Flow cytometry

Prof. Dr. Andrei Leitão

Fluorescence & flow cytometry

Molecular Probes Tutorial Series—Introduction to Fluorescence.mp4

Molecular Probes Tutorial Series—Anatomy of Fluorescence Spectra.mp4

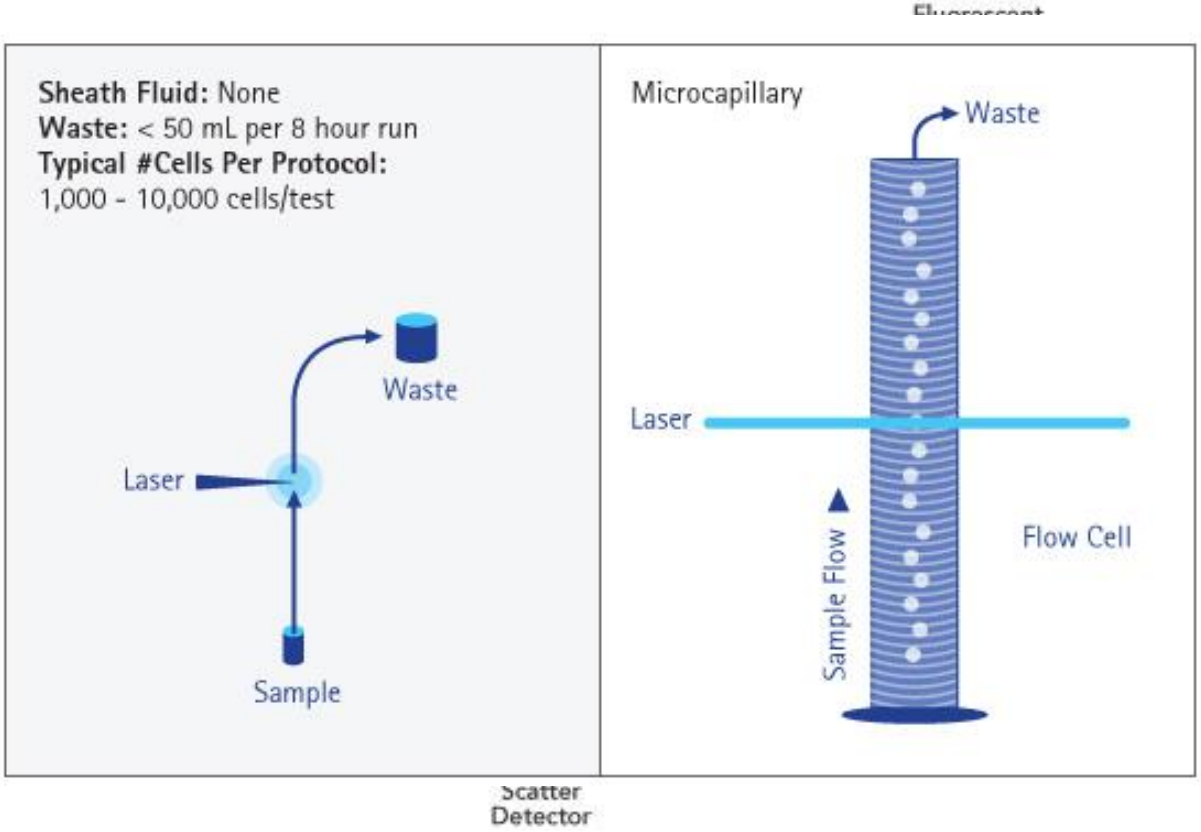
[Spetraviewer]

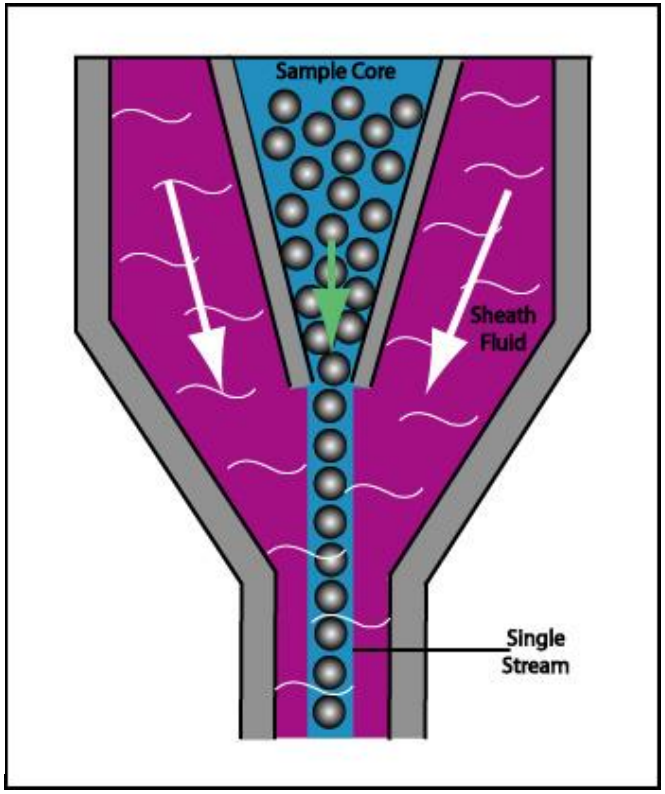
Molecular Probes Tutorial Series—Overview of Filters and Light Sources.mp4

