents. A charge applied across a lead-zirconia-titanate (PZT) crystal deforms the crystal and bends a zirconia diaphragm near the nozzle orifice (Le, 1999). The action of the diaphragm ejects a droplet from the nozzle. Solvents of almost any boiling point can be ejected. Oligo synthesis requires a solvent of very low volatility so that the coupling step can complete before significant evaporation occurs. The thermal print head used in bubble jet printers is more popular in the home/office printer, however, it is only suitable for fairly volatile solvents. It uses a small heating element in the capillary to vaporize solvent and create a bubble. The rapid expansion of the bubble ejects a droplet. Although they are unsuitable for de novo array synthesis, thermal or "bubble" jets have been used successfully to deliver aqueous solutions of oligonucleotide and of protein for microarraying (Stimpson et al., 1998; Roda et al., 2000).

Servo control.

The three-axis positioning system utilizes Parker-Daedal™ 506-series ball screw linear actuators driven by NEMA SM23 servo motors. The motors are, in turn, powered by GV digital servo amplifiers on command from a 6K4 multi-axis motion controller. The controller interfaces to the PC by Ethernet triggers the actual jetting through one of its eight digital output lines. This system can move the print head over a wide 50x60 cm range with 5 micron repeatability.

Microarrayer electronics.

Circuit schematics for the print head, solenoid, and droplet detection interfaces appear in Figure 3. Two PCI interface boards, the DIO-32HS and the MIO-16E are used (National Instruments, Austin, TX).

The F057020 print head requires three data, one clock, one latch, and one piezoelectric drive signals. Each of the three data lines programs two banks of 32 nozzles. Data is sent serially in 64-bit words to the nozzle selection register and latched with a single pulse of the latch signal. The clocking frequency used here is 500 kHz, though 3 MHz is possible. Digital signals are TTL-compatible and active high. The piezoelectric drive signal resembles a trapezoidal waveform, rising from 0V to 28V in 5μs, holding at 28V for 5μs, stepping up to 30V for 10μs, and falling back to 0V linearly over 20μs. Each waveform pulse results in the ejection of one droplet from each selected nozzle. Pulse frequencies as high as 14.4 kHz may be used. The digital signals are transmitted directly from DIO-32HS output port D. Unused digital lines in port D allow for an additional print head to be added, if needed. The waveform output is provided by output port C and converted to analog by the DAC0802 integrated circuit (IC). These two 8-bit ports are configured as a single 16-bit output port referred to, in software, as Group 1.

The six PTFE solenoid valves (Model 190224S30, Angar, Florham Park, NJ) used to control reagent flow are normally closed and require 24V to open. Three digital output lines of DIO-32HS port B are used to select the active solenoid via the 3-to-8 decoder IC. The solenoid driver DRV101 is a low-side power switch employing a pulse-width modulated (PWM) ouput. The driver provides a strong initial 100 ms pulse to open the solenoid valve, followed by a PWM square wave with a 20% duty cycle to hold the solenoid open without generating significant heat. During oligo synthesis, only one solenoid need be open at any given time. The two solenoid valves (Model ET-2-6-H, Airtronics, Bellevue, WA) controlling nitrogen flow are normally closed and require 3V to open. Two output lines of the MIO-16E digital port are used to activate the two TTL-compatible relays that power the solenoids.

Proper function of each of the 192 piezoelectric nozzles is verified by the laser droplet detection subsystem. A red laser diode is mounted orthogonally to the direction of print head motion such that the droplet stream of each bank of nozzles can intersect the beam. The print head is positioned 10 mm above the beam. Nozzles are fired in series through the beam and the forward scattering of each droplet is detected by the photodiode. A red bandpass filter is used with the photodiode. The output signal is amplified, highpass filtered, and converted to a digital signal by threshold comparison. Nozzles fail due to bubbles or plugging and are taken offline during synthesis. The 10 mm distance is chosen so that partial nozzle occlusion, which deflects droplet trajectory, also registers as a nozzle failure.

***Phosphoramidite delivery*.**

The six fluid channels of the inkjet print head each contain a needle inlet, a filter, an ink cavity, and a nozzle plate. The needle inlet normally punctures a protective membrane beneath the ink cartridges. The volume between the inlet and the nozzle is approximately 25 µL. In the design presented here, two multi-well ink cartridges are replaced by six glass vials. The vials are 1 mL, clear, conical Reacti-vials, and capped with silicone/Teflon septa. Thick-wall Teflon tubing, 15 cm in length, conducts the phosphoramidite or tetrazole solutions from the vials to the needle inlets. The tubing connects to the needle inlets with 1.6 mm I.D. PharMed® tubing and to the vials with 20 gauge non-coring stainless steel needles, 5 cm in length. The vials are at neutral pressure during printing.

Reagent/solvent storage

Reagents and solvents are stored in 500 mL glass bottles with GL45 screw-top caps, except for the 2 L acetonitrile and waste bottles. All tubing and bottle cap surfaces exposed to chemical vapors or liquid are Teflon. Pressurizing nitrogen enters the reagent and solvent bottles through PTFE check valves (Vari-bore type, Omnifit, Rockville Centre, NY) that have a 5 torr (0.1 psi) cracking pressure. The waste bottle is negatively pressurized with 380 – 650 torr (15 – 25 inHg) vacuum.

Nitrogen supply and system enclosure.

Inert gas and pressurization is supplied to the system by a liquid nitrogen dewar. Regulators are set to 50 psi for the inert atmosphere supply, to 30 psi for the slide drying jet, and to 3 psi for the reagent/solvent reservoirs. The microarray area is enclosed with a custom acrylic cover with a 89 x 104 cm footprint and an internal volume of approximately 510 liters. The cover is transparent and airtight. It has a single access door providing a 40 x 70 cm opening. A glove panel also allows the operator to work inside the enclosure without opening the access door. The high pressure inert gas is controlled by a 0-100 lpm valve/rotameter (Mini-Master type, Dwyer, Michigan City, IN). This gas supply serves dual roles: it displaces the air inside the enclosure and it powers the “air amplifier,” (1.5” Conveyvac, ARTX, Cincinnati, OH) our internal circulation apparatus. The apparatus circulates the internal atmosphere through a series of activated charcoal and desiccant filters.

The system is flushed with nitrogen at a 40 lpm flow rate for 40 minutes prior to oligo synthesis. Assuming good mixing of inert gas with air, the time constant (τ) for the flushing process would be approximate 7.9 minutes. The observed τ is slightly shorter, τ =5.6 minutes for oxygen depletion because much of the internal volume of the enclosure is occupied by hardware. The air amplifier circulates gas in a horizontal loop. The nitrogen fills the enclosure from bottom to top. Drying takes a little more time than oxygen depletion, τ = 6.8 minutes. While the use of desiccant accelerates the process, much water is adsorbed to the surface of the hardware if the enclosure door has been open for a significant period of time. Relative humidity was measured using a capacitance hygrometer (Model 4187, Control Company, Friendswood, TX; this has since been replaced by the smaller Humirel HM1520) and oxygen was measured using an Oxy-Plus electrochemical device (Brandt Instruments, Slidell, LA).

***Slide holder*.**

The slide holder was custom machined from solid 30 x 30 x 5 cm block of PTFE. Slides are arranged in three rows of nine with a center-to-center spacing of 31.75 mm (1.25”). The slides are secured by vacuum. Two 9.52 mm (0.375”) 0-rings form a gas-tight contact the bottom surface at the ends of the slides. Three inclined channels underneath the slides collect the reagent wash waste. (While this design works for us, it could be improved. We rarely synthesize more than eight microarrays at a time. A redesign of the slide holder is planned for the near future.)

GETTING STARTED

BEFORE BEGINNING ASSEMBLY, CONSIDER THE LOCATION OF THE ARRAY MACHINE. ALTHOUGH WE HAVE ATTEMPTED TO KEEP THE PHYSICAL DIMENSIONS OF THE MACHINE REASONABLE, IT REQUIRES A FAIR BIT OF OPEN FLOOR SPACE. TO BE ACCESIBLE FROM ALL SIDES, PROVIDE AN AREA AT LEAST 7 FEET BY 9 FEET. THE MAIN TABLE IS AN OPTICAL BREADBOARD 4 FEET WIDE BY 3 FEET DEEP. WE PACKAGED THE ELECTRONICS AND THE SUPERVISORY PC IN A 19-INCH FREE STANDING RACK ABOUT 2 FEET WIDE AND 2 FEET DEEP.

Consult with your facility manager about providing electricity, high-purity nitrogen, vacuum, and fume ventilation. The electrical needs are easily met with a 120 volt, 15 amp connection. A nitrogen dewar may be placed adjacent to the POSaM, but that requires additional floor space. We recommend placing two or more nitrogen dewars in a separate room and plumbing the inert gas into the arraying room. The dewars can be connected by a manifold which will automatically switch between tanks, minimizing waste. Our facility-wide vacuum system provides a reliable 15-20 inches of water negative pressure. If this is unavailable to you, consider a dedicated vacuum system. It is also useful to locate the POSaM near a fume hood, but the POSaM requires its own ventilation plumbing. Provide a 1.5 inch exhaust ventilation pipe and set the flow rate above 80 liters per minute.

Numerous small tools and fittings are required by this project. While we have tried to be detailed in our parts listings, it is likely that some small items are not listed but are commonly available around the laboratory or at the local hardware store. Useful tools include adjustable wrenches, combination wrenches, metric and English hex drivers, slotted and Phillips drivers, a hack saw, a file, a “Dremel” -style rotary tool, a sheet metal nibbler, a drill, a level, soldering irons of 15 and 35 watt size, and a multimeter. Useful supplies include wire (14 gauge, single and multiple strand), heat-shrinkable tubing, wire labels, drill bits, cap screws (¼ inch with 20 threads per inch, or “quarter twenties” of various length), a screw kit of various sizes between #4 and #8, and corresponding washers and nuts.

We have attempted to use commercial, off-the-shelf, components wherever possible. Nevertheless, some of the components must be custom-fabricated. For the print head bracket and the slide holder, this requires the services of a machine shop. For the cover, we use the services of Clear Cut Plastics of Seattle, Washington, although any local plastic fabrication shop should work. CAD drawings are available for downloading. For the printing of the three circuit boards, we use the services of ExpressPCB (www.expresspcb.com). These boards may be purchased online and delivered within the week, ready for soldering.

ASSEMBLING THE ARRAYER

[1] Assemble the table

Unpack and assemble the leveling stand. Set the optical breadboard on the stand. (This will require at least two people.) Place a level along the long direction of the table (from this point on, the “X” axis) and adjust the leveling screws. Place the level along the short direction of the table (the “Y” axis) and, again, adjust. Place the electronics rack next to the table, preferably to its right. (We previously had a short electronics rack that fit underneath the arrayer. The PC sat on a table nearby.)

Figure 1. We recommend placing the electronics rack to the right of the table. This allows the operator to access the front door and the gloves on the left.

[2] Place servo positioners

Place the servo positioners on the table using the coordinates given in Figure 2. First, mount the two vertical brackets using four screws in positions A-D. Fasten the Y axis positioner (the 24-inch stage) to the brackets with four screws. Attach the Z axis positioner to the moving face of the Y axis positioner with four screws. This will require turning the Z axis lead screw by hand a few times each direction to expose the mounting holes. The motor mount of the Z axis positioner points upward. Place the X axis positioner (the 20-inch stage) on the table underneath the YZ axis assembly. Elevate the positioner one inch above the table surface using eight one-inch aluminum spacer bushings. Fasten the positioner down with eight screws. Attach the SM23 servo motors to the positioners according to the manufacturer's instructions.

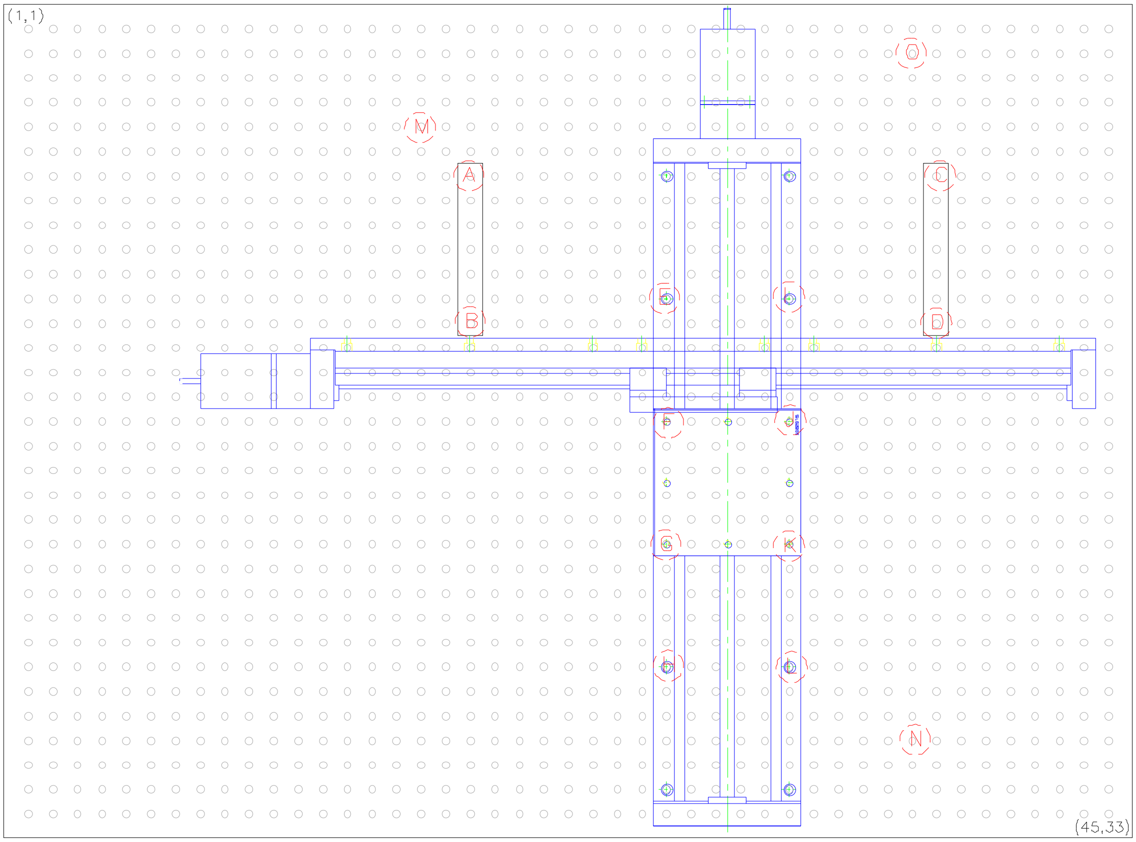


Figure 2. Layout of the servopositioners and other components on the optical table. The coordinates of the left-rear mounting hole are (1,1) and right-front are (45,33). Theses threaded holes are on a one-inch grid.

Point	What	Coordinates
A	Screw point for XZ axis support bracket	(19,7)
B	Screw point for XZ axis support bracket	(19,13)
C	Screw point for XZ axis support bracket	(38,7)
D	Screw point for XZ axis support bracket	(38,13)
E	Screw point for Y axis	(27,12)
F	Screw point for Y axis	(27,17)
G	Screw point for Y axis	(27,22)
H	Screw point for Y axis	(27,27)
I	Screw point for Y axis	(32,12)
J	Screw point for Y axis	(32,17)
K	Screw point for Y axis	(32,22)
K	Screw point for Y axis	(32,27)
M	Soleonid tower	(17,5)
N	Photodiode post	(37,28)
O	Laser post	(37,2)

Table 1. Coordinates of mounting screws on the optical table given in 1-inch units.

[3] Place servo controller and digital servo amplifiers

Mount the 6K4 servo controller and its 24VDC power supply inside the electronics rack and connect the power supply wires according to the manufacturer's instructions. We cut 18 inches of aluminum DIN mounting rail and fastened it near the inside top of the rack with self-tapping screws. The controller and the power supply both clip onto the rail. We placed an inline fuse holder between the power supply and the controller. Place a rack-mount shelf one foot below the rail to hold the supervisory PC.

Mount and wire the three GV servo amplifiers according to the manufacturer's instructions. Mount them to a large 19" rack panel that can accommodate the amplifiers, an emergency shutoff switch, two panel-mount fuse holders, and an optional neon power indicator lamp. Place the panel near the bottom of the rack. Connect the servo amplifiers to the servo controller. It is convenient to place a rack-mount 120V outlet power strip and surge protector just below this panel.

[4] Connect PC and configure servo system

Prepare the supervisory PC. First, install Windows 2000, if necessary. Install the National Instruments PCI-DIO-32HS and the PCI-MIO-16E cards and the supporting Ni-daq software according to the manufacturer's instructions. Configure the PCI-DIO-32HS as device number 1 and the PCI-MIO-16E as device number 2. Install and configure two ethernet interfaces. (*Yes, this open-source project requires some proprietary elements! This decision was made early in the project to take advantage of the drivers and setup tools from National Instruments and Compumotor. There probably wouldn't be any serious problems converting to all open-source elements. The OS could be Linux and the interface cards could now use the Comedi drivers. The servo controller has standard ethernet and RS-232 interfaces. Lombardi could be ported to C++ or Java with a Java Native Interface. Of course, this might take some time.*)

Configure the three servo amplifiers according to the manufacturer's instructions. Install Compumotor Motion Plannner Software on the PC. One at a time, connect the PC to the servo amplifiers using a crossover RS-232 cable. Download the configuration script using Motion Planner. An suitable configuration script can be found in the appendix.

[5] Assemble printed circuit board 1501

The smallest circuit board, 1501, is an adapter to connect a DB25 cable to the flexible flat cables of the Epson print head. This circuit board is mounted on the back of the X-axis positioner and the flexible cable stretches to the print head. (Perhaps it would be better to mount it right next to the print head.)

Download ExpressPCB software from http://www.expresspcb.com and the latest circuit board files (1501.pcb, 1501.sch, 1804.pcb, 1804.sch, 1901.pcb, and 1901.sch) from the ISB website (http://projects.systemsbiology.net/inkjet). Order two replicates of each board online with ExpressPCB. Order the necessary components for all three boards from a vendor such as DigiKey.

Assemble the circuit board using the layout information in the 1501 schematic (.sch) and circuit board (.pcb) files. First, screw four stand-offs into the corners of the board. Second, insert the components and check that everything fits properly. Third, solder the components in place. Use a solder iron of smaller wattage with a fine tip.

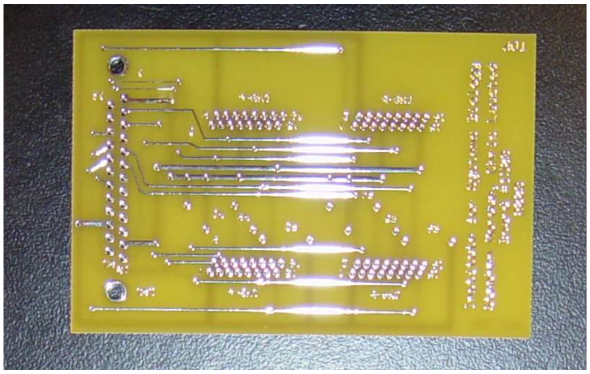


Figure 3. Top view of the 1501 board as it arrives in the mail.

Note that there are 16 holes for the small, white 15-pin flexible cable connectors. While there are probably lots of errors and omissions yet in the manual, this isn't one of them! At one time, only 16-pin connnectors were available in the U.S, and that is what we used. We simply inserted a 15-conductor cable into the first 15 positions of the 16-conductor connector. You may solder the 15-pin connector into the 16-hole position. Also note that there are four such connectors when only two are needed. The second pair of connectors are reserved for the future use of a second print head. As shown in the photo below, you do not need to insert the second pair of connectors.

