Electronic Supplementary Material (ESI) for Analyst.

Supporting Material

for

Paper-based Transwell Assays: An Inexpensive Alternative to Study Cellular Invasion

Rachael M. Kenney,[a^] Adam Loeser,[a^] Nathan A. Whitman,[a]
and Matthew R. Lockett [a,b] *

[a] Department of Chemistry, University of North Carolina at Chapel Hill, 125 South Road, Chapel Hill, NC 27599-3290

[b] Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, 450 West Drive, Chapel Hill, NC 27599-7295

^ indicates shared-authorship

* mlockett@unc.edu, 919-843-9440

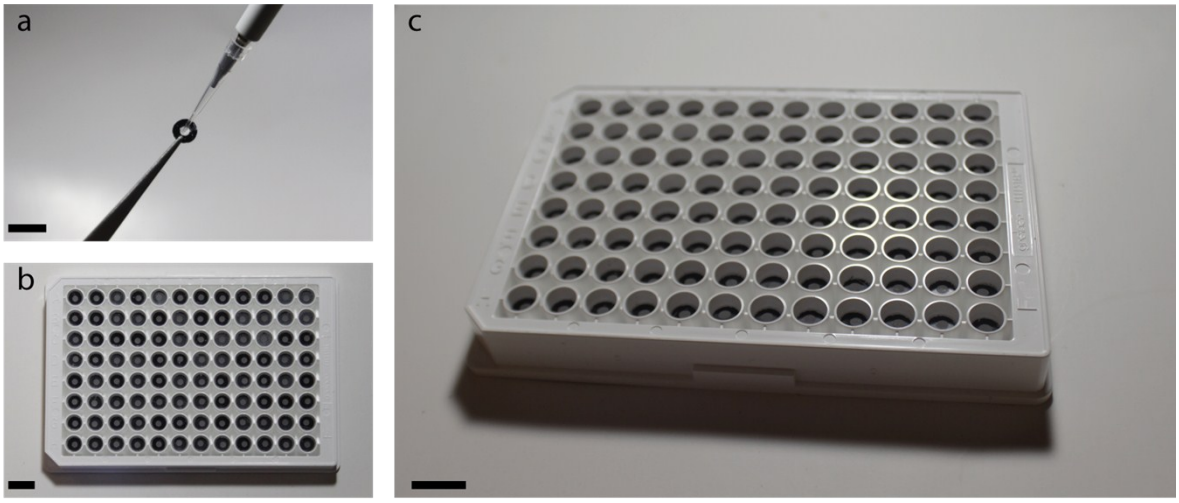


Figure S1. Photographs of a single zone, paper-based Transwell assay format. (a) Seeding a single zone paper scaffold with cells suspended in collagen, using a 2.5 µL pipette. (b) Top and (c) angled view of single zone scaffolds floating at the air-liquid interface of individual wells of a commercially available 96-well plate. Scale bar 10 mm.

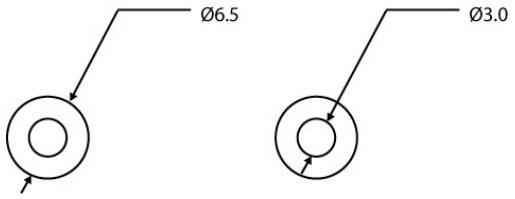


Figure S2. Schematics of the single zone, paper-based scaffolds. Each zone was wax-printed using a Xerox ColorQube®8870 printer. The outer circle is black wax and the inner circle is wax-free. The inner circle is referred to as a “zone” and supports the cell-laden hydrogels. All numbers are in units of mm.