Molecular Probes Tutorial Series—Introduction to Flow Cytometry.mp4

Molecular Probes Tutorial Series—Analyzing Flow Cytometry Data.mp4

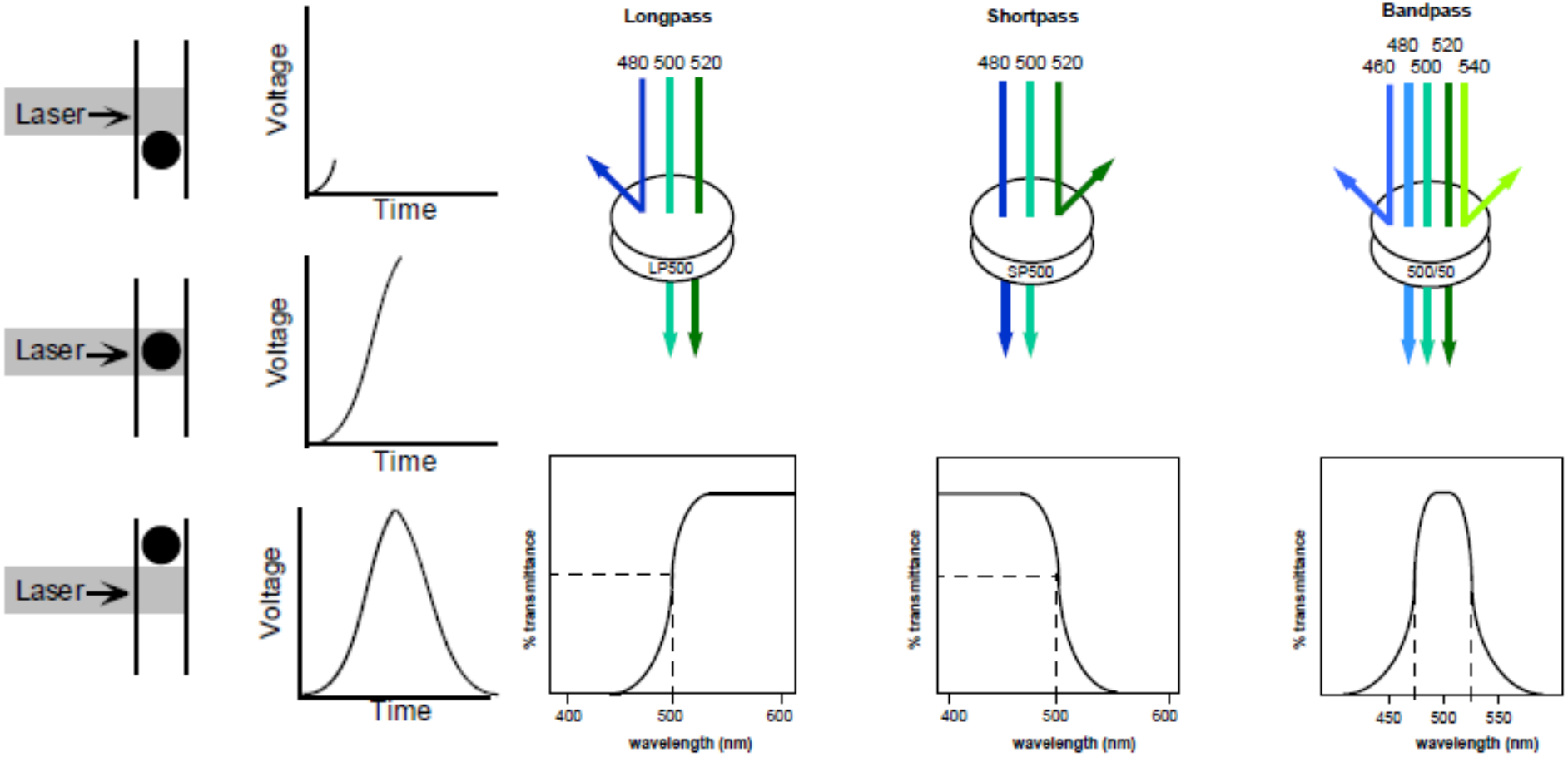
Flow cytometer





https://www.semrock.com/flow-cytometry.aspx
http://www.selectscience.net/flow_cytometry_buying_guide.aspx
http://www.merckmillipore.com/