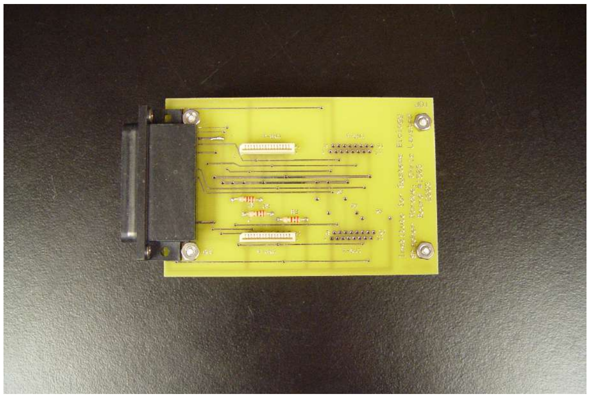


Figure 4. Top view of the 1501 board after assembly. Note that only the two necessary Molex connectors have been installed.

[6] Assemble printed circuit board 1804

In the same manner as the previous step, assemble the circuit board using the layout information in the 1804 schematic (.sch) and circuit board (.pcb) files. This board connects to the PCI-DIO-32HS board and contains the piezoelectric driver circuit, the solenoid driver circuits, and an optoisolator for the jetting trigger signal. There is an large empty area of solder pads at the upper right corner available for prototyping.

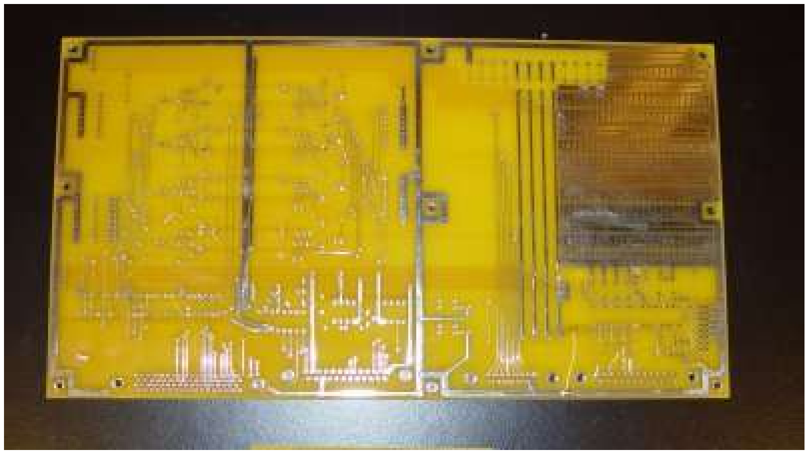


Figure 5. Top view of the 1804 board as it arrives in the mail.

This board is considerably larger and so it has holes for attaching nine stand-off posts. Be careful when attaching the cable connectors at the bottom edge of the board. The high-density 68-pin connector has a conducting metal shell that should not touch the copper pads and tracings of the circuit board's top layer. Separate this connector and the three "D" style connectors from the board by using nylon washers as spacers between the mounting screw holes. This will hold the connectors up off the board. Do not solder ICs, resistor arrays, or fuses directly to the board. Use sockets instead. After testing the board for printing errors and short circuits with a multimeter, insert these components into the sockets.

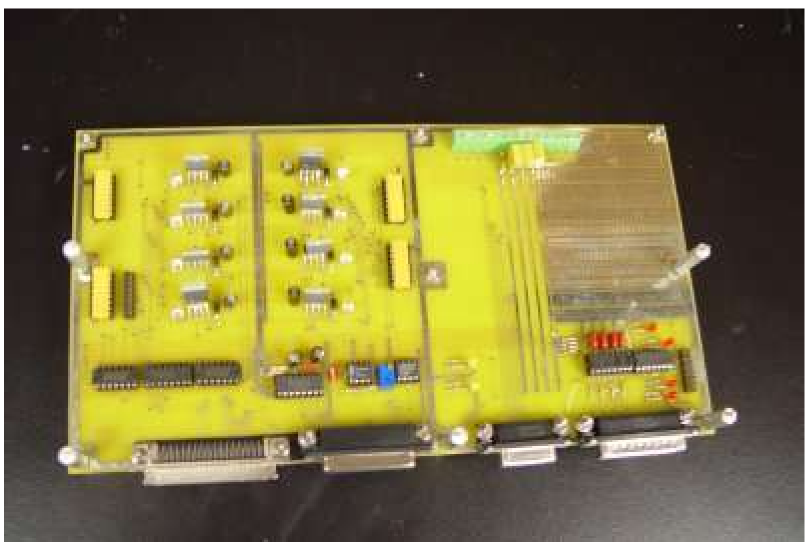


Figure 6. Top view of the 1804 board after assembly.

[7] Assemble electronics box 1

Package board 1804 into an aluminum rack-mount enclosure. First, drill or punch holes in the front panel for the 120V power switch and three power indicator LEDs. Optionally, punch two holes for BNC outputs, eight holes for solenoid indicator LEDS, and three holes for signal indicator LEDs. Next, punch holes for the three "D" style connectors of the circuit board. Cut square holes for the 68-pin connector of the circuit board and the 120V combination receptacle/fuse holder. Drill mounting holes for the circuit board, for the +/-5VDC power supply, and for the +30VDC power supply. Mount the circuit board, power supplies, DPDT power switch, LEDs and BNCs.

The photo below shows the circuit board alone mounted in place. Also shown are the cables connecting the board to the 1501 and the print head.

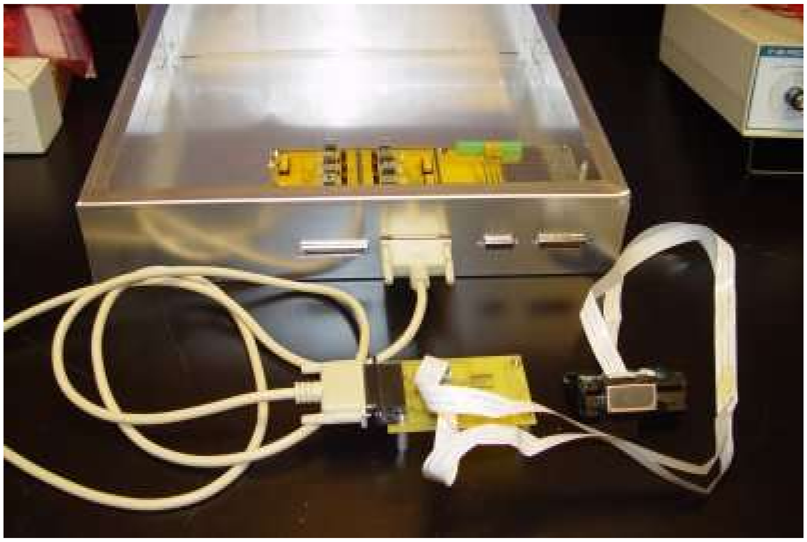


Figure 7. View of the 1804 board installed in a 19" rack, before installation of the power supply, power connectors, switch, and front panel diodes. The 120VAC receptacle, the power supplies, and the front panel parts are not yet installed.

Next, finish the internal connections. This will take a bit of soldering. Connect the 120VAC socket neutral terminal to the neutral posts of the power supplies using 18 gauge stranded wire. Connect the 120VAC receptacle live terminal to the live posts of the power supplies via the DPDT power switch. For the other connections, smaller wire gauges (22-26) may be used. Connect the +5, -5, and +30VDC power supply output posts to the appropriate circuit board screw terminals. The -30VDC screw terminal is unused. Connect the +5VDC and -5VDC indicator LEDs to the +5VDC and -5VDC power supply output terminals through ~1k current limiting resistors. Connect the +30VDC indicator LED to the +30VDC power supply output terminals through a ~6k current limiting resistor.

The other LEDs are connected to the circuit board without using any extra current limiting resistors. Connect the eight solenoid indicator LEDs to connector CN6. The BNCs and the other LEDs may be connected to various optional points in the circuit board such as screw terminal position 3 (piezoelectric pulse) and CN7 (6K4 outputs, printhead signals), respectively.

Insert the box into the electronics rack between the PC shelf and the servo amplifier panel.

[8] Assemble printed circuit board 1901

In the same manner that board 1804 was finished, assemble the circuit board using the layout information in the 1901 schematic (.sch) and circuit board (.pcb) files. This board connects to the PCI-MIO-16E multifunction board and contains the droplet detection circuit, an encoder signal conditioning circuit, and a set of relays. Although it is large in size, there isn't much on it. It primarily provides input/output connections and a lot of prototyping area. The encoder signal conditioning circuit is not required by the current Lombardi software. Screw terminals allow additional voltage signals to be connected to the POSaM. This board uses two +5VDC power supplies. Most components use the +5V supplied by the PC. The droplet detection circuit uses a separate +5VDC supply.

[9] Assemble electronics box 2

Package board 1901 into an aluminum rack-mount enclosure. As with the previous box, drill or punch holes first, insert the board, insert sockets, LEDs, and the power switch, and make the internal connections.

The inside of the box contains only the circuit board, a 5VDC power supply, and two solid-state relays. Mount the relays to the side wall of the box and make a thermal connection with heat-conducting grease. Connect the relay to screw terminals 4 & 5 (signal inputs) and 3 & 6 (signal ground). Connect the 120VAC output sockets to the 120VAC receptacle via the solid-state relays. The front panel requires a SPST 120VAC power switch and a power indicator LED. The back of the box requires the following holes for the 1901 board: two “D” connectors, a 68-pin connector, a BNC, and two round sockets. The box requires holes for the 120VAC input receptacle/fuse holder and two 120VAC solid-state relay output sockets. A connector for the droplet detector laser 5DVC supply is also usefule. (The photo below shows all of the connectors at the rear, although some placement has changed with the current board, 1901.)

Insert the box into the electronics rack between the PC shelf and the servo amplifier panel.

Figure 8. View of the rear of the rack-mount enclosure housing the predecessor of the 1901 board. From the left: one 120VAC receptacle, two 120VAC sockets, hygrometer “D” connector, Unused “D” connector, gas solenoid power jacks, 5VDC supply for the droplet detector laser, 5VDC BNC for the the droplet detector photodiode, and 68-pin interface board connector.

[10] Place reagent solenoids.

Construct a the solenoid tower and place at position M: coordinates (17,5) of Figure 2. To construct the tower, cut a 12 inch length of 1 inch square aluminum tubing. Using the base of an Angar teflon solenoid as the template, drill and tap #4-40 threads for mounting holes. Mount six solenoids with #4-40 screws. Cut a 14 inch length of ¼-20 threaded rod. Use the rod, a 1½ inch fender washer, and a ¼-20 nut to fasten the tower in place.

[11] Place gas solenoids

Make a 2”x6” mounting plate for the ET-2? solenoids from ¼” thick PVC sheet. Drill and counterbore four mounting holes for the two solenoids in the plate. Drill two ¼” holes 5 inches apart for mounting the plate to the optical table. Mount the two solenoids to the plate. Fasten the plate to the left rear corner of the optical table, outside the area enclosed by the acrylic cover.

[12] Place slide holder

Mount the slide holder to the Y-axis positioner using four 2 ½” long ¼-20 screws. Use four spacer bushings (½” thick, ¼” hole) between the positioner and the holder. This gap will allow the bellows of the Y-axis positioner to flex without touching the holder. Orient the slide holder so that the waste liquid will drain from right to left.

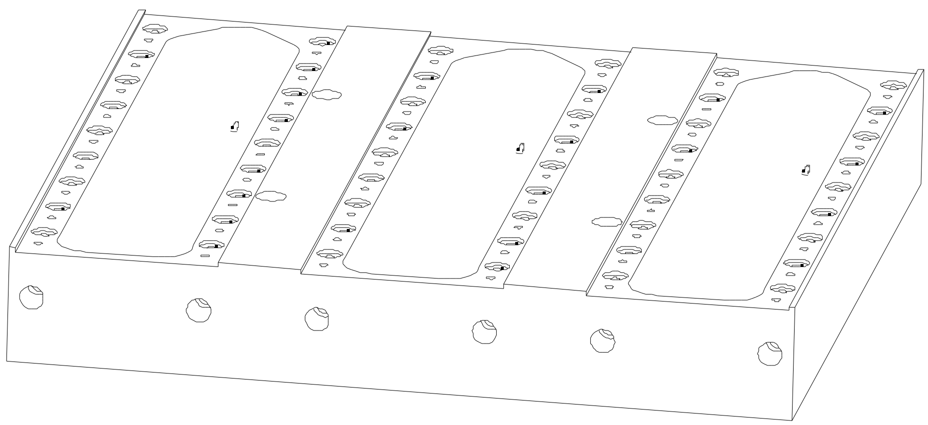


Figure 9. Teflon slide holder. The slide holder is mounted on the X axis positioner.

[13] Install print head brackets

Use four ¾ “ long ¼-20 screws to mount the left and right printhead bracket pieces to the Z-axis positioner.

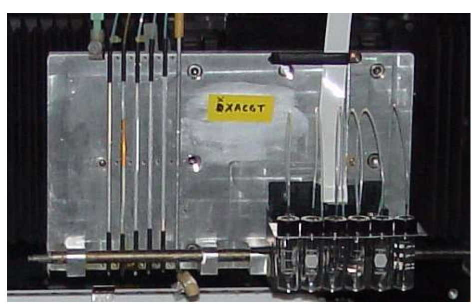


Figure 10. Print head brackets shown with inkjet, vials, slider rod, and teflon tubing installed.

[14] Install print head and phosphoramidite vials

Extract the black plastic print-head/ink-cartridge holder from an Epson Stylus Photo 700 printer. Also extract the stainless steel slider rod from the printer. Insert the holder between the mounting holes of the right printhead bracket piece. Insert the slider rod through all four mounting holes of the left and right printhead brackets, passing through the holder. Secure the rod with two set screws. Cut six 1” lengths of flexible 3/16” OD Pharmed tubing. Attach them to the inkjet inlet spikes. Cut six 7” lengths of 1/16” ODthick-wall teflon tubing. Insert them in the Pharmed tubing. Cut the luer ends off six 20 gauge non-coring 4” needles, leaving the needle 3” in length. Deburr the needles and wash in an ultrasonic bath. Insert the cut end of the needles into the teflon tubing. Carefully insert the needles into the phosphoramidite vials. In the illustration below, we have made a reagent rack from a sheet of aluminum. The vials may also be placed in the ink holder.

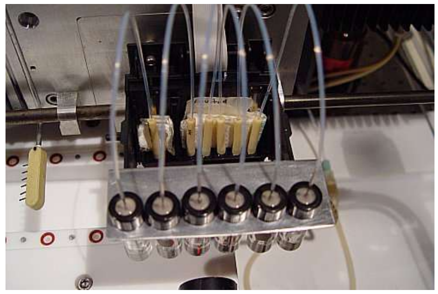


Figure 11. Phosphoramidite is fed from the vials through 20 gauge needles and teflon tubing to the inkjet. Pharmed tubing is used to attach the teflon tubing to the inkjet.

[15] Assemble pressurized gas and liquid plumbing

Gather together the components needed to complete the POSaM fluid system diagrammed below. Most of the components are listed in Appendix C6 but part numbers for metal and plastic tubing, fittings, connectors, and adapters will vary between hardware stores. (We purchased an assortment of 1/4 and 1/8 NPT brass fittings, "T" connectors, adapters, hose barb connecters, compression fittings, and quick-disconnect couplings from our local Mom-and-Pop hardware store. It's useful to have extra supplies. We took 100 feet of 3/8" O.D. polyethylene tubing rated with a working pressure of 87 psi. We also got our 1.5" I.D. flexible tubing there—for use as an exhaust hose and as cable conduit.)

[15a] Install pressure regulator and rotameter

The POSaM requires a high-purity inert gas source capable of providing 30 psi of pressure at flow rates of at least 40 lpm. We recommend installing a pair of liquid nitrogen dewars connected by an automatically switched manifold in a separate room. This allows the gas provider to replace an empty dewar without interrupting DNA synthesis. The output of the manifold is regulated at 60 psi. Copper pipe carries to gas to a wall-mounted, valved outlet in our array room. Mount a regulator (MG06) to the breadboard stand (PR13) and connect its input port to the nitrogen supply. Set the regulator to 30 psi. Alternatively, a single dewar can be placed next to the POSaM and regulated to 30 psi. Connect a "T" to the regulator output. Mount the 120VAC solenoid (MG07) on the breadboard stand. We also attached a heatsink to the solenoid with thermoconducting grease. Mount the rotameter to the breadboard stand and connect its input to an output from the "T" via the solenoid. Run a length of 3/8" O.D. tubing from the rotameter output to the left-read corner of the breadboard. This tube will be used to fill the working area of the POSaM with inert gas.

[15b] Install the air amplifier

The inert gas enters the POSaM cover through the air amplifier (MG11). The air amplifier is a flow-powered blower that uses the inflow of fresh gas to recirculate the POSaM atmosphere through a filter of calcium sulfate and activated charcoal. Our home-built filter tower is constructed from three black ABS plastic 1½" pipe pieces: a "T" (MG13), a 4" adapter (MG14), and an end cap (MG15). The ABS pieces fit together with friction. The air amplifier needs a rubber seal to friction-fit into the "T". We pour Drierite dessicant and pet-store variety charcoal into low-resistance porous filter bags and pack the bags into the top funnel of the filter tower. Place the tower in the left-rear corner of the breadboard and connect the 3/8" gas supply tube to the small barbed inlet of the air amplifier. When gas flows into the barbed inlet at 10 lpm, gas circulates through the tower at rates up to 100 lpm.

[15c] Connect reagent bottles

Reagents are delivered to the print head assembly by gas pressure of 3 psi. Add a second "T" to the outlet of the 30 psi regulator and feed this pressure into the 3 psi regulator (MG04). We mounted this regulator at the exposed breadboard top at the left rear edge of the table. Connect five check valves (MG05) to the 3 psi pressure outlet using flexible 3/16" OD tubing and nylon hose barb fittings and connectors. The check valves prevent reagent fumes from reaching other bottles or reaching the regulator. Place four 500mL bottles (MR02) and one 2L bottle (MR03) in the front-left corner of the POSaM. We place them inside a polypropylene tray to prevent any spillage from falling into the screw-holes of the breadboard. Fit the bottles with 3-port GL45 caps (MR01). Use 1/8" OD teflon tubes to deliver pressure to the bottles. Connect the bottles to teflon solenoids (MR09) numbers 1 to 5 using 1/16" OD teflon tubing. Be sure that the tubing reaches to the bottoms of the bottles.

Run approximately 2 meters of 1/16" OD tubing from the five solenoids to the print head assembly. Use a 1 meter length of flexible 1½" flexible tubing as conduit to hold the cabling to the print head assembly as well as the bundle of reagent tubes. Label all five tubes where they emerge from the conduit. Insert the tubes into sleeves of 1/16" ID (1/8" OD) flexible tubing. Press the sleeves into the left five grooves of the print head assembly. Hold the sleeved tubing in place with #4-40 screws and washers.

The solenoids may now be connected to board 1804. Solder a 10-conductor cable approximately 3m in length to pins 1-9 of a DB15 connector (PLUG06). Pins 2 through 6 will connect to reagent solenoids 1-5. Pin 7 will connecto to waste solenoid 5. Pins 1 and 8 are currently unused. Pin 9 is common (ground) to all solenoids.

[15d] Install the slide dryer

Microarray slides are blown dry during synthesis by a six-jet nitrogen manifold (MG10). Bend a 90^{o} angle in a 12" long aluminum tube (MG09) using a tubing bender to avoid kinking. The bend should be 1.25" from the end of the tube with a radius of about ½". Insert the tube into the sixth groove of the print head assembly. The bend should be orthogonal to the print head assembly. Coat the inlet tube (the large one) of the manifold with flexible silicone sealant or another viscous, weak glue. Insert the inlet into the bottom of tube MG09. Angle the manifold jets (outlet tubes) downward at 45^{o}.