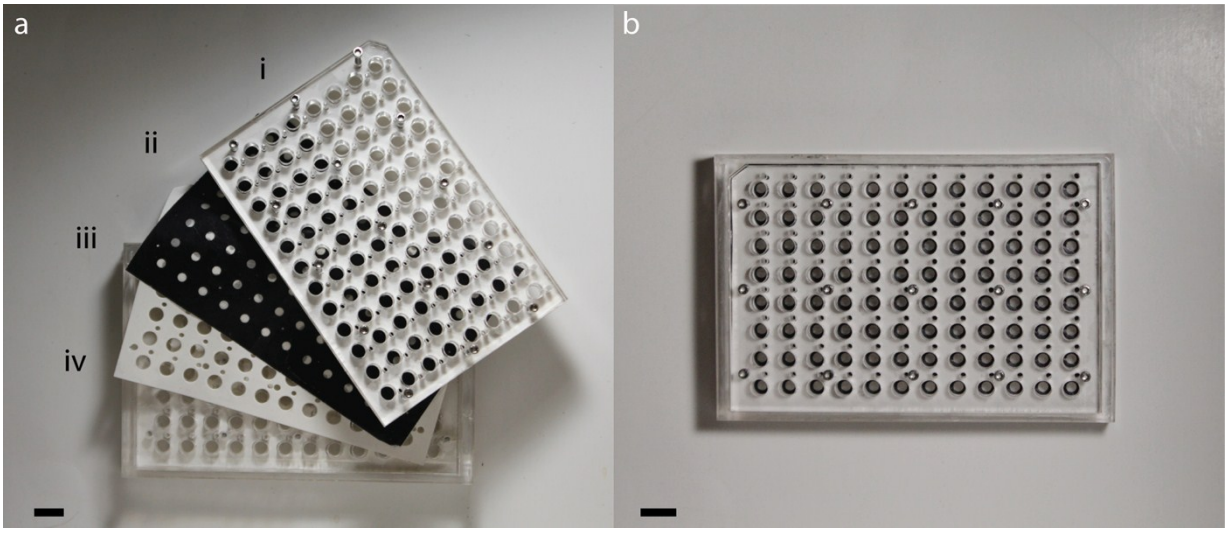


Figure S3. Photographs of the 96-well paper-based Transwell format. (a) The individual components of the 96-zone format include: (i) an acrylic top insert with a silicone gasket, (ii) a paper-based scaffold, (iii) a silicone gasket, and (iv) an acrylic bottom insert with a silicone gasket and bottom sheet of acrylic. The device components were laser cut (Universal Laser Systems, ILS9.75) food-grade silicone rubber 40A (1/32") and cast acrylic (1/4" or 1/8"). Two rubber gaskets were cut in the pattern detailed in Figure S5a. The third rubber gasket was cut in the pattern detailed in Figure S5b. The rubber gaskets were laser cut using the following parameters: Power (P) = 100, Speed (S) = 10, Pulses per inch (PPI) = 1000. The two acrylic inserts (1/4") were cut in the pattern detailed in Figure S5a. The acrylic base plate (1/8") and surrounding border (1/4") were cut in the pattern detailed in Figures S5b and S5c. The acrylic was cut using P = 100, PPI = 1000, and S =2 or S = 10 for 1/4" and 1/8" sheets, respectively. The individual components of the 96-zone Transwell device were held in conformal contact, once assembled, fifteen 3/4" 2-56 stainless steel screws. (b) The fully assembled 96-zone Transwell device. Scale bar 10 mm.

