Electronic Supplementary Material (ESI) for Lab on a Chip.

Supporting Information

3D Material Cytometry (3DMaC): A Very High-replicate, High-throughput Analytical Method using Microfabricated, Shape-specific, Biomaterial Niches

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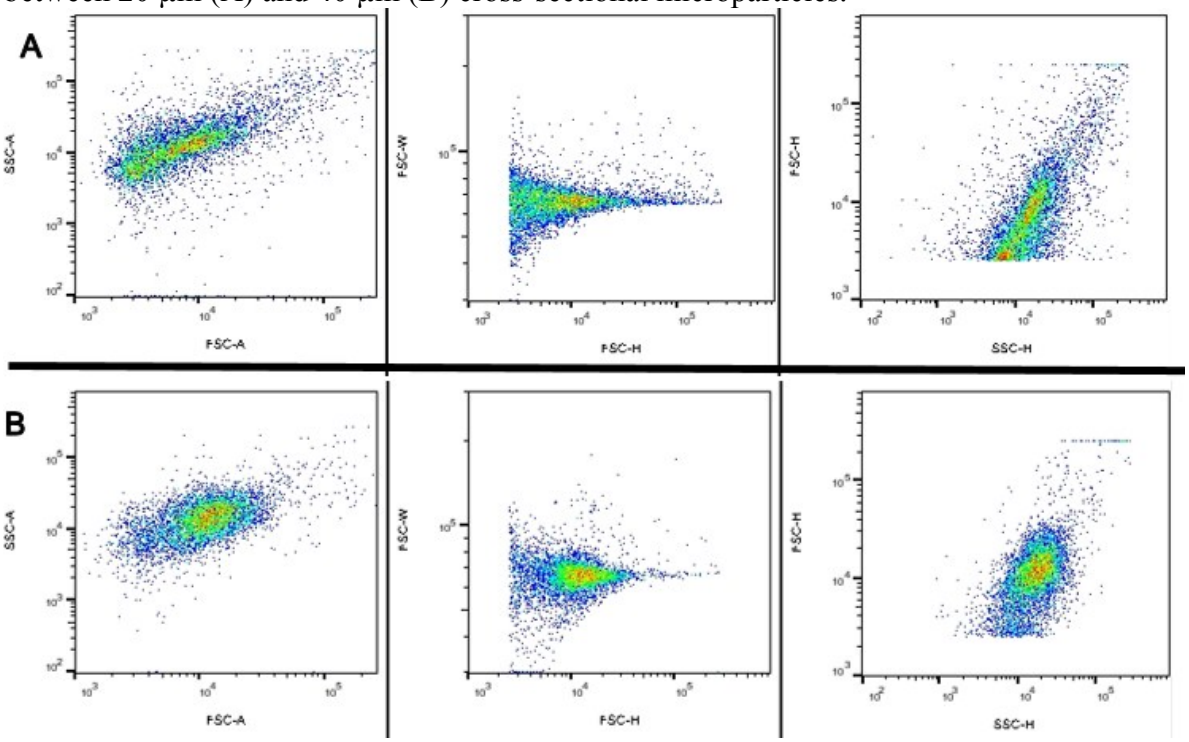
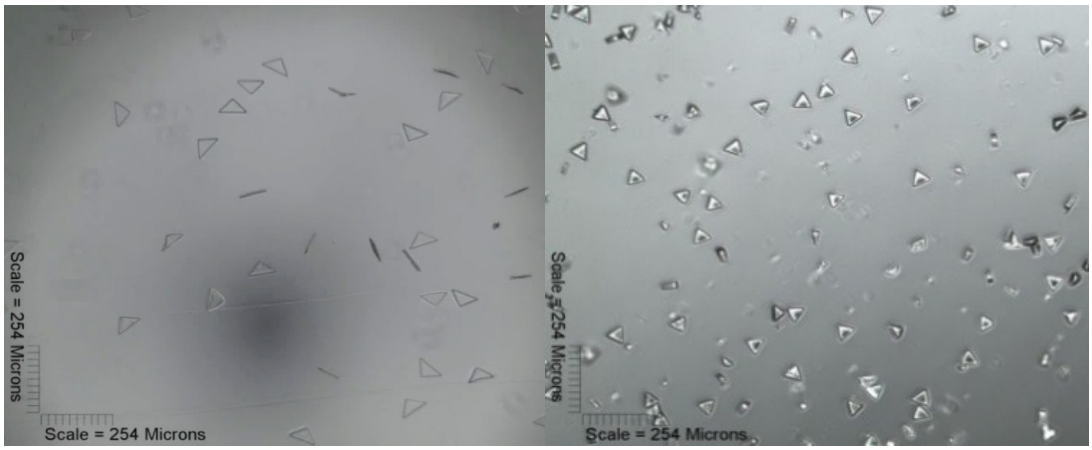
Supplementary Figure 1. Non-image Flow Cytometry Analysis Non-imaging flow cytometers, such as the BD LSR Fortessa used here, can also benefit from additional barcodes based on shape-multiplexing. Here a small shift can be seen in FSC vs. SSC scatterplots between 20 µm (A) and 40 µm (B) cross-sectional microparticles.