Mount two solenoids (MG03) near the 3 psi solenoid (MG04). Feed 30 psi pressure into the solenoids. The second solenoid is reserved for future use. The first is used to control the slide dryer. Connect the solenoid output to the top of tube MG09 using 3/16" OD flexible tubing. Route the tubing through the conduit, as in the previous step.

Make two two-conductor cables to connect the solenoids to connectors 7 & 8 of board #1901. Use two 1mm power plugs (PLUG03).

[16] Connect the vacuum components

Vacuum is used both to chuck the slides into the slide holder and to suck waste from the bottom of the slide holder. This requires the use of teflon tubing and fittings, a 2L liquid waste bottle (MR03), a 500mL trap (MR02), and a charcoal filter cartridge (MM10). We monitor vacuum visually with a dial gauge (MG02). (A voltage-output pressure transducer is planned for the future.)

[17] Install hygrometer

Connect the Humirel hygrometer (HU1) to a DB9 connector (PLUG02) via a 2m length of shielded cable. Connect wire 1 (white) to pin 2 (ground), wire 2 (blue) to pin 3 (voltage source), and wire 3 (yellow) to pin 1 (humidity signal). Mount the hygrometer on the XZ axis vertical bracket (PR10) near the slide holder. Plug the sensor in to board 1901.

[18] Install droplet detection laser and photodiode

The droplet detection laser sends a beam from behind the print head to the photodiode detector in front of the print head. The beam is aligned so that all droplets fired downward from the 32 nozzles in any one bank will intersect the beam. Light from the beam is normally blocked from reaching the photodiode by a black tape blocking strip. Light refracted by the droplets passes around the strip and reaches the detector.

Place a post mount (PO03) to support the photodiode at Position N of Figure 2: coordinates (37,28). Insert a 8” post (PO12) and attach a right angle clamp (PO06). Attach a ring clamp (PO09) to the end of a 4” post (PO11) and insert the post in the clamp. Insert the photodiode (PO07) into the bottom of the 1” SM1 barrel (PO10) and secure it with a ring nut (PO08). Use a pair of ring nuts to secure the red filter (PO01) in the middle of the barrel. Use another pair of ring nuts to secure the lens (PO02) at the end of the barrel. Since a large area photodiode is used, precision focusing and alignment is not necessary. Cut a srip of electrical tape 6mm thick and 50mm long. Place the strip on the end of the SM1 tube barrel as shown below. Place the barrel in the ring clamp and tighten the clamp. The lens faces the rear of the POSaM.

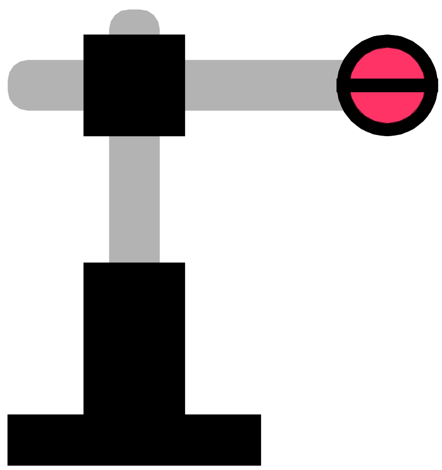


Figure 12. Droplet detection photodiode assembly as seen by the laser.

In the same manner, mount the laser diode at the rear of the POSaM at Position O of Figure 2: coordinates (37,2). Attach the laser to a 5VDC power supply. Align the laser with a bank of 32 nozzles. The laser needs to point at the center of the blocking strip. Focus the laser just below the print head nozzles. The laser elevation should be close to 7.5 inches.

[19] Install cover

The custom plexiglas (acrylic glass) cover is delivered in four pieces: the main body, the front panel door, the left panel (with glove holes) and the right panel. Installation of the cover involves making a seal to match the main body base, placing the main body, making panel seals, attaching the panels, and attaching gloves.

The main body has a footprint 41 inches wide and 35 inches deep, compared with the optical table dimensions of 47 inches wide and 35 inches deep. The cover and the table are aligned on their right edges, leaving 6 inches of table width exposed on the left side. Prepare the seal between the table and the cover. Attach two continuous strips of 5/16" thick weatherstrip foam (MC06) to the table top to match the flanged base of the cover main body. On the right rear corner of the table where the cables are routed, use polymer clay instead of weatherstrip to form the seal. On the left rear corner of the table, or where-ever gas tubing or other wires are routed, also use polymer clay. Carefully set the main body on the weatherstrip surface. Holes in the flanged base match the 1 inch grid pattern of the optical table. Gently fasten down the main body using ¼-20 screws and 1 inch fender washers (MC04). Only tighten the screws finger-tight to avoid damaging the flange.

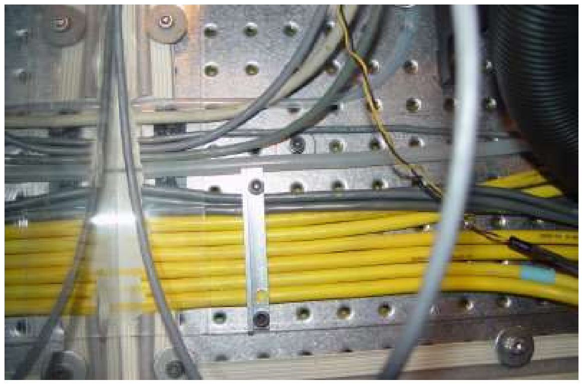


Figure 13. All wires between the rack and the POSaM interior are routed underneath the lower right rear corner of the cover (shown here as the lower left corner of the picture).

Next, attach the panels. Apply two strips of "D" profile weatherstrip (MC07) around the perimeter of the three holes in the main body. Attach the left (glove hole) and right (no glove holes) panels with ¾" long #6 screws. Seal the screw holes by covering them (inside the main body) with polyethylene tape (MC09). The front panel door attaches with three hinges on the top and has five latches around the sides and bottom. Attach the hinges (MC03) and latches (MC01) with more #6 screws (from MC02) and seal the screw holes with polyethylene tape.

Last, attach gloves and the exhause valve. Attach the gloves (MC08) to the left panel with large worm-screw clamps (large versions of those used for automotive radiator hose clamps). A very low resistance valve (MC10) is needed for the exhaust port at the upper left corner of the cover. We extracted ours from a dust mask sold by the local hardware store. It is 30mm in diameter and fits tightly inside the cover exhaust port tube. The mask comes with three such valves. We replace them every three months.

[20] Connect fume exhause hose

Connect the exhaust port at the upper left corner of the cover to your fume exhaust system using flexible conduit (MM01). Overlap the exhaust tube with about 30 mm of conduit and fasten with a worm-screw hose clamp. Cut a hole 20mm in diameter in the conduit immediately next to the exhaust port tube. This leak prevents your building's exhaust system from creating a vacuum inside the POSaM cover. Place a cork in the inlet port at the lower left-rear corner of the cover. (To rapidly remove fumes from inside the cover, open the inlet port and close the leak hole.)

THE SOFTWARE

Two software tools can be used to run the inkjet arrayer: Arrayer and Lombardi. Both require two types of input data files to work. A *Lineup* file describes the DNA sequences to be printed on the slide. A *Playbook* file is used to describe the procedure used to synthesize the DNA. Both programs were developed in Visual Basic 6.0 on Windows NT 4.0 (And we've recently started using it on Windows 2000) Both require that Compumotor's Motion Planner and National Instruments NIDAQ (Nidaq32.dll, specifically) be installed. A Pentium III 500 MHz with 256MB RAM is sufficient.

Using Lombardi

Lombardi, currently on version 0.7.3, is intended for the routine production of microarrays. Its main advantages are a simple playbook language and the ability to print different sequences on each slide. It can print up to 9800 spots (70 by 140) in 90 DPI mode. We don't recommend trying 180, 270, or 360 DPI mode. (We do not yet have a substrate that allows us to use 180 or more DPI. We once tried a set of amino slides with 180 DPI density but it resulted in about 20% malformed features.) Since the print head has 32 rows of nozzles, choose a number of rows that is equal to, or slightly less, than a multiple of 32. For example, 70 by 64 or 70 by 128 are recommended for faster cycle times.

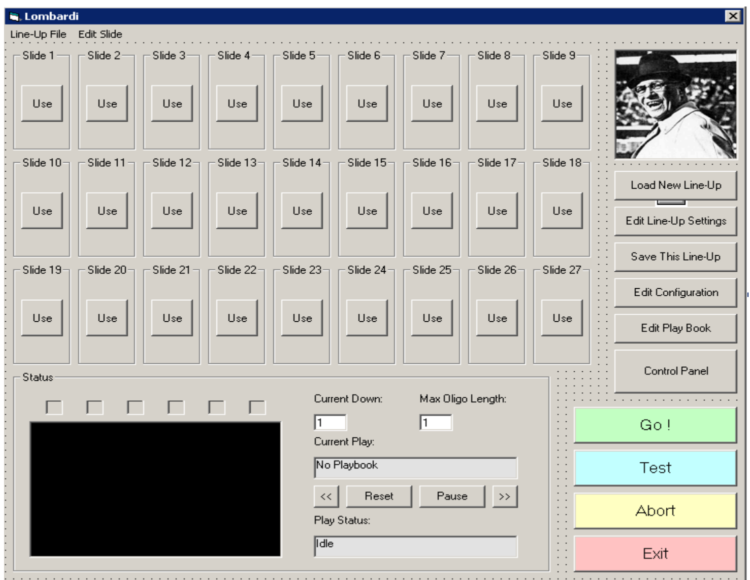


Figure 14. The Lombardi main panel. Clicking one of the 27 slide buttons brings forth a display of the array contents. Clicking the "Go" button starts synthesis.

Upon startup of the software, the main panel pictured here is displayed. The user should first open a lineup (*.lnp) file with the "Load New Line Up" button. A Lombardi lineup file also contains information about which bank each phosphoramidite or catalyst is loaded into (required) and which wash channel each large-volume reagent is loaded into (optional). The first letter of each phosphoramidite is used as a unique designator. The catalyst, tetrazole, is usually designated with an X. An unused bank is usually designated with a U. The six boxes in the lower left corner of the panel indicate the bank assignments. If bank one is unused, bank two holds tetrazole, and the remaining four banks hold the phosphoramidites, then the boxes will read U, X, A, C, G, and T, respectively. The black rectangle below the boxes will indicate which nozzles are believed to be functional with colored dots. If all nozzles are working, 192 (6 columns by 32 rows) dots will be displayed.

The user will next load a playbook (*.plb) file. A playbook can be created or loaded and edited by using the "Edit Play Book" button. The buttons and text boxes in the lower center of the panel can be used to view each command in the playbook file. They are also used to set the starting point of synthesis when the "Go" button is clicked. Usually, the synthesis will begin from the first "play" of the first "down". They play is the command indicated on a line in the playbook file, such as “PRINT” or “WASH.” The down refers to which base (monomer) is being synthesized. The first, or 3’, base is coupled on first down. The second base is coupled on second down. A 25-mer oligo will require 25 downs. (The sports analogy breaks down here.) Synthesis can be paused by using the “Abort” button. Synthesis can be resumed because the last down and the last play are remembered. If synthesis is to be restarted from the beginning, the current down and the current play must be manually reset by the user.

Buttons representing each of the 27 slide positions occupy most of the space on this panel. Clicking on a button will open the slide editor. This is mostly used to view the slide and ensure that the correct lineup file has been selected. The user also has the ability to edit the contents of a slide. Each spot, or feature of the slide, can be selected and the sequence modified. The slide editor also shows the spot density chosen. This is usually 90 DPI. The higher resolutions have not been well tested and should be avoided.

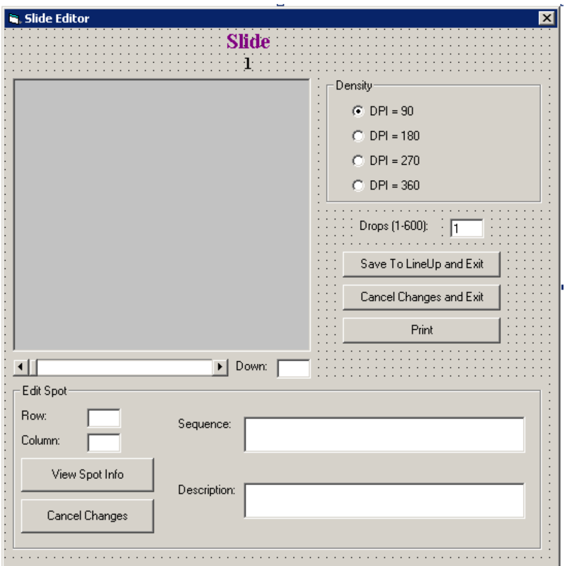


Figure 15. The slide viewer and editor.

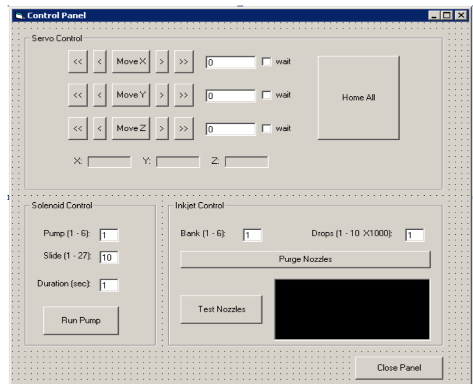


Figure 16. The Lombardi control panel allows robot movement, reagent delivery, and nozzle testing.

The control panel is used to manually jog the print head and slide holder. No damage can be done in the X, Y, or +Z (up) directions. WARNING: It is possible to crash the print head into the slide holder with the -Z (down) control. The control panel is also used to purge air out of the Teflon nozzles when reagents have been changed. Dispense liquid for about 4 seconds to completely change the volume in the tubes. The solenoids are identified by number only. Usually, 1-3 correspond to acetonitrile, oxidizer, and dichloroacetic acid, respectively. Solenoids 4 and 5 are intended for capping reagents. Solenoid 6 is treated differently by the control panel. It doesn’t move the solenoid’s dispensing nozzle to the specified slide location because it is used for vacuuming waste from the slide holder. The control panel can also be used to purge thousands of droplets from the piezoelectric nozzles. When the “Purge Nozzles” button is clicked, the entire bank ejects droplets through the laser beam. This is also a way to check the proper operation of the nozzles and the alignment of the laser.

Lombardi File Formats

The following example lineup file has the required information to make an array with four features (2 spots by 2 spots). Comment lines start with the “#” symbol. The six banks of the piezoelectric are next assigned their reagent. “XTetrazole” indicates the position of the catalyst. (If an “X” appears in a sequence, two drops of tetrazole will be delivered, but no base.) The slide printed will be in position 12. This is the position third from the left, in the middle row. The spot density is 90 DPI. Rows “1a” and “2a” contain oligo sequences. Rows “1b” and “2b” contain the corresponding descriptions of the sequences. The “b” rows are optional. As many as 27 slides may be included in a file. (In the future, we will be using a format interchangeable with our generic array data processing pipeline desribed at http://db.systemsbiology.net/software/ArrayProcess/index.html.)

```
# This is an example lineup file.
BANK1=Unused
BANK2=XTetrazole
BANK3=Adenine
BANK4=Cytosine
BANK5=Guanine
BANK6=Thymine

SLIDE12
DPI=90
1a:TTTTTCTGGAGGGCCTGTGCGTGAA,TTTTTCTGGAGGGCCTGTGCGTGGA
1b:Gene001,Gene002
2a:TTTTTCTGGAGGGCCTGGGCGTGGA,TTTTTCTGGAGGGAATGTGCGTGGA
2b:Gene003,Gene004
# End of file.
```

The following playbook file describes the standard synthesis procedure. Comments, again, begin with the "#" symbol. Lombardi has the habit of stripping off the comments when the file is opened and re-saved with the playbook editor. Using "##" prevents this. The first command, "WASH," dispenses acetonitrile from nozzle 1 for 0.7 seconds. It executes this action over slide 15, then 14, then 13, then 12. The next command, "DRY" uses the blower to remove the liquid from the same slides. The "PRINT" command takes no parameters. The "WAIT" command takes a time parameter in units of seconds. This procedure is repeated N times to make an N-mer.

```
# This is an example playbook file.
##WASH WITH ACETONITRILE
WASH 1, 0.7, 15, 14, 13, 12
DRY 15, 14, 13, 12
##PRINT
PRINT
WAIT 50
##WASH WITH ACETONITRILE
WASH 1, 0.7, 15, 14, 13, 12
WAIT 2
DRY 15, 14, 13, 12
##DOUBLE PRINT
PRINT
WAIT 50
##WASH WITH ACETONITRILE
WASH 1, 0.7, 15, 14, 13, 12
WAIT 2
DRY 15, 14, 13, 12
##OXIDIZE
WASH 2, 1.2, 15, 14, 13, 12
WAIT 30
DRY 15, 14, 13, 12
##WASH WITH ACETONITRILE
WASH 1, 0.7, 15, 14, 13, 12
DRY 15, 14, 13, 12
##DEPROTECT
WASH 3, 0.9, 15, 14, 13, 12
WAIT 30
DRY 15, 14, 13, 12
##WASH WITH ACETONITRILE
WASH 1, 0.7, 15, 14, 13, 12
DRY 15, 14, 13, 12
```

Using Arrayer

Arrayer, currently on version 0.9.0, is a more flexible controller intended for testing improvements to the machine. Its main advantage is the greater control offered by its playbook language. It can print up to 9800 spots (70 by 140). It also has a tool for finding the center of the laser beam used in droplet detection.

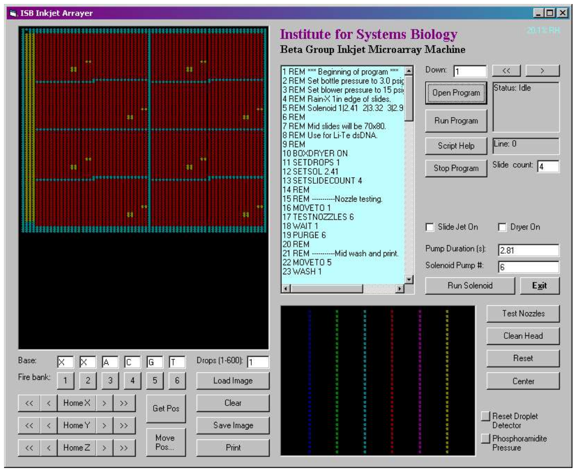


Figure 17. Main panel of Arrayer v.0.9 software.

The main panel pictured above is displayed at start-up. The left side of the panel contains printing functions. The large black box displays the image currently available for printing. The servo jog buttons are below. The center of the panel contains the playbook functions. The program, or playbook file, can be viewed here. Oligo synthesis programs can be started and stopped with these buttons. The right side contains droplet detection and reagent dispensing functions.

The user should first open a lineup file with the “Load Image” button. The user can edit the image displayed in the large black box by clicking. A left-click increments the bank number used to hit that spot. For example, a spot that had contained an A from bank 2 will be changed to a B from bank 3. A right-click resets the spot to zero, or no drop. The user must check that each bank is assigned a letter. In the example above, banks 123456 are assigned letters MACGTN. The letters used in the lineup file must all be from this set of six letters. It does not matter which symbol corresponds to the catalyst. The printing of tetrazole is done explicitly in the playbook using the bank number. The user can position the print head over a slide by using the jog buttons. The “<< >>” buttons move the servos farther than the “< >” buttons. The three axes can be homed individually by using the “Home*” buttons. Once the print head is over a slide, the user may click “Print” to print the image currently displayed. The number of drops printed is adjustable. If the number specified is one drop, then fast “fire-on-the-fly” printing is used. The image displayed is a snapshot of the lineup at a given down. To look at the bases deposited on a different down, the user can change the value in the “Down” text box.

