Supplementary Figure 1. Cellular viability in reformulated culture media

Cells were cultured in serum containing DMEM culture medium supplemented with 4%PEG and 5%DEX for 24hrs. Cellular viability was evaluated using a kit containing calcein acetoxyethyl (calcein AM) and ethidium homodimer-1 (EthD-1) to stain live and dead cells, respectively.

(a) A representative image of stained cells cultured in DMEM +5%DEX. Cellular viability was above 98%.
(b) A representative fluorescent image of stained cells cultured in DMEM+4%PEG. Cellular viability was above 96%.
Supplementary Figure 2. Aqueous two-phase system (ATPS) comprising Polyethylene glycol (PEG) and Dextran (DEX) as phase forming polymers

Phase diagram of an ATPS describes the composition of each phase and the range of concentrations that results in phase separation. Only those combinations of the two polymers P (PEG) and Q (DEX) above the binodal curve give an ATPS. Point A represents the initial concentration of each polymer in the entire solution whereas points B and C describe the compositions of bottom and top phases in equilibrium, respectively. Line BC represents a tie line and is a unique property of the given ATPS.
Supplementary Figure 3. Design principles regarding the resolution of arbitrarily shaped patterns

The resolution of arbitrary patterns can be enhanced by reducing the size of the dispensing tip used. The “UMICH” pattern in Fig. 1 of the manuscript was generated with a relatively large gel pipette tip (500 µm tip size). A homemade glass pipette with a small 150 µm tip size enabled generation of much finer patterns stably with lateral dimensions of ~300-350 µm using the same 4%PEG-5%DEX system as used in Fig. 1 of the manuscript. This is about 60-70% smaller than the lateral size of the patterned “UMICH” in the manuscript.

Interfacial tensions of a system have large effects on the patterning resolution. Here, another consideration is how interfacial tension affects the stability of a pattern. This is because unlike in the case of circular droplet patterns where curvatures and hence pressures are similar everywhere, these arbitrary shapes have local curvatures and pressures. The Laplace equation of capillarity \[ \Delta P = \gamma_{12} \left( \frac{1}{R_1} + \frac{1}{R_2} \right) \]
relates pressure difference (\(\Delta P\)) across the interface of the two aqueous phases to the corresponding interfacial tension (\(\gamma_{12}\)) and the principal radii of curvature (\(R_1\) and \(R_2\)). To maintain arbitrary shapes stably, the pinning interfacial tension forces between cell monolayer and each aqueous phase (\(\gamma_{1C}\) and \(\gamma_{2C}\)) must be able to withstand forces exerted by local pressure differences. Thus, there is a limit in terms of local radii of curvature below which line features would break up into droplets. Our experiments in Fig 1 and line writing demonstrations above used liquids with very low \(\gamma_{12}\) enabling formation of thin lines (~300 µm width).

We anticipate these fundamental rules to be applicable to other aqueous two-phase systems beyond the PEG-DEX system we specifically describe.
Supplementary Figure 4. Long term stability of patterned reagents
Complexes of a transfection reagent, Lipofectamine 2000, and dsRed or eGFP genes were mixed with the DEX phase and patterned in different shapes of diamond, triangle, and square on HEK293H immersed in the PEG phase. Cells were incubated at 37°C and 95% humidity for 8hrs. The two-phase patterns were washed away and replaced with regular growth media. Cells were incubated for 48hrs and then imaged with a fluorescent microscope. Cells showed localized expression patterns of dsRed and eGFP genes that exactly mimic the shapes of printed reagents. These experiments confirmed that the patterned reagents remain stable over long time periods.
Supplementary Figure 5. Stability of printed patterns

Pattern stability is due to a cooperative effect of a low interfacial tension between aqueous polymer phases ($\gamma_{12}$) and roughness of the cell monolayer and associated interactions between DEX and the surface of living cell.

(a) To pin down the effect of low $\gamma_{12}$ on pattern stability, we used a two-phase system with higher concentrations of polymers, i.e. 7%PEG/12%DEX. The interfacial tension between the resulting two aqueous phases is two orders of magnitude larger than that used to form the “UMICH” pattern (Fig. 1 of the manuscript). We formed linear patterns of the DEX phase on a cell monolayer immersed in the PEG phase and found that the patterns do not remain stable and immediately bead off at several places. This confirms that low interfacial tension $\gamma_{12}$ is a key factor for the stability of printed patterns on cells.