Supplementary Figure 2. Further increases in throughput capability. (A) Microgel generation can be made more high-throughput by directly exposing spincoated polymer precursor of controlled thickness to UV light through a photomask. Shown are two populations of different size and thickness microhydrogels suspended in PBS after swelling. (B) Using wafers to increase the replicate number also increases the level of significance that can be discerned in the study. (C) Suspensions of microhydrogels show the high replicate number achieved from a single mold after hydration.

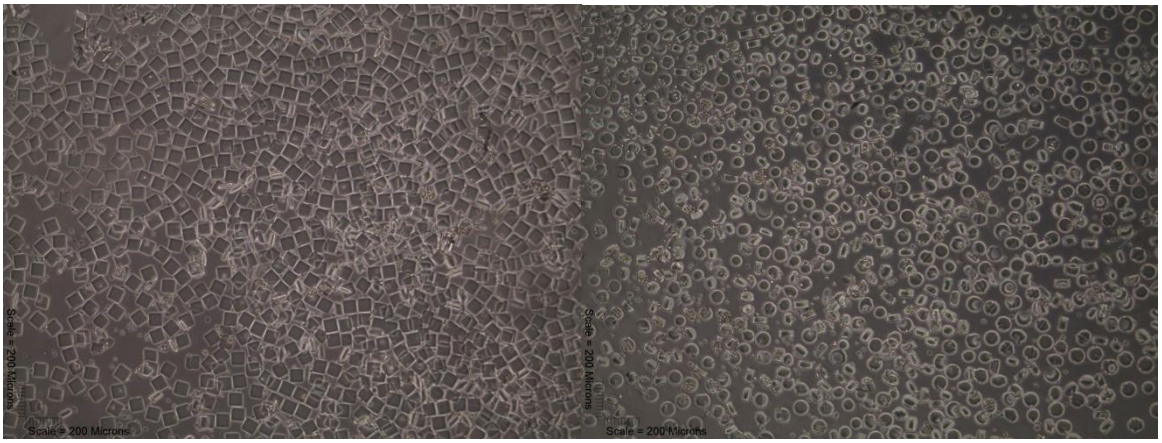
A



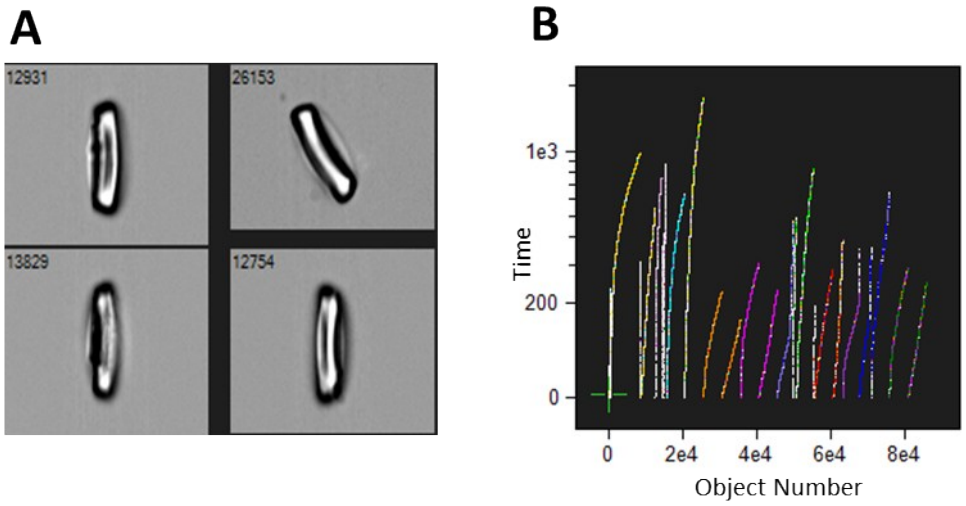
B

Mold Type	Array Area (μm^2)	Microhydrogel Unit Dimension (μm)	Microhydrogel Mold Area (μm^2)	Replicate Number	Level of Significance (p)
Test 1.5cm Array	$2.25*10^8$	20	900	250,000	0.001
		40	2500	90,000	0.0016
		60	4900	45,918	0.0023
10cm Wafer	$78.54*10^8$	20	900	8,726,666	0.00017
		40	2500	3,141,600	0.00028
		60	4900	1,602,857	0.00039