Since the jog buttons can be very tedious, eight frequently used positions can be saved in the “Important Coordinates” dialog box. The first three positions have fixed definitions. The droplet detector position (1) must be located where droplets pass through the laser beam. The purging area (2) must be chosen to avoid piezojetting onto a slide. The cleaning start point (3) is currently not used. The other five points are user defined. Positions 4-6 should correspond to the right-most slides in each row. When synthesizing more than one slide, operations can be performed on a row of slides, from right to left. Before using these coordinates, the servos must be homed. Homing is not done automatically. The Arrayer software may be exited and restarted without re-homing.

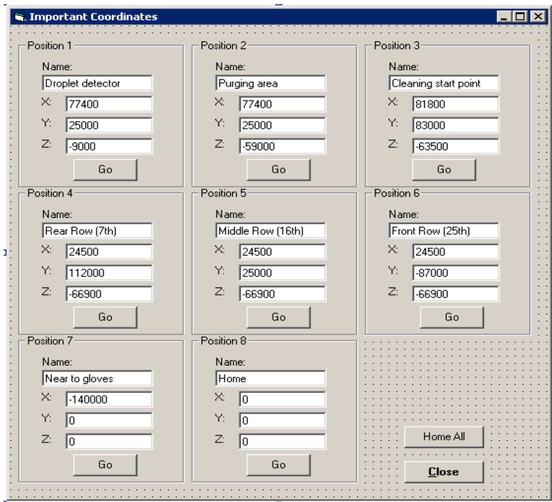


Figure 18. This dialog, activated by the "Move Pos..." button, allows the user to rapidly mvoe the print head to saved coordinates.

Arrayer File Formats

The file formats used by Arrayer are not compatible with those used by Lombardi. The lineup file is a simple file of comma-separated values (.CSV). This file must contain 70 (or fewer) columns and 140 (or fewer) rows of oligo sequence text.

A sample playbook and a table of commands follow below. The Arrayer playbook language is very primitive. Each line contains exactly one command and exactly one parameter. There is one looping command which must be used in oligo synthesis. The LOOP command increments the Down number and restarts execution of the program until the down number exceeds the LOOP count parameter. In this example, five slides are synthesized. They have been placed all in the same row. Position 5 has been defined with the coordinates of the right-most slide. An ftp script has been written to upload the error log to an http server.

```
REM *** Beginning of program ***
REM Set bottle pressure to 3.0 psig.
REM Set blower pressure to 15 psig.
REM Rain-X 1in edge of slides.
REM Solenoid 1|0.71  2|0.15  3|1.13  4|1.04  5|0.15
REM
REM Slides will be 32x50. Hyb to our 12mer and H. influenza.
REM File InkjetErrorLog.txt gets uploaded via ftp.  Be sure FtpScript.txt exists.
REM
SETDROPS 4
SETSOL 0.71
SETSLIDECOUNT 5
REM
REM -----------Nozzle testing.
MOVETO 1
TESTNOZZLES 6
WAIT 1
PURGE 6
REM
REM -----------Wash and print.
MOVETO 5
WASH 1
DRY 0
PUMP 6
WAIT 1
MOVEABSZ -69500
PRINT Bank1
PRINT Default
MOVEABSZ -66000
```

```
WAIT 60
REM
REM -----------REPEAT
REM -----------Nozzle testing.
MOVETO 1
TESTNOZZLES 6
WAIT 1
PURGE 6
REM -----------Wash and print.
MOVETO 5
WASH 1
DRY 0
PUMP 6
WAIT 1
MOVEABSZ -69500
PRINT Bank1
PRINT Default
MOVEABSZ -66000
WAIT 60
REM -----------Oxidize.
MOVETO 5
SETSOL 1.13
WASH 2
MOVEY 25000
WAIT 25
REM -----------Wash and acid.
MOVETO 5
DRY 0
SETSOL 0.71
WASH 1
DRY 0
SETSOL 2.06
PUMP 6
SETSOL 1.04
WASH 3
MOVEY 25000
WAIT 25
REM -----------Extra wash.
MOVETO 5
DRY 0
SETSOL 0.71
WASH 1
DRY 0
SETSOL 2.06
PUMP 6
SETSOL 0.71
REM
REM ----------FTP the error log.
REM
SHELL ftp -s:FtpScript.txt
REM
REM ----------Looping.
REM
LOOP 25
REM
REM ----------Extra wash at end.
REM -----------Mid wash.
MOVETO 5
DRY 0
SETSOL 0.71
WASH 1
DRY 0
SETSOL 2.06
PUMP 6
SETSOL 0.71
REM -----------Maintanence every 20 min.
MOVEX 25000
MOVEY -10000
PURGE 0
WAIT 1200
PURGE 0
WAIT 1200
PURGE 0
WAIT 1200
```

The contents of FtpScript.txt:

```
open ftp.mywebsite.org
myname
mypassword
cd public_html
put InkjetErrorLog.txt
bye
```

Command	**Parameter**	**Acceptable values**
PRINT	catalyst bank	1, 2, 3, 4, 5, 6, or 16
PRINT	current image	Default
LOADIMAGE	filename	*.csv
WASH	pump number	1, 2, 3, 4, 5, or 6
DRY	use manifold (0) or point (1)	0 or 1
WAIT	duration	real number
SETDROPS	number of inkjet drops	0..600, integer
SETSOL	solenoid on duration in seconds	0..15, real number
SETSLIDECOUNT	number of slides	0..9, integer
BLOW	on or off	ON or OFF
MESSAGE	text to display	string
MOVEX	relative distance	integer
MOVEY	relative distance	integer
MOVEZ	relative distance	integer
MOVEABSX	absolute position	integer
MOVEABSY	absolute position	integer
MOVEABSZ	absolute position	integer
HOME	Axis	X, Y, or Z
PUMP	pump number	1, 2, 3, 4, 5, or 6
PURGE	number of banks	1, 2, 3, 4, 5, or 6
SPEED	change servo velocity	1..25 or 0=default (10)
LOOP	number of downs to loop	integer
REM	comment text	string
IFFSKIP	If no new nozzle failures, skip this number of lines	integer
TESTNOZZLES	number of banks to test	1, 2, 3, 4, 5, or 6
SHELL	DOS shell command	string
CHECKHEAD	(deprecated function)	1, 2, 3, 4, 5, or 6

Table 2. Commands used by the Arrayer script. Each command takes exactly one argument.

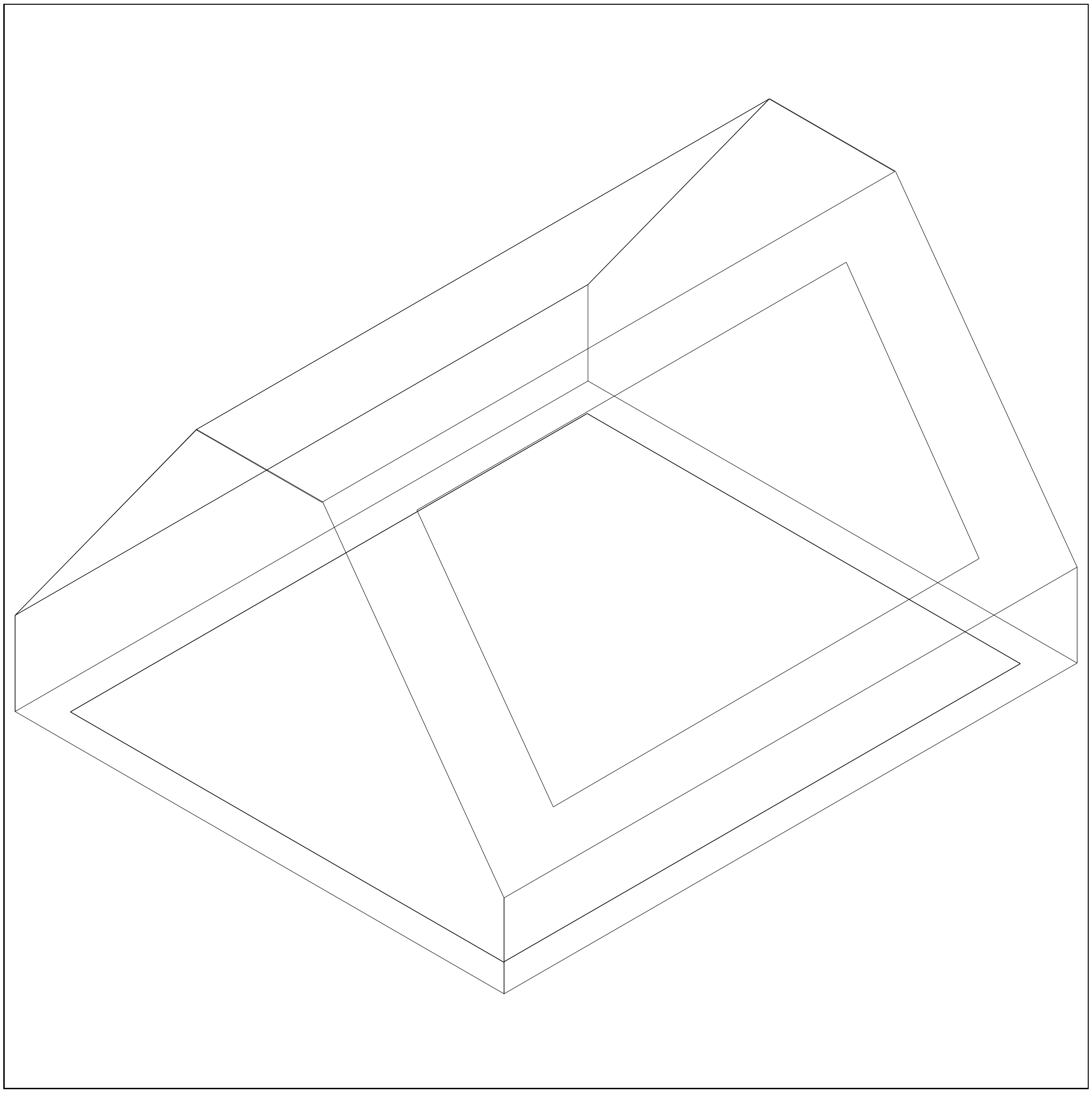
WHAT'S NEXT?

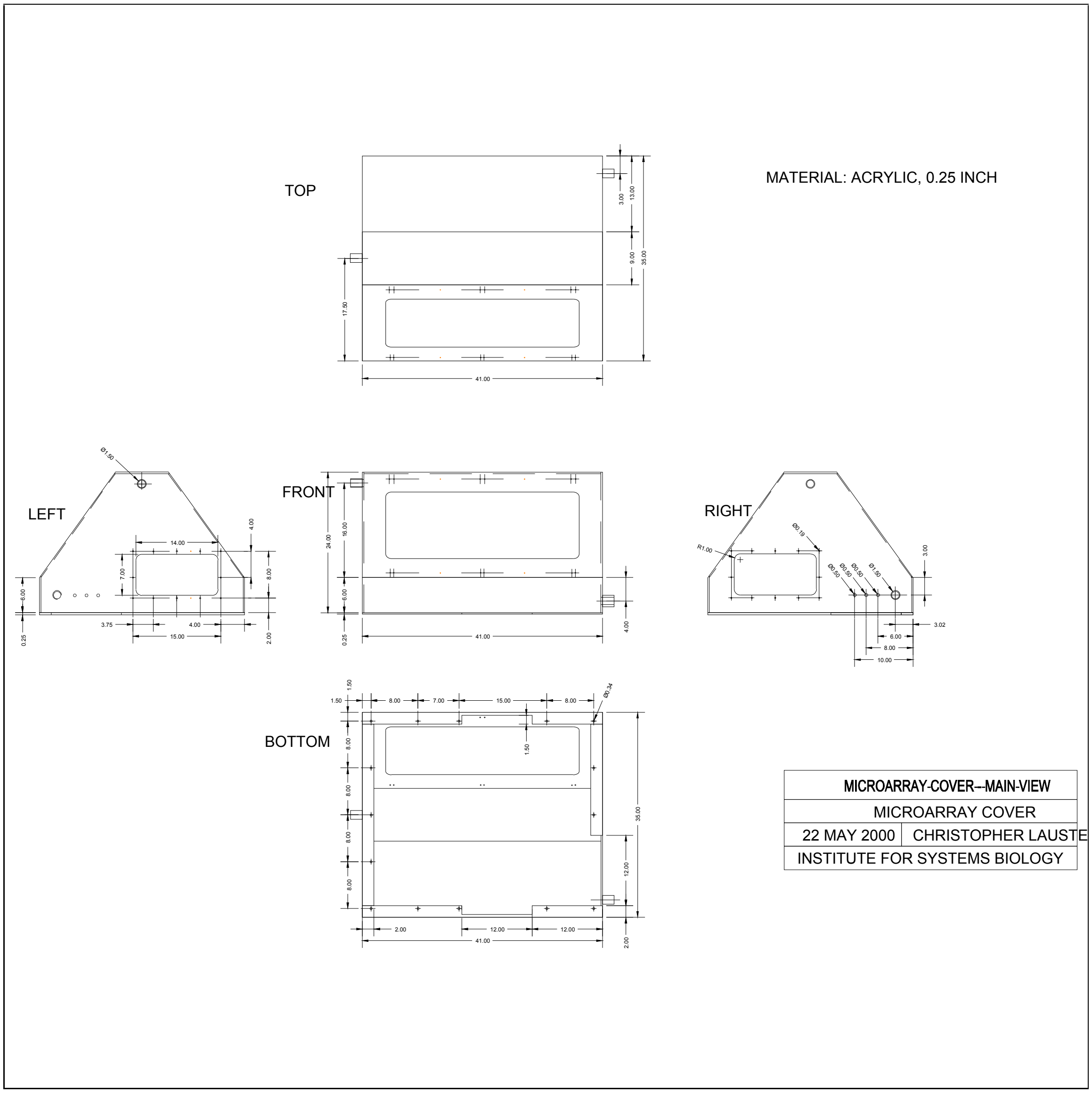
Improvements to the POSaM hardware and software are underway. We are currently adding a new quality control measure to the print head. As you may have noted, the droplet detector checks which nozzles are working before every round of printing. It isn't yet possible to track at what exact instant a nozzle fails. So for now, we always need to double-print. Better droplet monitoring could eliminate this need. We are also planning a new slide holder and wash area. Our current design holds more slides than we need, and the wash/dry steps are not as fast as we would like. We are working on a new design that might only hold eight slides, but will significantly speed washing and drying. Changes in the control software are underway. The code for Lombardi and Arrayer is—let's be honest—somewhat ugly. Also, some routing actions are cumbersome. For example, swapping different microarray designs in and out of a Lombardi lineup is slow. The scripting language could use some new features. We will soon be unveiling a new control program called “Pogo.” Pogo will be faster, easier to use, and it will understand our upcoming hardware changes. So before you build, be sure to check out our website for the latest versions of the documentation, the schematics, and the software.

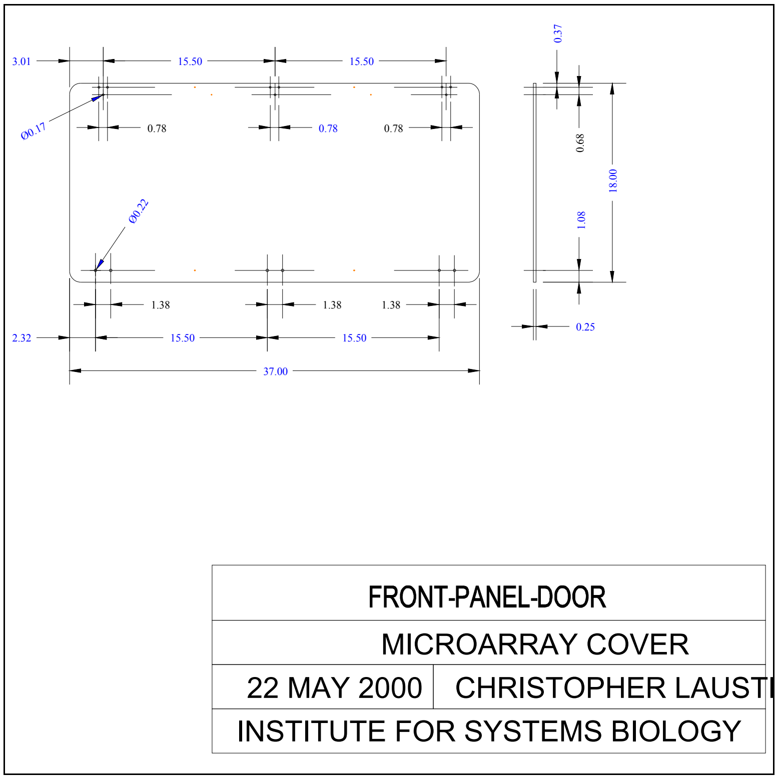
APPENDIX A: MECHANICAL DRAWINGS

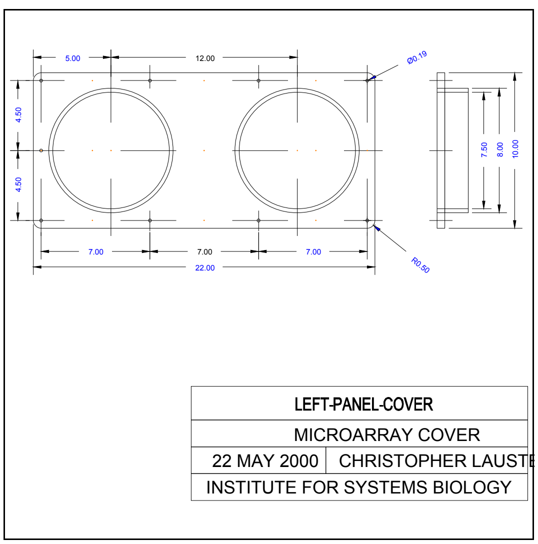
[A1] Array Cover

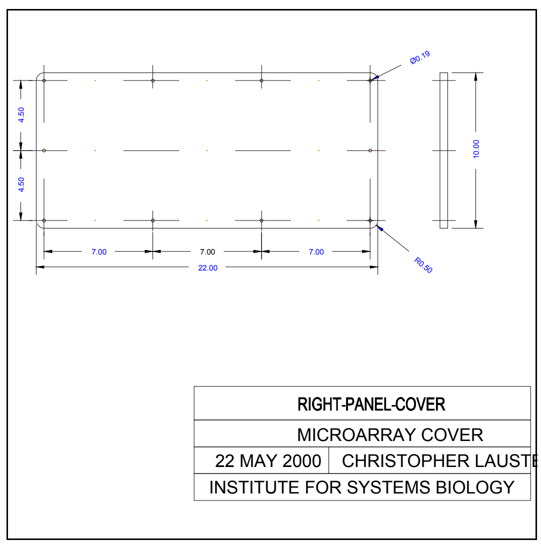
Not shown: Front door, exhaust port (top left, 1.5” dia.), inlet port (bottom rear, 1.5” dia.), removable glove panels (left and right sides).