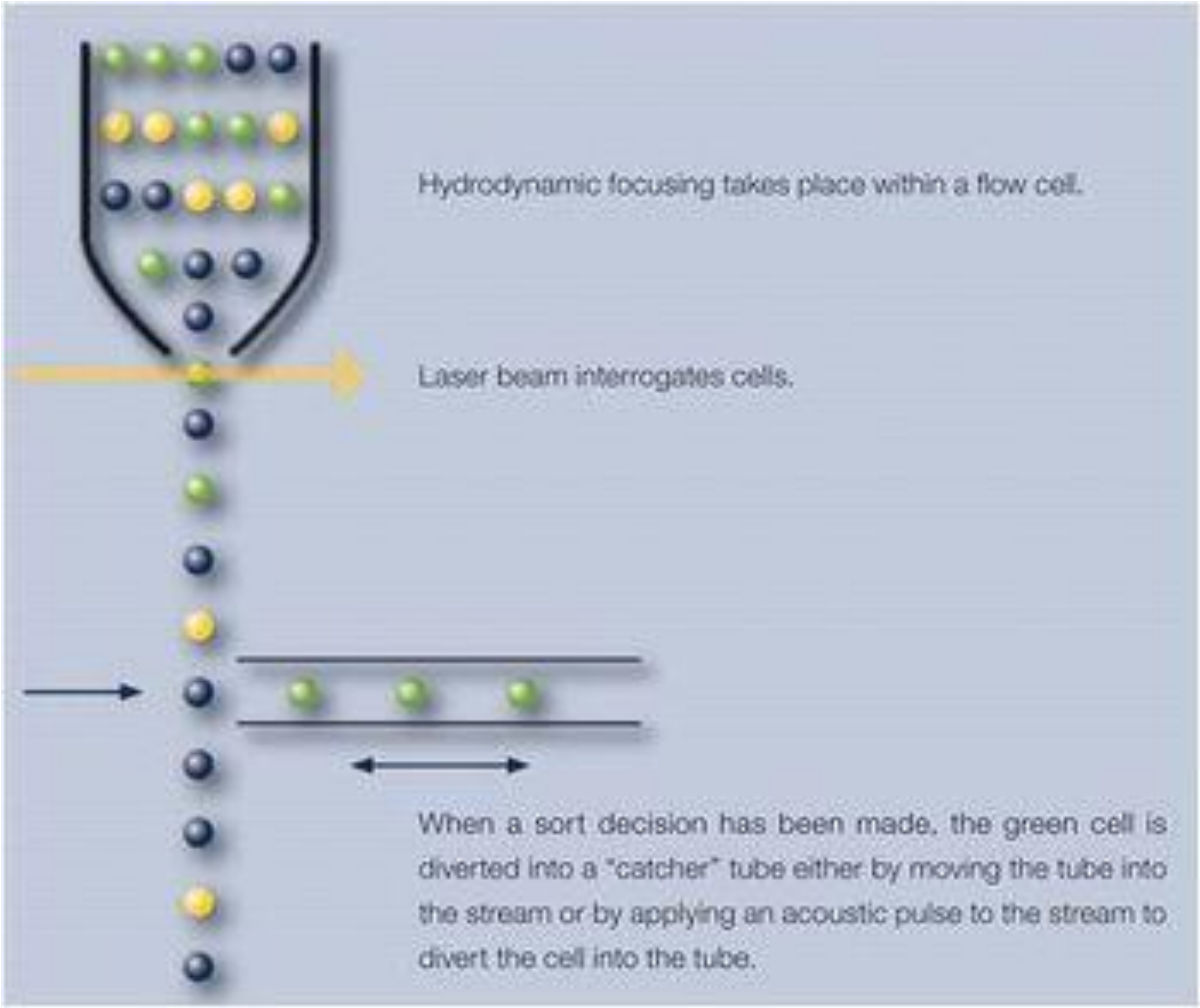
Mechanisms of detection



http://www.labome.com/method/Flow-Cytometry-A-Survey-and-the-Basics.html

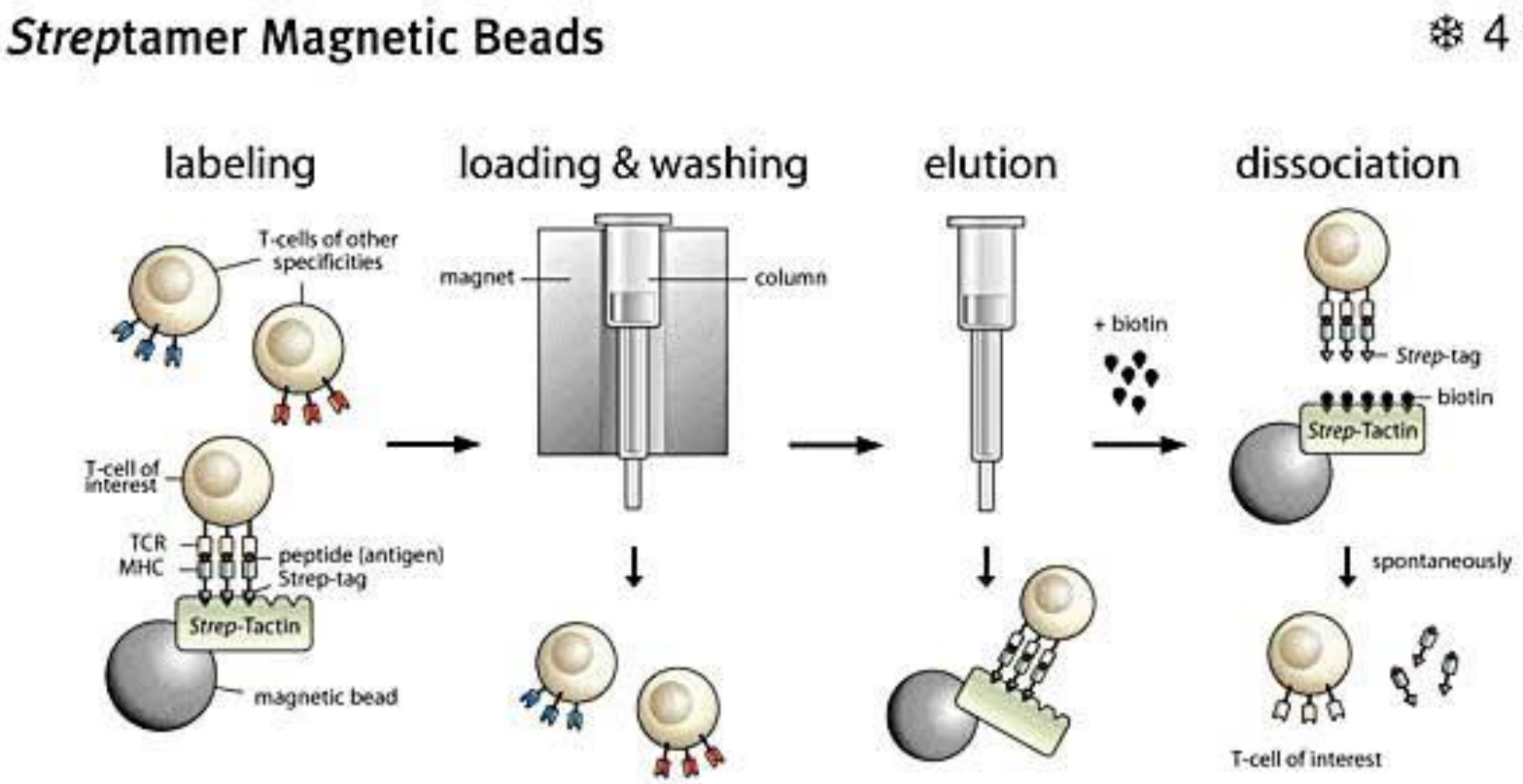
Fluorescence activated cell sorting (FACS)

Electrostatic and mechanic sorting



http://www.flow-cytometry.us/index.php?page=cell-sorting

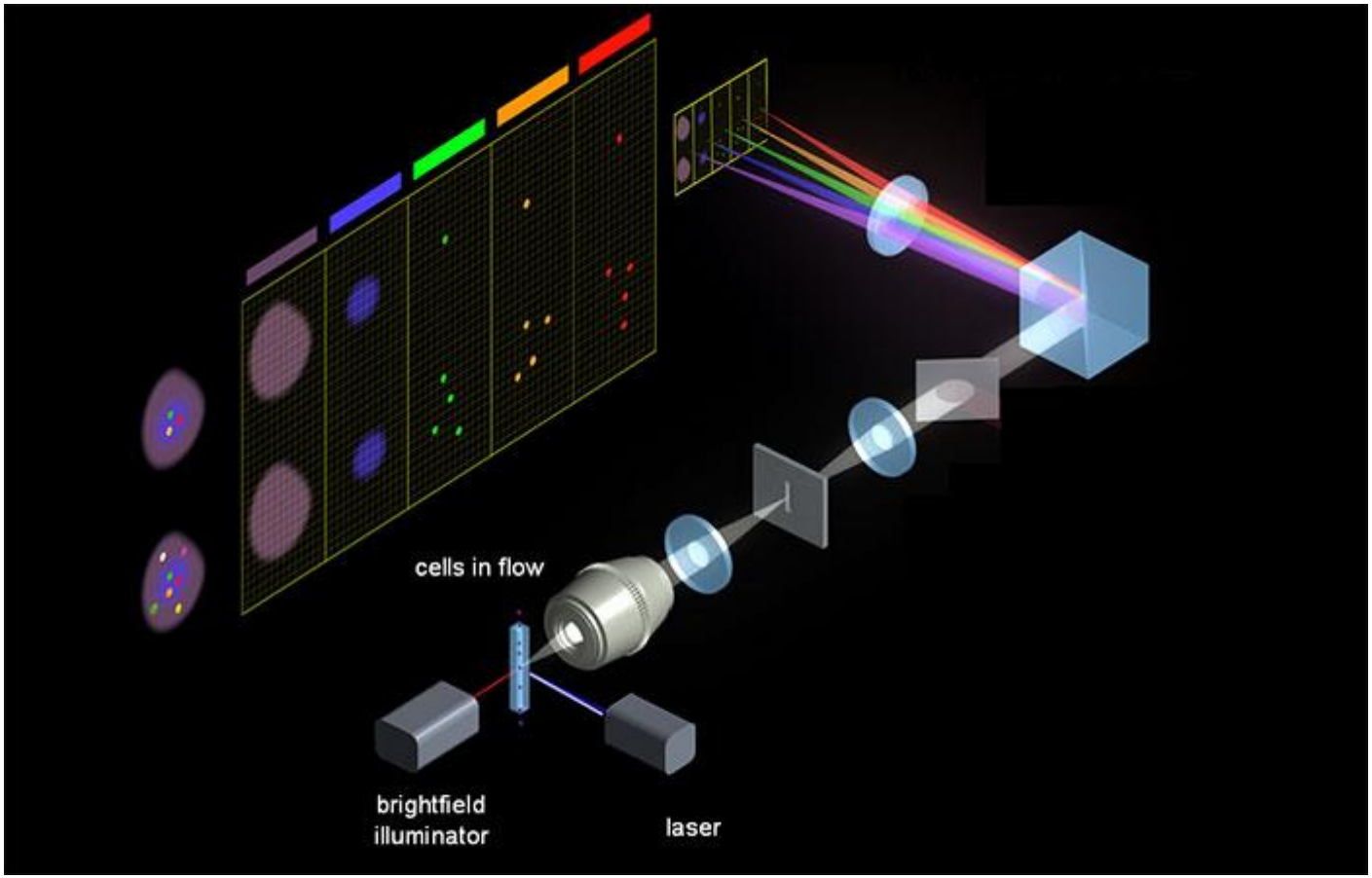
Magnetic activated cell sorting (MACS)



