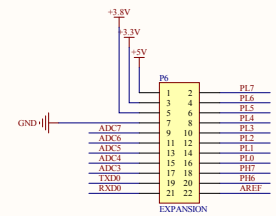
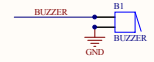
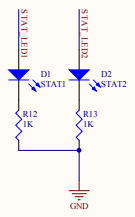
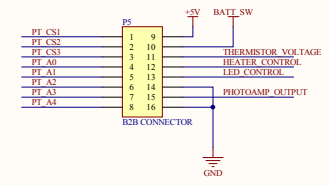
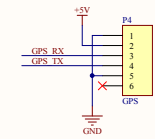
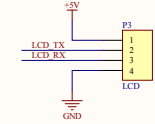
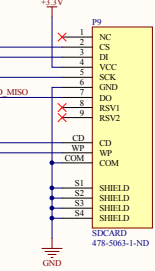
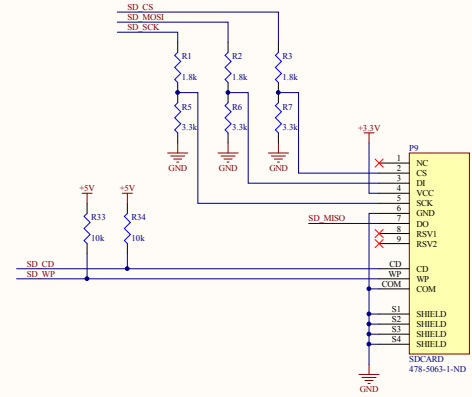
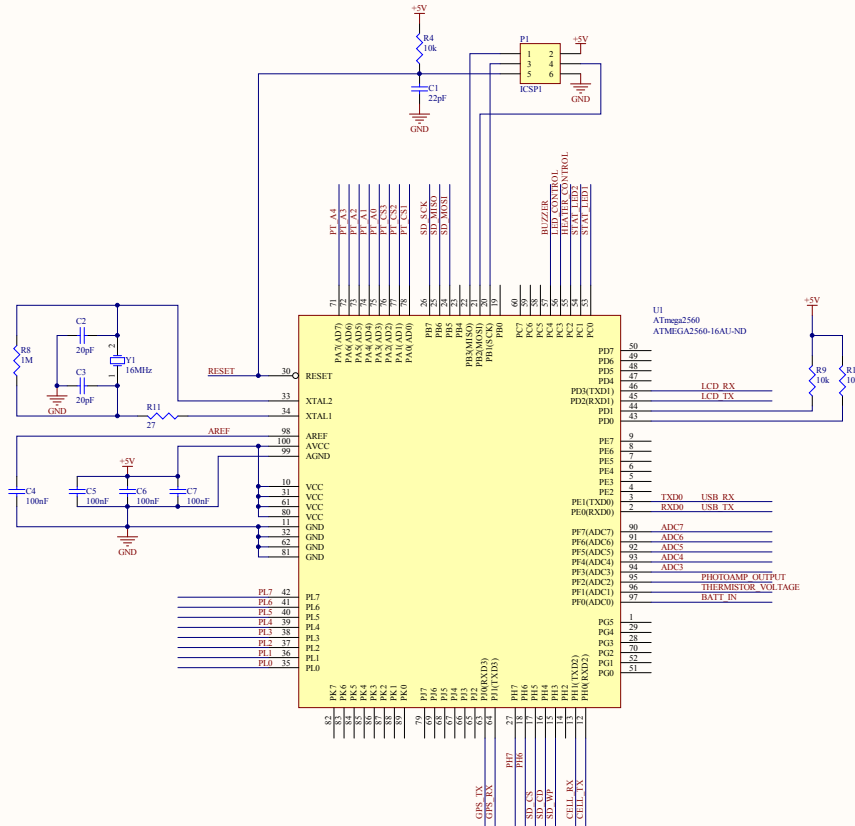
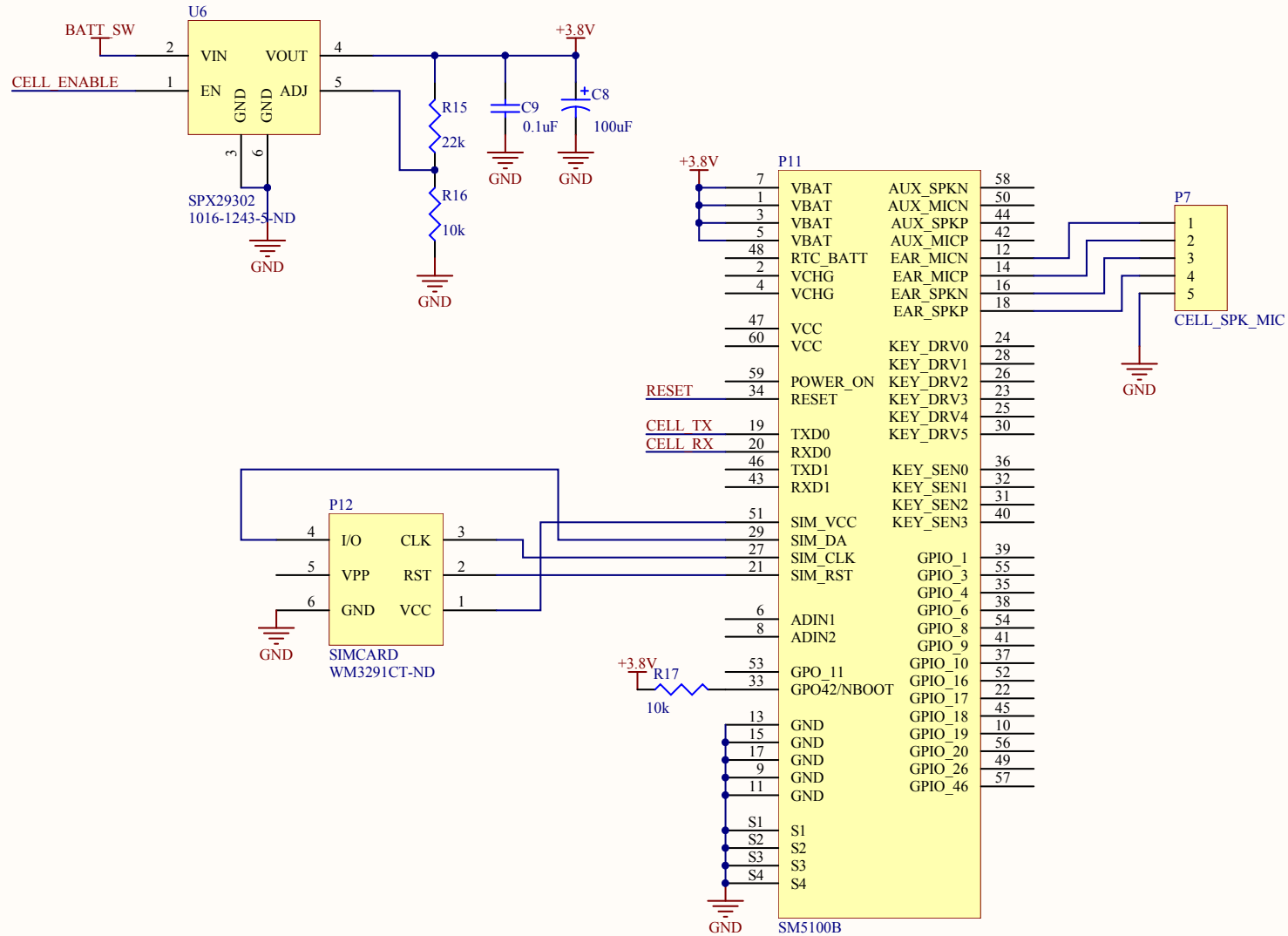


Figure S1 - Microcontroller Board Schematics and PCB Layout  
 a) Microcontroller and Peripheral Connectors