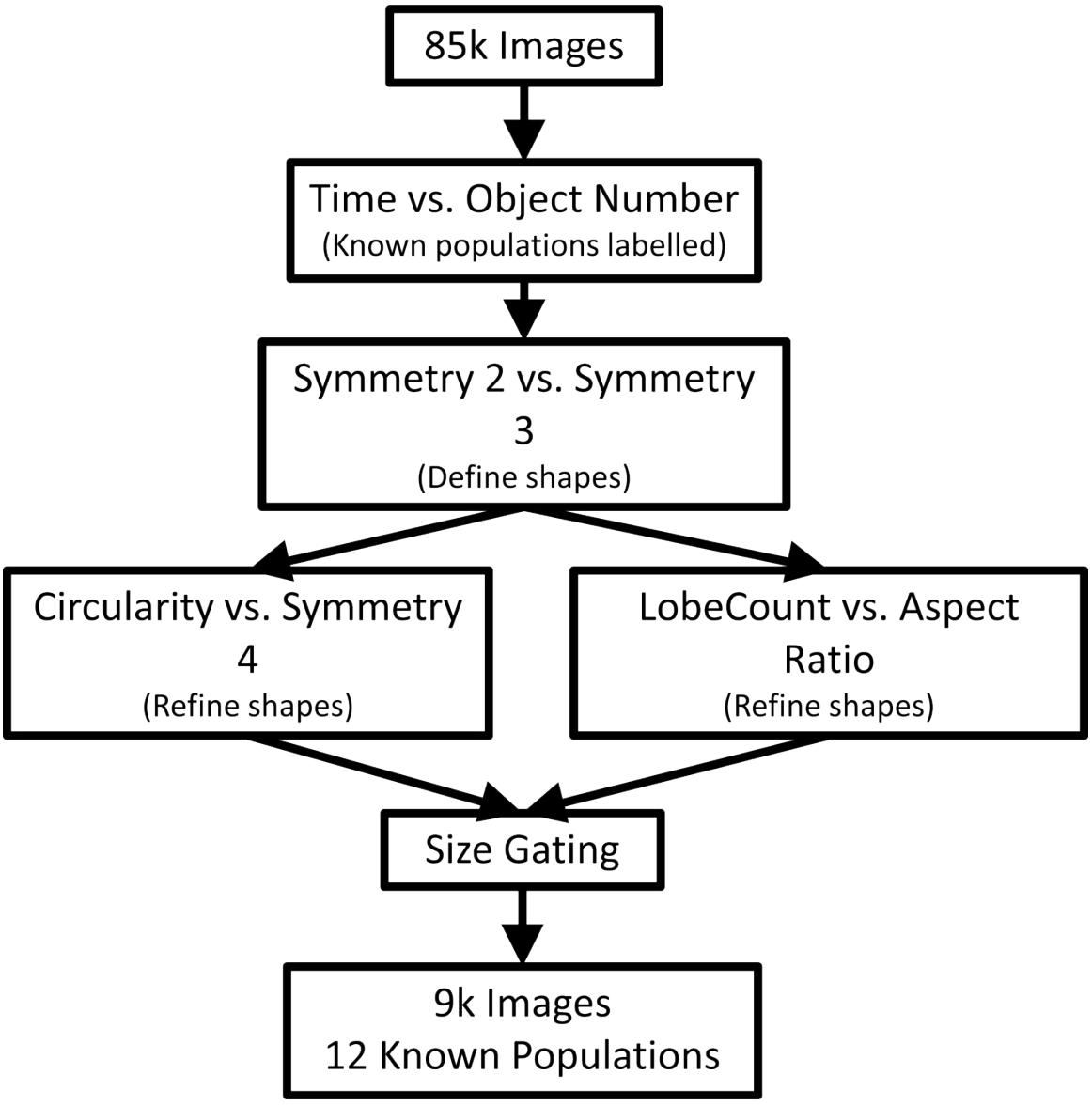
C



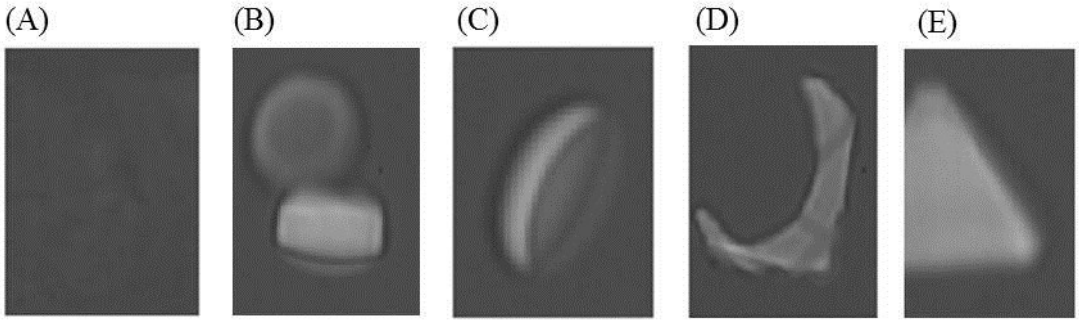
Supplementary Figure 3. ImageStream Runtime Analysis. (A) Microgels tilted relative to the camera such that the shape barcode cannot be read. These were successfully removed by gating on Aspect Ratio (B) Scatterplot of all sample runs shows similar data collection rates across samples