https://www.beckmancoulter.com/wsrportal/wsrportal.portal

Imaging flow cytometry

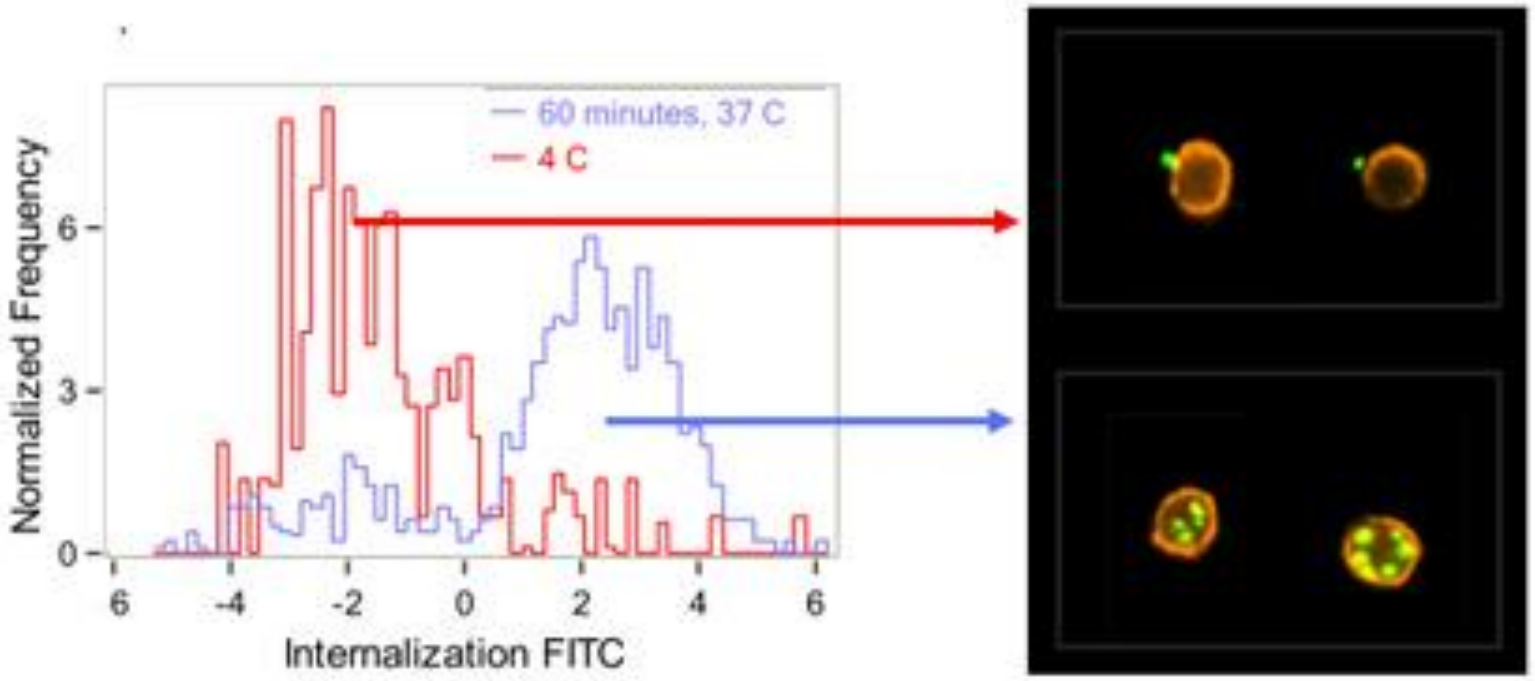
It acquires up to twelve images simultaneously of each cell or object including brightfield, scatter, and multiple fluorescent images at rates of up to 5000 objects per second with high photonic sensitivity.



http://www.merckmillipore.com/BR/pt/life-science-research/cell-analysis/amnis-imaging-flow-cytometers/how-it-works/mulitspectral-imaging/ALib.qB.5yUAAAFLbNUp.zIH,nav

Imaging flow cytometry

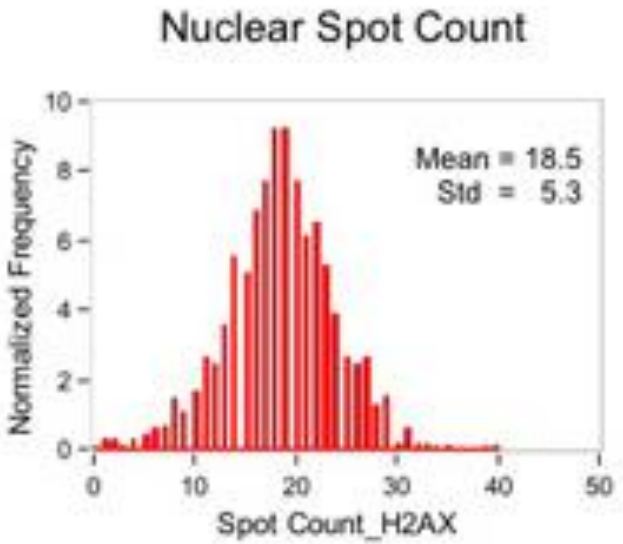
Internalization of zymosan (green) by murine RAW cells (orange) identified by immunophenotyping. Phagocytosis is measured as the percentage of cells with internalized zymosan at 15, 30 and 60 minutes at 37 ^{o}C

