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Byunghang Ha

Stanford University <https://orcid.org/0000-0002-4491-4627>

Tae Jin Kim

Luca Medical Systems

Eui Jung Moon

Stanford University

Amato Giaccia

Stanford Medicine

Guillem Pratx (✉ pratx@stanford.edu)

Stanford University <https://orcid.org/0000-0002-0247-6470>

Article

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Flow Radiocytometry Using Droplet Microfluidics

Byunghang Ha¹, Tae Jin Kim², Eui Jung Moon³, Amato J. Giaccia³, and Guillem Pratx^{3,*}

¹*Department of Mechanical Engineering, Stanford University, Stanford, CA 94305-3011, USA*

²*Luca Medical Systems, 992 San Antonio Rd, Palo Alto, CA 94303-4917, USA*

³*Department of Radiation Oncology, Stanford University, Stanford, CA 94305-5847, USA*

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ABSTRACT

Flow-based cytometry methods are widely used to analyze heterogeneous cell populations. However, their use for small molecule studies remains limited due to bulky fluorescent labels that often interfere with biochemical activity in cells. In contrast, radiotracers require minimal modification of their target molecules and can track biochemical processes with minimal interference and high specificity. Here, we introduce flow radiocytometry (FRCM) that broadens the scope of current cytometry methods to include radiotracers as probes for single cell studies. FRCM uses droplet microfluidics and radiofluorogenesis to translate the radioactivity of single cells into a fluorescent signal that is then read out using a high-throughput optofluidic device. As a proof of concept, we quantitated [¹⁸F]fluorodeoxyglucose radiotracer uptake in single human breast cancer cells and successfully assessed the metabolic flux of glucose and its heterogeneity at the cellular level. We believe FRCM has potential applications ranging from analytical assays for cancer and other diseases to development of small-molecule drugs.

INTRODUCTION

Single-cell analysis methods such as flow cytometry are widely used to investigate the functional and phenotypic heterogeneity of cell populations¹. However, in many instances, the use of bulky fluorophores is prone to interfering with the process being studied². In contrast, radiotracers require minimal modification of their target molecules and are capable of tracking biochemical processes with minimal interference and high specificity. In recent years, new approaches such as radioluminescence microscopy³ have been developed that can perform single cell analysis using clinical radiotracers, i.e. radioactive probes used for imaging scans and cancer therapies. However, none of the existing methods can analyze more than ~100 cells at a time, preventing statistically robust characterization of highly heterogeneous population of cells. Here, we report a drop-based microfluidic platform named flow radiocytometry (FRCM) which measures radioactivity of single cells with high-throughput and could potentially be integrated with downstream cell sorting.

Radiotracers have found wide usage throughout medicine due to their unique advantages. In addition to their ability to closely target specific molecular pathways, radiotracers can be imaged deep within the tissues of humans and animals. As an example, a fluorine-18-labeled glucose analog, 2-deoxy-2-[¹⁸F]-fluoro-D-glucose (FDG), serves as an imaging agent for positron emission tomography (PET) to track glucose consumption in-vivo⁴. FDG-PET enables a direct, real-time measurement of the activities of membrane transporters and downstream cellular enzymes. Owing to the superior specificity and sensitivity of the radiotracer, FDG-PET is routinely used in hospitals to assess organ function or locate disseminated tumors⁴⁻⁶. Another prominent advantage of radiotracers is that there are about 50 FDA-approved radiotracers available for human use⁷. These have been proven to be reliable and effective from decades of use in hospitals. In addition, PET has served a crucial role for precise pharmacology studies of human subjects in later-stages of drug development⁸. Moreover, novel radiopharmaceuticals are actively developed for cancer theranostics and many are in the pipeline pursuing regulatory approvals⁹⁻¹².

Despite the unique strength and utility of radiotracers, their use for single-cell analysis has been limited due to the poor resolution of conventional radionuclide imaging methods. PET has spatial

resolution of millimeters at best due to the fundamental resolution limit imposed by positron range¹³. Recently, researchers have worked to develop on-chip radiometric imaging platforms to overcome the fundamental challenge and achieve single-cell resolution. This research field emerging over the last decade has recently been reviewed by Liu and Lan¹⁴. A notable work in the field is radioluminescence microscopy (RLM) developed in 2012 at Stanford^{3,15}. The proposed technique combines scintillator-mediated radioluminescence and epi-fluorescence microscopy to visualize radiotracer uptake of living single cells¹⁵⁻¹⁷. RLM offers the best resolution (i.e., tens of microns) for radionuclide imaging to date¹⁴, and several RLM studies have successfully assessed heterogeneity of cellular uptake of various clinical radiotracers^{3,18-20}. Although RLM offers high spatial resolution, its use remains limited because it can analyze only 40 – 200 cells at a time, and each acquisition requires at least 5 minutes, and can last as long as an hour²¹. In fact, the limited throughput is fundamentally related to the stochastic nature of radioactive decay. Radioactive decay events are spontaneous and random, in contrast to fluorescence which can be measured on demand upon stimulation. Therefore, long exposures are required to detect a statistically robust number of radioactive events and achieve sufficient signal-to-noise ratio. RLM is also challenging to integrate with cell sorting for post analysis, and all these shortcomings preclude any large-scale application of RLM.

We developed FRCM using radiofluorogenesis and microfluidic technology to achieve high-throughput single cell radiometry and enable future on-chip integration with downstream cell sorting. Radiofluorogenesis converts dim and discontinuous radioactive signals from single cells into integrated bright and continuous fluorescence. The radiofluorogenic translation is realized using molecular probes which irreversibly convert to fluorophores in response to ionizing radiation. Using a microfluidic platform, we encapsulated radiolabeled single cells together with radiofluorogenic probes in water-in-oil drops (Fig. 1a). Within the drop, the ionizing particles (positrons, electrons, or alpha particles) emitted from an encapsulated single cell produce reactive oxygen species (ROS) via water radiolysis (Fig. 1b). These ROS can instantaneously react with radiofluorogenic probes, generating a fluorescent signal proportional to the level of radioactivity. The translation of radioactivity into fluorescence enabled a 2-order-of-magnitude reduction in acquisition time per cell, which made possible high-throughput single-cell radiometry by measuring the fluorescence of individual drops using a continuous-flow

optofluidic platform. The strategy of co-encapsulating single cells and ROS probes in microdrops was first proposed and attempted by Gallina et al. in our group²¹. Although Gallina et al. demonstrated the potential feasibility of the approach, their assay suffered from significant cross-contamination between drops, polydispersity in drop size, and low readout throughput.

