

Selective Laser Gelation

SUPPLEMENTAL FIGURES

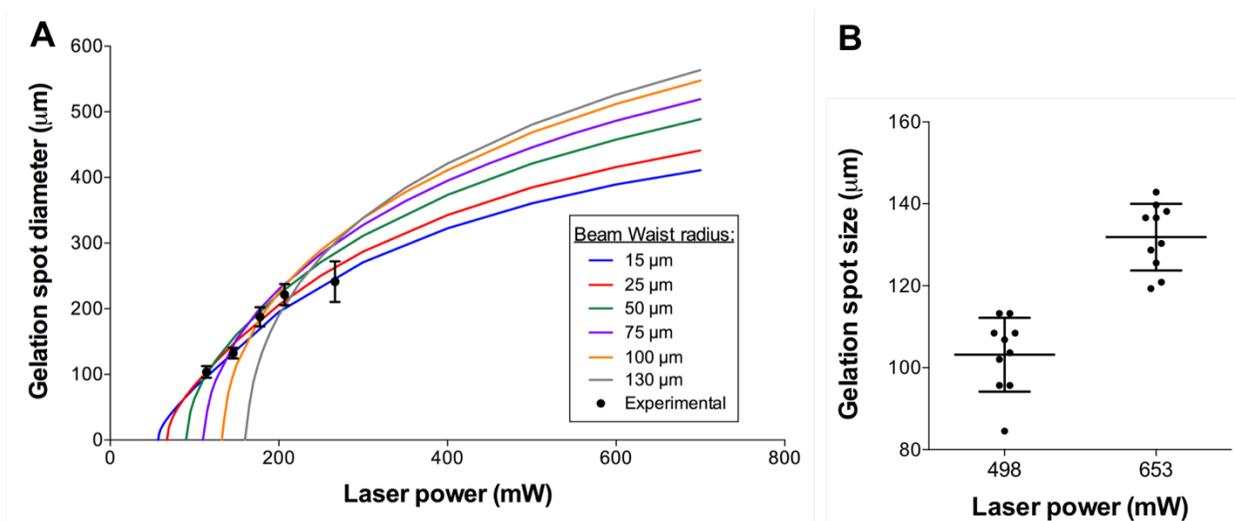


Fig. S1. A: Experimental and simulation results of gelation spot diameter as a function of laser power. **B:** Spot size of hydrogels formed in methylcellulose using a focused 1550 nm laser spot at 498 mW and 653 mW. Error bars show standard deviation.

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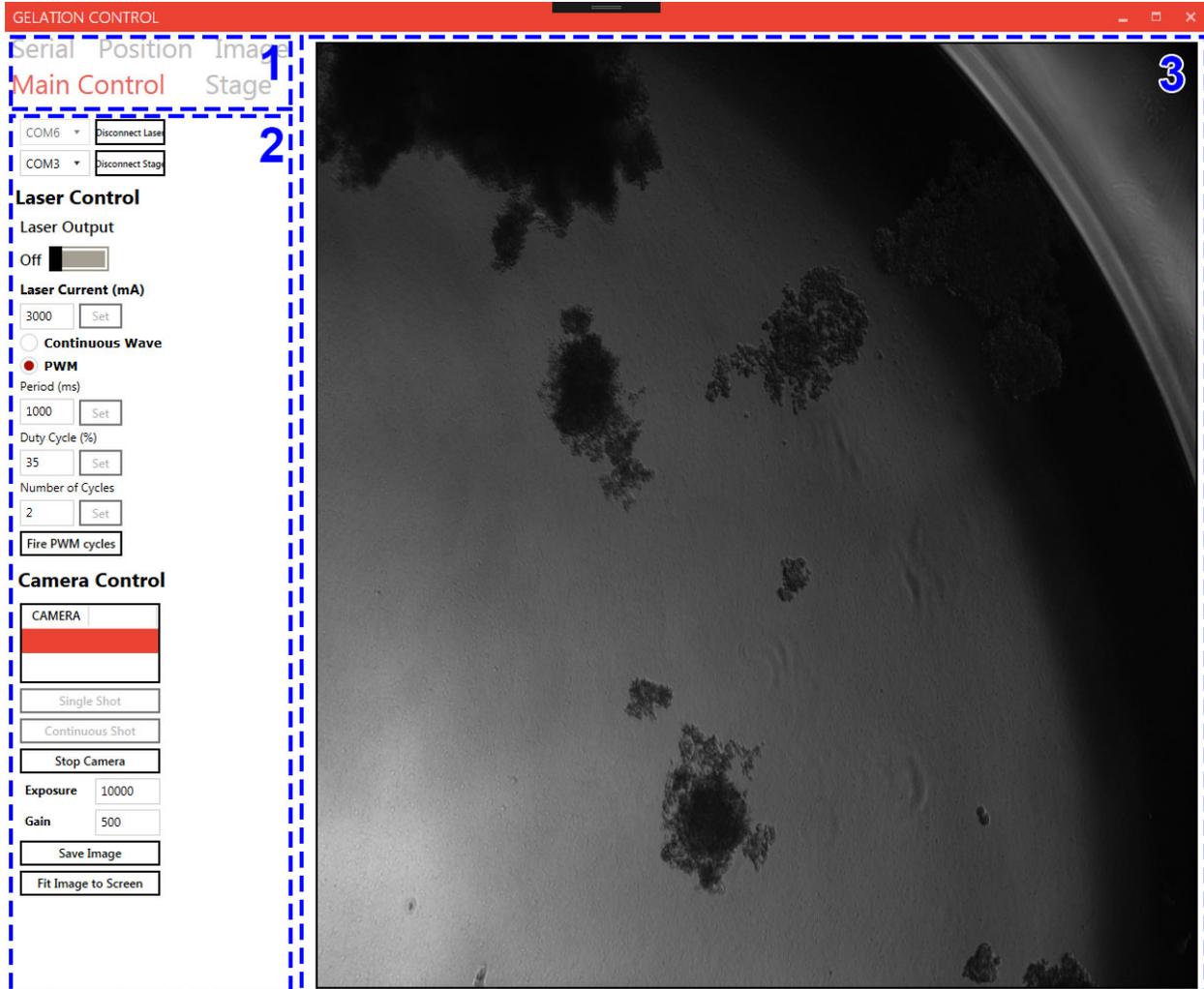