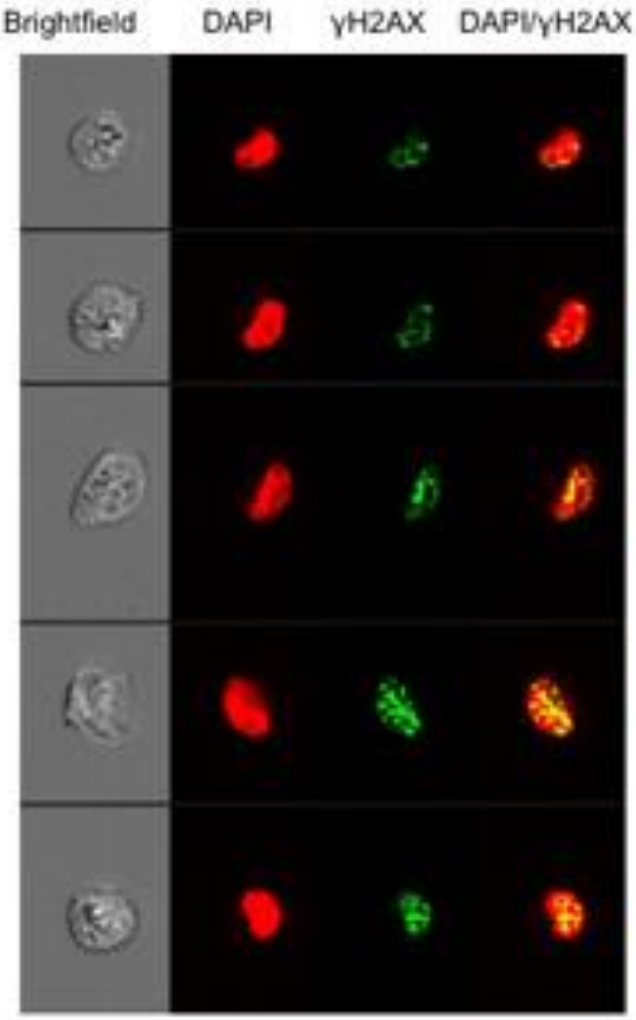


https://www.merckmillipore.com/BR/pt/life-science-research/cell-analysis/amnis-imaging-flow-cytometers/Q6ub.qB.m3UAAAFLCKIp.ygJ,nav?bd=1

Imaging flow cytometry

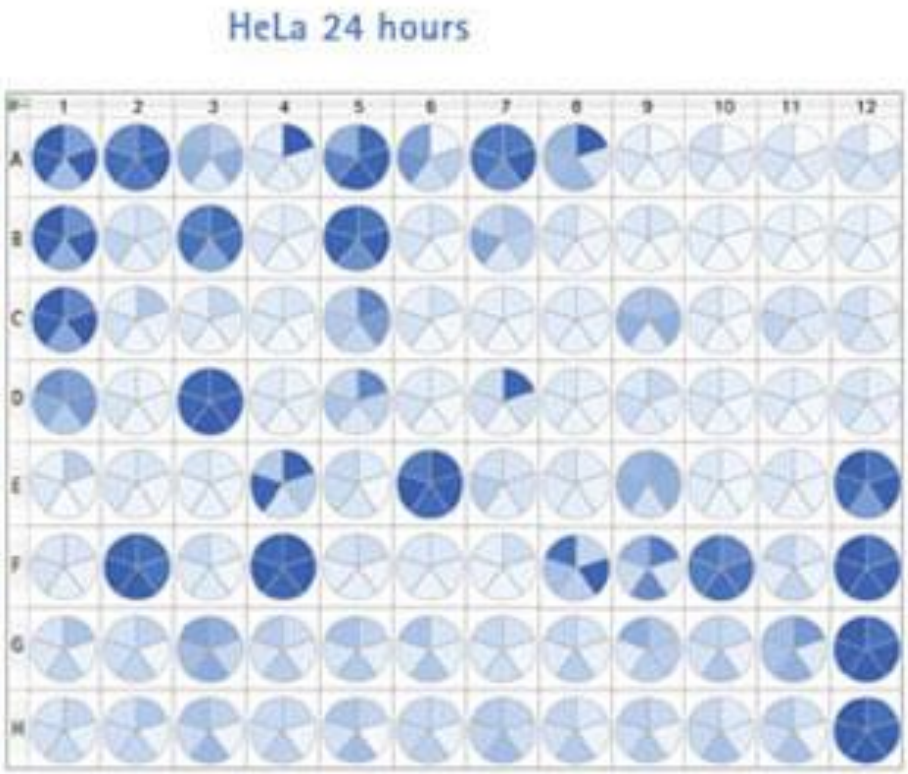
Phosphorylated H2AX (γ-H2AX) facilitates recognition and repair of DNA double strand breaks (DSBs) that may occur from exposure to ionizing radiation. Staining irradiated cells for γ-H2AX reveals nuclear foci that are readily observed microscopically in a dose response manner.

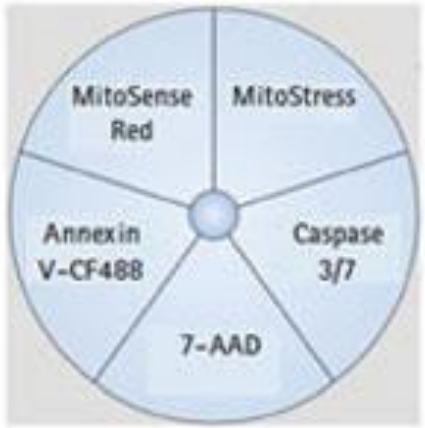




https://www.merckmillipore.com/BR/pt/life-science-research/cell-analysis/amnis-imaging-flow-cytometers/Q6ub.qB.m3UAAAFLCKIp.ygJ,nav?bd=1