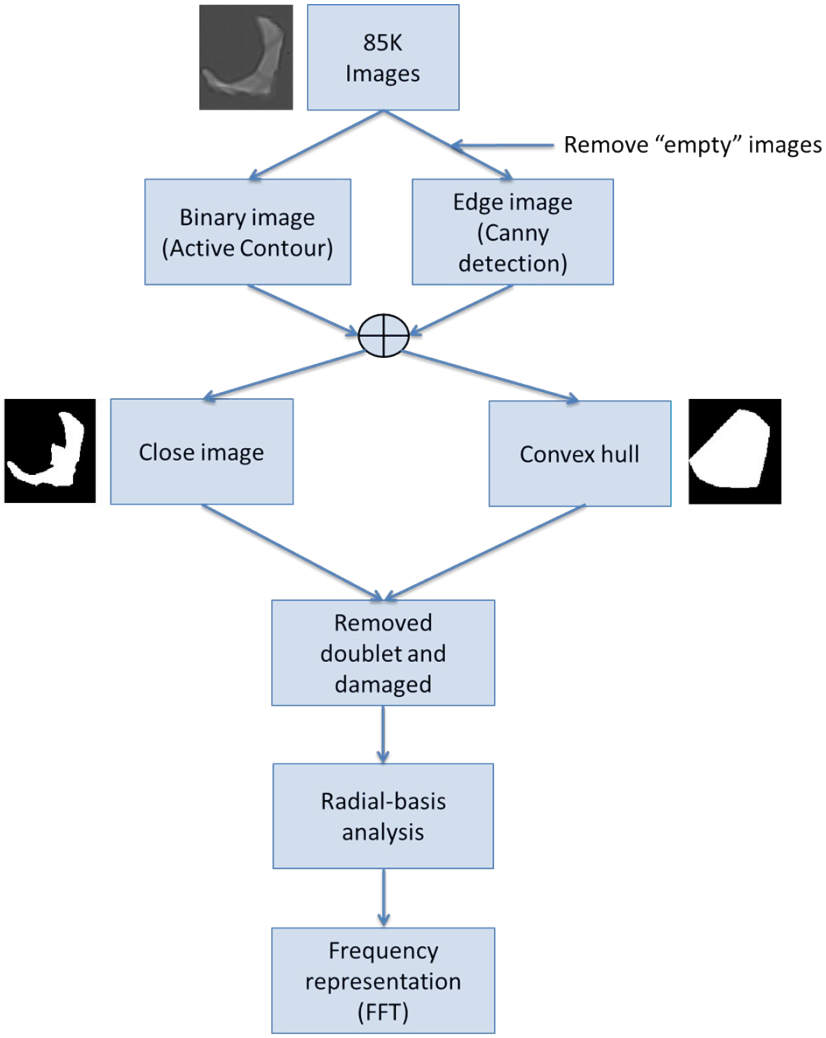
Supplementary Figure 4. ImageStream Data Processing. Process flow for IDEAS software analysis used to define the gating template.



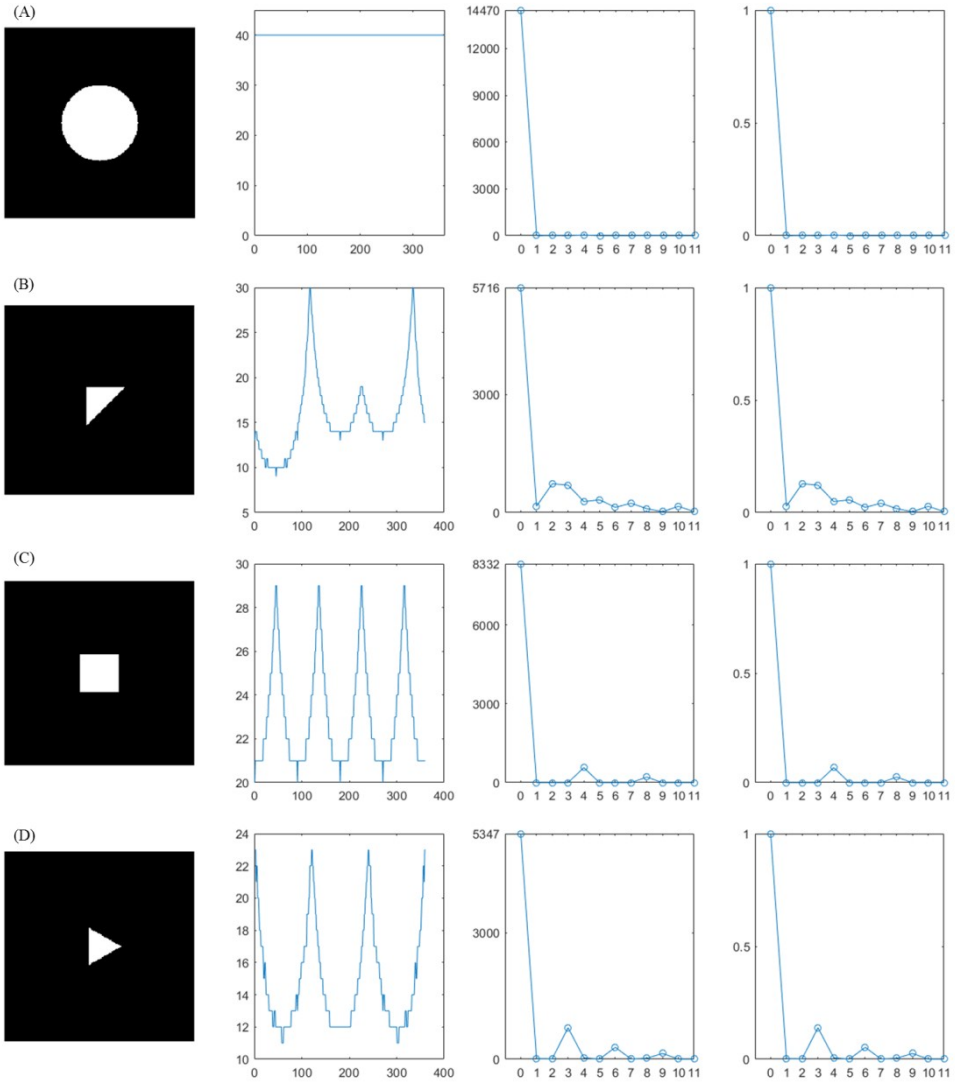
Supplementary Figure 5. Examples of low quality images: (A) empty, (B) doublet /overlapped, (C) tilted, (D) damaged, and (E) truncated. An empty image was formed because of bubbles within the microgel. These images can be removed based on standard deviations of their pixel intensities. Doublet/overlapped and damaged microgels are typically non-convex objects after the images were converted into binary. They were identified and removed by comparing the convex hull images and close images (obtained in the preprocessing step with dilation followed by erosion). To detect and remove truncated images, the percentage of foreground pixels along the image boundary was computed.



