Materials and Methods

Beta camera

Custom electronic readout circuitry for signal processing and data was acquired with a computer based data acquisition card running LabVIEW software (National Instruments). The raw images produced from the PSAPD using this positioning algorithm have an inherent pincushion spatial distortion which needs to be corrected to ensure spatial linearity in the final images (Supplemental Fig. 1b). Using a known input source pattern, the spatial distortions can be imaged to create a correction map that can be applied to all subsequent images (Supplemental Fig. 1c). It was also demonstrated that the spatial resolution of the beta camera can sufficiently discern ^{18}F positron sources separated by 1mm distance.

The PSAPD is placed in a large block of aluminum (100 × 100 × 7 mm^3) to maintain a constant temperature at 37˚C for live cell culture imaging. Thermal conductive silicon paste was placed between the PSAPD and the aluminum block to improve heat conduction. Heating elements were inserted into the block and a thermocouple was inserted directly below the PSAPD to monitor and control the block temperature to within ± 1˚C. The gain of the PSAPD detector in the beta camera system is inversely proportional to its temperature. In order to maintain a constant gain at varying temperatures the high voltage bias must be increased as a function of temperature (Supplemental Fig. 1d). Supplemental Table 1 shows some of the performance characteristics of the PSAPD at both room temperature and 37˚C.

For co-registration of the radioassay images with the microchambers we used a monochrome CCD camera (DMK 31AU03, The Imaging Source) with a resolution of 1024 × 768 pixels. The camera was placed overhead to capture optical photographs of the microfluidic platform. The beta camera and CCD images were then imported into the data analysis software AMIDE (A Medical Imaging Data Examiner)[1] where the pixel size and image centers could be adjusted to register the two images.

Microfluidic chip fabrication

Two different molds were first produced by photolithography to create fluidic channels (channel width ranging from 300 to 700 μm, channel height: 40 and 100 μm) and control channels (channel width: 25 μm, channel height: 35 μm). The molds utilized for fabrication of

the fluidic channels were made by two-step photolithography. In the first step, a 100 μm thick negative photoresist (SU-8 2100) was spin-coated onto silicon wafers. After UV exposure and subsequent development, rectangle-profiled patterns for culture chambers were obtained.

In the second step, a layer of 40 μm thick positive photoresist (AZ 50XT) was spin-coated onto the same wafer. Prior to UV exposure, the second mask was aligned by a mask aligner to ensure a good match between the previous and newly fabricated patterns. Once the positive photoresist was developed, the wafer was heated to 115°C which is above the glass transition temperature of the positive photoresist. As a result, the cross-section of the patterned positive photoresist was transformed into a round profile, while the rectangular profile of the negative photoresist remained unchanged. The round-profiled patterns were transferred into PDMS substrates to form the round-profiled fluidic channels, which ensured complete sealing of integrated pressure-driven valves.

The molds for control channels were made by introducing a 35 μm thick negative photoresist (SU-8 2025) pattern on silicon wafers. In order to achieve reliable performance of each valve, the widths of the control channels were set at 260 μm in locations where the valve modules were located.

Prior to chip making, both the fluidic and control molds were exposed to trimethylchlorosilane (TMSCl) vapor for 5 min. A well-mixed PDMS pre-polymer (Sylgard 184, A and B in 10 to 1 ratio) was poured onto the fluidic mold located in a Petri dish to give a 5 mm thick PDMS fluidic layer. Another portion of PDMS pre-polymer was spin-coated onto the control mold (2000 rpm, 30 sec, ramp 5 sec) to obtain a 100 μm thick PDMS control layer. The thick fluidic layer and thin control PDMS layers were cured in an 80°C oven for 15 min. After incubation, the thick fluidic layer was peeled off from the mold, and holes were introduced into the fluidic layer for the access of reagent solutions. The fluidic layer was then trimmed, cleaned and aligned onto the thin control layer. After baking at 80°C for 30 min, the assembled layers were peeled off from the control mold, and another set of holes were punched for the access of control channels. These assembled layers were then placed on top of a 150 μm thick glass slide that was coated (6100 rpm, 1 min, ramp 5 sec) with the PDMS pre-polymer that had been cured for 10 min in the 80°C oven. The microchips were ready for use after baking at 80°C for 24 hr.