Fig. S2. User interface for the software developed to control and automate the SLG process. Section 1 allows users to select the panel for various controls in Section 2, which include laser control (Main Control), stage control (Stage), serial communications (Serial), target selection (Position), image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

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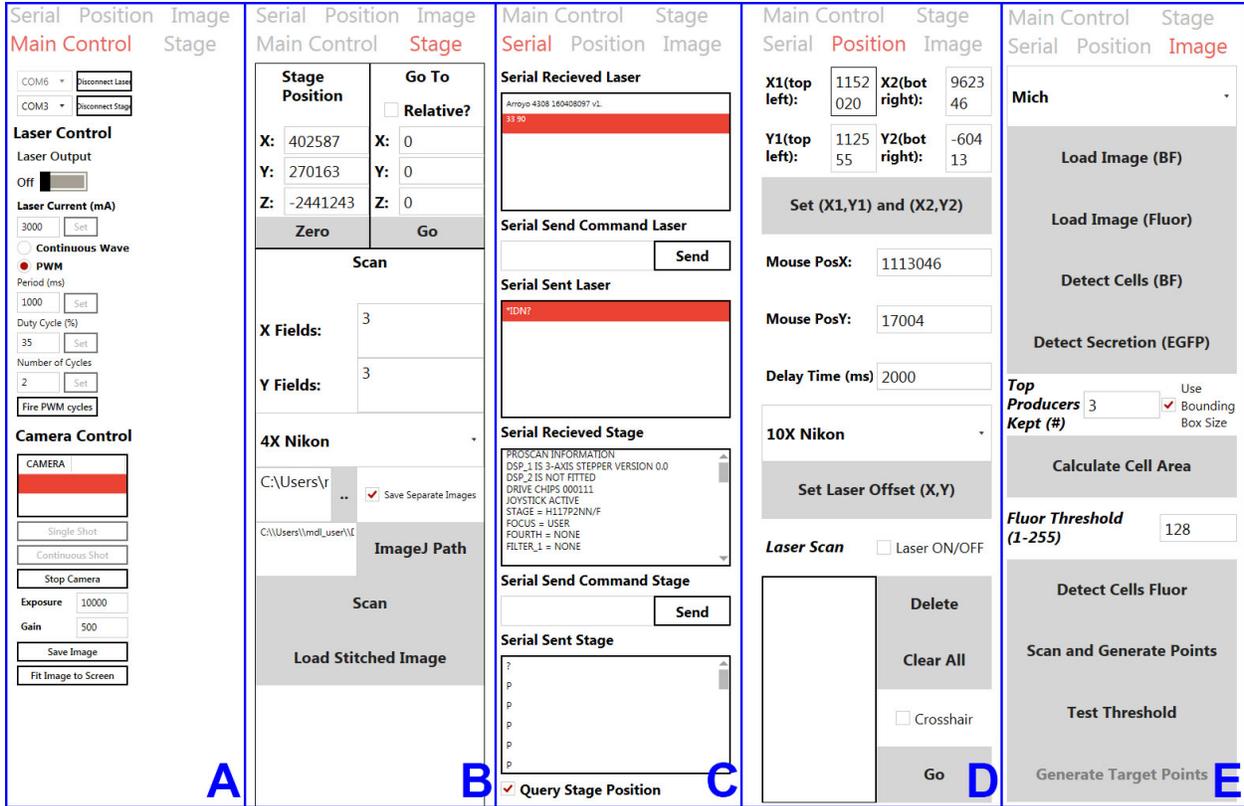


Fig. S3. Details of the five control panels for the SLG control software. **A:** The Main Control panel allows the user to connect to the stage, the laser controller, and the camera, as well as to adjust parameters for the camera and laser. **B:** The Stage control panel includes many parameters relating 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.