This paper reports several significant improvements to realize the high-throughput FRCM. First, we present a comprehensive study of a radiofluorogenic probe suitable for use with water-in-oil microemulsions. Optimal working conditions for the radiofluorogenic chemistry were established based on X-ray and FDG exposure. Second, we present microfluidic and optofluidic device designs for efficient drop generation and read. We also demonstrate the use of a 2-photon lithography system for 3D printing of complex microfluidic channel molds. To our knowledge, this is the first reported use of such a system for fabricating the mold of entire microchannels including inlets and outlets. Third, we present the design, fabrication, and operation of an incubation system for radiofluorogenic drops that minimizes physical crosstalk. Finally, as a proof of concept, FRCM was performed to compare FDG uptake by control and GLUT1-knock-down cells, which successfully quantitated the difference in the metabolic flux of glucose in the two biologically different samples and assessed cellular heterogeneity on the level of single cells. We anticipate that, with further integration with downstream cell sorting, this platform will constitute a high-throughput method for studying the distribution of a wide range of radiolabeled molecules in single cells using fluorescence detection.

Figure 1. Illustrations showing production of reactive oxygen species (ROS) from radioactive beta decay and radiofluorogenic conversion from the ROS. (a) A single radioactive cell is encapsulated into a water-in-oil drop. As the radiotracer decays, the emitted beta radiation creates ROS such as hydroxyl radicals, which in turn mediate the radiofluorogenic conversion. (b) Proton (P) decays into neutron (N), emitting positron (e^+) and neutrino (ν). The emitted positron travels a finite distance, interacting with water molecules along its path and creating reactive oxygen species (ROS), until it annihilates with an electron (e^-), resulting in two antiparallel 511 keV photons. (c) Nonfluorescent dichlorodihydrofluorescein (DCFH) is converted into fluorescent dichlorofluorescein (DCF) after losing two protons from interacting with radiation-induced ROS.

II. RESULTS AND DISCUSSION

A. Experimental overview and design

The goal of FRCM is to measure the heterogeneous behavior of single cells on the basis of their transport and incorporation of radiolabeled substrates. For instance, the metabolic flux of glucose into single cells can be assessed by measuring their radioactivity after incubation with a radiolabeled glucose analog. In this work, we quantitated [^{18}F]FDG radiotracer uptake in thousands of single human breast cancer cells (MDA-MB-231). It is important to assess cellular heterogeneity of cancer considering that higher uptake of FDG is interpreted as a distinctive property of cancer tissues. Reports have shown that increased glycolysis in cancer is associated with higher cell proliferation, metastatic potential and therapeutic resistance²²⁻²⁴. This implies that FRCM may provide crucial information for cancer therapeutics and identification of rare cell subclones. The assessment of heterogeneity in FDG uptake from ~ 100 cancer cells has been done via RLM, but we here present statistically robust high-throughput data for thousands of cells.

a. Drop design and generation

In the FRCM assay, cells are incubated with a radiolabeled substrate of interest, then washed and analyzed following a three-stage process comprised of drop encapsulation, incubation, and read (Fig. 2a). Using a flow focusing microfluidic chip, called hereafter DropGen microchip, single radioactive cells were mixed with a radiofluorogenic probe (Fig. 2b) and encapsulated in water-

in-oil drops (Fig. 2c). The device generated 800 pL monodisperse drops at a throughput of 200 per second. In addition, since drops may be empty or contain multiple cells, we used fluorescent quantum dots (QDs) to label cells and allow them to be counted in each drop.

The assay is designed such that the fluorescence of a radiofluorogenic drop accurately represent the amount of radiotracer in the encapsulated cell. To achieve this goal, unwanted cross-contamination and nonspecific fluorescence activation of probe should all be minimized. Specifically, radiofluorogenic probe, radiotracers, and ROS should all be hermetically encapsulated to avoid cross-contamination between drops. In addition, the radiofluorogenic fluorescence activation should be driven predominantly by extracellular radiation-induced ROS, not by intracellular ROS either inherently present or generated from oxidative stress. To address these requirements, we selected a hydrophilic radiofluorogenic probe, dichlorodihydrofluorescein (DCFH), as the ROS sensor. This helped create a strict compartmentalization of the three reactants (i.e., DCFH, FDG, and ROS) in the water drop and cell (Fig. 1b). The hydrophilic probe DCFH was observed to be strictly confined within the water phase, preventing cross-contamination between adjacent drops. In addition, the hydrophilicity of DCFH limited its transport into cells, thus mitigating the nonspecific fluorescence activation by cell-intrinsic ROS. As for ROS, the major radiation-induced radical, $\cdot\text{OH}$, is short-lived (c.f., nanoseconds order of lifetime) and remains confined to the drops in which it is created. In contrast metabolism-driven intracellular ROS such as $\cdot\text{O}_2^-$ and H_2O_2 are long lived, and therefore cell-containing drops were kept ice-cold to suppress ROS production and diffusion. Additionally, we thoroughly optimized drop size and chemical composition of the water phase to achieve high signal-to-background ratio (SBR). These considerations are discussed in detail in Supplementary Material.

Figure 2. Device overview and design. (a) Schematics showing drop generation, incubation, and read. (b) Optical micrograph (top view) showing cell samples and radiofluorogenic probe solution being introduced and mixed in DropGen microchip (c) Optical micrograph showing the cross-junction microfluidic channels where the mixture (i.e., dispersed phase) including the cells was encapsulated in water-in-oil drops. (d) Optical micrograph of polytetrafluoroethylene tubing where drops were incubated. (e) Picture of the tubing and holder. (f) Optical micrograph of DropRead microchip in which drops were introduced to have their fluorescence measured after incubation.

b. Drop incubation

In the second stage, the generated drops were collected off-chip for incubation. During incubation, positrons emitted from the radioactive cells ionized surrounding water molecules and produced ROS within the drop. The ROS readily attacks nonfluorescent DCFH, abstracting two protons from it and turning the molecule into fluorescent dichlorofluorescein (DCF) as illustrated in Fig. 1c²⁵.

