Supplementary Information (SI) for Analyst.

Supplemental Information for

Noninvasive and in situ identification of the phenotypes and differentiation stages of individual living cells entrapped within hydrogels

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Supplemental Figures

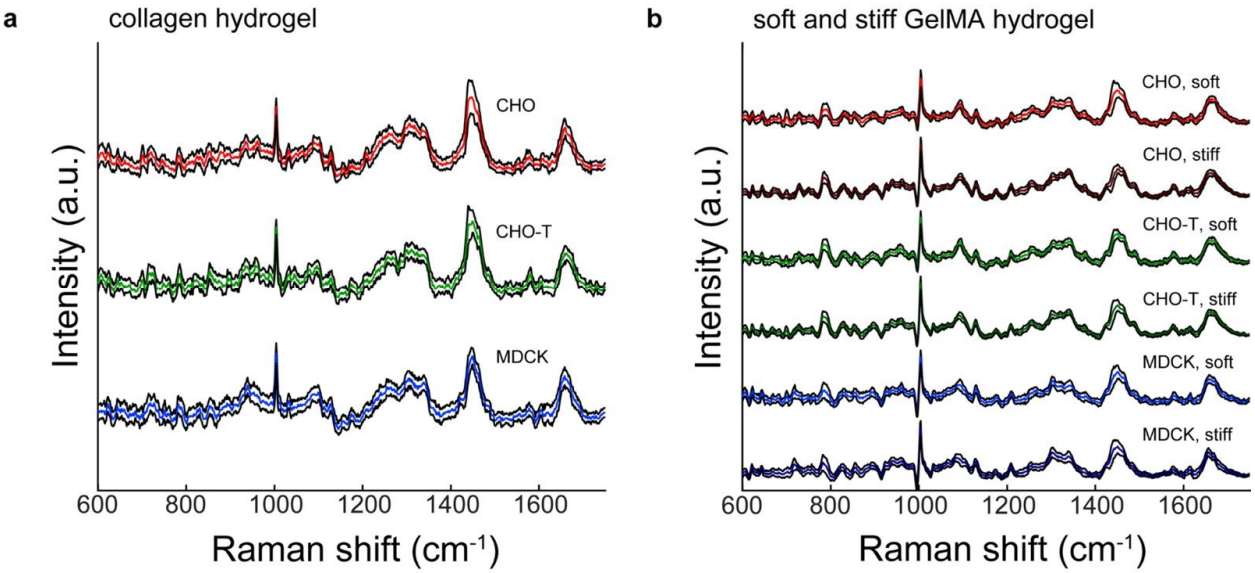

Figure S1. (a) Averaged preprocessed spectra acquired from individual, living CHO cells (red, n = 45), CHO-T cells (green, n = 45) and MDCK cells (blue, n = 39) within collagen hydrogels. (b) Average preprocessed spectra acquired from individual, living CHO cells within 4% (red, n = 61) and 7% (dark red, n = 62) gelMA hydrogels; CHO-T cells within 4% (green, n = 62) and 7% (dark green, n = 61) gelMA hydrogels; and MDCK cells within 4% (blue, n = 62) and 7% (dark blue, n = 57) gelMA hydrogels. Spectra preprocessing consisted of background subtraction, baseline correction using a weighted-least-squares method, normalization, and offsetting for visual clarity. Black lines in (a) and (b) represent the standard deviation from the average spectra.