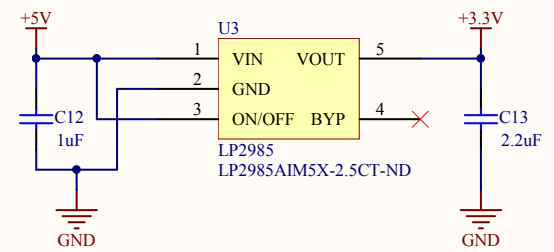
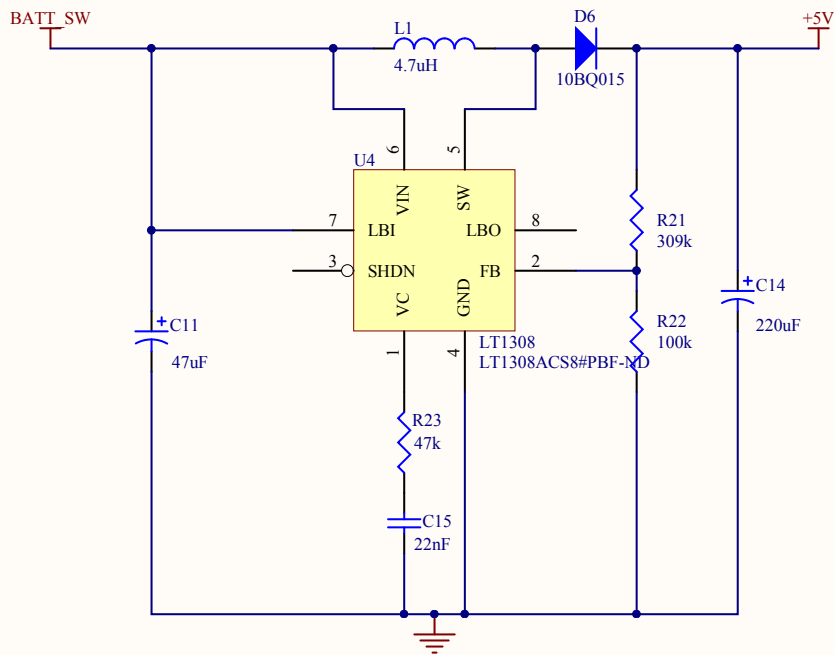
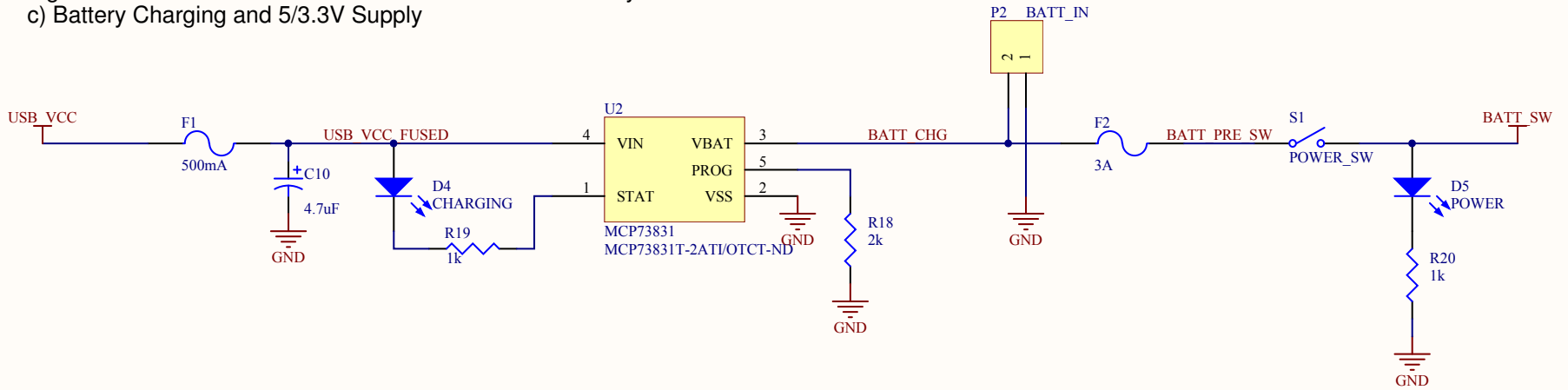
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Size	Number	Revision
C		
Date	6/5/2011	Sheet of
File	C:\Documents\Arduino Board SchDoc	Drawn By

Figure S1 - Microcontroller Board Schematics and PCB Layout  
 b) Cell Phone Module



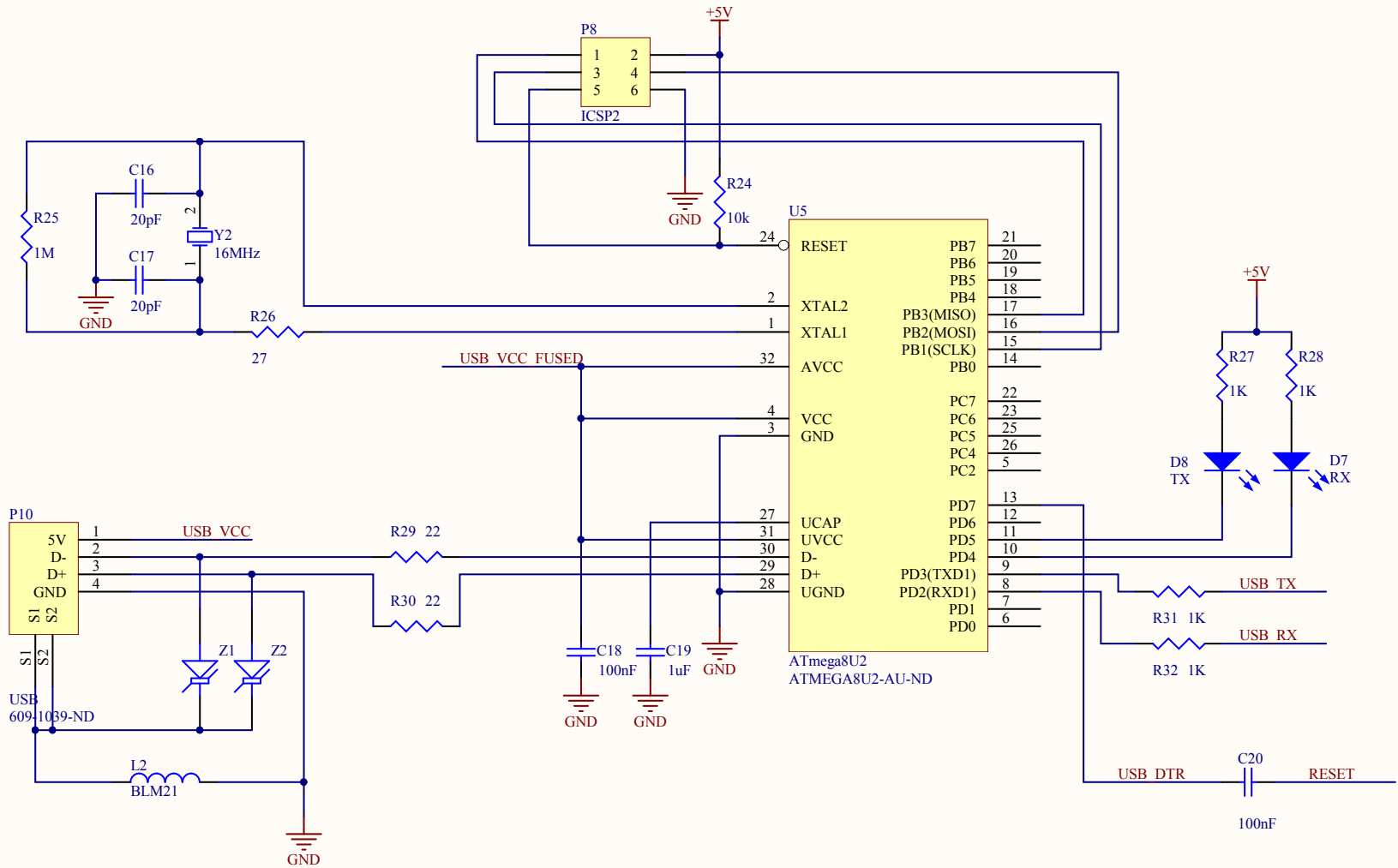
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Date:	6/5/2011	Sheet of
File:	C:\Documents\...\Cell Phone Module.SchDoc Drawn By:	

Figure S1 - Microcontroller Board Schematics and PCB Layout  
 c) Battery Charging and 5/3.3V Supply



Title		
Size	Number	Revision
A		
Date:	6/5/2011	Sheet of
File:	C:\Documents\...\Power Circuits.SchDoc	Drawn By:

Figure S1 - Microcontroller Board Schematics and PCB Layout  
 d) USB Interface



Title		
Size	Number	Revision
A		
Date:	6/5/2011	Sheet of
File:	C:\Documents\...\USB Host.SchDoc	Drawn By:

Figure S1 - Microcontroller Board Schematics and PCB Layout  
e) Board layout (2 layers, red=top, blue=bottom) with enclosure outline