We designed the incubation system such that radiofluorogenic conversion of a drop is proportional to the amount of radiotracer taken up by the encapsulated cell, with no unwanted

cross-contamination between adjacent drops. Considering that ^{18}F has a positron range of $R_{\text{mean}} = 0.6 \text{ mm}^{26}$ in water, it was important to separate the drops to avoid physical crosstalk. First, we kept the cell concentration in the sample as low as 13,000 – 25,000 cells per mL, such that only 5-9% of drops contained a cell. Second, we collected the drops into a 2.8 m-long microbore tubing for incubation. The narrow lumen (inner diameter of 500 μm) forced the drops to line up along a few rows (Fig. 2d). Third, the flow rate ratio of the continuous phase to the dispersed phase in drop generation was 3 to 1, and the larger volume of the continuous phase further spread out the drops in the incubation tubing. Together, these features of the assay provided significant spatial distancing between cell-containing drops, minimizing physical crosstalk. Other approaches were implemented for optimal incubation of radioactive cells within droplets, which are detailed as Supplementary Material including Fig. S1. After incubation, the box was placed upright to concentrate the drops by driving them upward through buoyancy along the inclining serpentine path (Fig. 2e). This drop packing procedure was crucial to achieve high throughput in drop reading by removing undesirable extra spacing between the drops.

c. Drop read

After incubation, we injected the drops into the second optofluidic microchip, called hereafter DropRead microchip, to measure the DCF fluorescence (Fig. 2f). The DropRead microchip was mounted on a microscope stage to monitor microfluidic operation and obtain brightfield and fluorescence images. We also used the microscope illumination for fluorescence excitation. A single excitation light source (480 nm wavelength) was sufficient to excite both DCF and QD at the same time. The emission peaks of DCF and QD were respectively 525 nm and 800 nm, and we did not observe any overlap between the two emission measurements.

The drops were arranged in a single file and spaced to be investigated one by one with no overlap. This spacing was achieved by adding a flow of continuous oil phase. The drops passed in front of two optical fibers, which were inserted into the DropRead microchip to collect fluorescence from QD and DCF, respectively. The blue excitation light was focused by a 40x objective lens onto the location shown in Fig. 2f, covering the two fiber ends. As the drops flowed in front of the optical fibers, QD fluorescence was collected via optical fiber 1 and measured by photomultiplier tube (PMT) 1, then, shortly afterwards, DCF fluorescence was

collected via optical fiber 2 and measured by PMT 2 (Fig. 3a). We synchronized the two PMT measurements in time using a common trigger signal. The plots in Fig. 3b show raw PMT signals as a function of elapsed time. Peaks and valleys were identified, and their heights were measured using custom MATLAB scripts (See Supporting Material including Fig. S2 for more details). Peak height was used as the signal (S) representing the fluorescence intensity of individual drops. Excluding outliers, valley heights were averaged to estimate background (B; i.e., intrinsic fluorescence of the continuous oil phase) and the normalized drop fluorescence was computed as $(S-B)/B$.

The fluorescence peaks from both PMTs were analyzed. Typically, the DCF fluorescence peak (PMT 2) had a width of 12 ms and appeared 4 ms after the corresponding QD peak (PMT 1). The combination of the two PMT signals enabled us to differentiate cell-containing drops from empty ones. Additionally, if two or more peaks were detected within 6 ms in the PMT 1 signal, we assumed the drop contained multiple cells and excluded the measurement from the dataset. The measured cell encapsulation probability in these experiments followed Poisson statistics, as expected for random encapsulation (see Supplementary Material including Tables S1 and S2 for more details).

The proposed optofluidic configuration offers crucial advantages in radiofluorogenic drop read over conventional epifluorescence microscopy. First, the proposed system is capable of detecting the dim fluorescence of empty drops and drops containing non-radioactive cells with high sensitivity. The measurement of these drops is necessary to accurately capture the full range of cellular heterogeneity, including cells taking up minimal amount of radiotracer. Minimal exposure of drop to excitation light is also important to minimize unwanted photo-activation of DCFH, which contributes to higher background. The resident time of each drop under excitation window was approximately 12 ms, which is much shorter than the time required to image a field of view using fluorescence microscopy. In addition, high sensitivity was achieved in our design by employing sensitive PMT photodetectors, which not only detected weakly radioactive single cells but also captured single cells labeled with minimal amount of QDs. Finally, thanks to precise microfabrication techniques, our system measures each drop at the same location, thus

improving reproducibility compared to microscope set-up that are subject to variations in excitation and light collection efficiency over the field of view.

Figure 3. Optofluidic configuration and measurement. (a) Schematic showing the optofluidic configuration. (b) The two PMT signals as a function of elapsed time.

B. Radiofluorogenic probe selection and optimization

a. Revisiting DCFH and its instability

We here describe why we chose DCFH for FRCM among many ROS probes available. Our foremost consideration was that DCFH is hydrophilic and, therefore, remained confined to the water drops and did not leak out to continuous phase, at least over 12 hours. In contrast, many other ROS probes (e.g., Amplex Red) are amphiphilic and thus prone to leaking out of aqueous drops. For example, Gallina et al. observed that DHRh123 partitioned to the oil phase and adsorbed onto the PDMS microchannel wall, reducing sensor concentration within the drop while increasing background fluorescence. Despite the notable advantage of using DCFH for a droplet assay, Gallina et al. ruled it out for use in their radiofluorogenic assay because the authors believed the sensitivity of DCFH to ionizing radiation drastically deteriorated over time²¹. However, repeating the same assay we achieved far more stable reagent than reported by Gallina et al. (Table 1). The data plots and linear regression analysis for the measured sensitivity and stability in Table 1 are respectively shown in Fig. S3. Detailed protocol is also discussed in Supplementary Material.

TABLE I. Stability and sensitivity measurements of DCFH

	concentration (μM)	Φ (Gy^{-1}) ^a	Φ_{4h} (Gy^{-1}) ^a	$\Delta\Phi\%_{4h}$ ^a
Gallina et al. ²¹	100	0.12	0.03	74
Ha et al.	400	0.126	0.124	1.2

^a Φ , sensor sensitivity; Φ_{4h} sensitivity after 4 hours; $\Delta\Phi\%_{4h}$, relative loss of sensitivity after 4 hours, i.e., instability.

b. DCFH sensitivity

We assumed that there would exist an optimal DCFH concentration relative to the sample radioactivity in achieving maximum sensitivity. This is because, if the sample had an excessive molar amount of DCFH relative to the molar amount of ROS produced by the radioactivity, then background fluorescence would be greater than desired due to nonspecific activation. On the other hand, if there were not enough moles of DCFH to measure all the ROS produced in sample, then the fluorescence signal would be saturated and fail to reach the desired value. To evaluate this effect, we dispensed 100 μL of mixture of FDG and DCFH (1:1 v/v) on a 96-well plate array with varying concentration of DCFH from 50 nM to 500 μM and a fixed radioactivity concentration of 160 MBq/mL. This radioactivity concentration corresponds to 12 Bq/cell contained within a drop of 113 μm diameter, and we thus believe the following spectroscopy results and analyses are suitable to understand to the drop-based FRCM experiments. We covered the wells with 100 μL paraffin oil on top to minimize nonspecific activation of DCFH by oxygen. The green fluorescence of DCFH, with and without FDG, was measured every 15 minutes using spectrometry. The measured background (I_0) and signal (I) fluorescence intensities are respectively plotted in Figs. 4a and 4b.