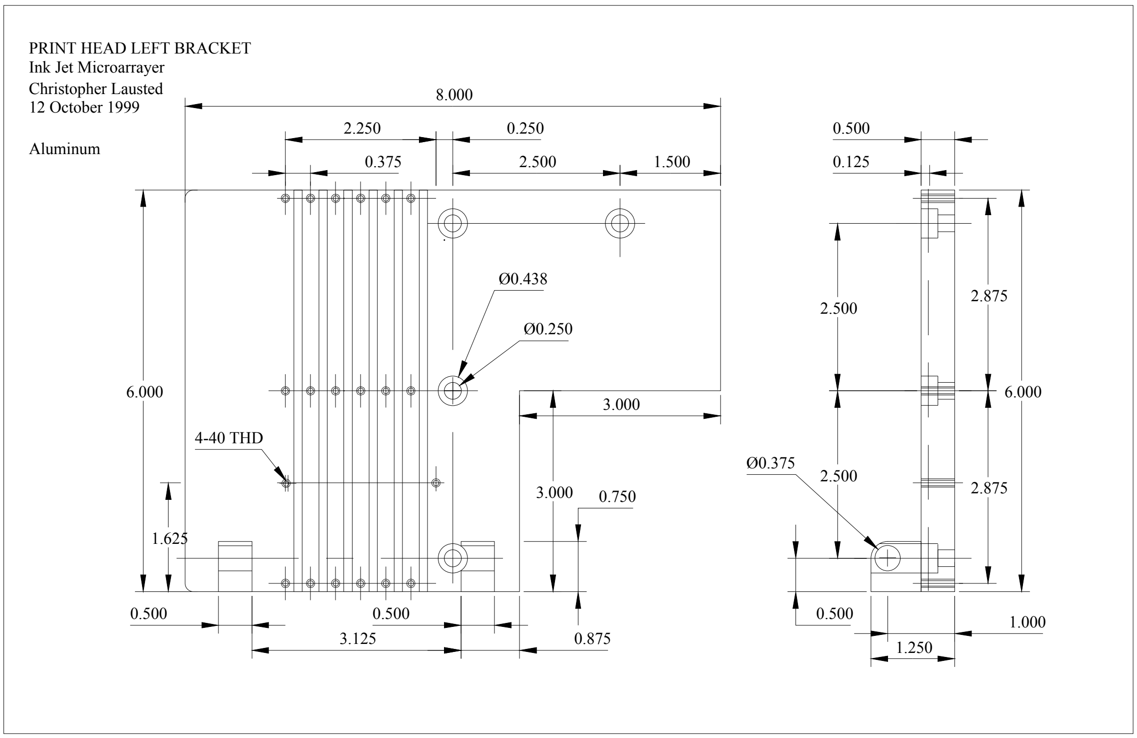

TOP
MATERIAL: ACRYLIC, 0.25 INCH
LEFT
FRONT
RIGHT
BOTTOM
MICROARRAY-COVER--MAIN-VIEW
MICROARRAY COVER
22 MAY 2000
CHRISTOPHER LAUSTE
INSTITUTE FOR SYSTEMS BIOLOGY

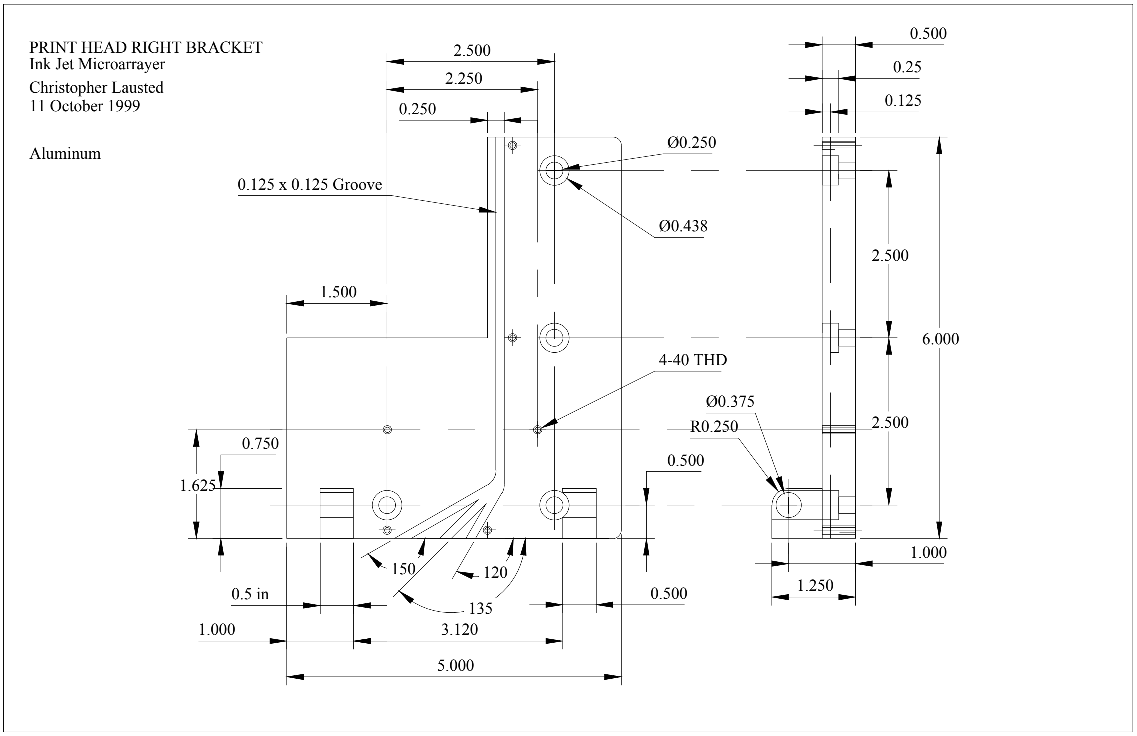

3.01
15.50
15.50
0.37
Ø0.17
0.78
0.78
0.78
0.68
18.00
Ø0.22
1.08
1.38
1.38
1.38
2.32
15.50
15.50
0.25
37.00
FRONT-PANEL-DOOR
MICROARRAY COVER
22 MAY 2000
CHRISTOPHER LAUSTI
INSTITUTE FOR SYSTEMS BIOLOGY


5.00
12.00
Ø0.19
4.50
4.50
7.50
8.00
10.00
7.00
7.00
7.00
22.00
R0.50
LEFT-PANEL-COVER
MICROARRAY COVER
22 MAY 2000
CHRISTOPHER LAUSTE
INSTITUTE FOR SYSTEMS BIOLOGY

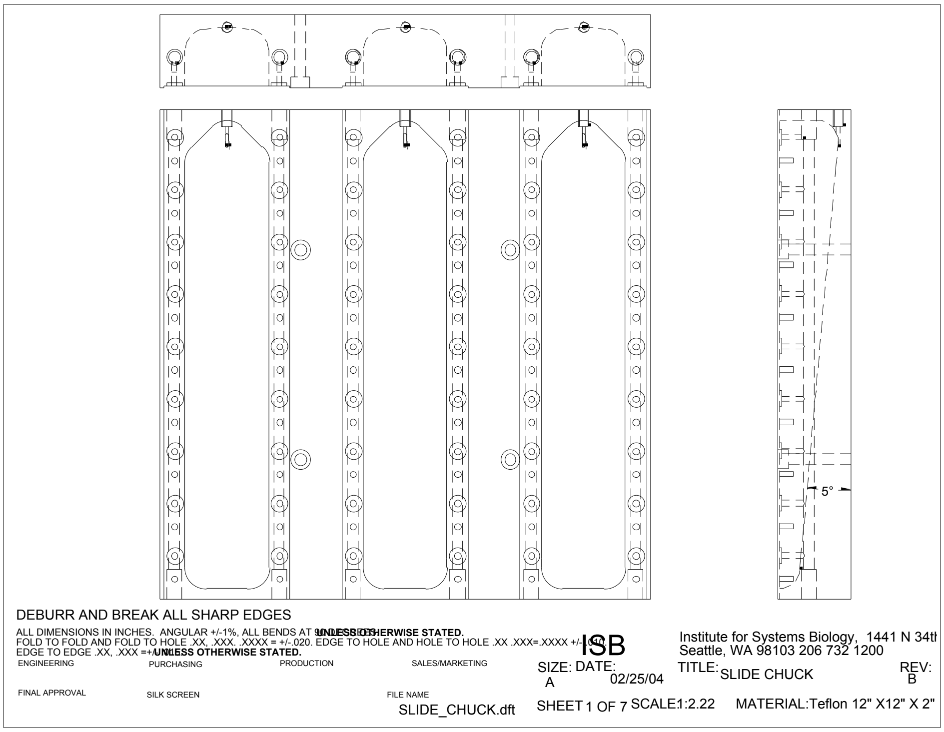

Ø0.19
4.50
4.50
10.00
7.00
7.00
7.00
22.00
R0.50
RIGHT-PANEL-COVER
MICROARRAY COVER
22 MAY 2000
CHRISTOPHER LAUSTE
INSTITUTE FOR SYSTEMS BIOLOGY

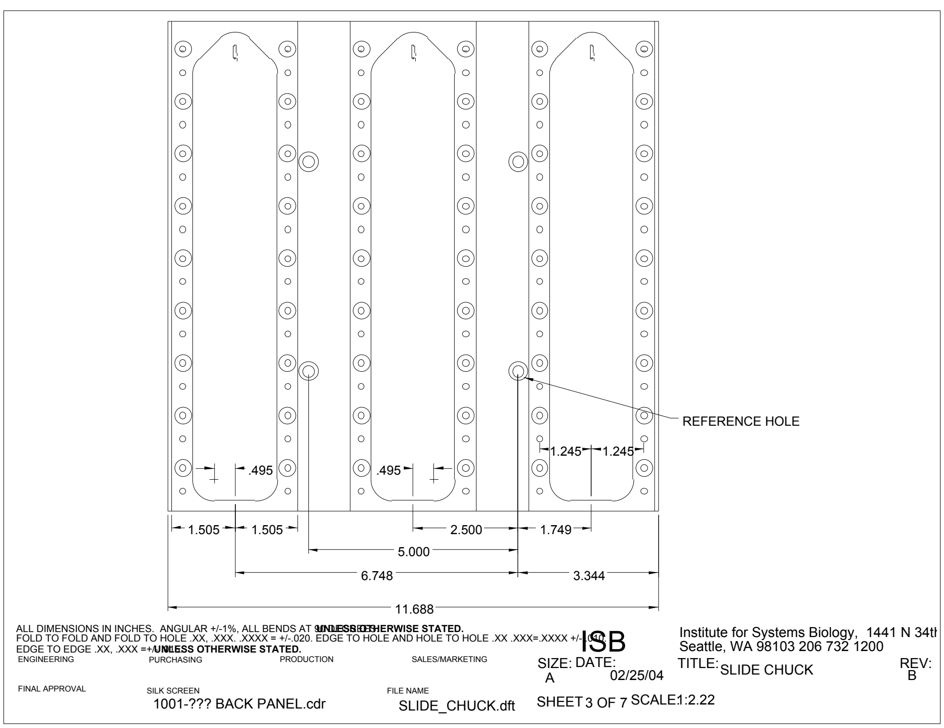
[A2] Print Head Mounting Brackets

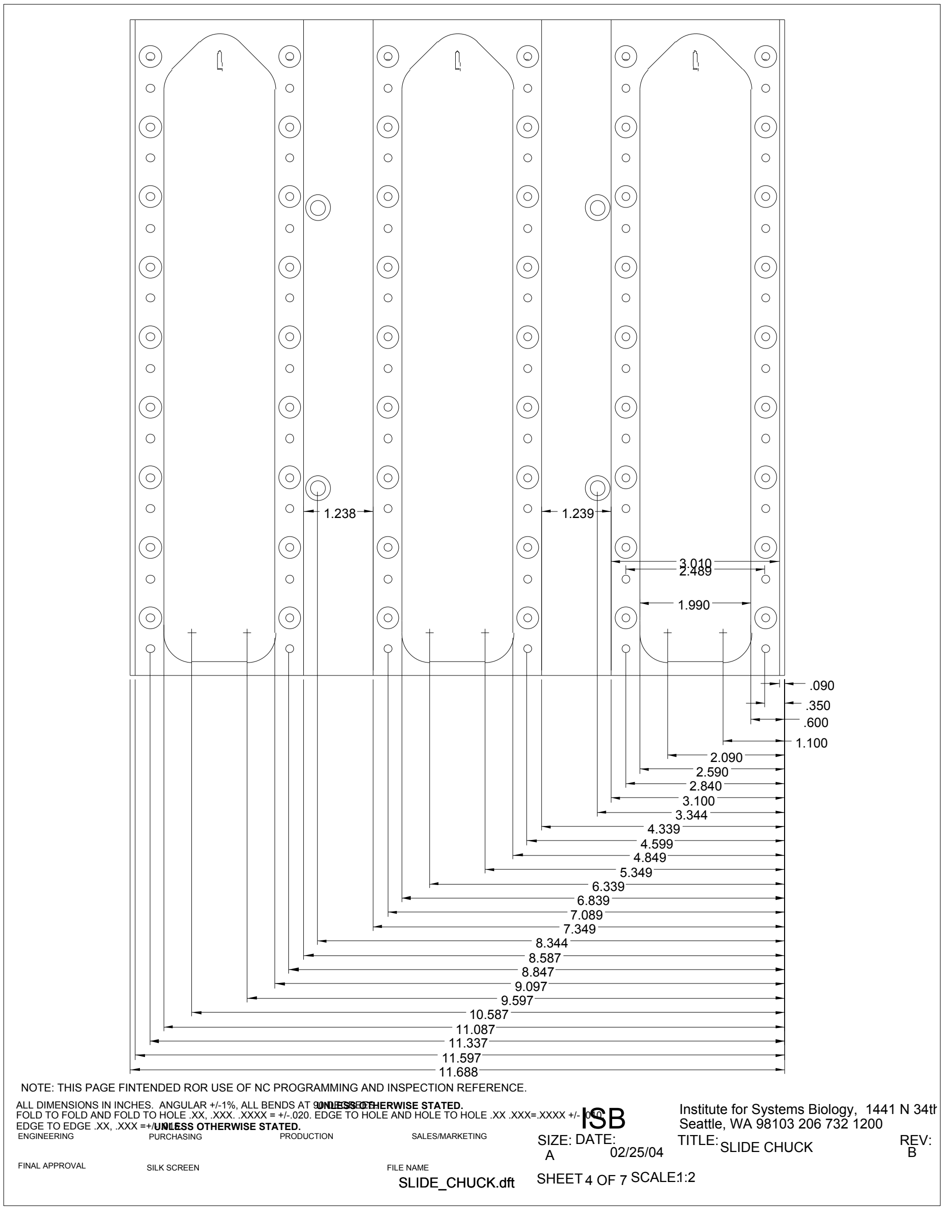



PRINT HEAD RIGHT BRACKET
Ink Jet Microarrayer
Christopher Lausted
11 October 1999
Aluminum
0.125 x 0.125 Groove
2.500
2.250
0.250
Ø0.250
Ø0.438
1.500
4-40 THD
Ø0.375
R0.250
0.750
1.625
0.500
150
120
135
0.5 in
0.500
1.000
3.120
5.000
0.500
0.25
0.125
2.500
6.000
2.500
1.000
1.250

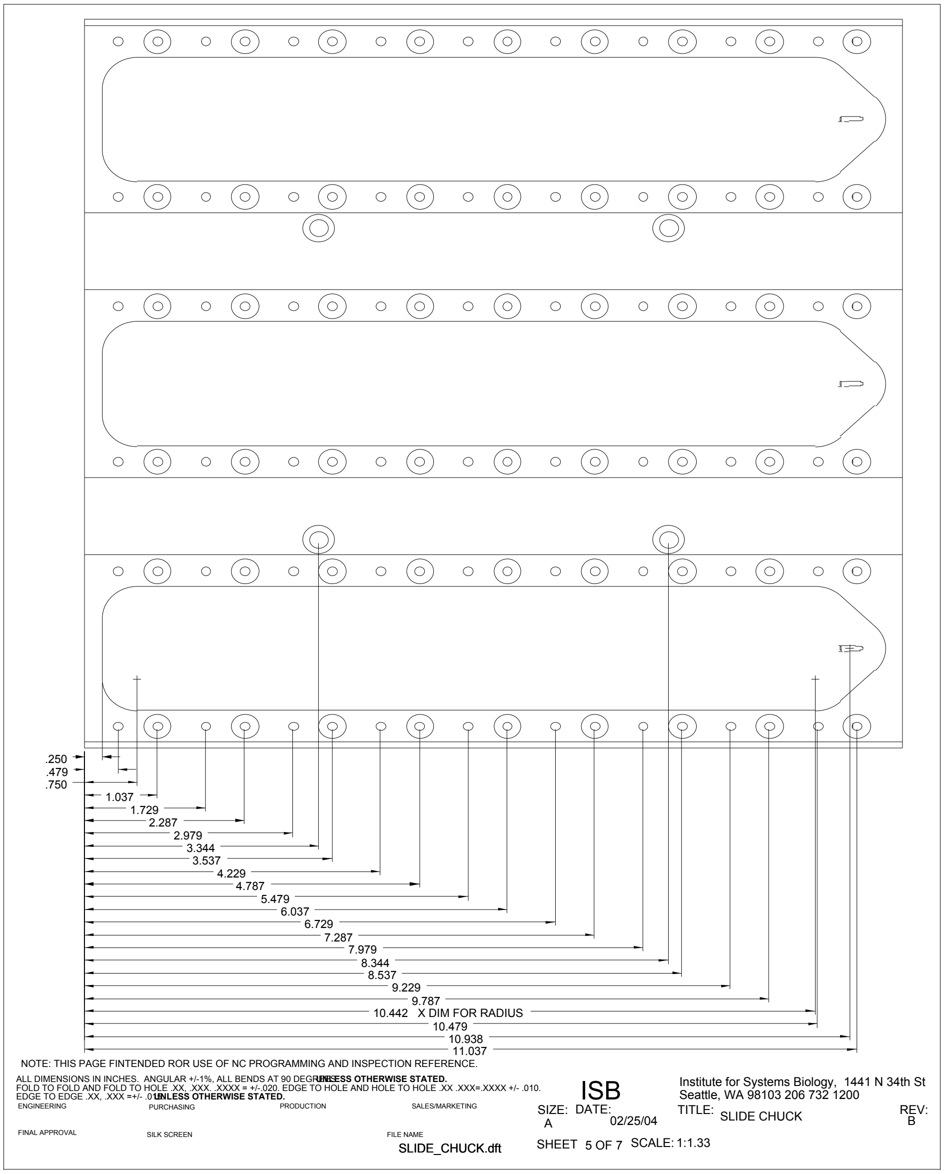
[A3] Slide Holder

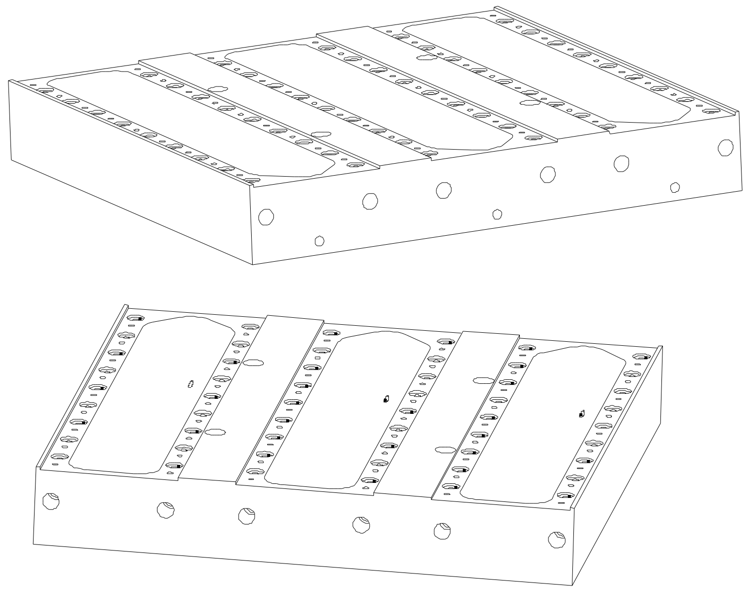






1.238
1.239
3.010
2.489
1.990
.090
.350
.600
1.100
2.090
2.590
2.840
3.100
3.344
4.339
4.599
4.849
5.349
6.339
6.839
7.089
7.349
8.344
8.587
8.847
9.097
9.597
10.587
11.087
11.337
11.597
11.688
NOTE: THIS PAGE FINTENDED ROR USE OF NC PROGRAMMING AND INSPECTION REFERENCE.
ALL DIMENSIONS IN INCHES. ANGULAR +/-1%, ALL BENDS AT 90 DEGREES UNLESS OTHERWISE STATED.
FOLD TO FOLD AND FOLD TO HOLE .XX, .XXX. .XXXX = +/-.020. EDGE TO HOLE AND HOLE TO HOLE .XX .XXX=.XXXX +/-.010.
EDGE TO EDGE .XX, .XXX =+/-.010 UNLESS OTHERWISE STATED.
ENGINEERING
PURCHASING
PRODUCTION
SALES/MARKETING
ISB
Institute for Systems Biology, 1441 N 34th
Seattle, WA 98103 206 732 1200
SIZE: A
DATE: 02/25/04
TITLE: SLIDE CHUCK
REV: B
FINAL APPROVAL
SILK SCREEN
FILE NAME
SLIDE_CHUCK.dft
SHEET 4 OF 7
SCALE 1:2