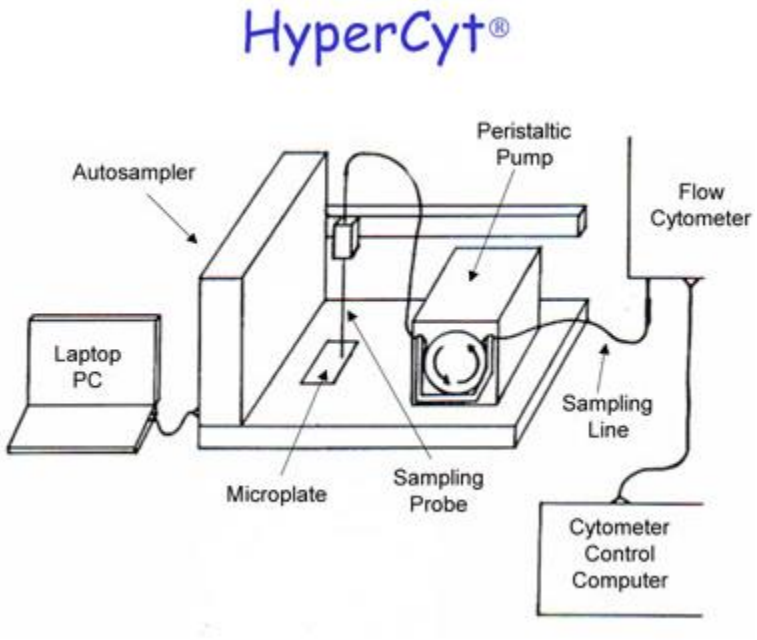
Cell screening

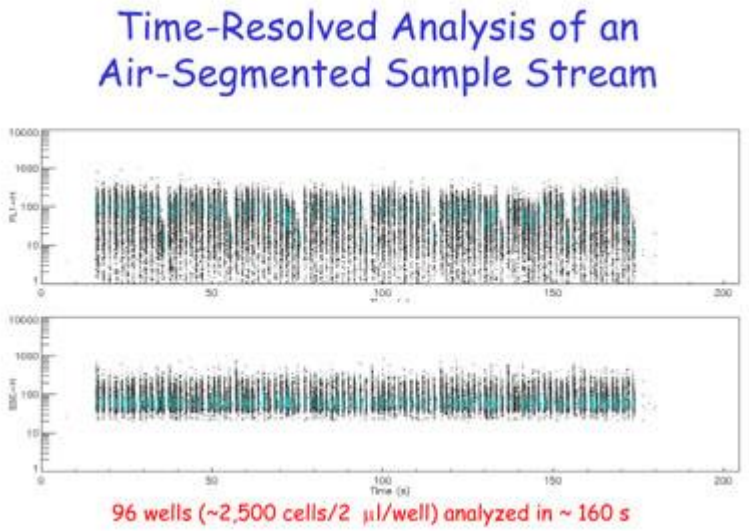


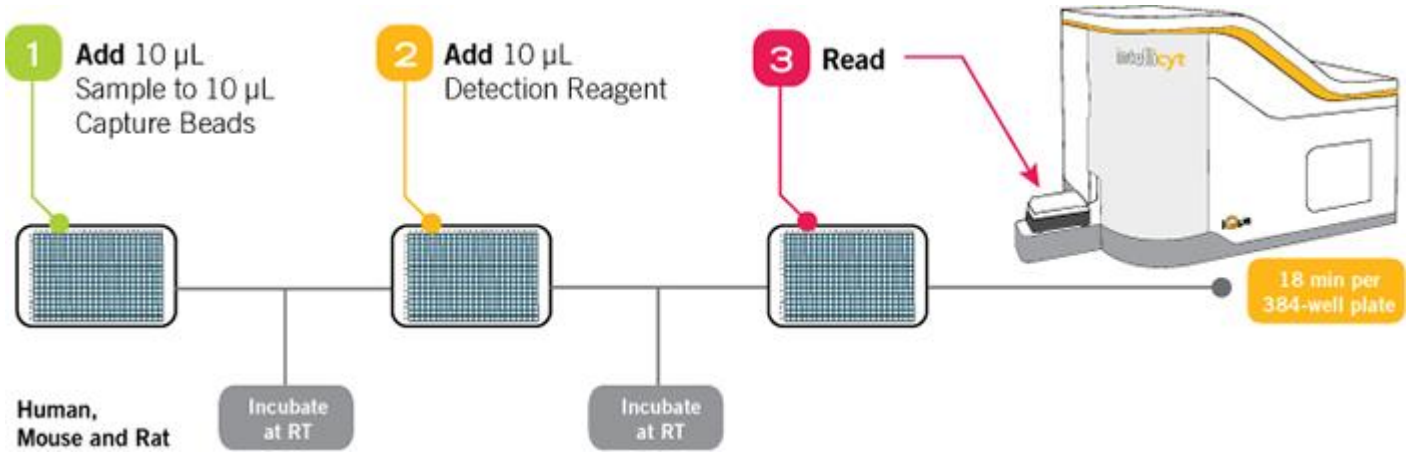


http://www.merckmillipore.com/BR/pt/life-science-research/cell-analysis/guava-easycyte-flow-cytometers/features/Ra2b.qB.ZF8AAAFBj5A7FnRa,nav

Cell screening







http://hsc.unm.edu/research/flowcyt/hypercyt.html
http://intellicyt.com/products/reagents/