In Fig. 4a, the background increases over time despite the absence of ionizing radiation, which we ascribe to nonspecific activation induced by excitation light and oxygen. In order to assess the photo-activation of DCFH induced by the excitation light, we performed the same kinetic spectroscopy and compared fluorescence increment rate for varying the time intervals between measurements and found that DCFH fluorescence increased 2.4 times faster when excitation light was given every 1 hour versus 20 hours (Fig. S4a). In addition, to assess the influence of oxygen on the nonspecific activation of DCFH, we compared two DCFH samples, one covered

with paraffin oil on top, and one with no oil cap so that oxygen could freely diffuse into the test solution. DCFH fluorescence intensities increased faster and reached larger values when the samples were not capped with oil (Fig. S4b). These two experimental results confirm that both light and oxygen contribute to the nonspecific fluorescence activation of DCFH, as also observed previously by others^{25,27}. We also note that the higher the DCFH concentrations, the larger the slopes (Fig. 4a). Linear regression shows that the slope is linearly proportional to the DCFH concentration (Fig. S4c).

When FDG is added to the solution, the fluorescence of the mixture increases more quickly than for the control DCFH samples (Fig. 4b). This is because radioactive decay of ^{18}F produced ROS, which oxidized nonfluorescent DCFH and converted it to fluorescent DCF. The fluorescence slopes gradually decreased over time because the rate of ROS production was reduced as a result of exponential decay of the ^{18}F radioactivity. We note that, for the low DCFH concentrations (e.g. 50 and 250 nM), the fluorescence of the samples reached a plateau, indicating that DCFH molecules in the samples were entirely converted to DCF. In contrast, the fluorescence of the relatively higher DCFH concentration samples continuously increased over 12 hours. This indicates that these samples had more DCFH molecules than could be converted within the time frame.

Figure 4c shows the SBRs, $(I - I_0)/I_0$, of samples with varying DCFH concentration as a function of elapsed time. This result confirms that there exists an optimal ratio of DCFH concentration to radioactivity in sample.

Figure 4d shows the SBRs measured at different elapsed times as a function of the ratio of radioactivity to DCFH concentration (GBq/nmole) in sample, which we denote as α . We observe that there exists an optimal incubation time to achieve maximal SBR. We assume the optimal incubation time is in the order of the half-life of the radionuclide in use, which is 110 minutes for ^{18}F . This is because the rate of fluorescence activation for the mixtures of DCFH and FDG decreases over time, while the non-specific activation of DCFH remains relatively constant. Given 4 hours of incubation time, an SBR higher than 10 is achieved for α between of 1.6 and 160 (GBq/nmole), with the peak value achieved for $\alpha = 16$ (GBq/nmole). Accordingly, the

optimal DCFH concentration is about 1 μM given 110 μm drop diameter and 20 Bq/cell radiolabeling. Detailed protocol of preparing the DCFH solution, including the selection of an optimal cell dissociation buffer for FRCM experiments, is described in Supplementary Material including Figs. S5 and S6.

Figure 4. Kinetic fluorescence spectroscopy measurements of (a) DCFH solutions and (b) mixture of DCFH and FDG. (c) Signal to background ratio (SBR) as a function of elapsed time. (d) SBR measured at increasing elapsed times as a function of the ratio of radioactivity to DCFH concentration.

C. FRCM of live cancer cells

The FRCM pipeline was then applied to measure the uptake of FDG by live human breast adenocarcinoma cells (MDA-MB-231). To estimate the contribution of intrinsic cell metabolism and other non-specific processes to fluorescence activation of DCFH, a control FRCM experiment was first performed with non-radiolabeled cells. The fluorescence micrograph of control droplets shows that drop fluorescence was uniformly low, regardless of the presence of

cells (Fig. 5a). No significant increase of background fluorescence was observed in the water-phase, suggesting minimal leakage of cellular ROS. We observed that some encapsulated cells exhibited visible fluorescence, and we attribute this to DCFH which was partitioned into the cell and converted to DCF in reaction to cell-intrinsic ROS. In contrast, when cells were incubated with FDG, we observed a large difference in fluorescence intensity between empty drops and cell-containing drops (Fig. 5b). PMT measurements acquired using FRCM show a similar trend: for non-radioactive cells, we found little difference between drops containing cells and empty drops (Fig. 5c), whereas for cells incubated with FDG, there is a clear difference in terms of peak heights between empty drops and cell-containing drops. (Fig. 5d). The results clearly demonstrate that the increase in drop fluorescence measured by FRCM is directly caused by the radiotracers taken up by the cells.

Figure 5. Fluorescence measurements of drops containing non-radiolabeled cells (control) vs. radiolabeled cells (sample). (a,b) Micrographs of drops at the inlet of DropRead microchips: (a) control and (b) sample; bright field image on the left and FITC fluorescence image on the right. (c,d) PMT measurements as a function of time for (c) control and (d) sample assays.

To further evaluate the performance of FRCM for single-cell analyses, we performed another experiment using two different cell populations that, by design, expressed different levels of glucose transporters and were incubated with the same amount of FDG. Our goal was to show

that the signal measured by FRCM reflects true biological processes in cells. Here, MDA-MB-231 cells were transfected to silence the expression of *SLC2A1*, the gene encoding GLUT1 protein, using short-hairpin RNA (shRNA). While there exist 14 different types of GLUT protein, GLUT1 is one of the major transporters that is expressed in all cell types to facilitate basal glucose uptake²⁸. GLUT1 expression is known to be one of the primary mediators of FDG uptake by cells. Once FDG is taken up, it becomes phosphorylated by hexokinase and effectively trapped inside the cell. The efficacy of *SLC2A1* knockdown in the cells (shGLUT1) was confirmed by GLUT1 protein expression using Western blot and compared to a scrambled shRNA control (shSCR; see Fig. 6a and Fig. S7).

Figure 6. (a) Western blot comparing *SLC2A1* knockdown (shGLUT1) to scrambled shRNA control (shSCR) in MDA-MB-231 cells. (b) Averaged radioactivity of shSCR and shGLUT1 samples measured using bulk dose calibrator and hemocytometry. The errors are standard deviation and associated with cell counting. (c) Half-violin plot summarizing the drop read results. Each dot in the scatter plots on the left-hand side represents a single drop measured by FRCM. The frequency distribution corresponding to the scatter plot is shown on the right-hand side. Grey: non-radioactive cell sample (i.e., control), red: FDG-labeled control shSCR cell sample, and blue: FDG-labeled shGLUT1 cell sample.