.250
.479
.750
1.037
1.729
2.287
2.979
3.344
3.537
4.229
4.787
5.479
6.037
6.729
7.287
7.979
8.344
8.537
9.229
9.787
10.442 X DIM FOR RADIUS
10.479
10.938
11.037
NOTE: THIS PAGE FINTENDED ROR USE OF NC PROGRAMMING AND INSPECTION REFERENCE.
ALL DIMENSIONS IN INCHES. ANGULAR +/-1%, ALL BENDS AT 90 DEGREES UNLESS OTHERWISE STATED.
FOLD TO FOLD AND FOLD TO HOLE .XX, .XXX. .XXXX = +/-.020. EDGE TO HOLE AND HOLE TO HOLE .XX .XXX=.XXXX +/- .010.
EDGE TO EDGE .XX, .XXX =+/- .015 UNLESS OTHERWISE STATED.
ENGINEERING
PURCHASING
PRODUCTION
SALES/MARKETING
FINAL APPROVAL
SILK SCREEN
FILE NAME
SLIDE_CHUCK.dft
ISB
Institute for Systems Biology, 1441 N 34th St
Seattle, WA 98103 206 732 1200
SIZE: A
DATE: 02/25/04
TITLE: SLIDE CHUCK
REV: B
SHEET 5 OF 7
SCALE: 1:1.33



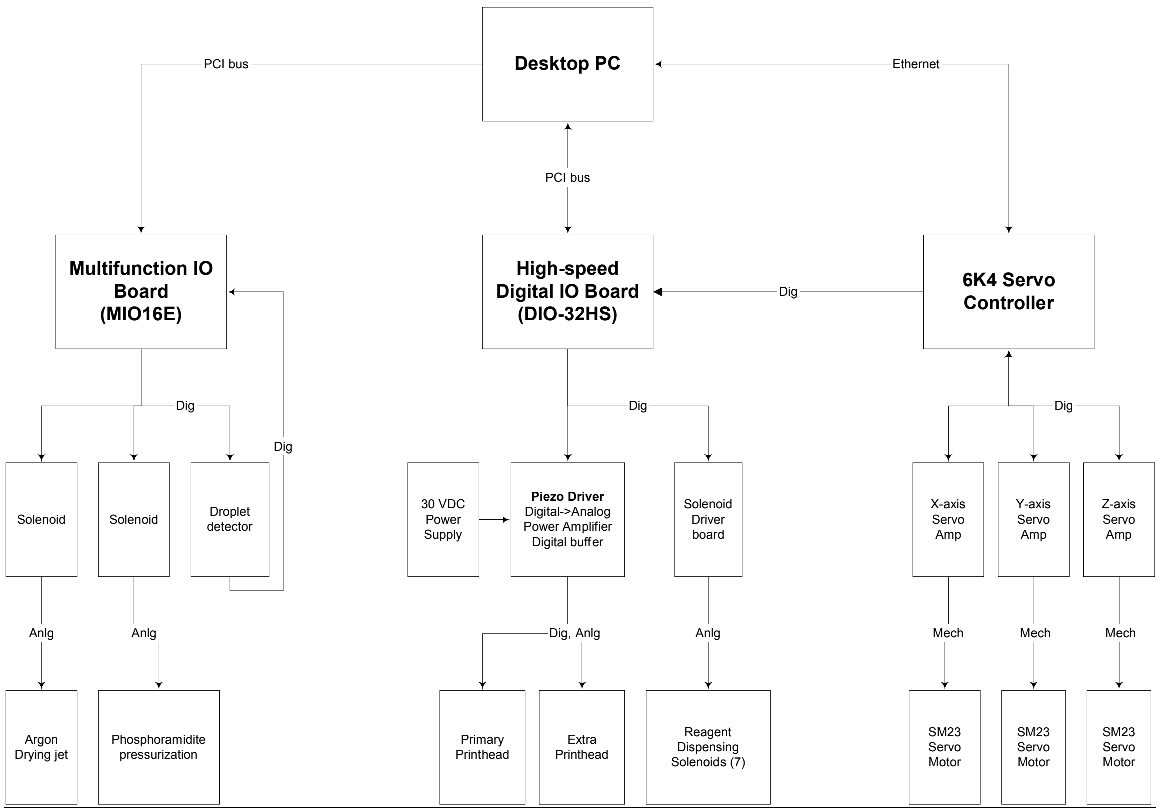
ALL DIMENSIONS IN INCHES. ANGULAR +/-1%, ALL BENDS AT 90 DEGREES UNLESS OTHERWISE STATED.
FOLD TO FOLD AND FOLD TO HOLE .XX, .XXX. .XXXX = +/-.020. EDGE TO HOLE AND HOLE TO HOLE .XX .XXX=.XXXX +/-.010
EDGE TO EDGE .XX, .XXX =+/-.010 UNLESS OTHERWISE STATED.

ENGINEERING	PURCHASING	PRODUCTION	SALES/MARKETING	ISB	Institute for Systems Biology, 1441 N 34th Seattle, WA 98103 206 732 1200
FINAL APPROVAL			FILE NAME SLIDE_CHUCK.dft	SIZE: A DATE: 02/25/04 SHEET 7 OF 7 SCALE:	TITLE: SLIDE CHUCK REV: B

APPENDIX B: ELECTRICAL

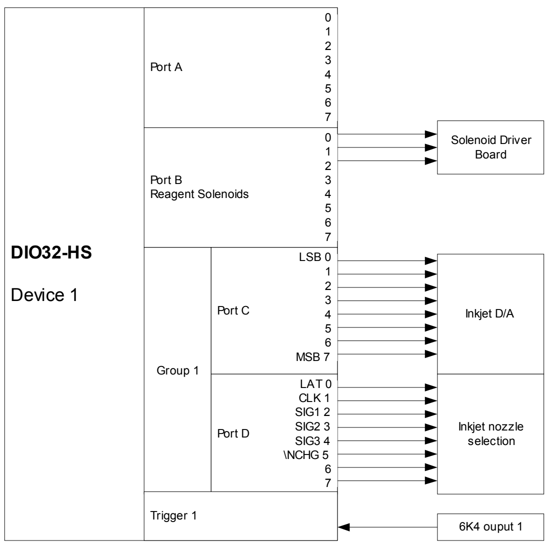
[B1] Electronics Overview

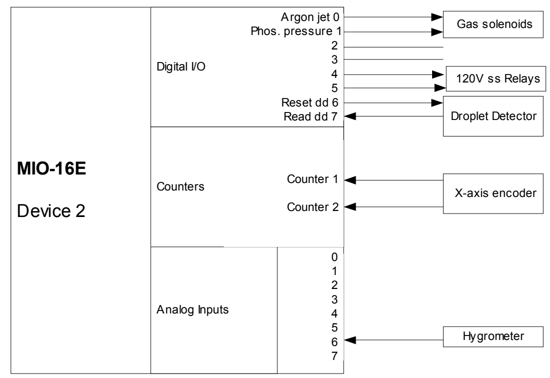
The diagram below provides an overview of the various POSaM electrical interfaces. The supervisory computer controls the piezoelectric printhead, the solenoid valves, and the droplet detection system via two PCI bus interface cards (DIO-32HS and MIO-16E). The computer commands the three-axis positioning system via Ethernet connection to the servo controller (6K4). Trigger positions are programmed in to the 6K4 and piezoelectric pulsing is triggered by digital signaling from the 6K4 to the DIO-32HS. (The solenoid labeled “phosphoramidite presssurization” is currently unused.)



[B2] PCI Card Interfaces

Two National Instruments interface cards are installed in the PCI slots of the PC. The high-speed digital card (DIO32-HS) controls the inkjet print head and eight 24VDC solenoids. (Six Angar solenoids are actually used.) It also receives a digital trigger signal from the 6K4 motion controller. This signal is used to trigger a piezo jet event. Thirteen digital lines remain available for future use. The multipurpose card (MIO-16E) connects to the nitrogen solenoids, the 120VAC solid-state relays, the droplet detector, and the X-axis precision linear encoder. It monitors analog voltage levels from the hygrometer and the droplet detector power supply. Four digital lines and six analog inputs remain available for future use. We intend to use the additional analog inputs to monitor pressure levels at the source dewar, the reagent bottles, and the vacuum.