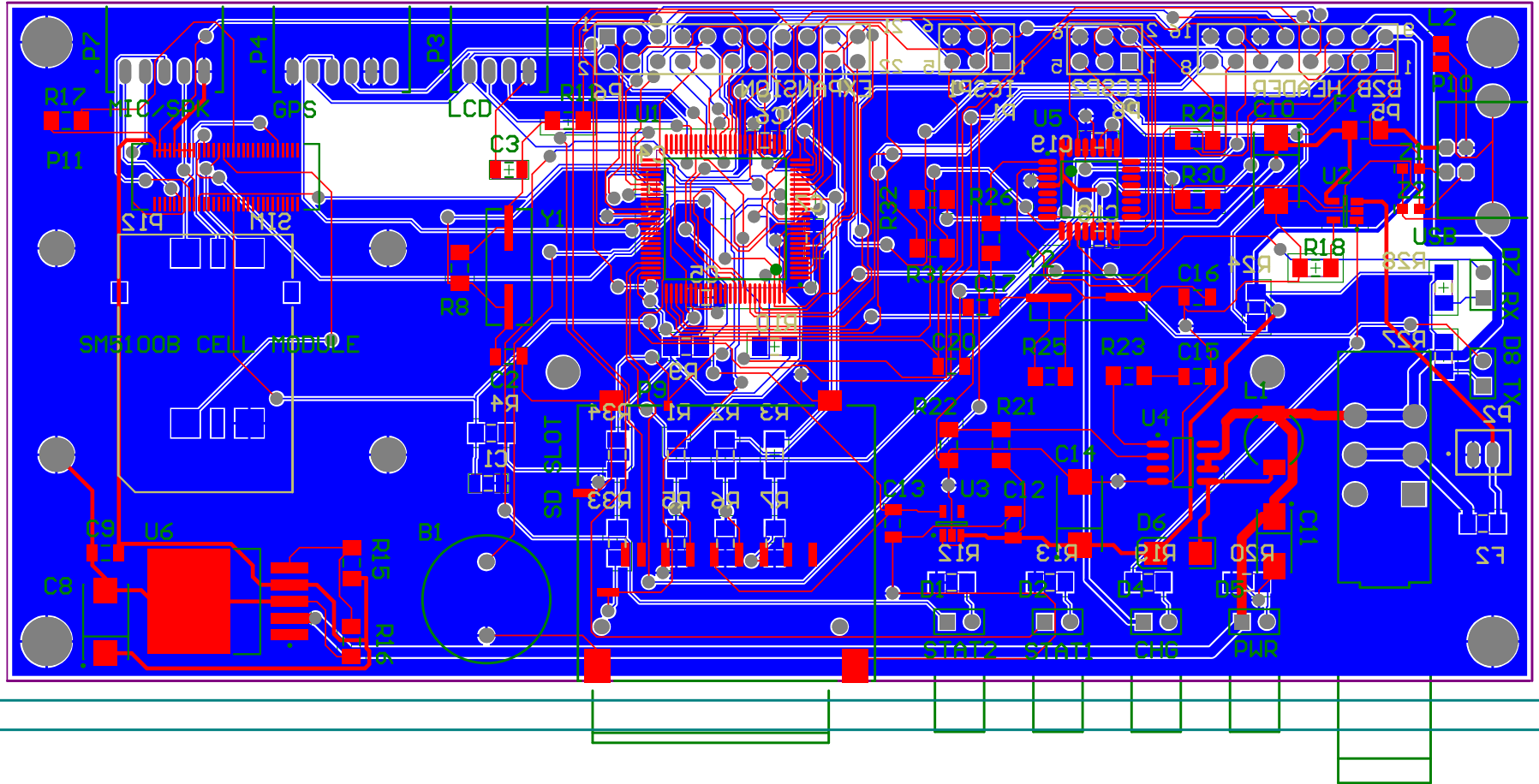
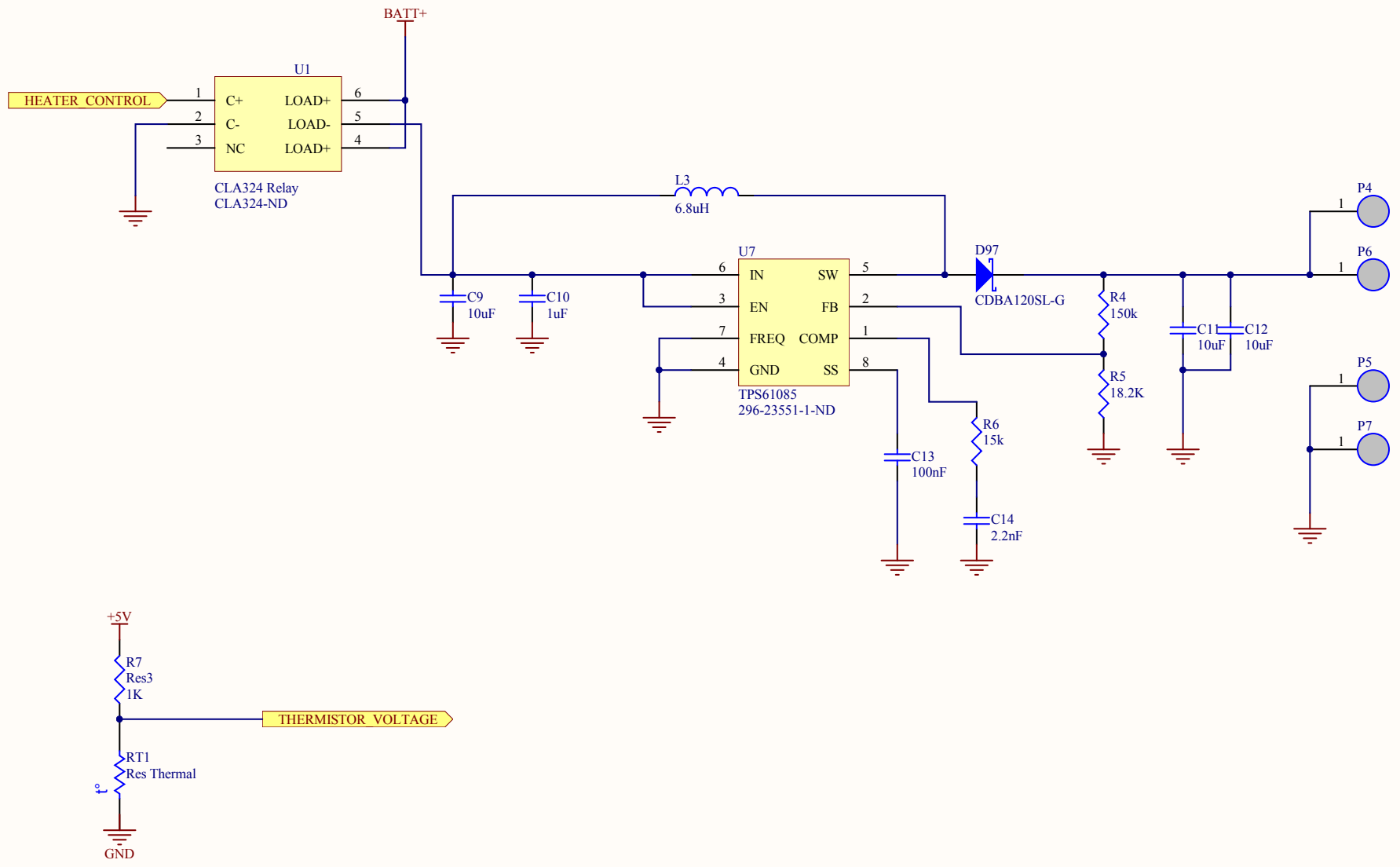
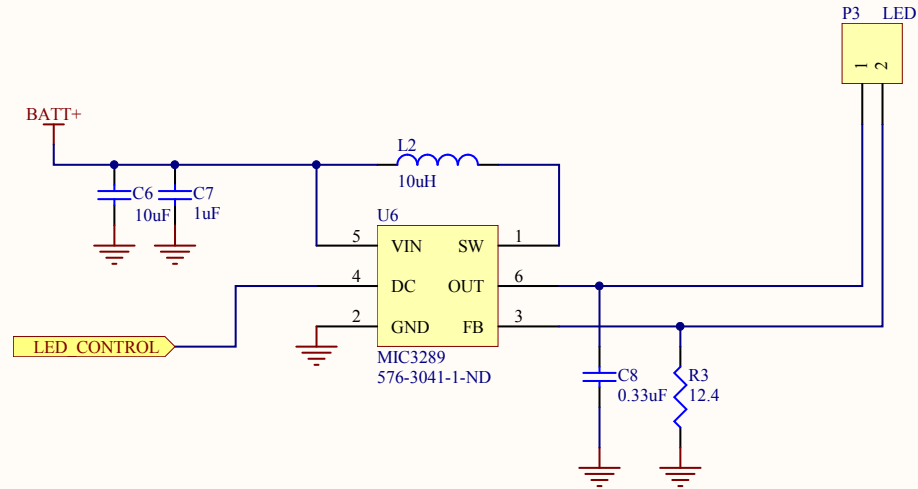


Figure S2 - Chip Reader Board Schematics and PCB Layout  
a) Heater Controller



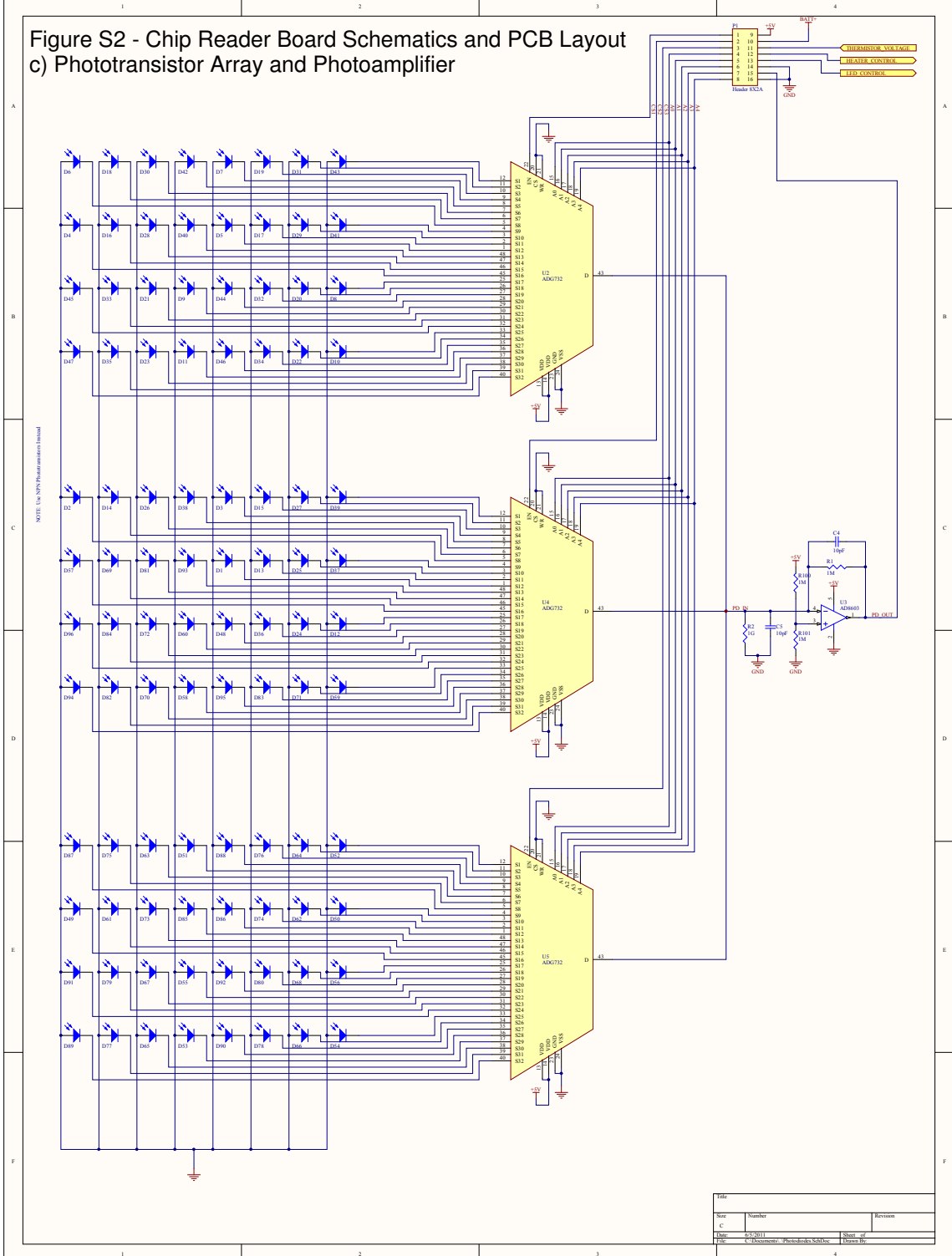
Title		
Size	Number	Revision
A		
Date:	6/5/2011	Sheet of
File:	C:\Documents\...\Heater Driver.SchDoc	Drawn By:

Figure S2 - Chip Reader Board Schematics and PCB Layout  
 b) LED Controller



Title		
Size	Number	Revision
A		
Date:	6/5/2011	Sheet of
File:	C:\Documents\...\LED Driver.SchDoc	Drawn By:

Figure S2 - Chip Reader Board Schematics and PCB Layout  
 c) Phototransistor Array and Photoamplifier



Title		
Size	Number	Revision
C		
File	S25011	Sheet of
File	C:\Documents - PhotoReader SMDs	Drawn By



Figure S2 - Chip Reader Board Schematics and PCB Layout  
d) Board layout (2 layers, red=top, blue=bottom) with enclosure outline

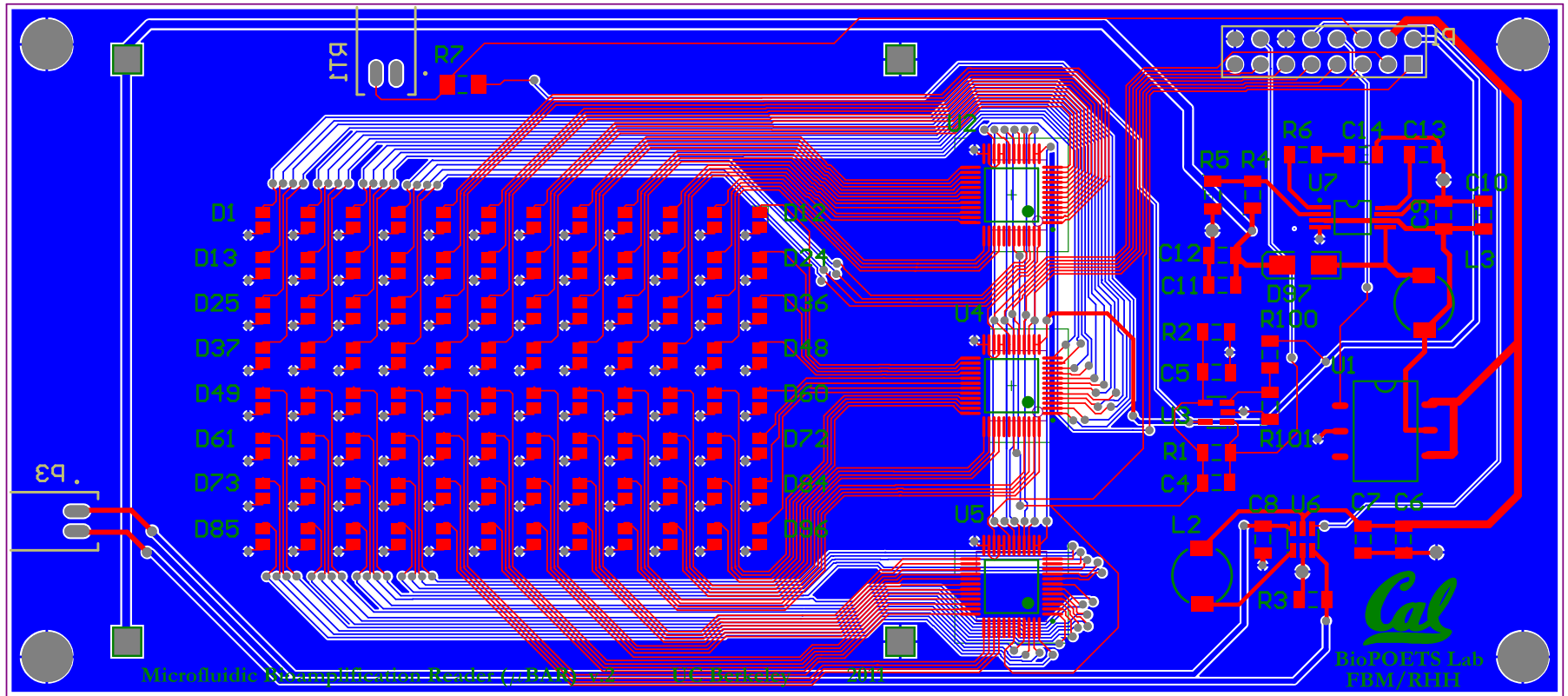


Table S1

MicroBAR v.2 Bill of Materials													
System	Board	Designator	Qty	Vendor	Part #	Mfg #	Description	Function	Unit Price (prev)	Unit Price (5-2020)	Est. Price (prev)	Est. Price (5-2020)	
ITO Heater	Top	U1	1	Digkey	480-5270-1-ND	LQWSP100M8N	INDUCTOR POWER 6.8UH 1A25MA 2220	Heater resistor	0.44	0.172	0.44	0.172	
	Top	U7	1	Digkey	296-2351-1-ND	TP5610BSPWR	IC STEP UP DC-DC CONV 8-TSOP	Heater boost converter	3.33	1.283	3.33	1.283	
	ITO Heater	Top	C01,C11,C12	3	Digkey	100F (1206)	100F (1206)	100F (1206)	Input/output filter caps	0.15	0.02	0.45	0.06
		Top	C13	1	Digkey	100F (1206)	100F (1206)	100F (1206)	output filter cap	0.15	0.02	0.15	0.02
		Top	C14	1	Digkey	2.2F (1206)	2.2F (1206)	2.2F (1206)	output filter cap	0.15	0.02	0.15	0.02
		Top	R4	1	Digkey	150R (1206)	150R (1206)	150R (1206)	0.05	0.01	0.05	0.01	
		Top	R5	1	Digkey	18.1K (1206)	18.1K (1206)	18.1K (1206)	0.05	0.01	0.05	0.01	
		Top	R6	1	Digkey	150R (1206)	150R (1206)	150R (1206)	0.05	0.01	0.05	0.01	
		Top	R7	1	Digkey	1A (1206)	1A (1206)	1A (1206)	0.05	0.01	0.05	0.01	
		Top	U3	1	Digkey	480-5270-1-ND	LQWSP100M8N	INDUCTOR POWER 6.8UH 1A25MA 2220	Half bridge for thermistor	0.44	0.172	0.44	0.172
		Top	U97	1	Digkey	641-1252-1-ND	CD8A1205L-G	DIODE SCHOTTKY 1A 20V SMA	6.8uA heater inductor heater diode	0.82	0.297	0.82	0.297
		Top	R11	1	Digkey	615-1043-ND	PRG10A180	DETECTOR RTD TF 1K OHM +/-0.0026	Thermistor	18.5	2.06	18.5	2.06
		Top	R11	1	Digkey	455-1718-ND	C28-K-6-QLFJFS	CONN HEADER PH SIDE SPOS 2MM	header for thermistor	0.1	0.047	0.1	0.047
		Top	R11	1	Digkey	455-1165-ND	PHR-2	CONN HOUSING PH 2POS 2MM WHITE	2-pin housing for thermistor	0.05	0.024	0.05	0.024
		Top	R11	2	Digkey	455-2148-1-ND	SPH-0027-P0.5L	CONN TERMINAL 28-24AWG TIN	JST PH pins	0.027	0.033	0.054	0.068
		Top	R11	1	Digkey	MC095-300-ND	38X095-300SF	CABLE 6 CONN 300FT FLAT GRAY	2-conductor ribbon cable (3 inches)	0.2	0.02	0.2	0.02
		Top	U1	1	Delta Tech	CB-50N-320S	CB-50N-320S	5-15 ohm, 50W/50V 5mm ITO slides	Heater	20	5	20	5
		Top	U1	1	Ace HW		Brass Bar Stock		Heater Contacts	0.05	0.01	0.05	0.01
		Top	U1	1	Fry's		Electrically conductive Epoxy		Heater Contacts	0.2	0.1	0.2	0.1
		Top	U1	1	Ace HW		Thermally conductive Epoxy		Glue for Thermistor	0.05	0.01	0.05	0.01
<b>TOTAL</b>													
<b>113.95</b>													
<b>113.95</b>													
LED Driver	Top	U6	1	Digkey	576-3041-1-ND	MC128P-2410C	IC LED DRIVER WHITE BCKGLT 150T-6	LED booster	1.9	0.973	1.9	0.973	
	Top	C5	1	Digkey	22F (1206)	22F (1206)	22F (1206)	0.15	0.02	0.15	0.02		
	Top	C7	1	Digkey	478-6028-1-ND	06032033M4AT2	CAP CER 330F 25V X5R 0603 (sub w/ 1206)	0.33uF	0.15	0.02	0.15	0.02	
	Top	C8	1	Digkey	478-6028-1-ND	06032033M4AT2	CAP CER 330F 25V X5R 0603 (sub w/ 1206)	0.33uF	0.15	0.02	0.15	0.02	
	Top	R3	1	Digkey	P12-4CCT-ND	ERI-6ENF1284V	RES 12.4 OHM 1/8W 1% 0805 SMD	current sense resistor	0.07	0.005	0.07	0.005	
	Top	U2	1	Digkey	490-5271-1-ND	LQWSP100M8N	INDUCTOR POWER 10UH 1250MA 2220	10uH LED inductor	0.44	0.172	0.44	0.172	
	Top	R3	1	Digkey	455-1718-ND	C28-K-6-QLFJFS	CONN HEADER PH SIDE SPOS 2MM	header for leds	0.1	0.047	0.1	0.047	
	Top	P3	1	Digkey	455-1165-ND	PHR-2	CONN HOUSING PH 2POS 2MM WHITE	2-pin housing for leds	0.05	0.024	0.05	0.024	
	Top	P3	2	Digkey	455-2148-1-ND	SPH-0027-P0.5L	CONN TERMINAL 28-24AWG TIN	JST PH pins	0.027	0.033	0.054	0.068	
	Top	R11	1	Digkey	MC095-300-ND	38X095-300SF	CABLE 6 CONN 300FT FLAT GRAY	2-conductor ribbon cable (3 inches)	0.2	0.02	0.2	0.02	
	Top	P3	3	Digkey	P14150CT-ND	LN923WBCRAJ	LED BLUE SIDE VIEW SMD	Blue LEDs	0.57	0.229	0.57	0.229	
	Top	U1	1	Ace HW		Liquid Electrical Tape		Optical shielding	0.05	0.01	0.05	0.01	
	<b>TOTAL</b>												
	<b>51.94</b>												
	<b>51.94</b>												
	Phototransistor Array	Top	U1, U2, U3	96	Digkey	478-1268-1-ND	SPH 1710-124	PHOTO TRANSISTOR ALS SMD CHENLEI	Phototransistors	0.64	0.43	54.08	36.29
		Top	U2, U4, U5	3	Digkey	ADG7328U2-ND	ADG7328U2	IC MULTIPLEXER 12X1 48TQFP	MUXes	12.13	6.62	36.39	18.18
		Top	U3	1	Digkey	AD6849AUZEEELCT	AD6849AUZEEELCT	IC IC OPAMP GP R-R CMOS TQFP23-5	transimpedance opamp	2.07	0.797	2.07	0.797
		Top	C4	1	Digkey				input capacitor	0.15	0.01	0.15	0.01
		Top	C5	1	Digkey				input capacitor	0	0	0	0
Top		R1	1	Digkey				1M	0.1	0.01	0.1	0.01	
Top		R2	1	Digkey				1G - not necessary?	0	0	0	0	
Top		R100-101	2	Digkey				bias resistors	0.1	0.01	0.2	0.02	
Top			1	McKesson	8647K108	Nagromax (EPOXY) 808, Soft, 1/2" Thick, 42" Width		Heat transfer	0.04	0.04	0.04	0.04	
Top			1	McKesson	778978 (899)	Light Fibers 20' x 24" Sheet		"Moist Green" gel #89	0.16	0.16	0.16	0.16	