Initial bulk radioactivity measurements confirmed that GLUT1 knockdown cells took up 53% less FDG compared to the control shSCR cells (Fig. 6b). However, unlike FRCM, bulk gamma counting does not provide information about heterogeneity at the cellular level. The radioactivity of the single cells from both the groups were therefore measured using FRCM. By doing so, we aimed to confirm our earlier results showing that the FRCM signal was specific to FDG uptake. Second, we aimed to demonstrate the radiometric accuracy of the proposed technique and show that it could differentiate between two different levels of radiotracer uptake by single cells.

The half-violin box plot in Fig. 6c summarizes the drop read results for all the experiments. In the plot, each dot corresponds to the pulse height measured by the PMT for a single fluorescent drop. The shaded area on the right-hand side shows the frequency distribution of the drop fluorescence measurements. The two grey-colored data sets are measured from the control experiment, which compared empty drops and drops containing non-radioactive cells. As previously mentioned, we attribute the small increase in fluorescence in cell-containing drops to DCFH partitioned into the cell and activated. We believe the any leakage of long-lived ROS out of an encapsulated cell was significantly suppressed as cells were kept ice-cold throughout the experiment. The red-colored data represent the FDG uptake of control cells treated with scrambled shRNA, and the blue-colored that of GLUT1 knockdown cells. As expected, on average, the drops containing radioactive single cells (i.e., radiolabeled shSCR and shGLUT1) exhibited much brighter fluorescence than the drops containing non-radioactive cells. In addition, the FRCM signal was significantly lower when GLUT1 was knocked down in cells (P value $< 10^{-4}$). On average, FRCM pulse height signal was 52% lower for the shGLUT1 cells compared to the control shSCR cells. This decrease is comparable to the 53% decrease in uptake measured using bulk gamma counting, suggesting that FRCM is a quantitative approach. This finding confirms that the increase in drop fluorescence was specifically due to FDG taken up by cells via GLUT1. This also demonstrated that the proposed technique has excellent radiometric accuracy, being capable of successfully differentiating the weak and strong levels of radiolabeling.

The FRCM results also highlight notable heterogeneity in cellular metabolism, as shown by the wide distribution of values in Fig. 6c. Using RLM, we previously found large heterogeneity in

FDG uptake, even among cells from the same population²⁹. This heterogeneity reflects the tremendous metabolic plasticity of cancer cells, which are able to use a variety of different metabolic substrates such as glucose and glutamine to satisfy their energetic requirements. To quantify the level of heterogeneity in these cell lines, we computed the coefficient of variation for both cell population and found it to be 50% for the control cells and 46% for the GLUT1 knockdown cells. Another remarkable feature of the data is the biphasic distribution of FDG uptake for shGLUT1 cells. This unusual distribution suggests that this sample is likely composed of two different sub-populations of cells. The first subpopulation of cells shows almost no detectable FDG uptake based on the pulse-height signal. This may be because GLUT1 expression was efficiently suppressed. These cells likely exist in a state of metabolic quiescence or may rely on other metabolic fuels such as glutamine. The second subpopulation contains cells that have higher FDG uptake, which may be due to the variable efficiency of GLUT1 silencing. These cells may also transport FDG through other glucose transporters such as GLUT3.

CONCLUSION

We present a method of quantifying radiolabeled small molecules in single cells through the permanent activation of radiofluorogenic droplets. By converting stochastic radioactive decays into a continuous fluorescence signal, this approach allows rapid, high-throughput radiometry of single cells. The use of radiofluorogenesis significantly reduced exposure time per cell, from 30 s/cell for RLM down to 100 ms/cell of the proposed FRCM. This 100-fold reduction in exposure time per cell allowed rapid, high-throughput radiometry. FRCM successfully measured over 500 single cells in less than a minute. Importantly, the continuous-flow platform also allows integration with downstream cell sorting for post-analysis assays such as RNA sequencing. Another notable advantage is that the proposed platform is versatile and compatible with any PET or SPECT radiotracer. In this proof of concept, we used FDG and measured the metabolic flux of glucose in single cells. But other radiotracers can be used to measure, for instance, radiolabeled drug incorporation, substrate transport, or therapeutic isotope uptake in single cells. We can also use additional fluorescent probes for multiplexing. Accordingly, the extent of applications of this technique is not limited to radiopharmaceutical studies but can also include additional fluorescence-based single cell assay. The platform would be useful for pharmaceutical study assessing cellular heterogeneity. For example, in-vitro tumor models are used to test the

efficacy of pharmaceuticals prior to clinical tests. These in-vitro models can be either 2D culture or 3D culture including patient-derived organoids or spheroids. In our work, as a proof of concept, we used 2D in-vitro tumor model to assess cellular heterogeneity in metabolism. For future work, primary single cells derived from human tumors or complex 3D tumor models can be investigated using our proposed technique. We believe that the approach has potential applications ranging from analytical assays for cancer and other diseases to the development of small-molecule drugs.

III. METHODS AND MATERIALS

A. Microchip fabrication

Microfluidic platforms capable of generating and reading droplets were designed and built. Polydimethylsiloxane (PDMS) microfluidic chips with channel depth modulations were fabricated using replica molding protocols. The reusable molds were fabricated using Nanoscribe (Nanoscribe Photonics Professional GT, Nanoscribe GmbH, Germany), a submicron resolution 3D printer based on two-photon polymerization of negative photoresist. We find a number of advantages in using Nanoscribe for microchannel mold fabrication over conventional photolithography methods. First, accurate channel depth modulation was easily achieved whereas the conventional way of spin coating liquid-type negative photoresist is influenced by slight differences in temperature, humidity and device control and thus prone to inaccurate and non-uniform depth modulation. Second, sharp rectangle cross-sectional shape of channels was easily achieved whereas a mask-based photolithography is prone to creating undesirable trapezoidal shapes.

We note that cleaning of silicon wafer substrate with 500W oxygen plasma treatment for 10 minutes (Drytek2 DRIE100 plasma etcher) prior to Nanoscribe fabrication significantly enhanced adhesion of photoresist onto the substrate surface and thus durability of the mold.

