SUPPLEMENTAL FIGURES



Fig. S1. A: Experimental and simulation results of gelation spot diameter as a function of laser power. **B:** Spot size of hydrogels formed in methylcellulose using a focused 1550 nm laser spot at 498 mW and 653 mW. Error bars show standard deviation.



Fig. S2. User interface for the software developed to control and automate the SLG process. Section 1 allows users to select the panel for various controls in Section 2, which include laser control (Main Control), stage control (Stage), serial communications (Serial), target selection (Position), image acquisition (Image). Section 3 displays the camera output in real time or results of the image processing steps.

Serial Position Image Serial Po	sition Image	Main Control Stage	Main Control Stage	Main Control Stage
Main Control Stage Main Con	trol Stage	Serial Position Image	Serial Position Image	Serial Position Image
COM6 * Disconnect Lase	Go To	Serial Recieved Laser	X1(top 1152 X2(bot 9623	
COM3 + Disconnect Stage	Relative?	Arroyo 4308 160408097 v1.	left): 020 right): 46	Mich
Laser Control X: 402587	X: 0		Y1(top 1125 Y2(bot -604	
Laser Output Y: 270163	Y : 0		left): 55 right): 13	Load Image (BF)
Off	3 7. 0		Set (V1 V1) and (V2 V2)	
3000 Set Zero	5 2 . 6	Serial Send Command Laser	Set (X1,11) and (X2,12)	Load Image (Fluor)
Continuous Wave	60	Send	Marria Darki Internet	
PWM Period (ms)	Scan	Serial Sent Laser	Mouse Posx: 1113046	Detect Cells (BF)
1000 Set	2	*IDN?		
Duty Cycle (%) X Fields:	3		Mouse Posy: 1/004	Detect Secretion (EGEP)
Number of Cycles	2			Detect Secretion (Lorry
2 Set Y Fields:	5		Delay Time (ms) 2000	Top Use
Fire PWM cycles				Producers 3 Bounding Kept (#) Box Size
Camera Control 4X Nikon	-	Serial Recieved Stage	10X Nikon	
CAMERA		DSP_1 IS 3-AXIS STEPPER VERSION 0.0 DSP_2 IS NOT FITTED		Calculate Cell Area
	 Save Separate Images 	DRIVE CHIPS 000111 JOYSTICK ACTIVE STAGE - H11702NN/F	25 000111 Set Laser Offset (X,Y)	
Single Shot C:\\Users\\mdl_user\	v	FOCUS = USER FOURTH = NONE		Fluor Threshold (1-255) 128
Continuous Shot	ImageJ Path	FILTER_1 = NONE	Laser Scan Laser ON/OFF	(=)
Stop Camera		Serial Send Command Stage		Detect Cells Fluor
Exposure 10000	Scan	Send	Delete	
Save Image		Serial Sent Stage		Scan and Generate Points
Fit Image to Screen	itched Image	?	Clear All	Stantana Scherate Fonds
		P P		
		P	Crosshair	lest Ihreshold
		P		
	B	✓ Query Stage Position	Go	Generate Target Points

Fig. S3. Details of the five control panels for the SLG control software. A: The Main Control panel allows the user to connect to the stage, the laser controller, and the camera, as well as to adjust parameters for the camera and laser. **B:** The Stage control panel includes many parameters relating to the motorized stage. **C:** The Serial communications panel includes the diagnostics for serial communication with the stage controller and laser. **D:** The Position control panel contains stage position coordinate info of the target well. **E:** The Image control panel includes individual buttons to load bright field (BF) and fluorescence (Fluor) images and to start the image processing pipeline.



Fig. S4. Example of a stitched well image after image processing. Aggregates of secreted antibodies are annotated using white pixels. All colonies are annotated using red rectangles.



Fig. S5. Image processing pipeline to detect and rank cell colonies secreting antibodies.

SUPPLEMENTAL TABLES

Table S1. Measured gelation spot diameter experimental data for various current inputs using 20X objective.

Current Input (mA)	Power From Diode (mW)	Estimated Power After System Losses (mW)	Average Gel Spot Diameter (μm)	Standard Deviation (µm)
2500	498.0	81.4	103.2	9.0
3000	635.0	103.9	131.9	8.1
3500	772.5	126.4	187.0	14.6
4000	900.0	147.3	220.5	15.9
5000	1160.0	189.9	240.2	31.0

Rank	Area	Protein Secretion (EGFP)	EGFP/Area	Centroid (X, Y)
1#	0.11	0.15	1.00	(1606, 1099)
2#	0.97	1.00	0.77	(3237, 798)
3#	0.23	0.18	0.57	(1592, 1134)
4	0.11	0.04	0.25	(2080, 3959)
5	0.06	0.01	0.15	(1806, 4106)
6	0.76	0.10	0.09	(3125, 4106)
7	0.06	0.01	0.09	(1146, 2308)
8	0.50	0.04	0.06	(3271, 4019)
9	0.21	0.01	0.05	(2338, 3839)
10	0.15	0.01	0.04	(1232, 2352)
11	0.10	0.00	0.03	(2652, 3786)
12	0.21	0.01	0.02	(2863, 4235)
13	1.00	0.02	0.02	(4134, 3722)
14	0.33	0.01	0.02	(2112, 4126)
15	0.09	0.00	0.01	(4205, 1695)
16	0.18	0.00	0.01	(1431, 3162)
17	0.09	0.00	0.01	(4296, 1734)
18	0.15	0.00	0.01	(1609, 1393)
19	0.10	0.00	0.01	(3059, 3990)
20	0.12	0.00	0.01	(3445, 1203)
21	0.82	0.01	0.01	(3920, 975)
22	0.13	0.00	0.01	(1678, 4174)
23	0.19	0.00	0.01	(3395, 3004)
24	0.16	0.00	0.01	(769, 1655)
25	0.11	0.00	0.01	(2949, 1411)
26	0.18	0.00	0.01	(1601, 2683)
27	0.55	0.00	0.00	(3781, 4010)
28	0.53	0.00	0.00	(1442, 1805)
29	0.34	0.00	0.00	(1904, 3728)
30	0.37	0.00	0.00	(4312, 2297)
31	0.43	0.00	0.00	(2013, 1153)
32	0.23	0.00	0.00	(2580, 4418)
33	0.10	0.00	0.00	(3188, 4193)
34	0.05	0.00	0.00	(1567, 4110)
35	0.07	0.00	0.00	(3298, 4090)

Table S2. Excerpt of the list of cell colonies and protein secretion (EGFP) detected in Fig. 2*.

*All values shown are normalized to the highest number in each column. The top 3 ranked cell colonies[#] are then removed from this list before the remaining centroids are used for the SLG process.