<b>TOTAL</b>													
<b>65.90</b>													
<b>65.90</b>													
Microcontroller	Bot	U1	1	Digkey	ATMEGA328P-16AU	ATMEGA328P-16AU	8-BIT AVR MICRO 28-PIN 1MHZ 100TQFP	Microcontroller	17.99	11.93	17.99	11.93	
	Bot	C1	2	Digkey	100F (1206)	100F (1206)	100F (1206)	decoupling caps	0.15	0.02	0.3	0.04	
	Bot	C2,3	2	Digkey	478-3735-1-ND	080514200ATZ1	CAP CERAM 20PF 5% 100V NPO 0805	0.23	0.038	0.46	0.076		
	Bot	C4,7	4	Digkey	100F (1206)	100F (1206)	100F (1206)	decoupling caps	0.15	0.02	0.6	0.08	
	Bot	R4	1	Digkey	10K (1206)	10K (1206)	10K (1206)	res on reset line	0.1	0.01	0.1	0.01	
	Bot	R8	1	Digkey	1M (1206)	1M (1206)	1M (1206)	res on vstl	0.1	0.01	0.1	0.01	
	Bot	R11	1	Digkey	27 ohm (1206)	27 ohm (1206)	27 ohm (1206)	res on vstl	0.1	0.01	0.1	0.01	
	Bot	R9-10	2	Digkey	XC128CT-ND	EC5-160-20-SF9	CRYSTAL 16.000MHZ 20PF SMD	0.56	0.26	0.56	0.26		
	Bot	P1	1	Digkey	609-3218-ND	67996-400HLF	CONN HEADER 6POS 100 STR TIN	pullups on PDI/POD	0.1	0.01	0.2	0.02	
	Bot	P1	1	Digkey				ICSP1 header	0.43	0.1	0.43	0.1	
<b>TOTAL</b>													
<b>20.67</b>													
<b>20.67</b>													
USB Interface	Bot	U5 </td <td>1</td> <td>Digkey</td> <td>ATMEGA328P-16AU</td> <td>ATMEGA328P-16AU</td> <td>8-BIT AVR MICRO 28-PIN 1MHZ 100TQFP</td> <td>usb controller</td> <td>1.9</td> <td>1.3</td> <td>1.9</td> <td>1.3</td>	1	Digkey	ATMEGA328P-16AU	ATMEGA328P-16AU	8-BIT AVR MICRO 28-PIN 1MHZ 100TQFP	usb controller	1.9	1.3	1.9	1.3	
	Bot	C16-17	2	Digkey	478-3735-1-ND	080514200ATZ1	CAP CERAM 20PF 5% 100V NPO 0805	0.23	0.038	0.46	0.076		
	Bot	R25	1	Digkey	10K (1206)	10K (1206)	10K (1206)	res on vstl	0.1	0.01	0.1	0.01	
	Bot	U2	1	Digkey	XC128CT-ND	EC5-160-20-SF9	CRYSTAL 16.000MHZ 20PF SMD	0.56	0.26	0.56	0.26		
	Bot	R24	1	Digkey	10K (1206)	10K (1206)	10K (1206)	res on reset line	0.1	0.01	0.1	0.01	
	Bot	R29-30	2	Digkey	27 ohm (1206)	27 ohm (1206)	27 ohm (1206)	USB data line resistors	0.1	0.01	0.2	0.02	
	Bot	U2	2	Digkey	F230ACT-ND	PH81020030M	SUPPRESSOR ESD 24VDC 0803 SMD	0.74	0.196	1.49	0.392		
	Bot	U2	1	Digkey	490-1038-1-ND	BR1424G1218	FERRITE CHIP 120 OHM 300MA 0805	ESD protection inductor	0.12	0.02	0.12	0.02	
	Bot	P10	1	Digkey	609-1039-ND	61729-00108LF	CONN REPT USB TYPE B A PCB	1.29	0.474	1.29	0.474		
	Bot	C18	1	Digkey	100F (1206)	100F (1206)	100F (1206)	0.15	0.01	0.15	0.01		
Bot	C19	1	Digkey	100F (1206)	100F (1206)	100F (1206)	0.15	0.01	0.15	0.01			
Bot	R27-28	2	Digkey	67-1046-ND	SSL-LX2575SD	LED 2X3MM RECTANGULAR GREEN DIFF	TX/RX pullups	0.1	0.02	0.2	0.04		
Bot	C7-8	2	Digkey	10K (1206)	10K (1206)	10K (1206)	TX/RX LEDs	0.137	0.02	0.274	0.04		
Bot	R11-32	2	Digkey	2	Digkey		data line resistors	0.1	0.01	0.2	0.02		
Bot	C20	1	Digkey	609-3218-ND	67996-400HLF	CONN HEADER 6POS 100 STR TIN	reset DTR line cap	0.15	0.02	0.15	0.02		
Bot	R8	1	Digkey	609-3218-ND	67996-400HLF	CONN HEADER 6POS 100 STR TIN	ICSP1 header	0.43	0.1	0.43	0.1		
Bot	U8	1	Digkey	Q31E1-ND	3021001-03	CR1 USB A-B CON 1' 28/28 AWG	T USB cable	3.15	1.21	3.15	1.21		
<b>TOTAL</b>													
<b>11.49</b>													
<b>11.49</b>													
SD Card Reader	Bot	R9	1	Digkey	478-5061-1-ND	185180003028	CONN FIBER SD CARD W/BOSS	SD card slot	4.7	3.93	4.7	3.93	
	Bot	R13-14	2	Digkey	10K (1206)	10K (1206)	10K (1206)	card detect / write protect pullups	0.1	0.02	0.2	0.04	
	Bot	R13	3	Digkey	1.8K (1206)	1.8K (1206)	1.8K (1206)	SD level shifter res	0.1	0.01	0.3	0.03	
	Bot	R5-7	3	Digkey	33K (1206)	33K (1206)	33K (1206)	SD level shifter res	0.1	0.01	0.3	0.03	
	Bot	R1	1	Digkey	296-18479-1-ND	UP2965A-1308VFC	IC LOGIC REE 13V 155MA SOT23-5	3.3V regulator	0.84	0.278	0.84	0.278	
	Bot	C12	1	Digkey	100F (1206)	100F (1206)	100F (1206)	input cap for 3.3v reg	0.15	0.02	0.15	0.02	
	Bot	C13	1	Digkey	2.2uF (1206)	2.2uF (1206)	2.2uF (1206)	output cap for 3.3v reg	0.15	0.02	0.15	0.02	
	Bot		1	Amzon				SD card	6	0	6	0	
	<b>TOTAL</b>												
	<b>12.64</b>												
<b>12.64</b>													
Cell Module	Bot	R11	1	Digkey	205-2977-1-ND	AXX190547C	CONN SOCKET BRG/BRD -SMM 0205	30-pin socket for cell module	6.79	1.54	6.79	1.54	
	Bot	R12	1	Digkey	WM1291CT-ND	47880001	150MM SMM CARD READER CONN	SMM card socket	1.51	0.986	1.51	0.986	
	Bot	C4	1	Digkey	1016-1243-5-ND	SPX292075A	IC REG LDO ADJ 3A TO263-5	3.3V 1A regulator	1.23	0.61	1.23	0.61	
	Bot	R15	1	Digkey	22K	22K	22K	3.3V voltage set res	0.1	0.01	0.1	0.01	
	Bot	R16	1	Digkey	478-1777-1-ND	TPSD10701086	CAP TANT LQSER 100UF 10V 10% SMD	3.3V voltage set res	1.13	0.381	1.13	0.381	
	Bot	C9	1	Digkey	0.1uF	0.1uF	0.1uF	output cap for 3.3V reg	0.15	0.02	0.15	0.02	
	Bot	R17	1	Digkey	10K (1206)	10K (1206)	10K (1206)	pullup for boot pin	0.1	0.01	0.1	0.01	
	Bot	R11	1	Digkey	455-1722-ND	S58-PH-K-QLFJFS	CONN HEADER PH SIDE SPOS 2MM	5-pin JST PH header for cell phone gpi/rtc	0.1	0.01	0.1	0.01	
	Bot	U1	1	Digkey	455-1165-ND	PHR-5	CONN HOUSING PH 5POS 2MM WHITE	5-pin housing - for micro/pcr cable	0.05	0.02	0.05	0.02	
	Bot	C1	1	SparkFun	CEL-00675	QWSD-band Cellular Duck Antenna SMA		GNSS/RF module - SIMU008	47.96	39.95	47.96	39.95	
Bot	C1	1	Digkey	CEL-00675	QWSD-band Cellular Duck Antenna SMA		Cell Antenna	7.95	6				