The mold surface was rendered hydrophobic with trichloro(1H, 1H, 2H, 2H-perfluorooctyl)silane (Sigma Aldrich, MO, USA). Then, the PDMS base (Sylgard 184, Dow corning) and curing agent were mixed in a 10:1 ratio, poured over the mold, degassed in vacuum chamber, and cured at $\sim 60^{\circ}\text{C}$ for over 4 hr. We peeled off the cured PDMS from the mold and punctured holes for inlet and outlet ports using a 1 mm diameter disposable biopsy punch (Integra Miltex, Integra York PA, Inc., PA, USA). The PDMS chips were then bonded to a 25 mm $\times$ 75 mm $\times$ 1.0 mm precleaned glass microscope slides (Corning® 2947-3x1, Corning Inc., NY, USA) with oxygen plasma treatment (Plasma cleaner PDC-32G, Harrick Plasma, NY, USA). Immediately after, microchips were placed on a 95°C hot plate for over 10 min to reinforce the bonding. The microchannel wall surfaces were rendered hydrophobic by flowing in

Novec^{TM} 1720 Electronic grade Coating (3M, MN, USA) through the channel and blow-drying with air.

B. Droplet generation and fluid flow operation

We generated 800 pL monodisperse droplets using the flow-focusing nozzle in our drop generation microchip. The cross section of the flow-focusing nozzle for all four channels was square with side length of 90 μm . The continuous phase was HFE 7500 perfluorinated oil containing 2% w/w of 008-fluorosurfactant (RAN Biotechnologies, MA, USA) as a surfactant to stabilize the droplets against coalescence. The disperse phase was a mixture of DCFH dissolved in phosphate buffered solution and Cell Dissociation Buffer Type II. Fluids were injected through the inlet ports and driven at flow rates of 36, 4.5, and 4.5 $\mu\text{L}/\text{min}$ using a syringe pump (neMESYS, Cetoni GmbH, Korbussen, Germany) for the continuous phase and the two dispersed phases, respectively. Drop diameters were measured using Leica microscope. The monodispersity of drop size was observed from the micrograph-based measurement and PMT-based fluorescence peak-width measurement.

C. Droplet incubation and incubator fabrication

The droplets were collected into a 2.8-meter long polytetrafluoroethylene (PTFE) tubing and incubated in the dark and 4°C for 4 hours. The inside of the tubing was rendered hydrophobic with Novec^{TM} 1720 Electronic grade Coating prior to use. The tubing holder and the box were 3D printed using Ultimaker S5 (Ultimaker B.V., Utrecht, Netherlands).

D. Droplet read and image acquisition

After incubation, we injected the droplets into another optofluidic microchip to read their fluorescence. The drops were arranged and spaced in a single file to be investigated one by one with no crosstalk. Red and green fluorescence emission from each droplet was collected through two optical fibers and measured using two photomultiplier tubes (PMTs). The red fluorescence emitted from quantum dots was delivered first through the 1st fiber, which was used to confirm single cell encapsulation. Within 15 ms, the green fluorescence emitted from radiofluorogenic probe was delivered through the 2nd fiber, which informed on the amount of FDG uptake of the corresponding single cell. Immersion oil was placed in between the fiber end and PDMS wall to

reduce scattering and reflection and achieve a good optical coupling. The microscope light was used as a fluorescence excitation source and focused on a droplet investigation area by 40x objective lens (as shown in Figure 2f). The red emission was detected using a Hamamatsu PMT (H7422P-50, Hamamatsu Corp., NJ, USA) and photon counting setup with 200 μ s gate time. The photon counting setup was composed of C9744 photon counting unit and C8855-01 counting unit. The C9744 photon counting unit was designed to convert single photoelectron pulses from a photomultiplier tube into 5 V digital signals by use of built-in amplifier and discriminator circuits. The C8855-01 counts the number of photons detected by C9744 within a gated time, and the minimum temporal resolution is 50 μ s. The green emission was detected using a Thorlabs PMT (PMM02, Thorlabs Inc., NJ, USA) and data acquisition board (USB-6002, National Instruments, TX, USA) with 5,000 Hz measurement frequency. The two PMTs were synchronized using a hardware-trigger given by a function waveform generator (SDG1025, Siglent Technologies, OH, USA). Both the PMT signals were read by a PC and recorded using Labview custom-purpose scripts. Peaks and valleys were identified from the PMT measurement data using an open-source MATLAB code. Erroneous peak measurements were found based on peak shape and width and excluded from data. The valleys were used to measure signal noise and background that were respectively standard deviation and average calculated from outliers-excluded valley height measurements. We developed a home-made MATLAB code to count the number of cell encapsulation from matching the peaks of red and green fluorescence signals.

E. Radiofluorogenic probe preparation

2',7'-dichlorodihydrofluorescein (DCFH) was prepared via chemical de-esterification of commercially available 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA; D6883-250mg, Sigma-Aldrich, MO, USA). To achieve the conversion of DCFH-DA to DCFH, we employed an alkaline hydrolysis protocol which we slightly modified from what was described by Sleiman et al.³⁰ That is, instead of 25 mM phosphate buffer, we used a mixture of commercially available phosphate buffered saline (PBS) to match the proposed buffer capacity: (a) 0.025 g of DCFH-DA was dissolved in 5 mL methanol. (b) By mixing 15 μ L of this 10 mM DCFH-DA stock solution and 60 μ L of 0.01 N NaOH, the de-esterification of DCFH-DA proceeded in the dark at room temperature for 30 min. (c) The mixture was then neutralized with 1 mL of 10x PBS at pH 7.4 and 13.25 mL of 1x PBS at pH 7.4. This solution was kept on ice in the dark until used. The

solution was prepared fresh and used immediately within one hour for all assays. The final concentration of DCFH-DA was 10 μ M. We caution that, as opposed to the protocol described by Sleiman et al.³⁰ and employed by us, the majority of literature describes preparation of 1 mM DCFH-DA stock solution and use of stronger base relative to the molar amount of DCFH-DA for de-esterification³¹.

F. FDG preparation

[¹⁸F]FDG was prepared through nucleophilic ¹⁸F-fluorination and hydrolysis of mannose triflate by the Stanford Cyclotron Radiochemistry Facility. ¹⁸F was made in a GE PETtrace cyclotron and the production was performed via cassette-based automated synthetic module (FASTlab, GE Healthcare). Quality control tests were performed according to USP823. The radiotracer was used within 8 h after production due to its short halflife ($\tau_{1/2}$ = 1.8 h). Radioactivity was measured using a dose calibrator (Atomlab 400, Biodex) prior to each experiment.