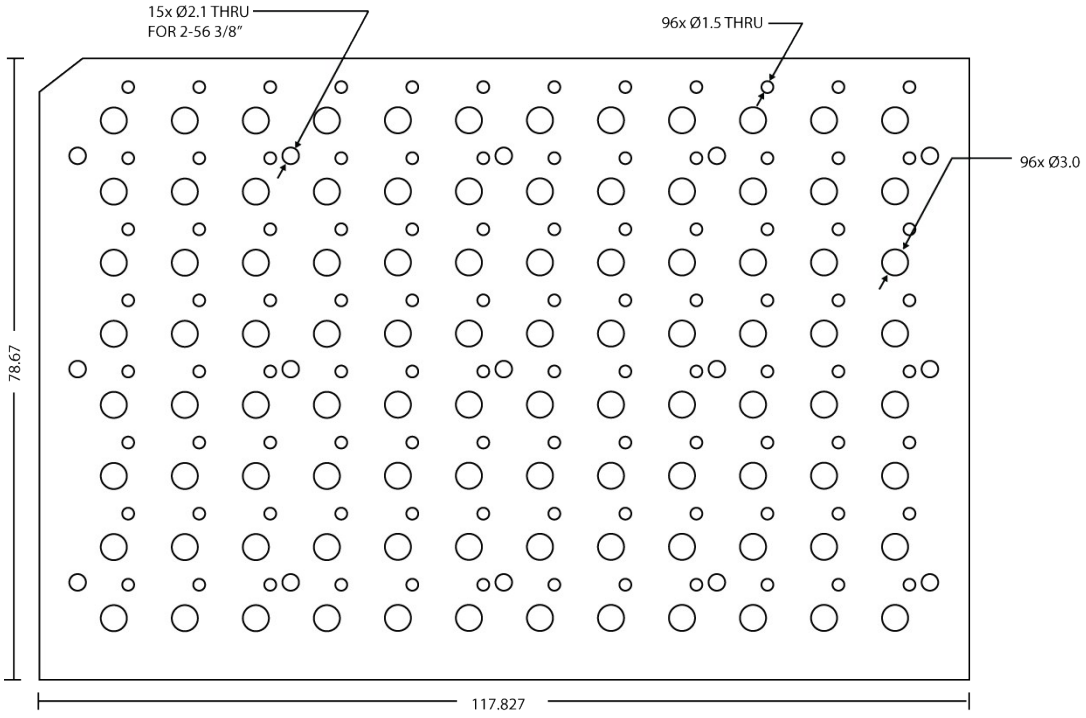
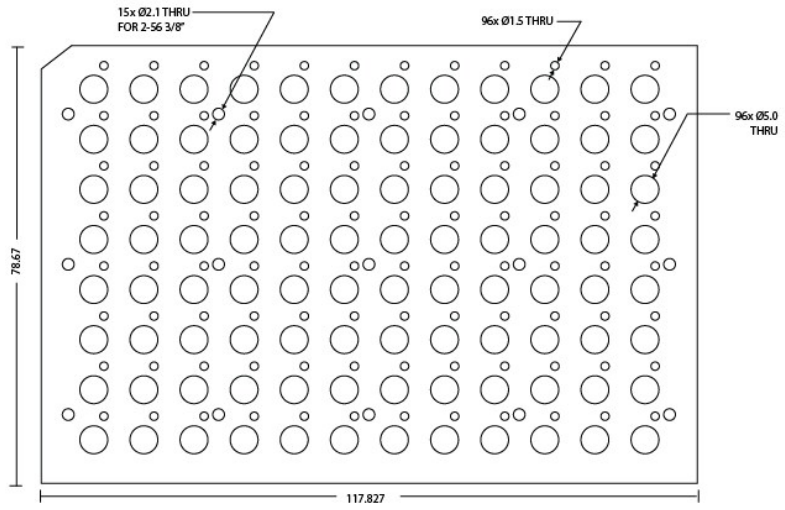
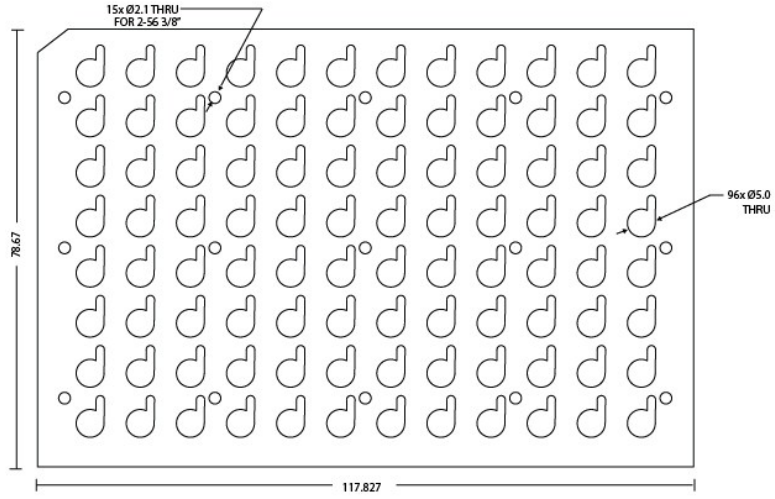


Figure S4. Schematic of the 96-zone, paper-based scaffold. Each scaffold was wax-printed using a Xerox ColorQube®8870 printer. All numbers are in units of mm.