Phenotypic assay - HyperCyt®

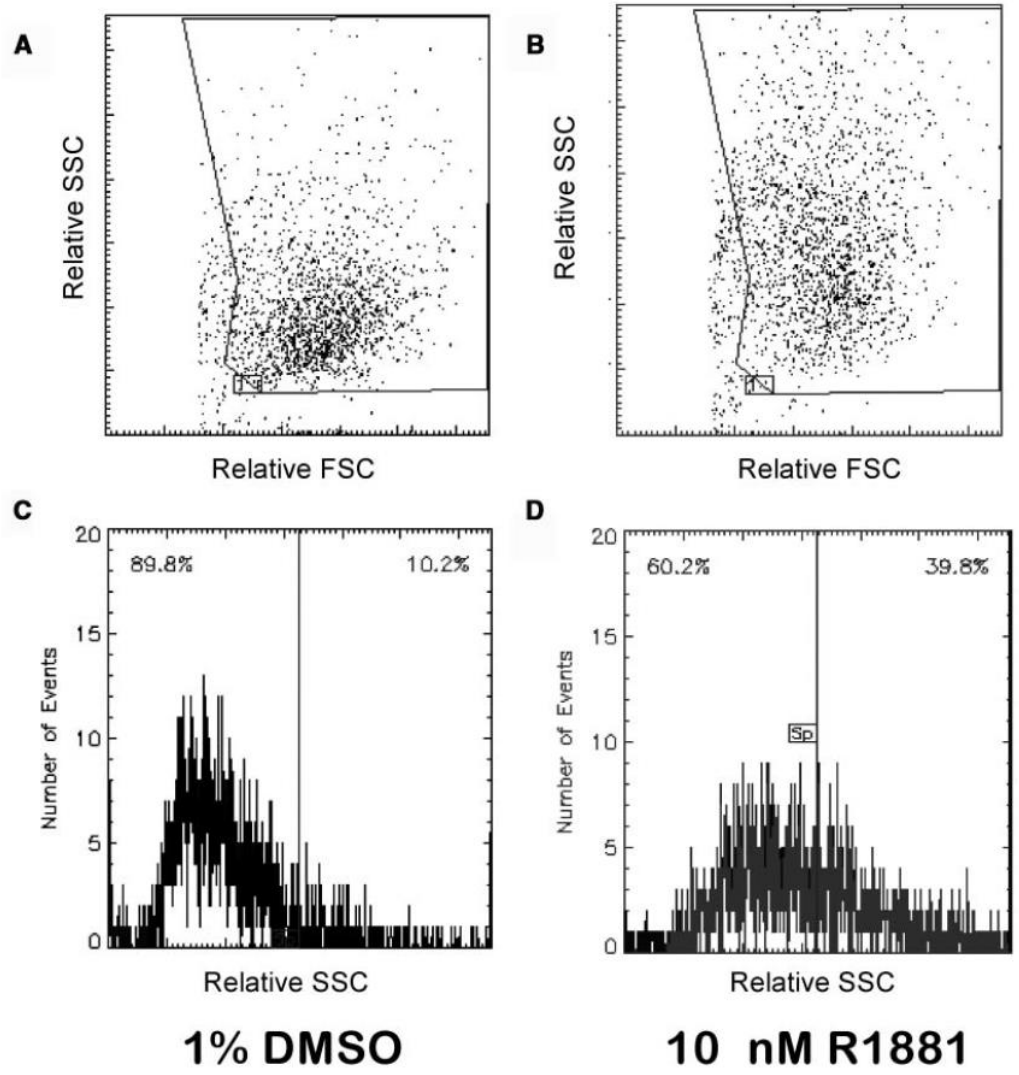


FIG. 1. The synthetic androgen R1881 causes increased intracellular granularity in LNCaP cells. Flask cultures of LNCaP cells were treated with 10 nM R1881 or 1% DMSO for 4 days followed by flow cytometric analysis. Plots of forward light scatter (FSC) versus side light scatter (SSC) (**A, B**) and SSC versus cell number (**C, D**) is shown. The 10 nM R1881 treatment (**B, D**) increased the mean SSC of the LNCaP population (28,900 v. 18,165) as well as the number of cells that exhibited increased SSC (10.2% v. 39.8%) when compared to 1% DMSO (**A, C**).

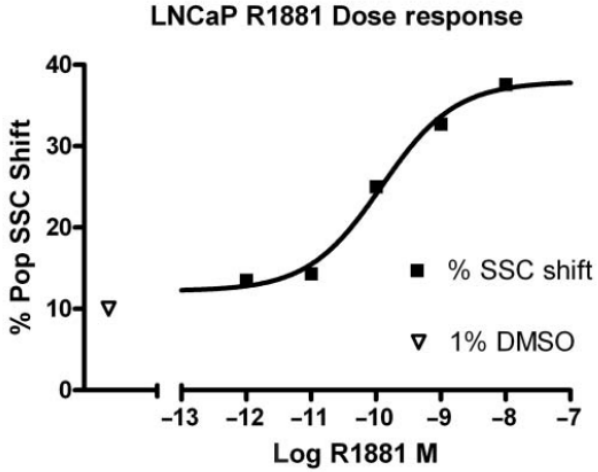


FIG. 2. Concentration response of LNCaP cells to R1881 treatment. A total of 75,000 cells per well were incubated for 4 days with 1% DMSO or varying concentrations of R1881 followed by flow cytometric analysis. The R1881 EC_{50} was 122 pM. SSC, side scatter.

Haynes, M.K.; Strouse, J.J.; Waller, A.; Leitão, A.; et al. *J. Biomol. Screen.* **2009**, *14*, 596-609.

Phenotypic assay

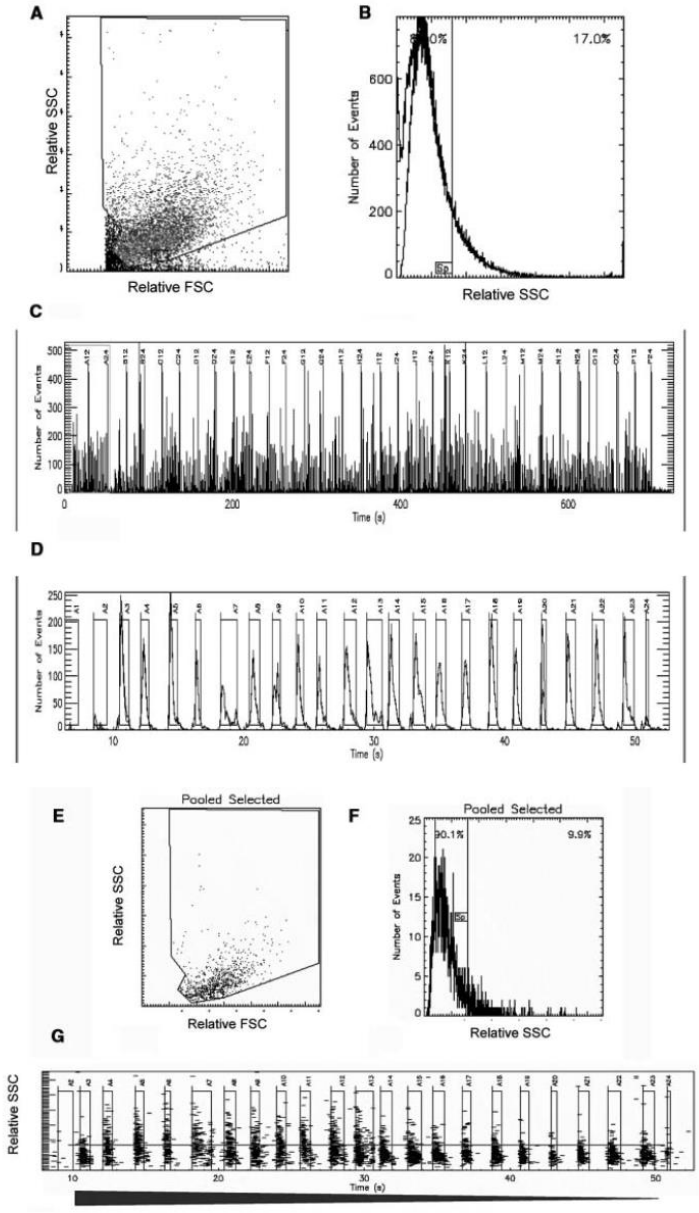


FIG. 3. HyperCyt® high-throughput flow cytometry. Individual wells of a 384-well microtiter plate were seeded at 10,000 LNCaP cells per well. Following overnight incubation, 2-fold serial dilutions of R1881 in DMSO were added to replicate wells (n = 8). Negative control wells (n = 8) contained 1% DMSO. Plots of forward light scatter (FSC) versus side light scatter (SSC) (**A**) and SSC versus cell number (**B**) represent the entire data set as a single file. The time-resolved format of the entire data set and one dose-response set of "binned" sample wells are shown in **C** and **D**, respectively. Pooled negative control samples (**E**, **F**) are used to set a 10% SSC split gate that is applied to the treated samples; the "binned" set shown in **D** is represented in **G**, where decreasing concentrations of R1881 (filled arrow) result in a decreased percentage of LNCaP cells exhibiting heightened SSC.