G. Media preparation

Cell culture medium was Dulbecco's Modified Eagle Medium (DMEM) containing high glucose (4.5 g/L), sodium pyruvate (110 mg/L), and GlutaMAX™ (Cat. No. 10569077, Gibco®, Grand Island, NY, USA) supplemented with 10% (v/v) fetal bovine serum (FBS; Cat. No. 15140122, Gibco®, Grand Island, NY, USA) and 1% (v/v) penicillin-streptomycin (PS; Cat. No. 15140122, Gibco®, Grand Island, NY, USA), called hereafter Complete Growth Medium. The cell culture medium used for radiolabeling was glucose-free DMEM (Cat. No. 11966025, Gibco®, Grand Island, NY, USA) supplemented with 10% (v/v) FBS and 1% (v/v) PS, called hereafter Glucose-free Medium. For single cell preparation, two types of media were prepared. The first type was Mg²⁺ Ca²⁺ free 1x PBS with 2 mM EDTA, 75 μ M bovine serum albumin (BSA) and 5 mM MgCl₂ · 6H₂O, called hereafter Cell Dissociation Medium Type I. The second was Mg²⁺ Ca²⁺ free 1x PBS with 3 μ M BSA and 2.5 mM MgCl₂ · 6H₂O, called hereafter Cell Dissociation Medium Type II.

All the DMEM, FBS, and PS were purchased from Thermo Fisher Scientific, Inc. and manufactured at Gibco®, Grand Island, NY, USA.

Cell Dissociation Medium Type I composition

37.22 mg EDTA (2 mM final concentration)

250 mg BSA (75.23 uM final concentration or 0.5%)

50.83 mg $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ (5 mM final concentration)

$\text{Mg}^{2+}\text{Ca}^{2+}$ free 1x PBS up to 50 mL solution

DNase I (10 units/mL or 25-50 ug/mL) added later to the mixture of sample and buffer

Cell Dissociation Medium Type I composition II composition

10 mg BSA (3.01 uM final concentration or 0.02%; 66463 g/mole; will become 1.5 uM in droplet when mixed 1:1 ratio)

25.42 mg $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ (2.5 mM final concentration)

$\text{Mg}^{2+}\text{Ca}^{2+}$ free *Degassed* 1x PBS up to 50 mL solution

DNase I (10 units/mL or 25-50 ug/mL) added later to the mixture of sample and buffer

H. Cell culture

Human breast adenocarcinoma cells (MDA-MB-231) were cultured at 37 °C and 5% CO_2 in Complete Growth Medium. Dr. Amato Giaccia at Stanford University kindly offered the cells of passage number 7. The passage number of the cell samples used for FRCM was in a range of 7-9.

I. Cell labeling with fluorescent quantum dots

Cells were labeled using fluorescent quantum dots (ex/em 405–760/800 nm; Qtracker® 800 Cell Labeling Kit, Q25079, Thermo Fisher Scientific, Inc.) to confirm single cell encapsulation during FRCM assay: (a) To prepare 15 nM labeling solution, we mixed 1.5 μL each of Qtracker® Component A and Component B in a 1.5 mL microcentrifuge tube. (b) We incubated the mixture for 5 minutes at room temperature and proceeded immediately to the next step. (c) We added 0.2 mL of fresh complete growth medium to the tube and vortexed for 30 seconds. (d) We added $\sim 2 \times 10^6$ cells (from a cell suspension at $\sim 1 \times 10^7$ cells/mL in growth medium) to the tube containing the labeling solution. (e) We incubated the sample at 37 °C for 45-60 minutes. (f) We washed the cells twice with complete growth medium and seeded them in a T25 flask.

J. Cell radiolabeling

In addition to labeling with fluorescent quantum dots, we labeled the cells with [^{18}F]-fluorodeoxyglucose ([^{18}F]FDG or FDG) radiotracer to assess heterogeneity of cellular metabolism using the proposed flow radiocytometry: (a) We washed the quantum dot-labeled cells in T25 flask twice with 1x PBS. (b) We added 2 mL of FDG dissolved in glucose-free complete growth medium to the flask and incubated the sample at 37 °C and 5% CO_2 for 45 min (Midi 40, Thermo Scientific, Inc.). The radioactivity of 2 mL FDG-dissolved medium was measured to be about 800 MBq. (c) We washed the cells twice with cold 1x PBS which was kept in ice. We assume the use of cold media reduces radial diffusion of FDG out of the cells by inducing an ordered packing arrangement of cell lipid bilayers, thus minimizing leakage of unphosphorylated FDG out of the radiolabeled cells.

K. Single cell preparation

High quality single cell preparation is essential for high efficiency single cell encapsulation in droplet. We needed to dissociate clumped cells into single cells prior to introducing them to DropGen microchip. Three factors commonly cause cell clumping and were addressed accordingly. First, calcium ions promote cell clumping by forming adherens junctions along with cadherin, a cell adhesion molecule. We used Mg^{2+} Ca^{2+} free PBS and added 2 mM EDTA to chelate any calcium ion present in cell media to disintegrate the adherens junctions and gently detach cells from each other. Second, DNA released from dead cells causes clumping by binding cells together. We added 10 units of DNase I per mL of sample to minimize this effect. To activate DNase I, we also added 5 mM magnesium, with the amount exceeding that of EDTA so that sufficient magnesium would be available for DNase activation even after some being chelated by EDTA. Third, over pelleting cause cells to clump. Gentle centrifugation of 100 $\times g$ was employed to minimize the mechanical clumping. We also pipetted cell sample suspension at least 20 times to promote the cell dissociation. Lastly, we used a mesh strainer to filter out any remaining clumps. Here, we describe detailed protocols for single cell preparation: (a) Immediately after radiolabeling, cells were treated with 1.5 mL Trypsin-EDTA 0.05% (Gibco, Thermo Fisher Scientific, Inc.) and incubated for 2 min at 37 °C. (b) After quenching trypsin digestion by adding 1.5 mL of cold complete growth medium, we centrifuged, washed with 1x PBS, and resuspended the cells in 3 mL of Cell Dissociation Medium Type I. (c) We added 30

units of DNase I to the sample and incubated at 37 °C for 10 min. (d) We exchanged the medium with 2 mL of Cell Dissociation Medium Type II via centrifugation for 5 min and added 20 units of DNase I. (e) We pipetted at least 20 times to help dissociate cell clusters into single cells and filtered out remaining cell clusters using a 40 µm-pored mesh strainer (make). (f) We measured cell density using hemocytometer and total radioactivity of the cell sample using a dose calibrator (Atomlab 400, Biodex) to estimate average radioactivity of radiolabeled single cell. (g) We added 1x PBS to achieve a cell number density of $\sim 1 \times 10^6$ cells/mL and 16% v/v OptiPrep density gradient medium (Sigma-Aldrich) to match the density of the final medium to 1.06 kg/m³ reported as the density of mammalian epithelial cells^{32,33}.