Table S2

## MicroBAR LAMP Reaction Cost Estimate

Reaction Volume: 6.28E-07 L  
 Reaction Cost: 0.012893607  
 Cost/uL: 0.020531221  
 Cost/chip 1.237786242

## Solids

Reagent	Molecular Weight	Mass (g)	Moles	Cost	Cost/mole	Concentration/Rea	Moles/reaction	Cost/reaction	Fraction of Cost
Tris Buffer	127.2	100	0.786163522	\$74	94.128	0.02	1.256E-08	1.18225E-06	0.01%
KCl	74.55	500	6.706908115	\$31	4.59228	0.01	6.28E-09	2.88395E-08	0.00%
MgSO4.7H2O	246.47	500	2.02864446	\$30	14.7882	0.008	5.024E-09	7.42959E-08	0.00%
(NH4)2SO4	132.14	100	0.756773119	\$14	18.76388	0.01	6.28E-09	1.17837E-07	0.00%
MnCl2	197.91	100	0.505280178	\$22	43.93602	0.001	6.28E-10	2.75918E-08	0.00%
Calcein	622.53	5	0.008031741	\$103	12761.865	0.00005	3.14E-11	4.00723E-07	0.00%

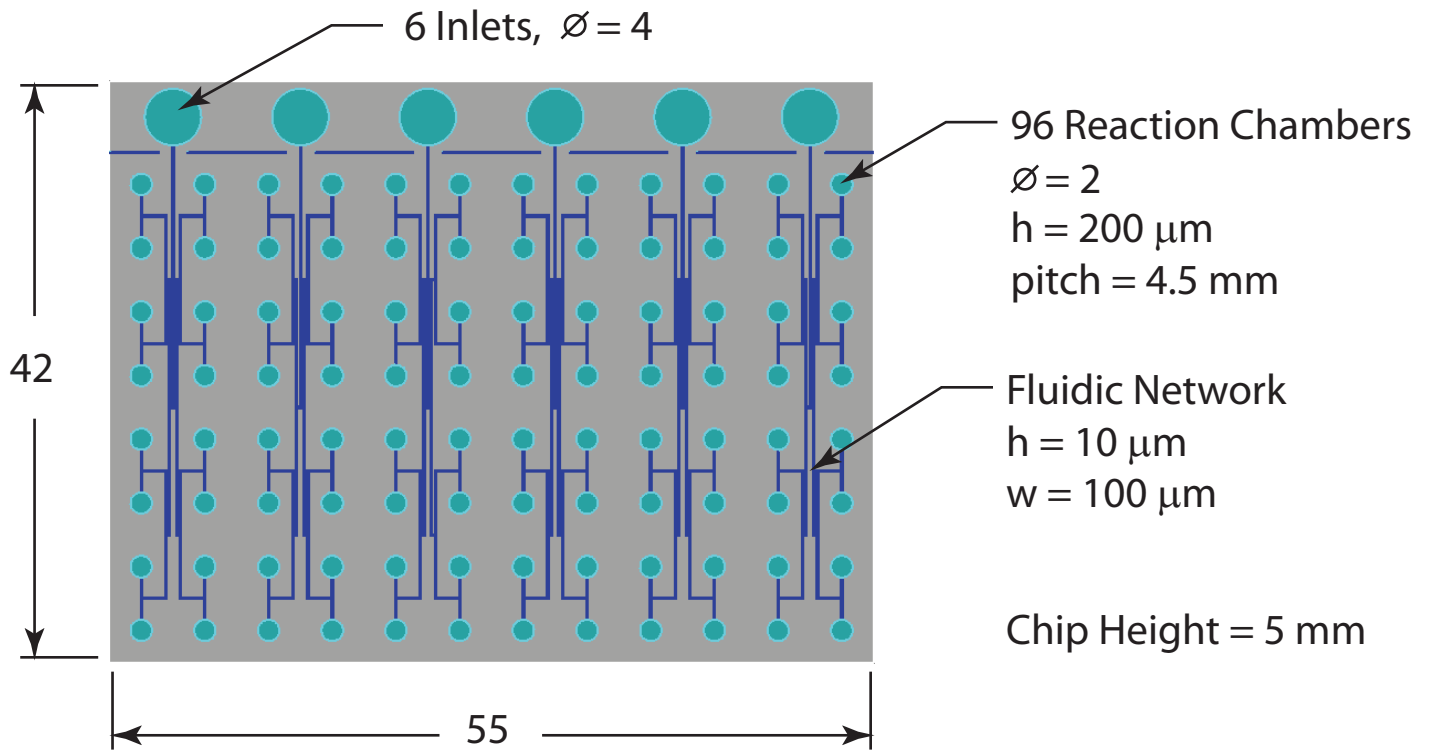
## Solutions

Reagent	Concentration (M)	Volume (L)	Moles	Cost	Cost/mole	Rxn Concentration	Moles/reaction	Cost/reaction	Fraction of Cost
Betaine	5	0.0015	0.0075	\$20	2666.66667	0.8	5.024E-07	0.001339733	10.39%
dATP	0.1	0.00025	0.000025	\$37	1470000	0.0014	8.792E-10	0.001292424	10.02%
dCTP	0.1	0.00025	0.000025	\$37	1470000	0.0014	8.792E-10	0.001292424	10.02%
dGTP	0.1	0.00025	0.000025	\$37	1470000	0.0014	8.792E-10	0.001292424	10.02%
dTTP	0.1	0.00025	0.000025	\$37	1470000	0.0014	8.792E-10	0.001292424	10.02%
Bst Polymerase	8000000	0.001	8000	\$244	0.0305	320000	0.20096	0.00612928	47.54%
Tween 20 (%)	1	100	100	\$27	0.273	0.2	1.256E-07	3.42888E-08	0.00%
Primers FIP/BIP			0.0000005	45	90000000	0.0000032	2.0096E-12	0.000180864	1.40%
Primers LoopF/B			0.0000002	10	50000000	0.0000016	1.0048E-12	0.00005024	0.39%
Primers F3/B3			0.0000002	16	80000000	0.0000004	2.512E-13	0.000020096	0.16%