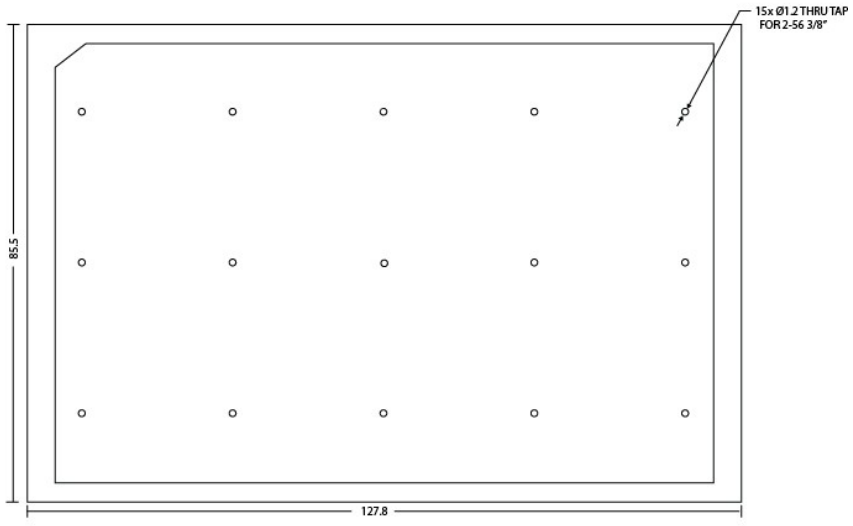
A


15x Ø2.1 THRU
FOR 2-56 3/8"
96x Ø1.5 THRU
96x Ø5.0
THRU
78.67
117.827

B


15x Ø2.1 THRU
FOR 2-56 3/8"
96x Ø5.0
THRU
78.67
117.827

C


15x Ø1.2 THRU TAP
FOR 2-56 3/8"
85.5
127.8

D

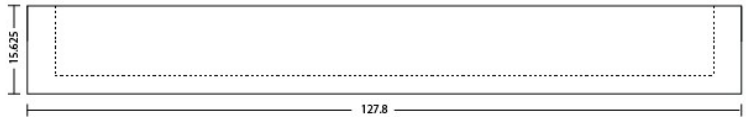

15.625
127.8

Figure S5. Schematic of the individual components of the 96-zone Transwell device. A) 96-zone insert containing through holes for fluid delivery. Two 1/4” acrylic inserts and two rubber gaskets are fabricated from this design. B) Bottom rubber gasket consisting of channels that connect the culture zones to the through holes for fluid delivery. C) Top view of the bottom plate, which contains 15 holes threaded for 2-56 screws and solvent-bonded borders. D) Side view of the bottom plate. The borders are solvent bonded to the base plate using 70% isopropyl alcohol and heating assembled unit to 68 °C for 30 min with compression. All numbers are in mm unless stated otherwise.