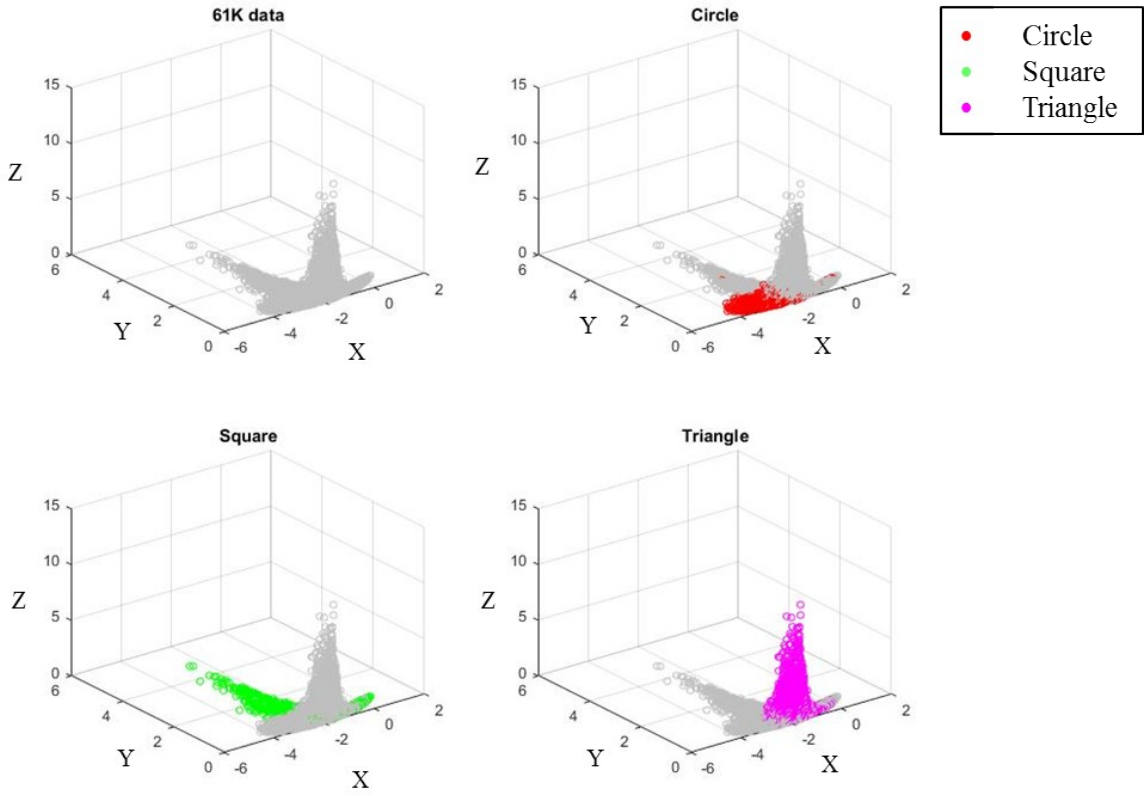
Supplementary Figure 6. Flow chart of preprocessing steps.