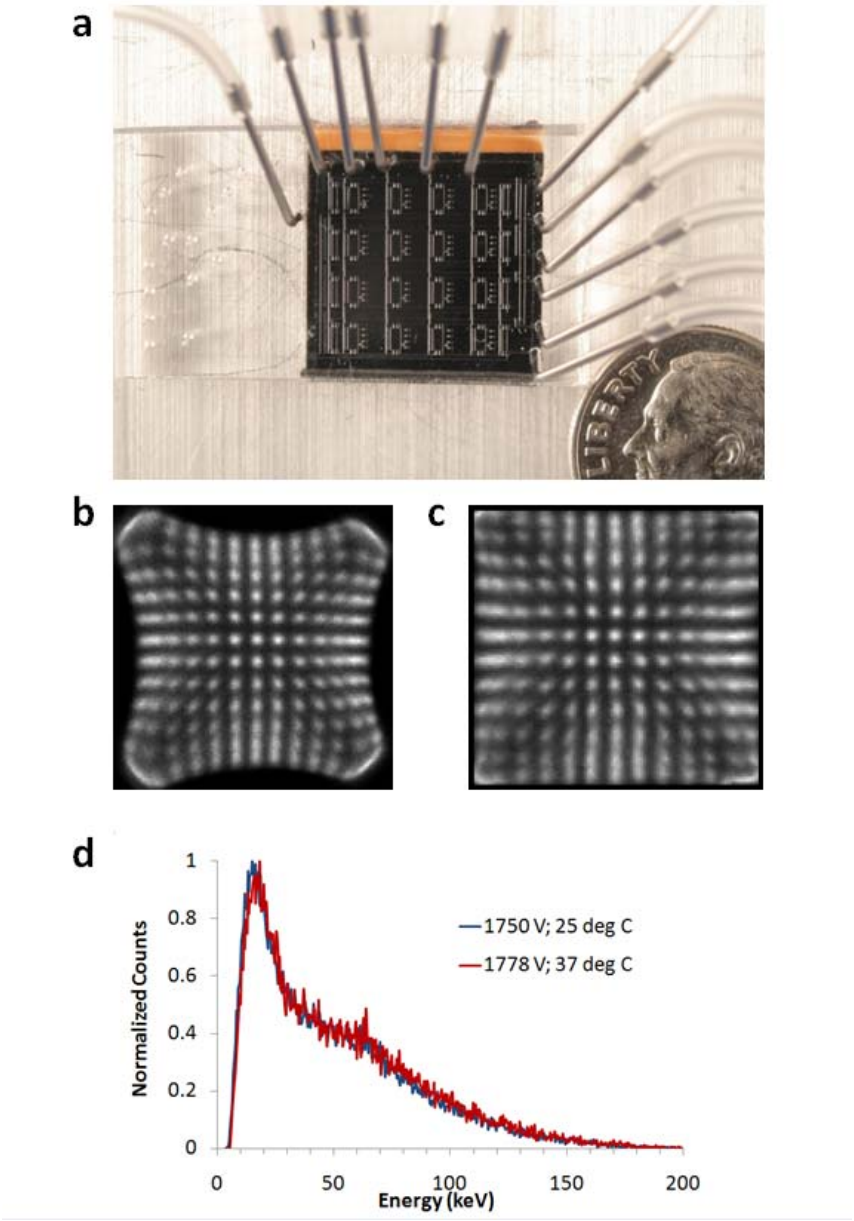
Cytotoxicity of melanoma cell lines to PLX4032

Human melanoma cell lines were cultured in RPMI 1640 with L-glutamine (Mediatech Inc., Manassas, VA) containing 10% (v/v) fetal bovine serum (FBS, Omega Scientific, Tarzana, CA) and 1% (v/v) penicillin, streptomycin, and amphotericin (Omega Scientific). Cells were kept in a humidified 37°C, 5% CO_2 incubator in filter-top T75 flasks (Techno Plastic Products, Trasadingen, Switzerland). All cell lines were mycoplasma free when periodically tested using a Mycoalert assay (Lonza, Rockland, ME). 5 x 10^3 cells/well were plated in flat-bottom 96-well plates (Techno Plastic Products) in 100 μL culture medium and rested for 24 hours. PLX4032 stock solution and parallel DMSO vehicle control were diluted in culture medium to the appropriate concentration and added in triplicates to the 96-well plates. After 120-hour incubation the number of viable cells was measured using a tetrazolium compound [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS)-based colorimetric cell proliferation assay (Promega, Madison, WI). Absorbance was measured after 2 hours incubation with the MTS agent at 490 nm on a DTX 880 multimode detector (Beckman Coulter, Fullerton, CA). Viability was calculated by dividing experimental wells with the corresponding vehicle control wells.