Reagents are from Sigma or New England Biosciences, Primers are from IDT

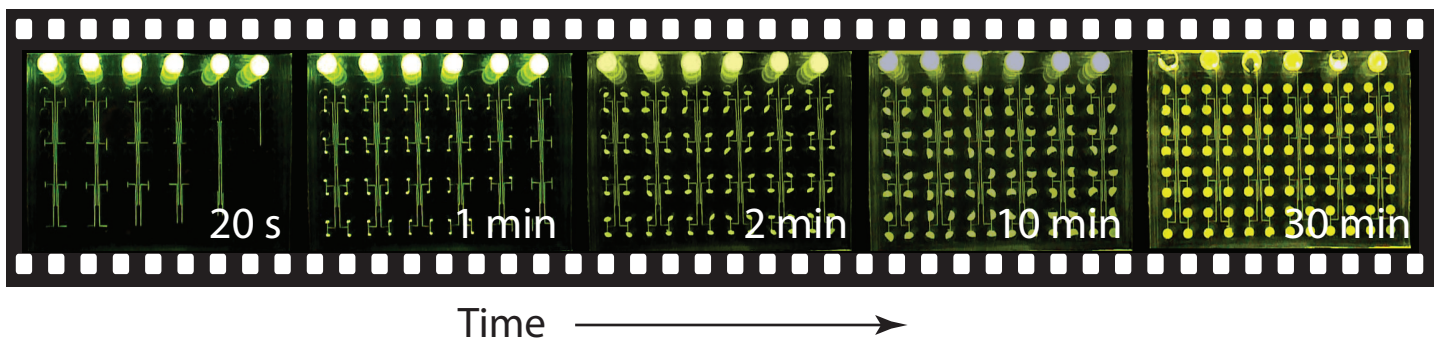
Figure S3

## Disposable Microfluidic Cartridge Layout



Dimensions in mm unless otherwise specified

## Passive Degas-Driven Sample Loading



# MicroBAR Assembly Procedure

Rev. 2013-06-21

1. Materials you need:
  - a. PCBs (order from Advanced Circuits)
  - b. SMT solder paste stencils (order from Pololu)
  - c. All components listed in bill of materials
  - d. Tube of solder paste
  - e. Trowel (glass slide will also work)
  - f. Skillet
  - g. Fine tipped tweezers
  - h. Tape and scrap PCBs
  - i. Power supply with a cable to plug into JST battery input connector
  - j. Conductive epoxy
  - k. Spray paint and primer
  - l. 2-part clear epoxy (fast drying)
  - m. AVR-ISP mkII in-circuit programmer
2. Printing 3D enclosure
  - a. Each piece is saved as a separate STL file. Load these all simultaneously into the Zprinter software and it should automatically arrange them. You can do a "fix" on the parts if there have been changes.
  - b. 3D print using default settings
  - c. After 24 hours or so, remove the parts and epoxy according to directions. Let sit overnight.
3. Tapping holes in 3D enclosure
  - a. In current version, I drilled pilot holes (no actual tapping) with a #43 bit in the PCB pillars and in the lid. But the material strips easily with these fine threads. In the next version, there shouldn't be any tapping (except maybe the lid). Rather, just use pockets for nuts/4-40 sleeves in the lid and base. So in all places where there should be a nut (8 in the lid and 4 in the base) that is epoxied in.
  - b. Countersink the heads so the lid closes flat (may also print that directly into future designs)
  - c. It's good to test fit the components. The display required some tweaking
4. Painting 3D enclosure
  - a. Sand the enclosure thoroughly with 100 grit sandpaper
  - b. Prime, sand, prime, sand. Be careful of rounding off edges. I applied 2-3 coats of primer, but only a little at a time. So in total I probably sprayed it 8 times with primer, and 8 times with paint, considering I also had to rotate it and get it from all sides.
  - c. Spray paint with semi-gloss black. Use thin coats and even strokes. No need to paint the interior, but definitely coat it with primer.
5. Populating PCBs (see Sparkfun tutorial on using Skillet for PCB reflow)
  - a. Tape several scrap PCBs to the table around the PCB to be populated so it fits snug without sliding
  - b. Align and tape the stencil over the PCB. The scrap PCBs are there so the trowel doesn't bow off the edges.
  - c. Squeeze out \*plenty\* of solder paste at one end of the board.
  - d. With one firm motion, smear the solder paste over the stencil, try to keep it very uniform and get paste in all the holes. Touchup if necessary
  - e. Remove the stencil with one motion.
  - f. Place all the components on the top side of the board. Ensure you've got the correct polarity on things. Most solder paste bridges will fix themselves, if there's not too much solder.
  - g. Plug in the skillet and turn it all the way to max. Place the board on the skillet. The warmest region is around the circular heating element.
  - h. After about 3 minutes, the paste will begin to reflow. Move the board around a bit if one part isn't getting hot enough. The whole process should take <5 minutes.

- i. When all the components have reflowed, turn off the skillet and carefully remove the board and set it down to cool.
  - j. Inspect all components for solder bridges, bad connections, etc.
  - k. Flip the board over and populate it with a regular tip iron
  - l. Populate all through-hole components by hand. If populating the cell phone module, take special care that the 60-pin socket doesn't have any solder bridges and the module is soldered down to the board securely.
  - m. Populate the rectangular LEDs, with special care to bend them at the correct position and solder them at the correct height.
  - n. Snip off the tip of the pushbutton - this will make assembly easier (only one detent is needed)
  - o. Note the PCB mods that I made in the "readme.txt" file and make these revisions (mostly adding jumper wires). These should be incorporated into the next version.
6. Initial testing
- a. Plug a power supply with current limiting capability to the battery in port and turn it all the way down.
  - b. Slowly ramp up the voltage on the power supply and make sure there isn't a significant current draw. If there is, you've likely got a solder bridge short.
  - c. Set the power supply to 4 V and probe the 5V, 3.8V, and 3.3V supplies on the board to make sure they're correct. Vary the power input and make sure they are stable
  - d. Turn off the power supply and connect the top board (screw in the standoffs). Repeat this procedure.
  - e. Connect a LiPo battery to the port and connect the USB port to a computer. Verify that the USB port does not overdraw current and that the battery voltage increases steadily (charging) when it is plugged in. Also verify that when you press the power switch, the computer recognizes new hardware (Atmel 8U2)
  - f. Dry fit the boards to the 3D enclosure (if available) and make any adjustments to the protruding components, if necessary.
7. Install battery and cell phone antenna
- a. Cut brackets out of brass for the cell phone antenna and the LiPo battery. Attach these to the bottom PCB and mount the antenna and battery. There should be a piece of foam between the battery and the bottom of the PCB to prevent the bottom components from digging into the battery.
8. Downloading the AVR firmware (USB controller and Arduino bootloader)
- a. Make sure drivers are installed for the AVR-ISP mkII (easiest way to do this is to install AVR Studio)
  - b. Connect the AVR-ISP mkII to the ICSP2 header to download the 8U2 DFU firmware.
    - i. Note that this firmware is the same as that available from the Arduino website, but I modified the descriptor string.
    - ii. Download with:
 

```
avrdude -p at90usb82 -F -P usb -c avrispmkii -U
flash:w:MicroBAR_dfu_and_usbserial_combined.hex -U lfuse:w:0xFF:m -U hfuse:w:0xD9:m -U
efuse:w:0xF4:m -U lock:w:0x0F:m
```
  - c. Make sure the board enumerates as "Berkeley MicroBAR 2"
    - i. Point Windows to the "Windows Driver" directory and install the driver
  - d. Plug in the mkII to the ICSP1 header and fire up the Arduino software. Make sure the correct board (Arduino 2560) is selected and correct COM port is selected, then go to "burn bootloader"
  - e. Unplug the mkII and power cycle the board.
  - f. Load the Controller.ino software onto the MicroBAR
9. Firmware testing
- a. When the MicroBAR restarts, you should hear two beeps and see the RUN/BAT lights flash. This indicates that the firmware is running.
  - b. Open the arduino serial monitor and verify that you get state data from the board. Then run the Processing UI and verify that you see data from the phototransistors and that you can tell the board to run an assay.

- c. Verify that all 96 phototransistors respond individually (block out all but one using black foam and observe that each one is uniquely addressed). If there is a problem, it may indicate a solder bridge on the addressing pins.
10. Assembling ITO heater
- a. Start w/ 75x50x0.5mm ITO coated glass slide from Delta Technologies (5-15 ohm/sq)
  - b. Verify which side is conductive
  - c. Cut ITO heater brackets
    - i. Start w/ ~100um brass bar from Ace HW, 3/4" wide, K&S Engineering 614121102330
    - ii. Cut to width of slide w/ tin snips (50mm)
    - iii. Mark 5mm line on one side
    - iv. Use tin snips to cut the pins (about 1.5mm wide)
    - v. Put in vice (pins down) and cut at 5mm line using Dremel cut-off wheel
    - vi. Use wire brush wheel to remove flashing and roughen back side (where epoxy will go)
    - vii. Put in vice and bend legs 90 degrees
    - viii. Make sure pins will insert into PCB.
  - d. Glue on brackets and thermistor
    - i. Lay brackets face down on kimwipe
    - ii. Dry fit slide, make sure spacing is correct, double check for conductive side
    - iii. Before gluing the slide, put a dot where the thermistor will go (middle of slide ~5mm in)
    - iv. Mix conductive epoxy and smear on bracket evenly. Cut a small piece of the brass to apply it to the slide.
    - v. Lay slide down (conductive side facing down) and push IN on brackets a bit so excess epoxy goes outside rather than smearing on the slide. Make sure it makes contact along entire length of bracket. Let it sit with a light weight on the slide for 10 minutes or so.
    - vi. Mix 2-part epoxy (Loctite quickset) and then pickup a small droplet (brass piece works for this) and apply on the slide.
    - vii. Use tweezers to place thermistor on slide, in the middle of the droplet
    - viii. Once glue has set (20 min) transfer to oven (60C) for 1 hr. Or let sit overnight.
11. Adding LEDs/waveguides
- a. Cut/spraypaint waveguides
    - i. Cut (2) 50x75x1mm glass slides and make 2 50x7mm strips to use as waveguides. Best to cut each waveguide from a separate slide.
    - ii. Use flat black spraypaint, lay waveguides on napkin flat, sandwiched between other glass slides to mask off the regions we don't want to spray paint. Best to use the slides you cut from, so the edge fits perfectly.
    - iii. Shake can and spray evenly, lightly so it doesn't drip on the sides
    - iv. Make sure to get both ends (small ends)
    - v. Once one side's dry, flip them over and do the other
    - vi. Use sandpaper if necessary to remove any paint on the clear sides
  - b. Glue on waveguides
    - i. Once spraypaint is dry (could put in oven to accelerate this), mix more 2-part epoxy and apply small droplets at the edges of the brackets.
    - ii. Set the waveguide down on top of the brass brackets. Note that the glass should extend over the ITO a bit (to avoid scattering into the ITO) and should be flush at either end of the brass brackets.
    - iii. Let glue dry
  - c. Glue on LEDs
    - i. Use helping hands to hold ITO slide vertically. Make sure the helping hands have lots of tape around them so they don't mark the ITO. Grip it by the brackets (not the waveguide).
    - ii. Pull out 3 LEDs and line them up in the same orientation (you'll want to get them quickly)
    - iii. Make sure all the polarities are the same (note the position of the green line on the back)
    - iv. Mix more 2-part epoxy and dip each LED into the epoxy (just barely, don't let the epoxy cover the contacts). Place them on the edge of the glass waveguide, 3 to a side. One should be in