Supplementary Figure 7. Reference images used to determine FFT feature characteristics. The first column shows images of the reference for each shape (A) circle (B) right triangle (C) square and (D) triangle. The second column represents centroid-contour distance vector, which is the distance from centroid to edge from 0 degree to 360 degree. Where x-axis is degree and y-axis is the centroid-contour distance. The third column represents twelve FFT (FFT0~FFT11) values with x-axis as frequency (FFT) and y-axis as its magnitude. The value of FFT 0 is size of the object. The forth column shows normalized FFT values (FFT1~FFT11). Each shape shows a distinctive pattern. The circle has low magnitudes in all frequencies and the right triangle has some magnitudes in almost every frequencies. The square has peaks at FFT4, and 8 and the triangle shows peaks at FFT 3, 6, and 9.



Supplementary Figure 8. Scatter plots for selecting shapes (circle, square and triangle). Based on the unique peak features, these three shapes can be selected. Circles tend to have close to 0 magnitudes at all 11 FFT features and Squares show peaks at FFT 4 and 8. Lastly, triangles have peaks at FFT 3, 6 and 9. X-axis represents $\log\left(\sum_{i=1}^{11} FFT_i\right)$, Y-axis represents sum of feature 4and 8 divided by sum of remaining 9 FFTs and Z-axis represents sum of feature 3, 6 and 9 divided by sum of the other 8 FFTs.



Supplementary Figure 9. Selecting right triangles. Calculating distances between normalized FFT values of data (~61,000) and four different references' normalized FFT values. Sorting distances between data and right triangle reference in ascending order and set threshold 75 % of intersect point between right triangle (red) and the other three shapes curves. Define all data, below the threshold (black straight line), as high quality right triangles.