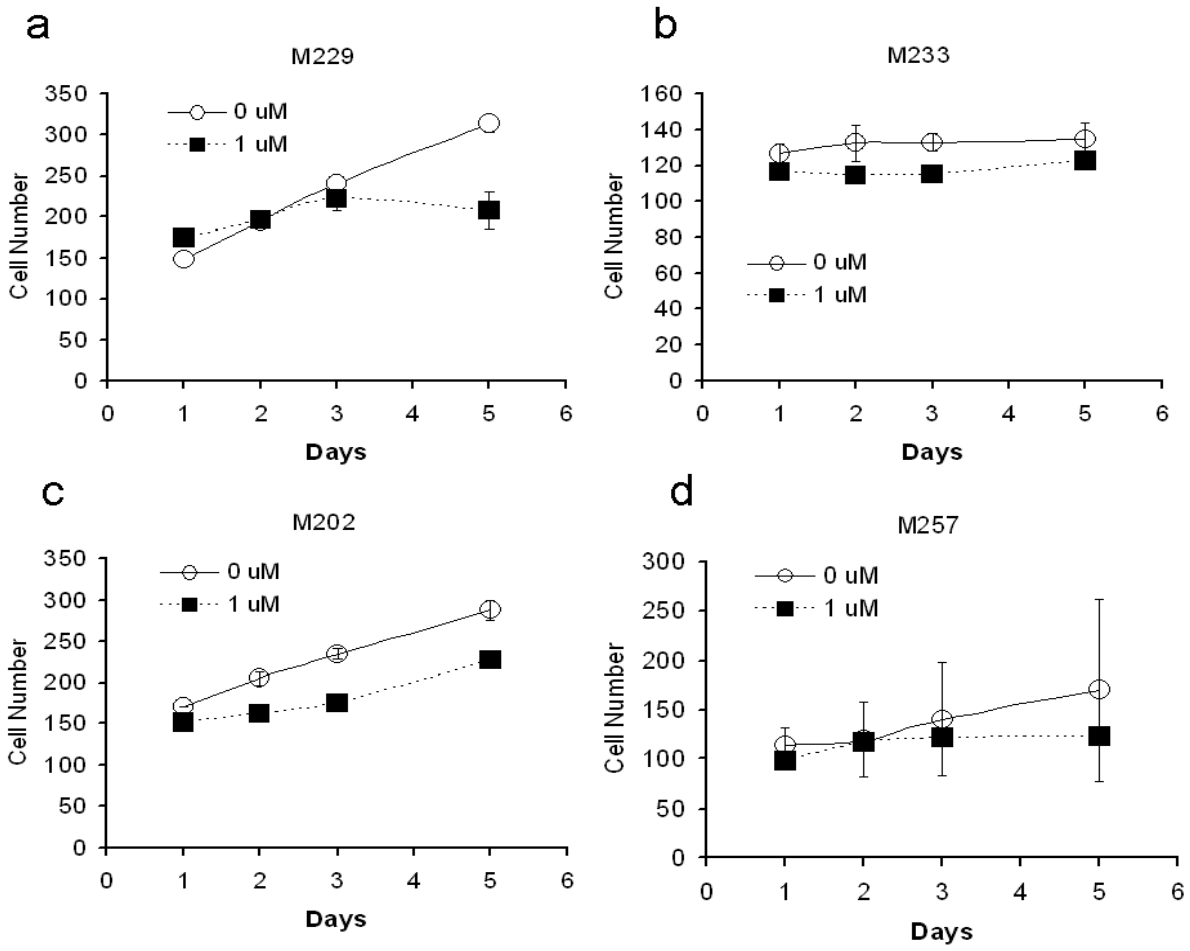
Macroscale radioassay

Filter bottom 96-well plates (multiscreen HTS GV 0.22 μm opaque, Millipore, Billerica, MA) pre-incubated with 0.001% poly-L-lysine (Sigma-Aldrich) were used for plating 10^4 melanoma cells per well. After resting the cultures for 24 hours the PLX4032 stock solution and parallel DMSO vehicle control were diluted in culture medium to a 1 μM solution and added in triplicates for 20 hours. After that, 5 μCi/mL of 2-FDG [5,6-^{3}H] (American Radiolabeled Chemicals Inc., St. Louis, MO) in glucose-free DMEM (Invitrogen) was added to each well for 1 hour. Extracellular metabolic tracer was washed off using a multiscreen HTS vacuum manifold system (Millipore) and 100 μL scintillation fluid (Perkin Elmer, Waltham, MA) was added to each well. Tritium count was measured on a 1450 microbeta trilux microplate reader (Perkin Elmer).