(b) The surface of cells possesses a rough structure of a few microns [Ref: J. Cell Sci. 1994, 107, 1105-1114]. Direct observation of the PEG-DEX interface on cells shows that the interface becomes hinged to cells at “node” points, suggesting that cell surface plays an important role in the stability of patterns.

(c) As a negative control to show lack of stability of patterns on a surface with nanometer scale roughness, we fabricated a surface with nanometer roughness obtained by molding polydimethy siloxane (PDMS) against a silicon nanostamp (LightSmyth Technologies). The resulting surface contains ridges of 340 nm with 2 µm spacing. A linear pattern of the DEX phase (5%) was formed in the PEG phase (4%) covering the surface (note: this two-phase system has the lowest $\gamma_{12}$ and always gives a stable pattern on cells). The pattern was unstable and quickly retracted to a circular shape (see Supplementary Video 1).
Supplementary Figure 6. Cellular viability post transfection
HEK293H cells were transfected with eGFP plasmid DNA both with two-phase and conventional transfection techniques. Cellular viability was evaluated 12hrs after the transfection complexes were removed. At this time point, cells had not divided yet, which ensured the results reflect the actual number of live and dead cells present after the transfection process. A kit containing calcein acetoxyemethyl (calcein AM) and ethidium homodimer-1 (EthD-1) was used stain live and dead cells, respectively.
(a) A representative image from staining of cells transfected with the two-phase technique. Green color corresponds to live cells and red color represents the dead cells.
(b) Fluorescent image of stained cells transfected with the conventional method. Green color corresponds to live cells and red color represents the dead cells.
(c) Quantitative comparison of cellular viability post transfection with the two techniques. The number of live and dead cells were counted from five different images and each bar represents the mean ±S.D. Viability is above 95% in both cases.
Supplementary Figure 7. Design principles regarding the resolution of circular patterns

(a) For a given volume of dispensed liquid, the size of DEX droplets is determined by the balance between the three interfacial tension forces: Interfacial tension between the two immiscible polymer phases, $\gamma_{12}$, and interfacial tension of each aqueous polymer phase and the cell monolayer, $\gamma_{1C}$ and $\gamma_{2C}$, respectively. While $\gamma_{2C}$ and $\gamma_{12}$ (through its horizontal component) decrease the contact area of the drop with the cell monolayer surface (and hence the droplet diameter), $\gamma_{1C}$ tends to increase it. From a design point of view, in addition to the possibility of using very small dispensing volumes (few nanoliters only), increasing the $\gamma_{12}$ component is another means to achieve higher resolution.

(b) The interfacial tension between two immiscible aqueous polymer phases ($\gamma_{12}$) directly correlates with the tie line length (TLL) through the following empirical relation: $\log(\gamma_{12}) = A + B \log(\text{TLL})$. Constants A and B depend on the two-phase system under consideration [Bamberger et al., J. Colloid Interface Sci. (1984) 99, 194-200]. Therefore on a phase diagram, longer tie lines correspond to larger $\gamma_{12}$ values. Since each tie line represents a different pair of concentrations (%w/w) of the two-phase system, increase in $\gamma_{12}$ can be achieved by increasing the concentrations of the phase-forming polymers.

We selected four different concentrations of phase-forming polymers PEG (Mw:8000) and DEX (Mw:500000) in a range of PEG 4-7 (%w/w) and DEX 5-12 (%w/w). The resulting TLLs were 11.8-28.5 (%w/w) (see figure below) corresponding to $\gamma_{12}$ of ~6 µJ/m$^2$ to ~146 µJ/m$^2$.

(c) For a given DEX droplet volume, droplet diameter decreases consistently with increasing TLL and hence with increasing $\gamma_{12}$. A 7%PEG-12%DEX system gives droplets with diameters ~40% smaller than that of the 4%PEG-5%DEX system. All polymeric aqueous two-phase systems follow this rule and in principle, this design rule is applicable to all such systems.

It should also be considered that if these aqueous polymer systems are used for cell studies, too much increase in polymer concentrations may adversely affect viability or function of certain cell types.
Supplementary Figure 8. Side view images of DEX droplets on a cell monolayer

Side view images of 500, 200 and 50 nl DEX droplets from a 4% PEG/5% DEX system formed on a monolayer of MDA-MB-231 cells. Please also Supplementary Video 2 of confocal microscopy of a 200 nl DEX droplet on cells.