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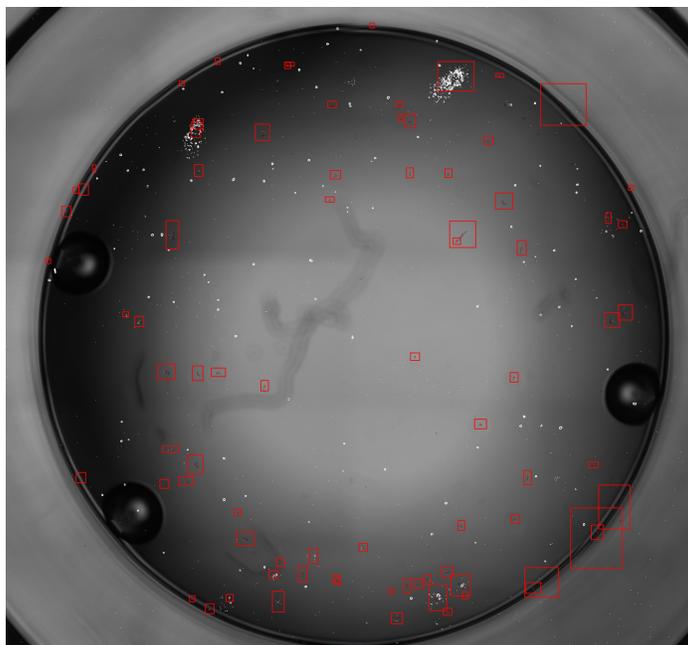


Fig. S4. Example of a stitched well image after image processing. Aggregates of secreted antibodies are annotated using white pixels. All colonies are annotated using red rectangles.

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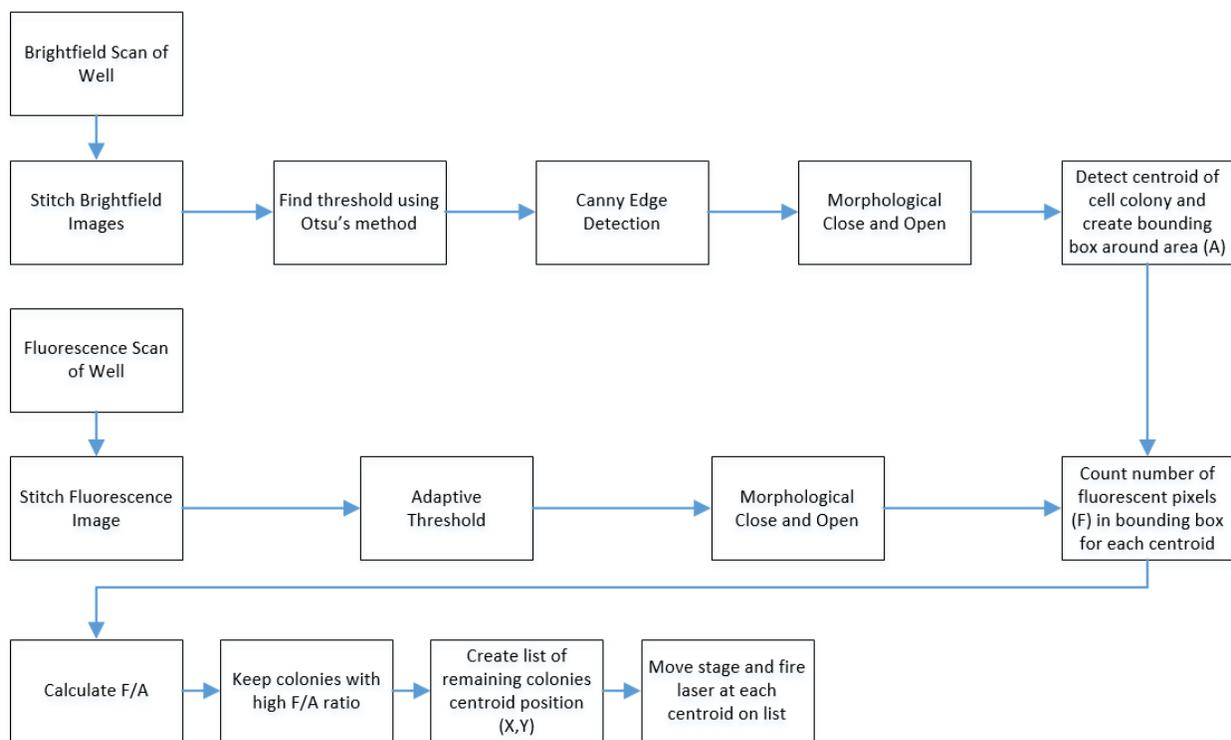


Fig. S5. Image processing pipeline to detect and rank cell colonies secreting antibodies.

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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 |

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

| 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) |
| ... | ... | ... | ... | ... |

*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.