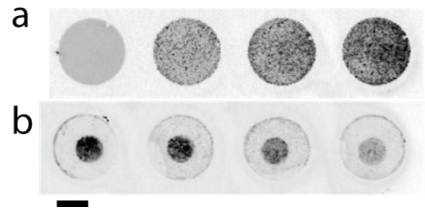
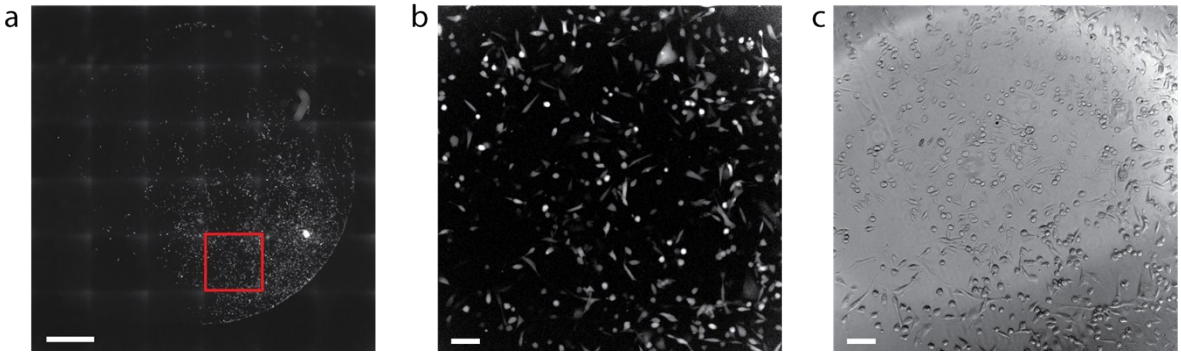
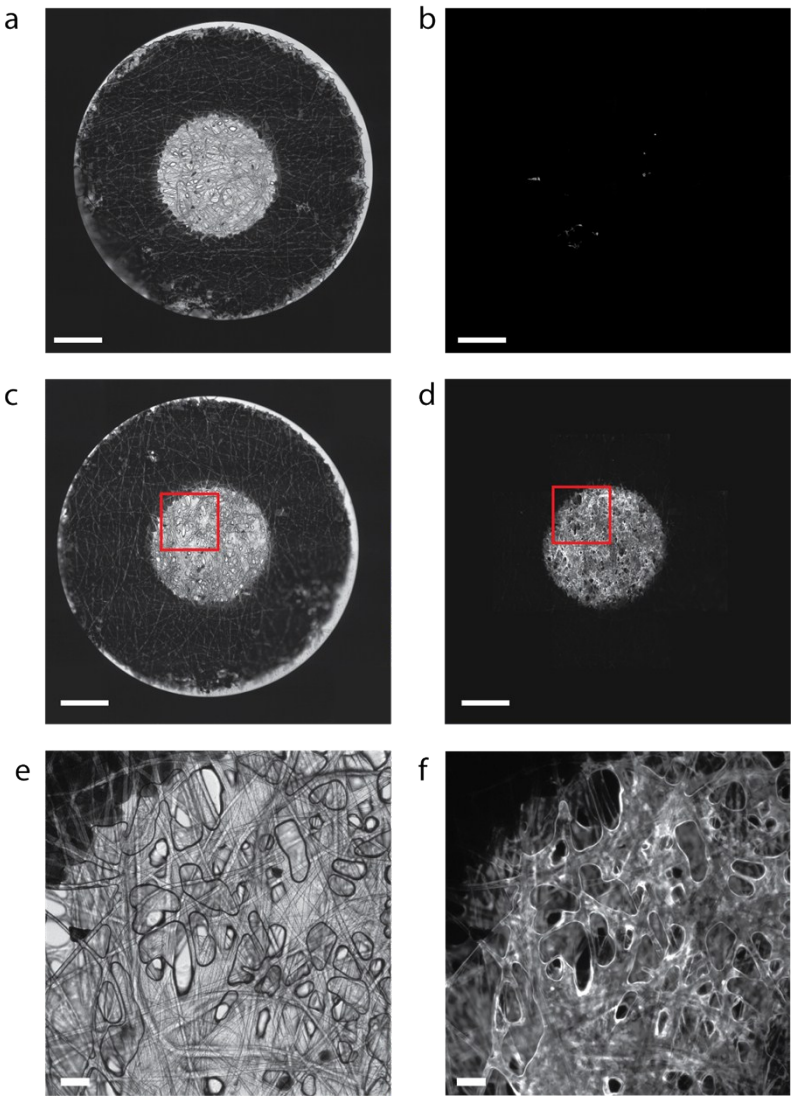


Figure S6. Representative fluorescence flatbed images of mCherry-expressing MDA-MB-231 cells after a 48 h paper-based Transwell assay. Images of the (a) wells and (b) paper scaffolds were obtained with a Typhoon 9400 scanner (GE Life Sciences) at a 100-μm resolution. The images show (from left-to-right) increasing concentrations of cells in the wells and decreasing concentrations of cells in the papers. Scale bar is 4 mm.



Figures S7. Representative images of mCherry-expressing MDA-MB-231 cells at the bottom of a 96-well plate obtained at the end of a paper-based Transwell assay (48 h). Images were obtained from an Olympus IX-70 fluorescence microscope equipped with a 10x objective. (a) Fluorescence image of the entire well, stitched together using FIJI. Lighter areas in the upper right corner of the stitched images are a result of stitching images with little detail and can be ignored. (b) Fluorescence and (c) brightfield images of the region highlighted with a red-colored box. Scale bar for (a) is 1 mm and (c, d) 100 □m.



Figures S8. Images of paper scaffolds (a, b) and mCherry-expressing MDA-MB-231 cells remaining in paper-based scaffolds (c, d, e, f) at the end of a paper-based Transwell assay (48 h). Bright field (a, c, e) and fluorescence (b, d, f) images were captured using an Olympus IX-70 fluorescence microscope equipped with a 10x objective. Close up view of the red boxes (c, d) are shown in (e) and (f), respectively. Scale bars for (a, b, c, d) are 1 mm and (e, f) 100 □m.