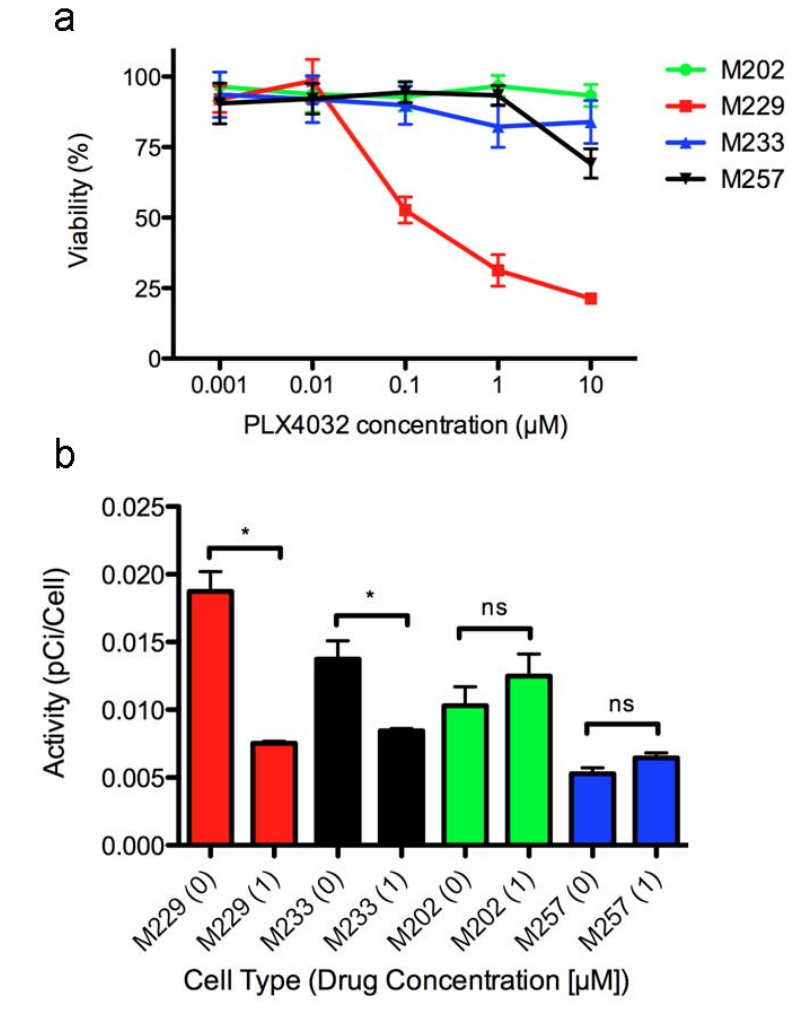
Supplemental Table 1. PSAPD Performance Characteristics		
Temperature	22° C	37° C
Leakage Current	1 μA	4 μA
Background Rate	4.28×10^{-4} cps/mm^2	4.34×10^{-4} cps/mm^2
Absolute sensitivity directly on PSAPD	41%	41%
Absolute sensitivity with Glass Layer	12%	12%
Absolute sensitivity with Glass Layer + 100μm Plastic	6%	6%



Supplemental Figure 1. PSAPD performance for *in vitro* ^{18}F positron imaging. **(a)** Photograph showing the microfluidic chip placed directly on top of the PSAPD. The dark area of the PSAPD indicates the active region. The PSAPD detector was embedded in a large aluminum plate and the temperature was regulated to 37°C. **(b)** Raw image produced by PSAPD shows the inherent pincushion distortion along the edges of the field of view. The image was produced with a ^{18}F-FDG-ink printed source showing a matrix of dots spaced 1mm apart center to center. **(c)** PSAPD image after applying the spatial linearity correction algorithm **(d)** ^{18}F-FDG energy spectrum acquired with beta camera at 25°C and 37°C. The gain of the PSAPD detector is inversely proportional to its temperature. In order to maintain a constant gain at varying temperatures the high voltage bias must be increased as a function of temperature.



Supplemental Figure 2. Proliferation rates of cells in each microfluidic chamber in response to 0 and 1 μM of PLX4032 over a period of 5 days. The cells were treated with 1 μM PLX4032 for 20 hours before day 1. The microfluidic radioassay 1 μM PLX4032 treatment was performed during days 1-3. Treatment with 1 μM PLX4032 was continued through day 4 and 5 with the cell populations counted on the last day. The data values represent mean ± SEM for each of the four cell lines (**a**) M229 (**b**) M233 (**c**) M202 (**d**) M257.



Supplemental Figure 3. Macroscale cell viability and radioassay of melanoma cell lines treated with PLX4032 *in vitro*. **(a)** Melanoma cell lines were treated with a range of concentrations of PLX4032 from 0.001-10 μM for 120 hours. Cell viability was determined using an MTS-based assay. Each concentration was repeated in triplicates and data points represent mean ± SEM. **(b)** Melanoma cell lines were treated with 1 μM PLX4032 for 20 hours and incubated in 2-FDG [5,6-^{3}H] PET tracer for 1 hour. Uptake was measured on a micro-beta reader.

Reference

1. Loening, A. M. & Gambhir, S. S. Amide: A free software tool for multimodality medical image analysis. *Molecular Imaging* 2, 131-137 (2003).