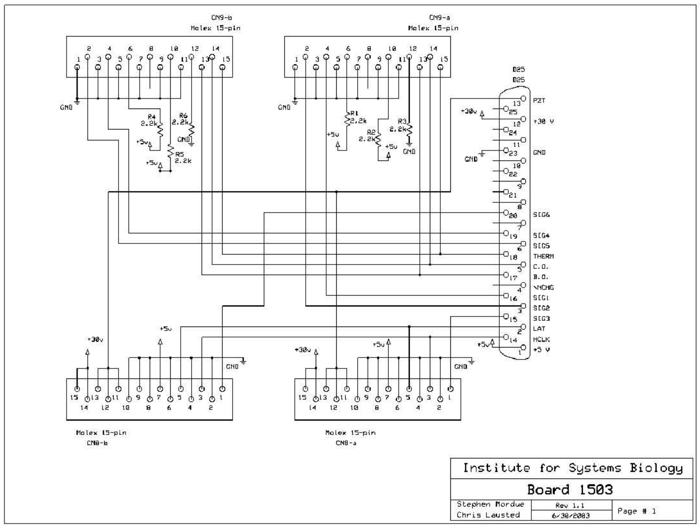


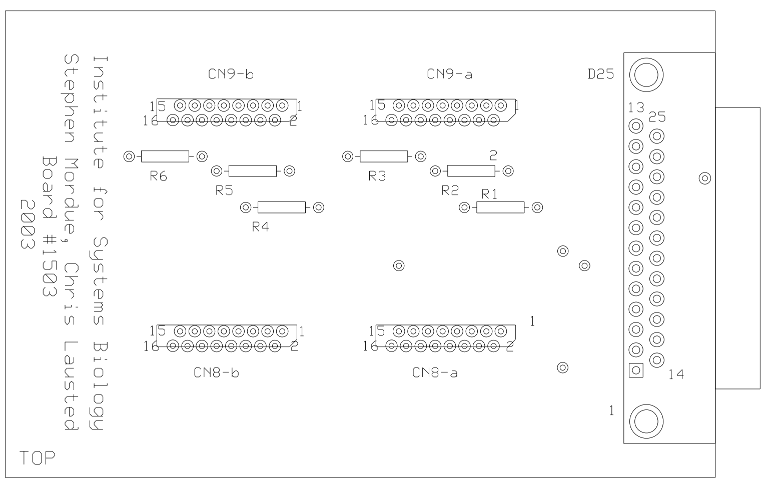


[B3] Board 1503

Circuit schematic

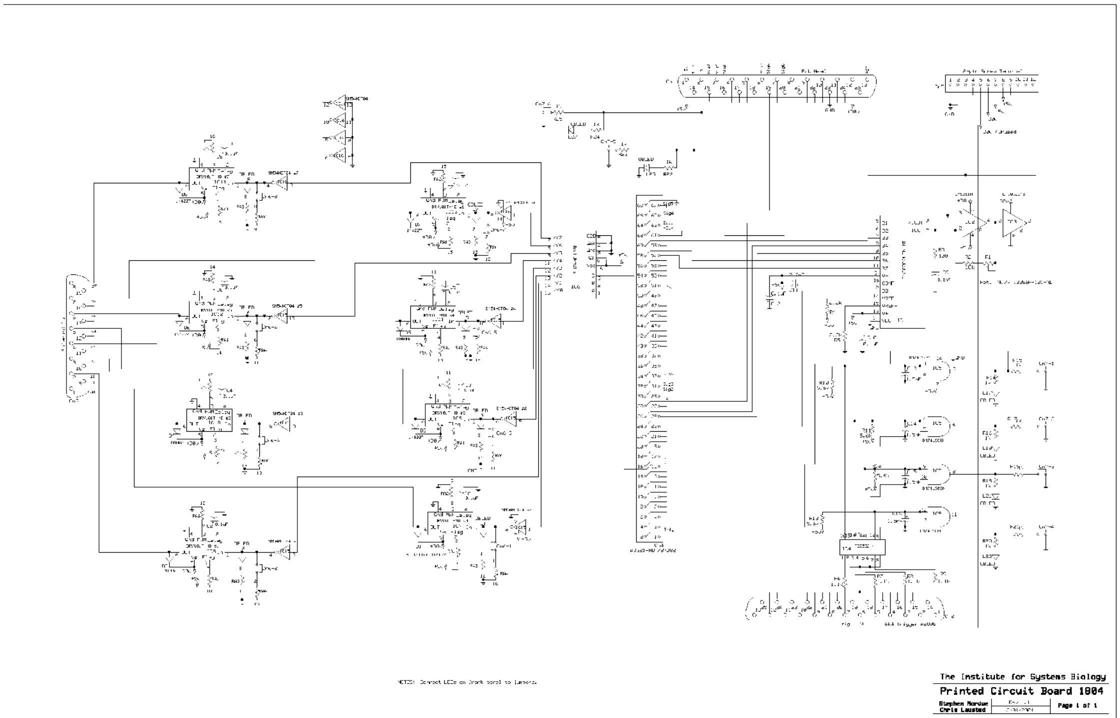
Top view of circuit board



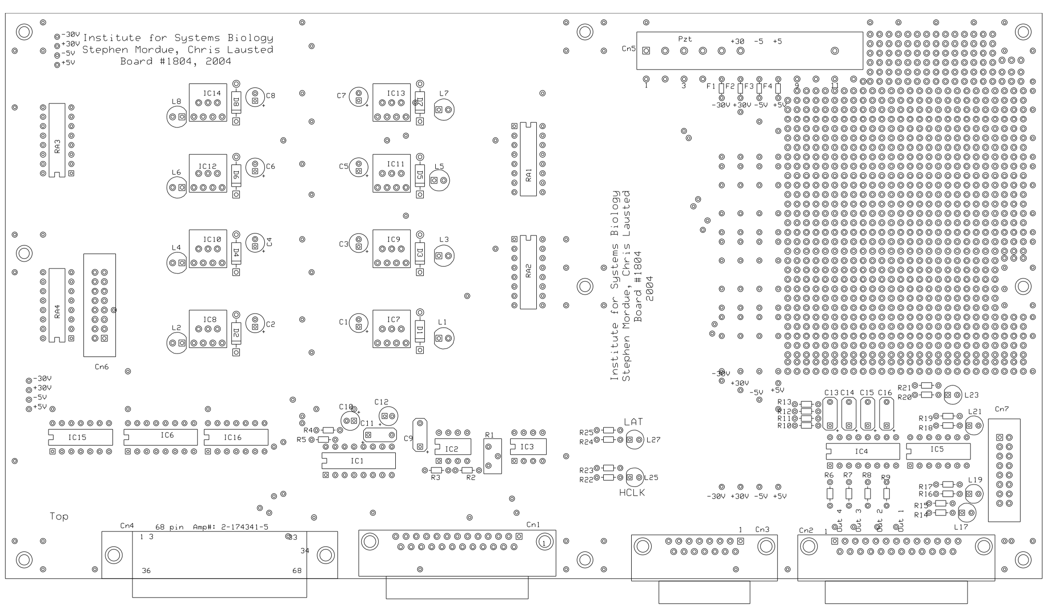


[B4] Board 1804

Circuit schematic

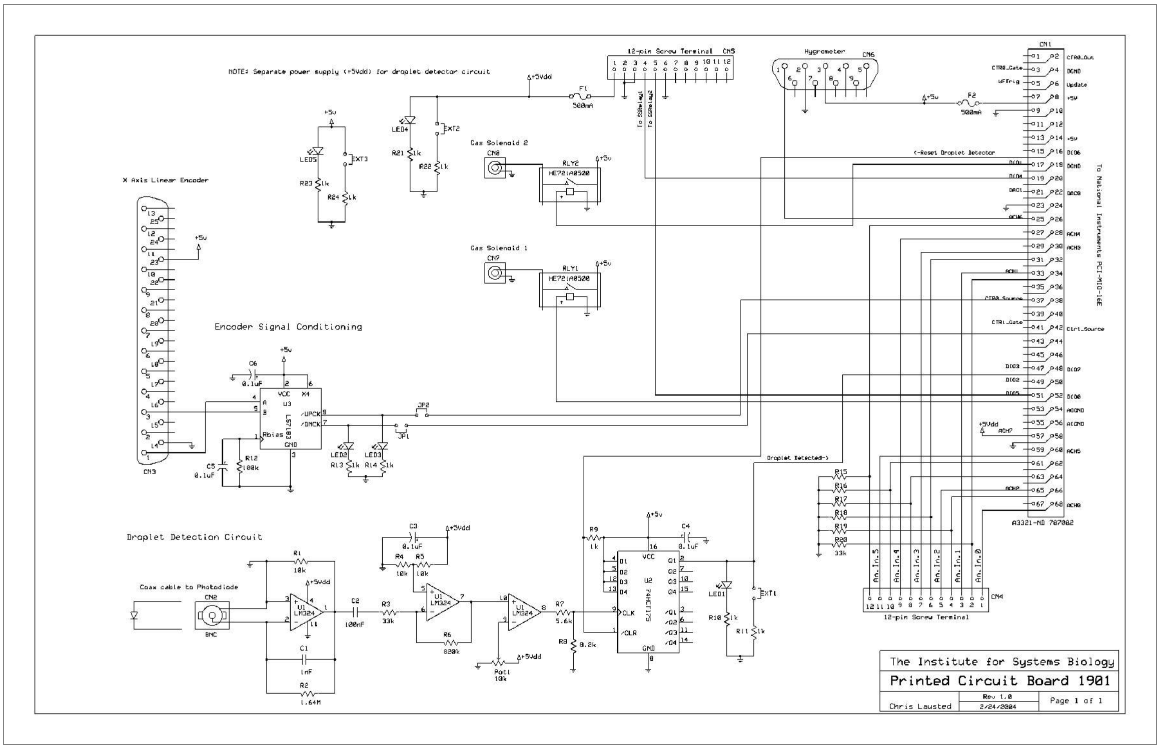


Top view of circuit board

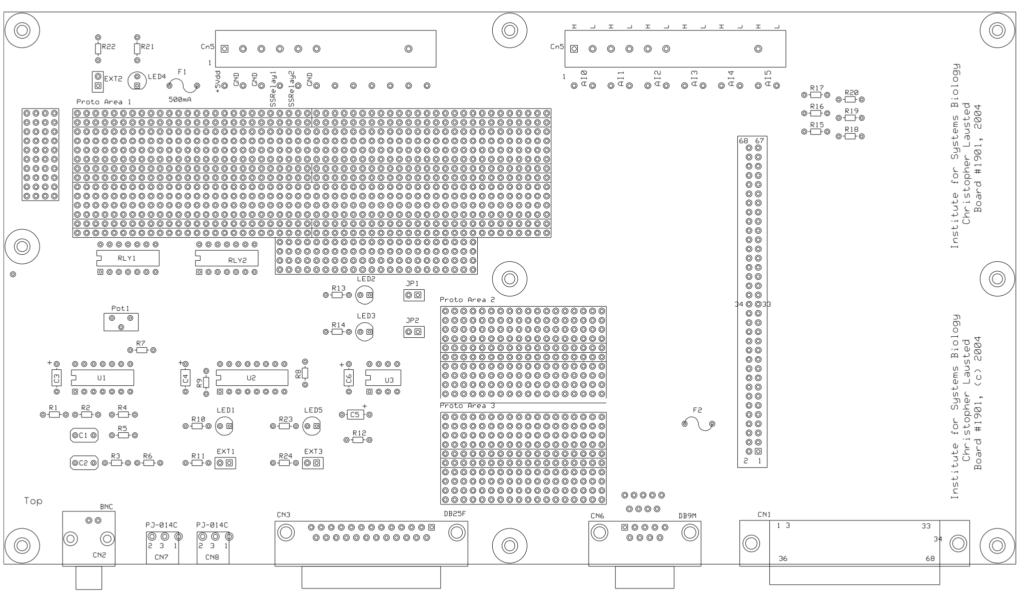


[B5] Board 1901

Circuit schematic

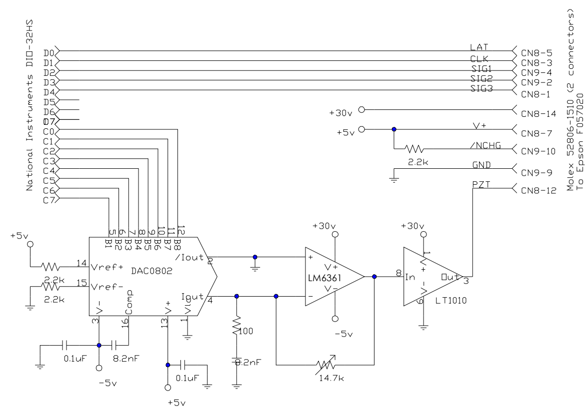


Top view of circuit board



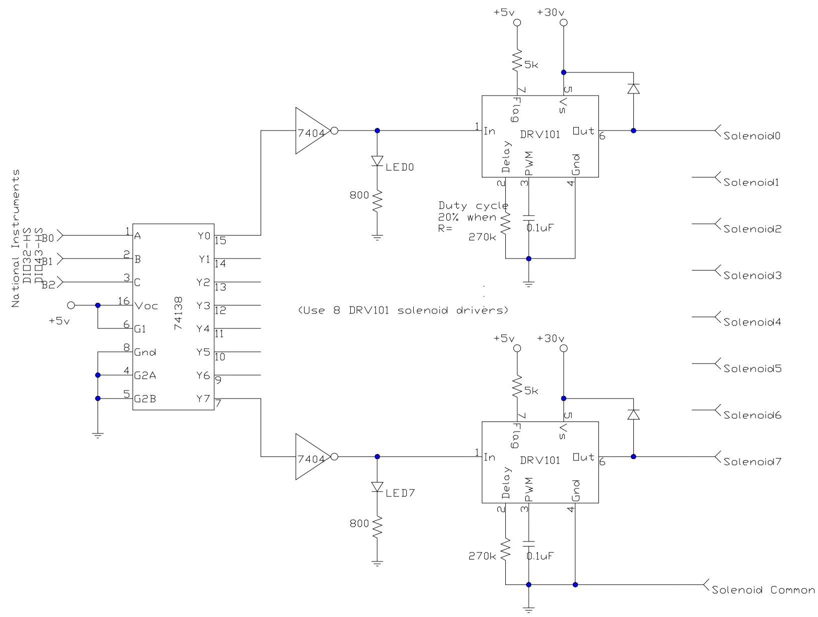
[B6] More circuit information for boards 1804 and 1901

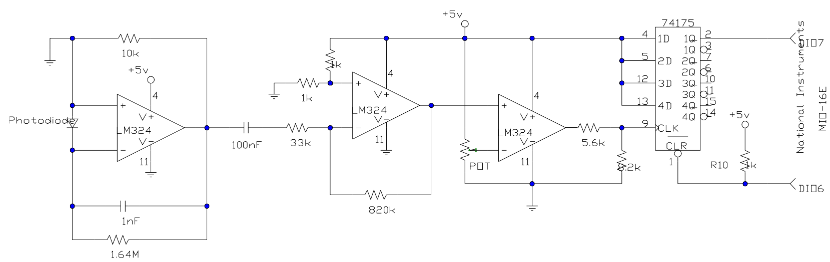
The print head drive circuit resides on board 1804. It uses two 8-bit ports of the DIO-32HS interface board. The lower eight bits connect to a high speed D/A converter (DAC0802, settling time of 100ns) which provides the piezoelectric pulsing voltage (0-30V). Current is boosted by a 150mA power buffer (LT1010). The higher bits provide the clock, latch and signal inputs to the print head.



The drive circuit for the teflon reagent solenoids resides also resides on board 1804. It uses three digital output lines to activate the seven solenoid valves which deliver washes, reagents, and vacuum to the arrayer work area. The driver IC (DRV101) provides a strong initial 100 ms pulse to open the solenoid valve, followed by a 24 kHz square wave with a 20% duty cycle.

The droplet detection circuit resides on board 1901. Droplet verification uses the high-frequency flash of forward-scattered laser light to indicate the presence of an ejected droplet. The output signal if the photodiode is amplified, high-pass filtered, and converted to a digital signal by threshold comparison. The state of the droplet detection is held in a D-type flip-flop. The circuit is reset just prior to droplet ejection and the state is read immediately after ejection.


+5v
+30v
5k
Flag
Vs
In
DRV101
Out
Delay
PWM
Gnd
7404
LED0
800
Duty cycle
20% when
R=
270k
0.1uF
Solenoid0
Solenoid1
Solenoid2
Solenoid3
Solenoid4
Solenoid5
Solenoid6
Solenoid7
National Instruments
DIO32-HS
DIO43-HS
B0
B1
B2
+5v
A
B
C
Vcc
G1
Gnd
G2A
G2B
74138
Y0
Y1
Y2
Y3
Y4
Y5
Y6
Y7
(Use 8 DRV101 solenoid drivers)
LED7
Solenoid Common


10k
+5v
Photodiode
LM324
1nF
1.64M
100nF
33k
1k
820k
POT
5.6k
8.2k
74175
1D
2D
3D
4D
CLK
CLR
R10
DIO7
DIO6
National Instruments
MIO-16E