Sensitivity of flatbed scanner vs. Cell Titer-Glo assay when determining cell number

To address differences in the sensitivity of measuring cell number with fluorescence images obtained on a flatbed scanner when cells were a monolayer at the bottom of the well plate vs. retained in the paper scaffolds, we repeated the experiment described in Figure 1c. Briefly, we seeded single-zone paper-scaffolds with mCherry-expressing MDA-MB-231 cells and placed them in wells containing culture medium with 0, 0.5, 1, or 5% FBS. After 48 h, we imaged the wells and scaffolds on our flatbed fluorescence scanner, and immediately ran a Cell Titer-Glo viability assay.

There was an increased number of invasive cells for increasing concentrations of FBS. We compared the fold change from 0% to 5% in the wells and the paper scaffolds, using both methods. When using the flatbed scanner, we observed larger fold changes in the paper scaffolds (a 1.91-fold decrease) than in the well (a 1.42-fold increase). The Cell Titer-Glo assay yielded comparable fold changes for cells in the paper scaffolds (a 1.78-fold decrease) and in the wells (a 1.78-fold increase). These datasets show that the flatbed scanner underestimates the number of cells in the scaffolds, which is likely due to the 3D nature of the scaffolds. Therefore, other assays, like Cell Titer-Glo can be used, as an alternative method should the user want to quantify cells remaining in the paper scaffold.

Data for these experiments are summarized in Figure S9.

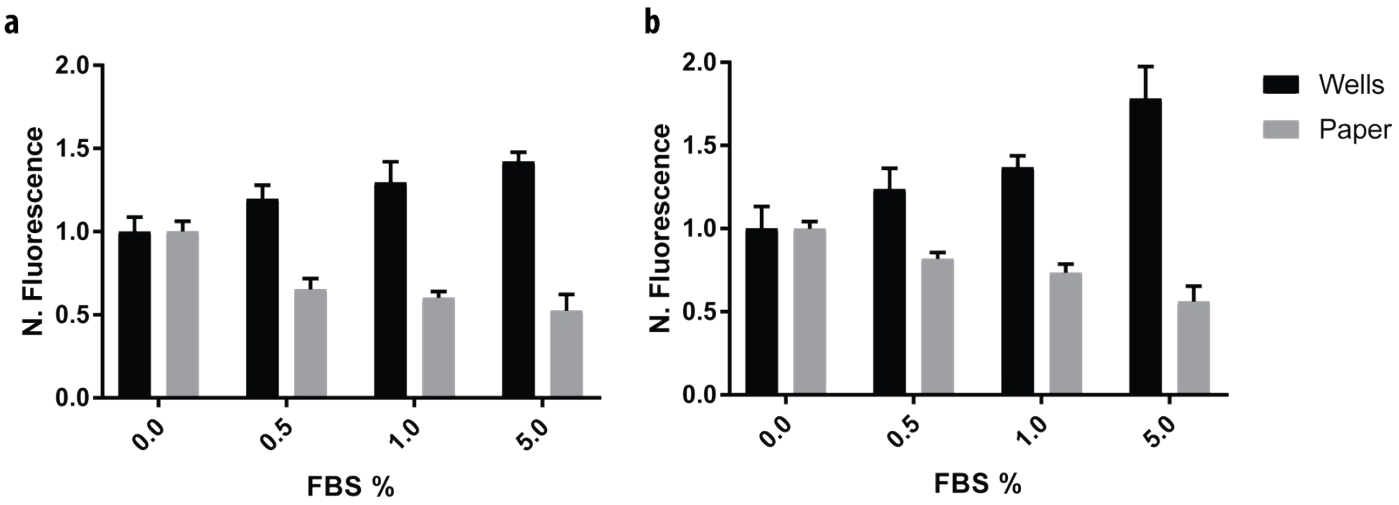


Figure S9. MDA-MB-231 cell invasion from collagen-containing paper scaffolds after a 48 h exposure to medium containing increasing concentrations of FBS. All signals were normalized to 0.0% FBS. Signal from (a) fluorescence flatbed scan of scaffolds followed by (b) Cell Titer-Glo viability luminescence. Each bar represents the average and standard deviation of n = 5 replicates.