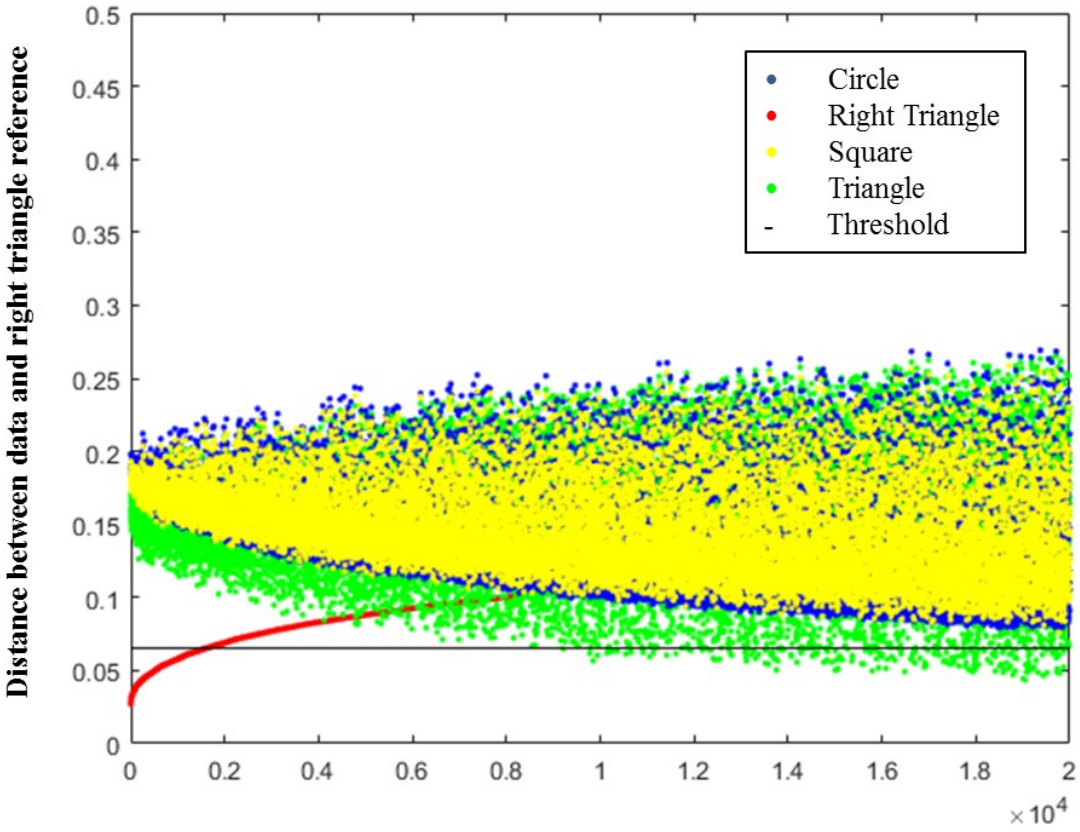
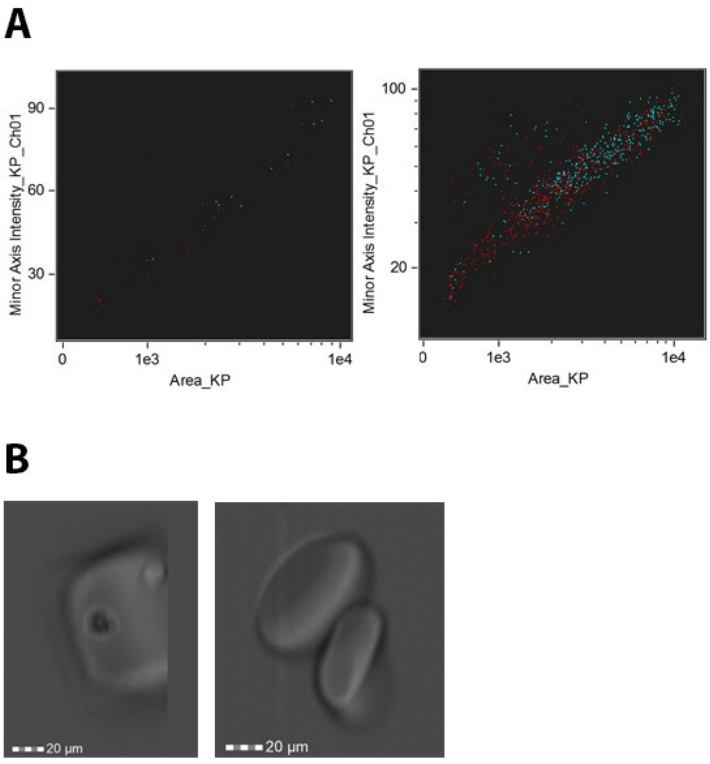


Table S1. Evaluation of manual gates derived from the centroid distance analysis. In the first row, percentages represent the number of events with correct known shapes divided by the total number of events in a gate. The remaining rows represent the performance after subsequent classification based on size.

Size (µm)	Circle	Right Triangle	Square	Triangle
	78.33%	97.23%	90.40%	95.94%
20	67.04%	86.86%	73.92%	86.06%
40	70.38%	79.76%	78.98%	79.97%
60	81.02%	96.74%	97.51%	98.78%

Supplementary Figure 10 Cell-containing Microgel Misclassification. (A) Clustering of shaped, cell-containing microhydrogels into Triangles (left) and Right Triangles (right) with the true Circle population displayed in red and the true Square population in blue. (B) Examples of the types of Square and Circle microhydrogels that are being misclassified as Right Triangles. The Triangle gating collected 34 events and the Right Triangle gating collected 801 events.



Supplementary Figure 11 Cell-containing Microgel Fluorescence Sorting. (A) Clustering of shaped microhydrogels that stained positively for fluorescence and were sucessfully gated into the final Circle or Square populations (true Circles in red and true Squares in blue). The Circle gating collected 203 events and the Square gating collected 138 events. (B) Ideal examples of correctly sorted Circles with high and low cell incorporation. (C) Ideal examples of correctly sorted Squares with high and low cell encapsulation.