[B7] Configuration file for Gemini drives

Download and install this file to all three Gemini digital servo amplifiers using Motion Planner or a serial terminal communication program. This requires an RS-232 cable to be connected, one at a time, to each amplifier.

```
;Gemini GV Servo Drive Setup
;File name 'Gemini_GV_SM231AE_config.prg'
;Motor Setup
DMTR 1303                 ;Motor ID (SM/SE231AE)
DMTIC 1.98                ;Continuous Current (Amps-RMS)
DMTICD 22.90              ;Continuous Current Derating (% derating at rated speed)
DMTKE 17.7                ;Motor Ke (Volts (0-to-peak)/krpm)
DMTRES 5.22               ;Motor Winding Resistance (Ohm)
DMTJ 51.970               ;Motor Rotor Inertia (kg*m*m*10e-6)
DPOLE 2                   ;Number of Motor Pole Pairs
DMTW 100.0                ;Motor Rated Speed (rev/sec)
DMTIP 5.94                ;Peak Current (Amps-RMS)
DMTLMN 2.2                ;Minimum Motor Inductance (mH)
DMTLMX 2.9                ;Maximum Motor Inductance (mH)
DMTD 0.000017             ;Motor Damping (Nm/rad/sec)
DMTRWC 0.85               ;Motor Thermal Resistance (degrees Celsius/Watt)
DMTTCM 20.0               ;Motor Thermal Time Constant (minutes)
DMTTCW 0.33               ;Motor Winding Time Constant (minutes)
DMTAMB 40.00              ;Motor Ambient Temperature (degrees Celsius)
DMTMAX 125.00             ;Maximum Motor Winding Temperature (degrees Celsius)
;Drive Setup
DMODE 2                   ;Drive Control Mode
DRES 4000                 ;Drive Resolution (counts/rev)
DPWM 0                    ;Drive PWM Frequency (kHz)
ERES 4000                 ;Encoder Resolution (counts/rev)
ORES 4000                 ;Encoder Output Resolution (counts/rev)
DMEPIT 0.00               ;Electrical Pitch (mm)
DMTLIM 1.2                ;Torque Limit (Nm)
DMTSCL 1.2                ;Torque Scaling (Nm)
DMVLIM 100.000000         ;Velocity Limit (rev/sec)
DMVSCL 100.000000         ;Velocity Scaling (rev/sec)
;Load Setup
LJRAT 0.0                 ;Load-to-Rotor Inertia Ratio
LDAMP 0.0000              ;Load Damping (Nm/rad/sec)
;Fault Setup
FLTSTP 1                  ;Fault on Startup Indexer Pulses Enable
FLTDSB 1                  ;Fault on Drive Disable Enable
SMPER 4000                ;Maximum Allowable Position Error (counts)
SMVER 0.000000            ;Maximum Allowable Velocity Error (rev/sec)
DIFOLD 0                  ;Current Foldback Enable
;Digital Input Setup
LH 0                      ;Hardware EOT Limits Enable
INLVL 11000000            ;Input Active Level
INDEB 50                  ;Input Debounce Time (milliseconds)
;Digital Output Setup
OUTLVL 0000000            ;Output Active Level
;Analog Monitor Setup
DMONAV 0                  ;Analog Monitor A Variable
DMONAS 100                ;Analog Monitor A Scaling (% of full scale output)
DMONBV 0                  ;Analog Monitor B Variable
DMONBS 100                ;Analog Monitor B Scaling (% of full scale ouput)
;Servo Tuning
DIBW 1000                 ;Current Loop Bandwidth (Hz)
DVBW 100                  ;Velocity Loop Bandwidth (Hz)
DPBW 25.00                ;Position Loop Bandwidth (Hz)
SGPSIG 1.000              ;Velocity/Position Bandwidth Ratio
SGIRAT 1.000              ;Current Damping Ratio
SGVRAT 1.000              ;Velocity Damping Ratio
SGPRAT 1.000              ;Position Damping Ratio
DNOTAF 0                  ;Notch Filter A Frequency (Hz)
DNOTAQ 1.0                ;Notch Filter A Quality Factor
DNOTAD 0.0000             ;Notch Filter A Depth
DNOTBF 0                  ;Notch Filter B Frequency (Hz)
DNOTBQ 1.0                ;Notch Filter B Quality Factor
DNOTBD 0.0000             ;Notch Filter B Depth
DNOTLG 0                  ;Notch Lag Filter Break Frequency (Hz)
DNOTLD 0                  ;Notch Lead Filter Break Frequency (Hz)
SGINTE 1                  ;Integrator Enable
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APPENDIX C: LISTS OF COMPONENTS

[C1] Robotics

Components necessary for POSAM robotics

Total: $23,206.00

Label	Component	Description	Supplier	Part no.	No.	Cost Ea.	Cost
PR01	Motion controller, 4-axis	Compumotor 6K	Olympic contrc	6K4	1	$2595.0000	$2,595.00
PR02	Servo drive, digital	Compumotor GV	Olympic contrc	GV-L3E	3	$845.0000	$2,535.00
PR03	Servo motor, NEMA 1-stack	SM23	Olympic contrc	SM231-AE-NGSN	3	$732.0000	$2,196.00
PR04	Cables, drive to motor	Yellow	Olympic contrc	23GS-CABLE10	3	$313.0000	$939.00
PR05	Cables, controller to drive	Black	Olympic contrc	71-016987-10	3	$150.0000	$450.00
PR06	Power supply, 24VDC, 2.5A	24V	Olympic contrc	PS60W	1	$250.0000	$250.00
PR07	Motion table, 24 inch	Daedal 506	Olympic contrc	506024ST-ES-D2L2C5M2E3	1	$4533.0000	$4,533.00
PR08	Motion table, 20 inch	Daedal 506	Olympic contrc	506020ST-ES-D2L2C5M2E3	1	$4372.0000	$4,372.00
PR09	Motion table, 4 inch	Daedal 105	Olympic contrc	106004BT-ES-D2L2C5M1E1	1	$1724.0000	$1,724.00
PR10	Brackets, vertical mounting	Anodized aluminum	Olympic contrc	4990-08	2	$250.0000	$500.00
PR11	Personal computer, Pentium III	256MB RAM, 4 PCI slots	various	various	1	$1000.0000	$1,000.00
PR12	Optical Breadboard, 35x47x2 inch	Lightweight	Coherent	61-9312	1	$1089.0000	$1,089.00
PR13	Breadboard stand	Leveling adjustment	Coherent	54-0930	1	$1023.0000	$1,023.00

[C2] Optical Mechanical

Droplet detector opto/mechanical parts

Total: $583.04

Label	Component	Description	Supplier	Part no.	No.	Cost Ea.	Cost
PO01	Filter, red glass	RG	Edmond Industrial Opti	K32-755	1	$19.0000	$19.00
PO02	Convex lens, 50 mm f.l.	1" dia	Edmond Industrial Opti	K32-478	1	$21.9000	$21.90
PO03	Post holder	½" dia	Edmond Industrial Opti	K02-656	2	$26.0000	$52.00
PO04	Laser LED, 670 nm, 3 mW	Red	Edmond Industrial Opti	K38-922	1	$175.0000	$175.00
PO05	Laser diode mount		Edmond Industrial Opti	K53-264	1	$90.0000	$90.00
PO06	Post clamp, right angle	½" dia	Edmond Industrial Opti	K53-357	2	$19.8500	$39.70
PO07	Photodiode, 1cm x 1cm square area		Edmond Industrial Opti	K53-373	1	$98.0000	$98.00
PO08	Ring nuts, 1"	1" dia	Thor Labs	SM1RR	3	$5.0000	$15.00
PO09	Mounting ring clamp, SM1 style	1" dia	Thor Labs	SMR1	1	$16.0000	$16.00
PO10	Barrel, SM1 style	1" dia	Thor Labs	SM1L20	1	$18.0000	$18.00
PO11	Post, 4" long	½" dia	Thor Labs	TR4	2	$6.5000	$13.00
PO12	Post, 8" long	½" dia	Thor Labs	TR8	2	$8.0000	$16.00
PO13	BNC cable		Digi-Key	290-1024-ND	1	$9.44	$9.44

[C3] PCB 1503

Components for printed circuit board 1503

Total: $86.52

Label	Component	Description	Supplier	Part no.	No.	Cost Ea.	Cost
-	Circuit board 1503	File 1503.pcb	Express PCB	File 1503.pcb	3	$20.6667	$62.00
--	Aluminum stand-off	#4-40, 1" long, threaded	Digi-Key	8405K-ND	8	$0.4850	$3.88
--	Aluminum stand-off	#4-40, 1" long, threaded	Digi-Key	8405K-ND	8	$0.4850	$3.88
CN8a, CN8b,	Molex micro connector, 15 conducter	15 pin	Mouser	538-52806-151	8	$0.7100	$5.68
D25	D-SUB 25-pin connector plug - male	D25	Digi-Key	A2098	2	$4.8400	$9.68
R1-R6	resistor	2.2 kOhm 1/8W	Digi-Key	2.2KQBK-ND	25	$0.0560	$1.40

[C4] PCB 1804

Components for printed circuit board 1804

Total: $703.03

Label	Component	Description	Supplier	Part no.	No.	Cost Ea.	Cost
-	Circuit board 1804	File 1804.pcb	Express PCB	File 1804.pcb	2	$88.6500	$177.30
--	Aluminum stand-off	#4-40, 1" long, threaded	Digi-Key	8405K-ND	27	$0.4850	$13.10
--	LED, Nice panel mount 3mm green	Threaded	Digi-Key	67-1148-ND	10	$0.8000	$8.00
--	LED, Nice panel mount 3mm red	Threaded	Digi-Key	67-1147-ND	10	$0.8000	$8.00
C13-C16	plastic film capacitor	1.5 nF	Digi-Key	PS1H152J-ND	8	$0.3500	$2.80
C1-C8 C10 C12	aluminum electrolytic capacitor	0.1 uF	Digi-Key	P5559-ND	20	$0.1520	$3.04
C9 C11	plastic film capacitor	8.2 nF	Digi-Key	PS1H822J-ND	4	$0.3700	$1.48
CN1	D-SUB 25-pin connector receptacle - female	Pzt Head	Digi-Key	182-825F-ND	2	$2.8700	$5.74
PLUG04	D-SUB 25-pin connector -male	Matches CN1	Digi-Key	1125M-ND	2	$3.6400	$7.28
CN2	D-SUB 25-pin connector plug - male	6k4 Trigger	Digi-Key	A2098	2	$4.8400	$9.68
PLUG05	D-SUB 25-pin connector – female	Matches CN2	Digi-Key	1125F-ND	2	$4.7600	$9.52
CN3	D-SUB 15-pin connector receptacle - female	Solenoid Out	Digi-Key	A2101	2	$2.8900	$5.78
PLUG06	D-SUB 15-pin connector – male	Matches CN3	Digi-Key	2215M-ND	2	$1.9100	$3.82
Cn4	Amp 68-pin connector, male	Amp #: 2-174225-5	Digi-Key	A29093-ND	2	$20.1900	$40.38
Cn5	12-pin Screw Terminal	Screw Terminal	Digi-Key	277-1257-ND	2	$4.3600	$8.72
D1-D8	diode	1N4007	Digi-Key	1N4007DICT-ND	16	$0.1360	$2.18
F1-F4	Fuse	Fuse	Digi-Key	283-2111-ND	16	$1.7300	$27.68
H1-H2	Breakaway header mounts	16-pin Header mount	Digi-Key	WM6880-ND	4	$4.4300	$17.72
IC1	Digital to analog converter	DAC0802	Digi-Key	DAC0802LCN-ND	2	$1.5800	$3.16
IC15-IC16	NOT gate, hex	7404	Digi-Key	296-1605-5-ND	4	$0.4400	$1.76
IC2	Op amp	LM6361	Digi-Key	LM6361N-ND	2	$2.9800	$5.96
IC3	Follower	LT1010	Digi-Key	LT1010CN8	2	$4.3800	$8.76
IC4	Optoisolation, quad, darlington	PS2502	Digi-Key	PS2502-4	2	$21.5000	$43.00
IC5	AND gate, quad	7408	Digi-Key	DM74LS08N	2	$0.6000	$1.20
IC6	Decoder	74138	Digi-Key	SN74HC138N	2	$0.4500	$0.90
IC7-IC14	Solenoid driver	DRV101T	Digi-Key	DRV101T-ND	16	$7.7000	$123.20
L17, L19 L21, L23 L25, L27	LED	LED RED T1-3/4 W/STD	Digi-Key	HLMP3750ACM-ND	12	$0.3500	$4.20
L18, L20 L22, L24 L26, L28	LED	LED 3MM FLUSH YELLC	Digi-Key	67-1149-ND	12	$0.8000	$9.60
L1-L8	LED	LED GREEN HI-BRITE T	Digi-Key	P461-ND	16	$0.2750	$4.40
L9-L16	LED	LED 3MM FLUSH GREE	Digi-Key	67-1148-ND	16	$0.8000	$12.80
PS1	+- 5V Power Supply	Linear Dual 5V @1.5 A	Digi-Key	271-2010-ND	1	$54.8100	$54.81
PS2	+- 30V Power Supply	Linear Single 28V@1A	Digi-Key	271-2017-ND	1	$37.0300	$37.03
R1	potentiometer	~4.7k Ohms	Digi-Key	3214J-103ECT-ND	2	$3.2200	$6.44
R10-R13	resistor	5.6 kOhm 1/8W	Digi-Key	5.6KEBK-ND	25	$0.0560	$1.40
R14-R25	resistor	1 kOhm 1/8W	Digi-Key	1.0KEBK-ND	25	$0.0560	$1.40
R2	resistor	10k Ohms 1/8W	Digi-Key	10KEBK-ND	25	$0.0560	$1.40
R3	resistor	100 Ohm 1/8W	Digi-Key	100EBK-ND	25	$0.0560	$1.40
R4-R5	resistor	2.2 kOhm 1/8W	Digi-Key	2.2KQBK-ND	25	$0.0560	$1.40
R6-R9	resistor	1.1 kOhm 1/8W	Digi-Key	1.1KEBK-ND	25	$0.0560	$1.40
RA1	resistor array	4.7 kOhms	Digi-Key	4116R-1-472-ND	2	$0.6000	$1.20
RA2	resistor array	270 kOhms	Digi-Key	4116R-1-274-ND	2	$0.6000	$1.20
RA3-RA4	resistor array	820 Ohms	Digi-Key	4116R-1-821-ND	4	$0.6000	$2.40
S1 S4 S6 S17-S20	Socket	16-pin Socket	Digi-Key	A9416-ND	14	$0.8700	$12.18
S2-S3	Socket	8-pin Socket	Digi-Key	A9408-ND	4	$0.5700	$2.28
S5 S15-S16	Socket	14-pin Socket	Digi-Key	A9414-ND	6	$0.9900	$5.94

[C5] PCB 1901

Components for printed circuit board 1901

Total: $482.08

Label	Component	Description	Supplier	Part no.	No.	Cost Ea.	Cost
-	Circuit board 1901	File 1901.pcb	Express PCB	File 1901.pcb	2	$89.2000	$178.40
--	Aluminum stand-off	#4-40, 1" long, threaded	Digi-Key	8405K-ND	27	$0.4850	$13.10
--	LED, Nice panel mount 3mm green	Threaded	Digi-Key	67-1148-ND	10	$0.8000	$8.00
--	LED, Nice panel mount 3mm red	Threaded	Digi-Key	67-1147-ND	10	$0.8000	$8.00
--	LED	LED 3MM FLUSH GREE	Digi-Key	67-1148-ND	10	$0.8000	$8.00
--	LED	LED 3MM FLUSH YELLC	Digi-Key	67-1149-ND	10	$0.8000	$8.00
C1	Ceramic Disc Capacitor (0.25in)	1nF	Digi-Key	P10461-ND	10	$0.3000	$3.00
C2	Radial Leaded Capacitor (0.2in)	100nF	Digi-Key	399-2143-ND	10	$0.1210	$1.21
C3-6	Electrolytic Capacitor (0.3in)	0.1uF, 50V	Digi-Key	P10967-ND	10	$0.3600	$3.60
CN1	Amp 68-pin connector, male	Amp #: 2-174225-5	Digi-Key	A29093-ND	2	$20.1900	$40.38
CN2	BNC connector	Amphenol 31-5431-2010	Digi-Key	ARF1065NW-ND	2	$7.5800	$15.16
CN3	D-SUB 25-pin connector receptacle-female	DB25 PCB mount	Digi-Key	182-825F-ND	2	$2.8700	$5.74
PLUG01	D-SUB 25-pin connector receptacle-male	DB25 solder	Digi-Key	1125M-ND	2	$3.4500	$6.90
CN4-5	12 pin Screw Terminal	Screw Terminal	Digi-Key	277-1257-ND	2	$4.3600	$8.72
CN6	D-SUB 9-pin connector plug-male	DB9 PC mount	Digi-Key	182-809M-ND	2	$1.8300	$3.66
PLUG02	D-SUB 9-pin connector plug-female	DB9 solder	Digi-Key	2209F-ND	2	$1.6200	$3.24
CN7-8	Connector power jack 1mm	CUI part # PJ-014C	Digi-Key	CP-014C-ND	4	$0.4100	$1.64
PLUG03	Connector power plug 1mm	Matches CN7 and CN8	Radio Shack	NA	4	$0.4900	$1.96
F1-2	Fuse(0.2in)	500mA, 250V	Digi-Key	283-2111-ND	10	$1.7300	$17.30
H1-5	Breakaway square header (80pin)	0.1inch	Digi-Key	WM6880-ND	2	$4.4300	$8.86
JP1-2	Closed end shorting jumper	0.1inch	Digi-Key	S9001-ND	10	$0.1160	$1.16
LED1-3	LED	LED GREEN HI-BRITE T	Digi-Key	P461-ND	10	$0.2750	$2.75
LED4-5	LED	LED RED T1-3/4 W/STD	Digi-Key	HLMP3750ACM-ND	10	$0.3500	$3.50
Pot1	Potentiometer	3386H series 10k	Digi-Key	3386H-103-ND	2	$1.2900	$2.58
R1, 4-5	Carbon Film 1/8W (0.25in)	10kOhm	Digi-Key	10kEBK-ND	10	$0.0560	$0.56
R12	Carbon Film 1/8W (0.25in)	100kOhm	Digi-Key	100kEBK-ND	10	$0.0560	$0.56
R13-14	Carbon Film 1/8W (0.25in)	1kOhm	Digi-Key	1.0kEBK-ND	10	$0.0560	$0.56
R15-20	Carbon Film 1/8W (0.25in)	33kOhm	Digi-Key	33kEBK-ND	10	$0.0560	$0.56
R2	Carbon Film 1/8W (0.25in)	1.64MOhm	Digi-Key	1.6MEBK-ND	10	$0.0560	$0.56
R21-24	Carbon Film 1/8W (0.25in)	1kOhm	Digi-Key	1.0kEBK-ND	10	$0.0560	$0.56
R3	Carbon Film 1/8W (0.25in)	33kOhm	Digi-Key	33kEBK-ND	10	$0.0560	$0.56
R6	Carbon Film 1/8W (0.25in)	820kOhm	Digi-Key	820kEBK-ND	10	$0.0560	$0.56
R7	Carbon Film 1/8W (0.25in)	5.6kOhm	Digi-Key	5.6kEBK-ND	10	$0.0560	$0.56
R8	Carbon Film 1/8W (0.25in)	8.2kOhm	Digi-Key	8.2kEBK-ND	10	$0.0560	$0.56
R9-11	Carbon Film 1/8W (0.25in)	1kOhm	Digi-Key	1.0kEBK-ND	10	$0.0560	$0.56
RLY1-2	Relay		Digi-Key	HE100-ND	4	$1.5900	$6.36
SO1-2	Socket (14-pin)	0.3inch	Digi-Key	A9414-ND	8	$0.7920	$6.34
SO3	Socket (8-pin)	0.3inch	Digi-Key	A9408-ND	4	$0.5700	$2.28
U1	Op amp, quad	LM324N	Digi-Key	296-1391-5-ND	1	$0.4900	$0.49
U2	D flip-flop, quad	74HCT175	Digi-Key	296-2101-5-ND	1	$0.5000	$0.50
U3	Encoder signal conditioner	LS7183	usdigital.com	LS7183	2	$3.0500	$6.10
HU1	Humidity Sensor	HM1520	Digi-Key	HM1520-ND	2	$49.5000	$99.00

[C6] Other Parts

Miscellaneous POSaM components

Total: $8,663.05

Label	Component	Description	Supplier	Part no.	No.	Cost Ea.	Cost
ME01	Rack mountable case, 19"	Electronics rack	Mouser (Manfield, TX)	63-CH-14404	2	$78.8000	$157.60
ME02	Chassis cover, 19"	Electronics rack	Mouser (Manfield, TX)	563-C-14432	2	$26.4000	$52.80
ME03	AC power inlet with fuse holder	Electronics rack	Mouser (Manfield, TX)	161-0717-187	4	$2.0900	$8.36
ME04	Power switch – SPST	Electronics rack	Mouser (Manfield, TX)	642-631H2	4	$5.0800	$20.32
ME05	Fuse 5x20mm 5A 250V	Electronics rack	Digi-Key (Thief River Fa	F952	10	$0.2300	$2.30
ME06	DIN rail	Electronics rack	Mouser (Manfield, TX)	651-5602100	1	$8.9100	$8.91
ME07	Power strip and surge protector, rack mount	Electronics rack	Mouser (Manfield, TX)	563-POS-1295-S	1	$49.5000	$49.50
ME08	Shelf, 19"	Electronics rack	Mouser (Manfield, TX)	563-SA1278-BT	1	$82.8000	$82.80
ME09	Panel, 19"	Electronics rack	Mouser (Manfield, TX)	546-PBPA19014BK2	1	$40.9900	$40.99
ME10	Connecter, DB-25M	Electronics rack	Digi-Key (Thief River Fa	1125M-ND	2	$3.2500	$6.50
ME11	Hood, DB-25 connector	Electronics rack	Digi-Key (Thief River Fa	970-25MP-ND	2	$5.8600	$11.72
ME12	Digital I/O board (PCI-6533 a.k.a. DIO-32HS)	Electronics rack	National Instruments (A	777314-01	1	$995.00	$995.00
ME13	Multi I/O board, (PCI-6040E a.k.a. MIO-16E)	Electronics rack	National Instruments (A	777383-01	1	$845.00	$845.00
ME14	Cable, 68 pin, for PCI-MIO-16E	Electronics rack	National Instruments (A	184749-01	1	$95.00	$95.00
ME15	Cable, 68 pin, for PCI-DIO-32HS	Electronics rack	National Instruments (A	183432-01	1	$125.00	$125.00
MC01	Draw Latch, Type 304 SS	Cover	Small Parts (Miami Lak	B-LD-1	6	$6.6000	$39.60
MC02	Hardware, stainless steel	Cover	Digi-Key (Thief River Fa	1607-KIT-ND	1	$39.9500	$39.95
MC03	Hinges	Cover			3		$0.00
MC04	Fender washers, 1" dia, ¼" hole, 25/pk	Cover	Small Parts (Miami Lak	B-WXF-1/4	1	$5.2000	$5.20
MC05	Fender washers, 1½" dia, 25/pk	Cover	Small Parts (Miami Lak	B-WXF-3/8	1	$6.9500	$6.95
MC06	Weather seal, 5/16" thick, 19/32" wide	Cover	Hardware store	#63669 M+D Building Products	4	$5.0000	$20.00
MC07	Weather seal, 5/16" thick, "D" profile	Cover	Hardware store	M+D Building Products or equi	4	$5.0000	$20.00
MC08	Box gloves and fastening clamps	Cover	VWR	56223-154	1	$100.5000	$100.50
MC09	Polyethylene tape, 18m roll 2" wide	Cover	Small Parts (Miami Lak	B-HTT-190532	1	$33.6500	$33.65
MC10	Valve, low resistance air, 22-30mm diameter	Cover	Instrumentation Industr	BE 131 or similar	10	$15.0000	$150.00
MC11	(Shop to fabricate cover)	Cover	Clear Cut Plastics (Sea	Contact: Dave Ryan	1	$800.0000	$800.00
--	Tubing, 3/8" OD, Polyethylene or copper	Gas pressure	Hardware store	Hardware store	1	$10.0000	$10.00
--	Misc. brass fittings, "T" connectors, and adapte	Gas pressure	Hardware store	Hardware store	1	$100.0000	$100.00
MG01	Nylon fittings	Gas pressure	Small Parts (Miami Lak	B-TCNY-K5	1	$98.5000	$98.50
MG02	Vacuum gauge, 0-30inHg range.	Gas pressure	Various	Ashcroft or equivalent	1		$0.00
MG03	Solenoid, ET-2, 6Volt	Gas pressure	Airtronics of Bellevue, \	ET-2-6-L	2	$12.0000	$24.00
MG04	Pressure regulator, 0-10psi range	Gas pressure	Various	8067 or equivalent	2		$0.00
MG05	Check valves, 0.1psi cracking pressure	Gas pressure	Omnifit	11340	6	$20.0000	$120.00
MG06	Regulator, 0-200psi	Gas pressure	Various	Controls Corp. 2023301-580M c	1	$120.0000	$120.00
MG07	Solenoid valve, large, 120VAC	Gas pressure	iprocessmart.com	Burkert 450914V	1	$68.0000	$68.00
MG08	Rotameter/valve 0-100 liters per minute	Gas pressure	Dwyer (Michigan City, I	VFA-27-BV	1	$26.2500	$26.25
MG09	Aluminum tubing 1/8" OD, 12" long	Gas pressure	Hardware store	NA	1	$4.0000	$4.00
MG10	Manifold	Gas pressure	Small Parts (Miami Lak	B-TCM-13-20/6	2	$10.8500	$21.70
MG11	Tube clamp valve for flexible tubing, 10/pk	Gas pressure	Small Parts (Miami Lak	B-DC-1-4	1	$3.7500	$3.75
MG12	Air amplifier (Blower)	Gas circulation	ARTX	Hardware store	1	$250.0000	$250.00
MG13	"T" joint, ABS plastic, 1½" size	Gas circulation	Hardware store	NA	1	$2.0000	$2.00
MG14	Adapter, ABS plastic, 4" to 1½" size	Gas circulation	Hardware store	NA	1	$2.0000	$2.00
MG15	End cap, ABS plastic, 11/2" size	Gas circulation	Hardware store	NA	1	$1.0000	$1.00
MR01	GL-45 ported caps	Reagent handling	Western Analytical	BC-1-72-24	6	$19.5000	$117.00
MR02	Bottle, GL45 cap, 500mL, case	Reagent handling	VWR	16157-169	1	$98.1200	$98.12
MR03	Bottle, GL45 cap, 2L, case	Reagent handling	VWR	16157-227	1	$317.4300	$317.43
MR04	1/8 OD Teflon tubing 50ft and fittings	Reagent handling	Upchurch Scientific	1509L	1	$147.0000	$147.00
MR05	Teflon fitting nuts, 10 pack	Reagent handling	Upchurch Scientific	P-306x	3	$10.0000	$30.00
MR06	Ferrules, 10 pack.	Reagent handling	Upchurch Scientific	P-300x	3	$10.0000	$30.00
MR07	1/16" OD Teflon tubing, 20ft	Reagent handling	Upchurch Scientific	1548	3	$40.0000	$120.00
MR08	Tefzel fitting nuts, 10 pack	Reagent handling	Upchurch Scientific	P-215RX	3	$10.0000	$30.00
MR09	Solenoid valve, Teflon, 14VDC	Reagent handling	Angar	190057	6	$325.0000	$1,950.00
MP01	Epson Stylus Photo 700 printer	Print head	Epson or Ebay	Photo 700	1	$100.0000	$100.00
MP01a	Cable, 15 cond flexible flat	Print head	Epson	Extract from Epson Stylus Pho	2	$0.0000	$0.00
MP01b	Print head & ink holder, black	Print head	Epson	Extract from Epson Stylus Pho	1	$0.0000	$0.00
MP01c	Print head slide, silver	Print head	Epson	Extract from Epson Stylus Pho	1	$0.0000	$0.00
MP02	Vial, 1mL clear, conical bottom, 12/pk	Print head	Fisher	06-444B	1	$57.5700	$57.57
MP03	Septa, Pierce "Tuf-bond" 72/pack	Print head	Fisher	PI12712	2	$47.0000	$94.00
MP04	Needles, non-coring, #20 x 4 inches	Print head	Fisher	14-825-15AD	1	$87.0000	$87.00
MP05	Piezoelectric print head	Print head	Agson	F057020	5	$85.0900	$425.45
MM01	Flexible conduit, 1½" I.D., 25ft length	Conduit	Hardware store	NA	1	$10.0000	$10.00
MM02	Aluminum tubing, square, 1" wide, 12" long	Solenoid tower	Hardware store	e.g. Small Parts LSAT-063/16-1	1	$2.5000	$2.50
MM03	Threaded rod, ¼-20, 14+inches long	Solenoid tower	Hardware store	e.g. Small Parts B-TRX-1420	1	$12.0000	$12.00
MM04	Bushing, ¼ " ID, 1" long	Spacer	Hardware store	e.g. Small Parts B-RSA-14/16	16	$1.7350	$27.76
MM05	Bushing, ¼ " ID, ½" long	Spacer	Hardware store	e.g. Small Parts B-RSA-14/8	8	$1.2950	$10.36
MM06	Teflon block 12x12x2" (raw material for machine	Slide holder	Port Plastics	28000190	1	$321.0000	$321.00
MM07	O-rings, 3/8" OD, 1/4" ID	Slide holder	Small Parts (Miami Lak	B-ORS-010	100	$0.1800	$18.00
MM08	Machine shop fabrication, slide holder	Labor					
MM09	Machine shop fabrication, print head holder	Labor					
MM10	Punch, arch, 3/8 inch	Tool	Small Parts (Miami Lak	B-ARCH-6	1	$9.9100	$9.91
MM11	Filter, Whatman P/N 6704-1500, a "Carbon Cap	Fume filter	Fisher	09-744-37	1	$79.1000	$79.10

References

AP Blanchard, R. J. Kaiser, and L. E. Hood.: **High-density oligonucleotide arrays.** *Biosensors & Bioelectronics.* 1996, **11**:687-690.

J DeRisi, V Iyer, PO Brown: **The MGuide: A complete guide to building your own microarrayer**. http://cmgm.stanford.edu/pbrown/mguide.

TC Islam, J Lindvall, A Wennborg, LJ Branden, H Rabbani, CI Smith.: **Expression profiling in transformed human B cells: influence of Btk mutations and comparison to B cell lymphomas using filter and oligonucleotide arrays**. *Eur J Immunol*. 2002, **32**(4):982-93.

WP Kuo, TK Jenssen, AJ Butte, L Ohno-Machado , IS Kohane: **Analysis of matched mRNA measurements from two different microarray technologies**. *Bioinformatics*. 2002, **18**(3):405-12.

HP Le:**Progress and trends in ink-jet printing technology.** In: *Book Recent Progress in Ink Jet Technologies II* (Editor E Handson). City: Springfield, VA; 1999.

J Li, M Pankratz, JA Johnson: **Differential gene expression patterns revealed by oligonucleotide versus long cDNA arrays**. *Toxicol Sci*. 2002, **69**(2): 383-90.

A Relogio, C Schwager, A Richter, W Ansorge, J Valcarcel: **Optimization of oligonucleotide-based DNA microarrays**. *Nucleic Acids Res*. 2002, **30** (11):e51.

A Roda, M Guardigli, C Russo, P Pasini, M Baraldini: **Protein microdeposition using a conventional ink-jet printer**. *Biotechniques* 2000, **28**:492-6.

AT Rogojina, WE Orr, BK Song, EE Geisert Jr.: **Comparing the use of Affymetrix to spotted oligonucleotide microarrays using two retinal pigment epithelium cell lines**. *Mol Vis*. 2003, **9**:482-96.

DI Stimpson, PW Cooley, SM Knepper, DB Wallace: **Parallel production of oligonucleotide arrays using membranes and reagent jet printing**. *Biotechniques* 1998, **25**:886-90.