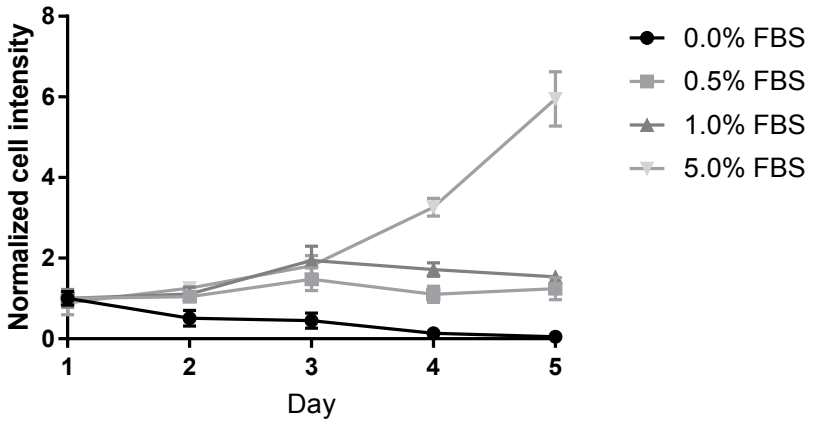


Figure S10. Proliferation of 5,000 mCherry-expressing MDA-MB-231 cells over five days. Cells were seeded in a 96-well plate on day zero. Wells contained DMEM medium with a particular concentration of FBS. The plate was incubated at 37 ^{o}C and 5% CO_2, and the medium exchanged every 24 h. Cells viability was evaluated using Cell Titer-Glo viability assay, following the manufacturer's protocol. Cells cultured in 1.0 % and 5.0 % FBS had a doubling time of 161 h and 30 h, respectively. There was no significant cell growth at 0.0 % or 0.5 % FBS. Each data point represents the average and standard deviation of n = 5 replicates.

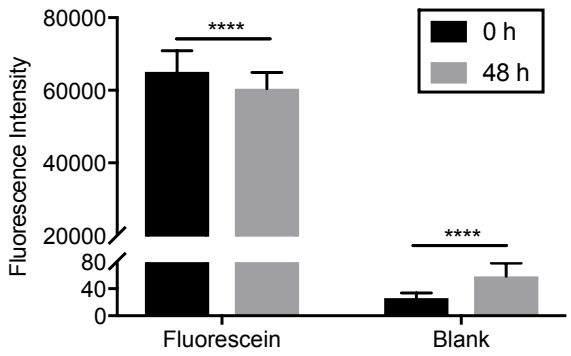


Figure S11. Results from a chemical cross-talk study between wells of the assembled 96-zone Transwell device. DMEM medium containing fluorescein was loaded into assembled device in a checkerboard pattern. The remaining wells were loaded with DMEM medium, free of fluorescein. The wells of the assembled device were imaged at 0 h and after a 48 h incubation at 37 ^{o}C and 5% CO_2. The fluorescence intensity of each well was compared at both time points. After 48h there is a significant decrease in wells originally loaded with fluorescein and a significant increase in signal from wells without fluorescein. Bars represent the average and standard deviation of n=48 replicates from a single setup. $p<0.0001$ ****.

Calculations to determine the cost of a single paper-based assay

1. GE Healthcare Whatman Lens Cleaning Tissue 09-800-973 (Fisher) $48.25/100 sheets

$$\frac{\$48.25}{100\ \text{sheets}} \times \frac{1\ \text{sheet}}{300\ \text{single zones}} \times \frac{1\ \text{zone}}{1\ \text{assay}} \times \frac{100\ \text{cents}}{\$1} = \frac{0.16^{01}\ \text{cents}}{\text{assay}}$$

2. Corning 96-well Clear Bottom White Polystyrene Plate 07-200-588 (Fisher) $840.53/100 plates

$$\frac{\$840.53}{100\ \text{plates}} \times \frac{1\ \text{plate}}{96\ \text{zones}} \times \frac{1\ \text{zone}}{1\ \text{assay}} \times \frac{100\ \text{cents}}{\$1} = \frac{8.75^{55}\ \text{cents}}{\text{assay}}$$

3. Black Xerox ColorQube 8570 Black Solid Ink Sticks 933450 (Staples) $193.99/4 cubes

$$\frac{\$193.99}{4\ \text{cubes}} \times \frac{1\ \text{cube}}{2150\ \text{pages}} \times \frac{1\ \text{page}}{300\ \text{single zones}} \times \frac{1\ \text{single zone}}{1\ \text{assay}} \times \frac{100\ \text{cents}}{\$1} = \frac{0.0075^{19}\ \text{cents}}{\text{assay}}$$