L. X-ray irradiation and fluorescence spectrometry

Stability and sensitivity of DCFH were assessed using kinetic fluorescence spectrometry. DCFH samples prepared in 5x PBS with concentration range of 50 nM – 500 µM were dispensed in 96-well plates in four aliquots, each with a volume of 100 µL. Paraffin oil with a volume of 100 µL was dispensed on top of DCFH to prevent DCFH in direct contact with air and minimize undesirable oxidation of DCFH. Samples were irradiated with an X-RAD 320 X-ray irradiator (Precision X-ray) with a source-surface distance of 45 cm and a dose output set to 504.40 cGy/min (320 kVp, 12.5 mA and 2 mm thick aluminum filter).

M. GLUT1 knock down cell preparation

To establish GLUT1 knockdown cells, shRNAs targeting *SLC2A1* (GLUT1) (Target sequence #1: TTGGCTCCGGTATCGTCAACA, #2: CCAAAGTGATAAGACACCCGA) as well as a nontargeting control were purchased from Millipore Sigma. For transfection, 1×10^6 HEK293T cells were seeded in 10 cm dish with DMEM supplemented with 10% FBS and 1% antibiotic-antimycotic. After 24-hour incubation, cells were washed twice with DMEM only and 5 ml of DMEM with 10% FBS were added. 5 µg of shRNAs were mixed with 2.5 µg of packaging plasmids, Δ8.7 and VSV.G and transfected into cells using 15 µL of X-tremeGENE™ 9 transfection reagent. Cell media containing virus particles were collected 48 hours after and filtered through 0.45 µm syringe filters. As a recipient cell line, 1.5×10^5 MDA-MB-231 cells were seeded into 6-well plate and incubated for 24 hours. Then cell media were removed, and 1 mL of virus particles were added with 1 µg/mL of polybrene and 1 mL of regular DMEM with

10% FBS and 1% antibiotic-antimycotic. After incubating for 48 hours, knockdown cells were selected with 2 µg/mL puromycin for 2 weeks. GLUT1 knockdown was confirmed by Western blot after removing N-glycans using PNGase F.

N. Western blot of shGLUT1 and shSCR

To determine *SLC2A1* knockdown in MDA-MB-231 cells, GLUT1 protein expression was evaluated using Western blot. Since GLUT1 is highly glycosylated, N-glycans were removed using PNGase F. As the first procedure, whole cell protein was extracted with RIPA buffer supplemented with protease inhibitor cocktail. Then, 10 µg of protein was mixed with 2 µL of PNGase F (NEB #P0707S) and incubated at 37°C overnight. After incubation, we added sample buffer and ran BOLT 4–12% Bis-Tris gel for western blot (Thermo Fisher Scientific #NW04120). After a transfer to nitrocellulose membrane using Trans-Blot Turbo transfer system (BioRad), the membrane was probed with anti-GLUT1 antibody (Abcam #ab115730).

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AUTHOR CONTRIBUTIONS

B.H., T.J.K., and G.P. designed the research. E.J.M. prepared the GLUT1 knockdown and control cell samples and performed the Western blot assays. B.H. performed all the other experiments. B.H. wrote the codes and analyzed the data. B.H., E.J.M., A.J.G., and G.P. wrote the manuscript.

COMPETING INTERESTS

G.P. is listed as inventor on a patent (US 20160025701 A1) related to this work. Other authors declare no conflict of interest.

CORRESPONDENCE AND REQUESTS FOR MATERIALS

Correspondence and requests for materials should be addressed to G.P.

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Figures

Figure 1

Illustrations showing production of reactive oxygen species (ROS) from radioactive beta decay and radiofluorogenic conversion from the ROS. (a) A single radioactive cell is encapsulated into a water-in-oil drop. As the radiotracer decays, the emitted beta radiation creates ROS such as hydroxyl radicals, which in turn mediate the radiofluorogenic conversion. (b) Proton (P) decays into neutron (N), emitting positron (e^+) and neutrino (ν). The emitted positron travels a finite distance, interacting with water molecules along its path and creating reactive oxygen species (ROS), until it annihilates with an electron (e^-), resulting in two antiparallel 511 keV photons. (c) Nonfluorescent dichlorodihydrofluorescein (DCFH) is converted into fluorescent dichlorofluorescein (DCF) after losing two protons from interacting with radiation-induced ROS.

Figure 2

Device overview and design. (a) Schematics showing drop generation, incubation, and read. (b) Optical micrograph (top view) showing cell samples and radiofluorogenic probe solution being introduced and mixed in DropGen microchip (c) Optical micrograph showing the cross-junction microfluidic channels where the mixture (i.e., dispersed phase) including the cells was encapsulated in water-in-oil drops. (d) Optical micrograph of polytetrafluoroethylene tubing where drops were incubated. (e) Picture of the tubing and holder. (f) Optical micrograph of DropRead microchip in which drops were introduced to have their fluorescence measured after incubation.

Figure 3

Optofluidic configuration and measurement. (a) Schematic showing the optofluidic configuration. (b) The two PMT signals as a function of elapsed time.

Figure 4

Kinetic fluorescence spectroscopy measurements of (a) DCFH solutions and (b) mixture of DCFH and FDG. (c) Signal to background ratio (SBR) as a function of elapsed time. (d) SBR measured at increasing elapsed times as a function of the ratio of radioactivity to DCFH concentration.

Figure 5

Fluorescence measurements of drops containing non-radiolabeled cells (control) vs. radiolabeled cells (sample). (a,b) Micrographs of drops at the inlet of DropRead microchips: (a) control and (b) sample; bright field image on the left and FITC fluorescence image on the right. (c,d) PMT measurements as a function of time for (c) control and (d) sample assays.

Figure 6

(a) Western blot comparing SLC2A1 knockdown (shGLUT1) to scrambled shRNA control (shSCR) in MDA-MB-231 cells. (b) Averaged radioactivity of shSCR and shGLUT1 samples measured using bulk dose calibrator and hemocytometry. The errors are standard deviation and associated with cell counting. (c) Half-violin plot summarizing the drop read results. Each dot in the scatter plots on the left-hand side represents a single drop measured by FRCM. The frequency distribution corresponding to the scatter plot is shown on the right-hand side. Grey: non-radioactive cell sample (i.e., control), red: FDG-labeled control shSCR cell sample, and blue: FDG-labeled shGLUT1 cell sample.

Supplementary Files

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