- the center and the other two should line up so they're between columns 3 and 4 on the chip (use a scrap chip to eyeball it)...or maybe measure this precisely.
- v. Once you've done 3, let the glue dry, flip over the ITO, and do the other side.
  - vi. Make sure the polarities of the other side line up, so a series string around all 6 will have them all in the proper polarity.
- d. Wire up LEDs
    - i. Use solid wire wrap wire (32 awg) and cut small pieces to connect between the LEDs. Bend the ends of the wire at 90 degrees, and solder them on one by one. The end of one side will connect with the beginning of the other.
    - ii. Use small stranded wire from ribbon cable (28awg) to make a connector tying the strings together and put a JST connector on it (make sure the orientation is correct).
  - e. Coat all LEDs/wires with liquid electrical tape and make sure no light can escape, except through the waveguide. Hold the slide such that whichever side you're currently coating is facing down, since the liquid electrical tape tends to drip. 3-4 coats seems to do the trick. Note that this is also strain relief for the leads.
  - f. Let it dry
  - g. Connect leads to thermistor. Use smallest heatshrink to protect leads from each other, and put a JST connector on the other end.
12. Plug in the ITO, thermistor, and LEDs and test them using the software. Check to make sure the ITO is getting hot, the LEDs can vary their brightness across all the levels, and the thermistor is reading approximately the correct temperature. The ITO should have approximately 10V across it. The LEDs should not flicker.
13. Cutting foam insert and gasket
- a. The thinner foam (1/16") is for the insert underneath the ITO, and the thicker foam (1/8") is for the light gasket.
  - b. These burn very easily, so cut them on the lowest power setting on the Versalaser (Paper). I used ~ 0.1mm for the 1/16" @ +10% or so, and 1mm @0% for the gasket (both on Paper).
  - c. Test fit both
14. Cut green gel to fit array and sandwich this between the ITO and foam. Get the height level and as close as possible to the PTs without the slide touching, then solder down the ITO.
15. Attaching light gasket
- a. Mix up more epoxy and smear it in the trench on the base, careful to avoid getting it around the sides. Insert the gasket. Do this quickly since the epoxy sets in 5 min. DO NOT put something heavy on the foam while it's drying or it will be permanently compressed.
16. Cutting battery and antenna brackets
- a. See current design. Antenna bracket is cut from brass, 9mm wide, with a 4-40 hole at one end and a 90 degree bend at the other for the SMA connector to thread through.
  - b. Battery bracket should fit between the holes under the board and be a little larger than the battery, so foam will fit snugly in there.
  - c. Attach the battery. Use a 4-40 nut/bolt. Cut a square of foam to go between the battery and PCB, and a strip of foam to go along the underside of the bracket.
  - d. Thread the SMA connector into the bracket.
17. Installing PCBs
- a. In this version, the clearances really aren't sufficient. The enclosure should be enlarged slightly.
  - b. Use a drill bit for 75% thread engagement for 4-40 screws and drill holes in the pillars. Note that this isn't ideal, and you should instead epoxy nuts in the pillars since the 3D printed material is prone to stripping.
  - c. Screw in the top board using the mid-size standoffs from Digikey). Put the board in and use a 4-40 screw to actually drive the standoff into the material (let the screw bottom out in the standoff). Then hold the standoff with pliers and back the screw out.
  - d. To get the bottom board in, make sure all the LEDs are bent so they will sit just behind the enclosure and bend the board-to-board header towards the hinge side so the pins are at an angle. Then you can wiggle the bottom board in and get it to mate while the USB port protrudes through. Pushing



- the switch in the ON position helps.
- e. Screw in 4 more standoffs (same length). Make sure to attach the antenna bracket to one of them. This is what the base will screw in to.
18. Installing LCD module
    - a. Burn LCD firmware on via USB port
    - b. Modifications to Amulet LCD
      - i. Remove RS-232 connector and power connector. I just used angle cutters to cut them apart and then desoldered the pieces from the board
      - ii. Cut the traces which go to the MAX232 on the board (these are clearly marked)
      - iii. Solder the TX/RX/+5/GND leads onto the board directly. The wire should be ~8" long
    - c. New enclosure version should have pillars for mounting the LCD with embedded 4-40 nuts. So you'll just screw it in. I just cut foam (2 thick pieces) to sandwich the display in. I cut out holes in the foam for all the components on the PCB.
  19. Installing GPS module
    - a. Use double sided tape or hot glue to glue the module into the lid.
    - b. Make a small JST connector w/ 5 wires coming off it.
    - c. Attaching lid pieces and verifying that hinge works
    - d. Use hot glue to attach the GPS receiver. The LCD can be floating (next version it should fit more snugly).
    - e. Thread the GPS/LCD cables through the hinge and put the hinge together
    - f. Screw the hinge together using wood screws (#4, 5x8")
    - g. Add JST connectors to GPS cable and LCD cable.
    - h. Plug GPS/LCD into PCB.
  20. Attaching base
    - a. Screw it in using 3/4" 4-40 screws. These should be a little more than finger tight...any more and you will strip out the pillars.

# MicroBAR Operating Instructions

Rev. 2013-06-21

## Making PDMS Chips

- 1) The chip mold is fabricated using standard SU-8 lithography techniques
- 2) Chips are molded from Sylgard 184 PDMS with a 10:1 curing weight ratio
- 3) After molding, remove the chips from the wafer and cut along the edges with a sharp straight razor blade. Note that initially we cut a little beyond the edge of the chip and we make the final edge cut after the "floor" of the chip is bonded.
- 4) A 4 mm hole punch is used to create the inlet wells (Harris Unicore, Ted Pella). Make sure to clean the punch with Eliminase.
- 5) The bottom of the chip is cut from Stockwell Elastomerics .010" Thick HT6240 sheets.
- 6) Both the PDMS channels and the "floor" sheet are treated with Eliminase to remove DNA, DNase, and RNase and then are rinsed with ultrapure water.
- 7) The two chip parts are bonded using oxygen plasma.
- 8) After bonding, the edges are cut to the precise dimensions

## Setting up a Chip

- 1) Currently, the LAMP reaction mix is prepared off-chip under sterile conditions. Template is added last.
- 2) Positive and negative reaction mixes are split into two tubes: one for the thermocycler and one for the MicroBAR
- 3) The MicroBAR cartridge is vacuum treated for > 1 hr prior to loading.
- 4) At this point, cartridges may be sealed for future use using a commercial food sealer (although they should be vacuum treated first with a more powerful vacuum as the food sealer isn't powerful enough to enable full vacuum loading).
- 5) 20 uL of each reaction mix is loaded into each inlet immediately after it is taken out of vacuum. It takes about 30 minutes for the reaction mix to fully load into all chambers.

## Operating the MicroBAR

- 1) Make sure you have a fully-charge battery or you have the instrument plugged in to wall power (note that we added a power plug in lieu of the LiPo battery so that the instrument could be powered continuously with a 5V wall-wart power supply. The power supply you use should have a rating of at least 2A.
- 2) During powerup, you should see the lights go through all 16 brightness levels, the run and bat lights flash once, and you should hear two beeps.
- 3) Plug the box into the USB port
- 4) Fire up the UI
- 5) Often, it doesn't connect the first time. Close the UI, unplug the USB and plug it back in again. That almost always fixes it.
- 6) Set your assay parameters (note that we almost always use the defaults, which are given in parentheses):
  - a. Step time (2000 ms)  
The time between samples. Note that internally, the MicroBAR averages 100 samples per sample that it returns.
  - b. Stabilization steps (10)  
The photodetector amplifier needs a little time to reach a steady state value, so we let the instrument run for a certain number of steps before we start recording.
  - c. Run steps (5000)  
This determines the length of the assay. Run steps \* step time = total assay time (although actually it's a little off due to the way it calculates things).
  - d. Temp (60 C)  
This is the run temperature
  - e. LEDs (1)

Sets the light level. These are logarithmically spaced intensity values from 1-15 (0 is OFF, 1 is lowest intensity). Values above 1 tend to saturate the detectors, so we stick with 1.

f. Start Well (1)

If you only want to sample a subset of wells, you specify the start and stop here.

g. End Well (96)

Stop well to sample.

- 7) Click "Set Assay Params" and verify that the unit responds with the correct parameters (the unit's state is printed on the right side)
- 8) Set the appropriate output directory. Note that it must already exist. Don't include a filename here, that will be automatically added (.log)
- 9) Insert chip and line it up with the phototransistors. Make sure that the bottom of the chip sits flat and the PDMS "wets" the surface of the ITO/glass slide. This will ensure that it doesn't flex during heating.
- 10) Close the lid gently and make sure it completely seals (might have to wiggle it back and forth a bit)
- 11) Click Start Assay and wait
- 12) The unit will emit 3 long beeps when it's done
- 13) To view the data, load the Matlab script and change the filename to your log file. It takes a long time to parse the log files, but it saves them as a MAT file so you can do this again and it's much faster.
- 14) Specify the colors of the wells you want to plot. PLOT\_COLORS tells the script what color to print (standard matlab 1-letter abbreviations). If you set a well to 0, it won't print.

## Summary of Files Available on GitHub

Rev. 2013-06-21

The GitHub repository contains all of the design and source code files for the MicroBAR in their native format. These include:

### Schematics and PCB Layout

Software: Altium Designer 10

This folder includes two separate projects for the two boards in the MicroBAR:

1. LAMP Chip PCB.PrjPCB
2. LAMP Microcontroller PCB.PrjPCB

### Microcontroller and LCD Firmware

Software: Arduino 1.0.4 and Amulet Technologies GEMcompiler 1.1.2.

This folder includes:

1. Amulet LCD GUI. The "mainmenu.htm" is the root page that should be downloaded to the LCD.
2. Atmel8U2 DFU USB Firmware. This should be programmed onto the 8U2 USB interface chip via AVR-ISP.
3. Arduino Sketches. These include the firmware code for the MicroBAR. The "controller.ino" is the root sketch. Code for hardware peripherals (LCD, Header, Lights, GPS, etc.) is included in separate sketch files.

### Enclosure CAD Files

Software: Rhinoceros 4

This folder includes the original enclosure design (enclosure.3dm) as well as exported STL files for 3D printing.

### PC Control and Analysis Software

Software: Processing 1.2.1 and MATLAB

This folder includes:

1. Windows Driver. Once the 8U2 firmware is loaded, Windows will be able to use this driver to communicate with the MicroBAR as a serial port.
2. Processing UI. This is the main control program for the MicroBAR, written in the Processing language. LAMP\_UI.pde is the main file.
3. MATLAB Analysis Scripts. All analysis and data visualization was carried out in MATLAB. plot\_data\_from\_log.m parses log files generated by the Processing UI and generates a series of plots. microbar\_calibration.mat includes the sensitivity coefficient matrix for the detector array.

### Microfluidic Chip Layout

Software: AutoCAD 2012

This folder includes the standard chip layout that we use in this manuscript, which features 96 reaction wells at 4.5 mm pitch, with 16 wells connected in parallel to each of 6 inlets.