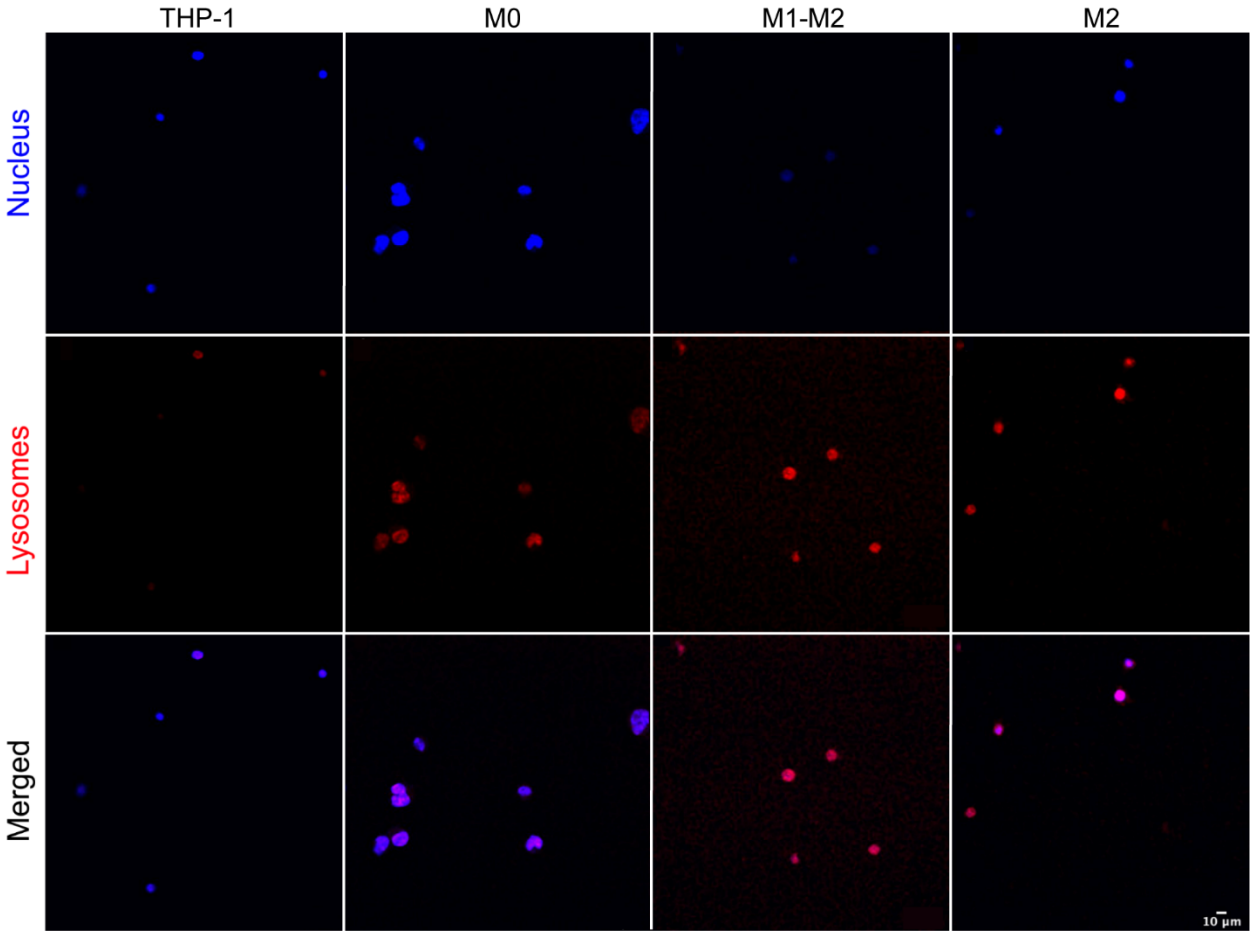

Figure S2. Fluorescence from Hoechst nuclear stain (top row, blue), LysoTracker™ Red DND-99 (middle row, red), and the overlay of both signals (bottom row) indicate the lysosomal compartments were smallest in the THP-1 cells and larger and in the M0 cells, M1-M2 cells, and M2 cells.

Supplemental Tables

Table S1. The number of latent variables (LVs), the variance they captured, and identification errors for PLS-DA model of single-cell spectra acquired from living CHO, CHO-T, and MDCK cells in collagen. The model that contained 4 LVs was employed.

Latent Variables	*Variance Captured (%)*	*Error of phenotype prediction (%)*		
		CHO	*CHO-T*	*MDCK*
1	32.79	30.5	35.9	60.4
2	62.74	18.3	10.5	2.6
3	77.91	3.5	4.5	2.6
4	86.68	1.2	2.4	2.6
5	90.67	3.5	2.4	2.6
6	93.51	3.5	4.9	2.6
7	95.01	1.2	3.7	2.6
8	96.48	2.4	2.4	2.6
9	97.15	2.4	2.4	2.6
10	98.05	4.7	4.9	2.6

Table S2. Number of latent variables with their respective captured variance and identification errors for PLS-DA model of CHO, CHO-T, and MDCK cells in soft and stiff gelMA. The model with 6 LVs was applied to the test set of spectra to identify each cell's phenotype.

Latent Variables	*Variance Captured (%)*	*Error of phenotype prediction (%)*		
		CHO	*CHO-T*	*MDCK*
1	25.73	29.9	34.4	14.6
2	46.81	11.2	24.8	13.3
3	61.29	13.2	14.5	6.3
4	69.42	12.4	10.3	5.0
5	76.12	7.4	8.3	2.5
6	79.55	1.2	5.0	2.5
7	83.28	1.2	5.8	1.7
8	86.09	2.5	3.3	1.7
9	88.61	2.5	2.5	2.5
10	90.45	3.3	2.5	1.2

Table S3. Number of latent variables, the variance they capture, and identification errors for PLS-DA model of THP-1, M0, M1 or M2, and M2 cells in soft gelMA hydrogels. The PLS-DA model with 6 LVs was employed to identify the lineage-specific differentiation stages of the cells in the test set.

Latent Variables	*Variance Captured (%)*	*Error of phenotype prediction (%)*			
		THP-1	*M0*	*M1-M2*	*M2*
1	27.72	7.7	35.1	55.4	14.9

2	51.77	3.7	5.4	55.8	11.3
3	70.10	3.7	4.4	4.0	5.2
4	75.11	3.1	4.2	1.7	5.2
5	78.87	1.5	2.3	0.8	8.0
6	82.95	0.8	2.3	0.8	4.8
7	86.52	0.8	0.5	0.8	4.8
8	88.65	0.8	0.5	0	4.8
9	90.26	0.8	0.5	0	5.2
10	91.58	0.8	1.0	0	5.2