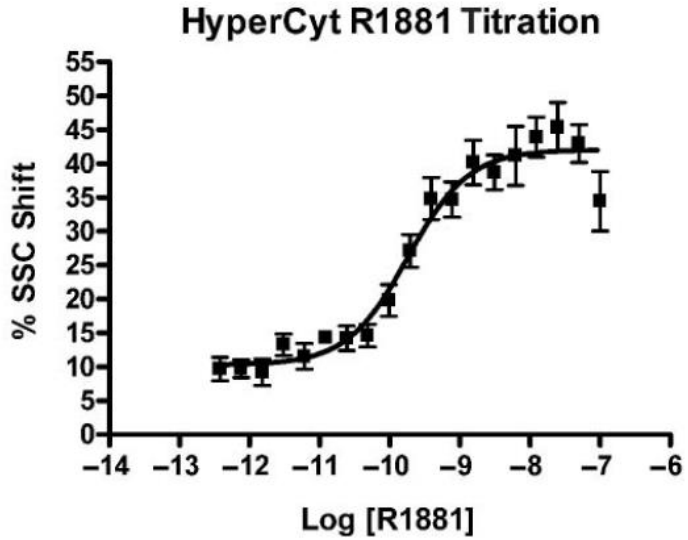


FIG. 4. HyperCyt® analysis of the concentration response of LNCaP cells to R1881. Individual wells of a 384-well microtiter plate were seeded at 10,000 LNCaP cells per well. Following overnight incubation, 2-fold serial dilutions of R1881 in DMSO were added to replicate wells (n = 8). Negative control wells (n = 8) contained 1% DMSO. The R1881 EC_{50} was 182 pM. SSC, side scatter.

Haynes, M.K.; Strouse, J.J.; Waller, A.; Leitão, A.; et al. *J. Biomol. Screen.* **2009**, *14*, 596-609.

Phenotypic assay – concentration-response

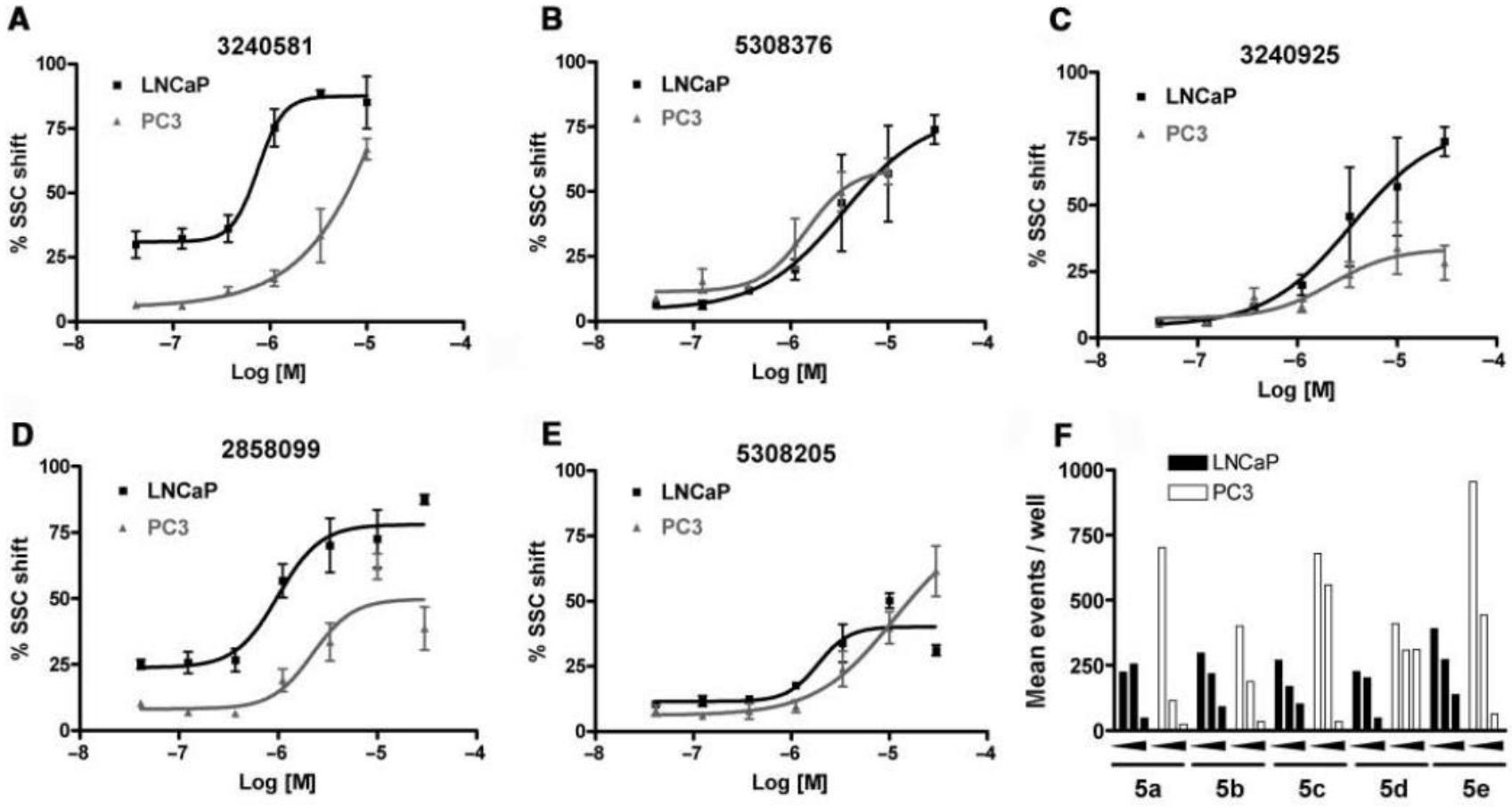


FIG. 5. Dose-response curves of the best representative compounds from the 5 families discussed in **Table 2**. LNCaP (closed squares); PC3 (closed triangles). Methods were essentially as described for **Figure 4** except that PC3 cells were seeded at 7500 cells per well. (**A**) LNCaP EC_{50} was 0.77 μM, and PC-3 EC_{50} was 6.5 μM. (**B**) LNCaP EC_{50} was 3.35 μM, and PC3 EC_{50} was 1.46 μM. (**C**) LNCaP EC_{50} was 3.45 μM, and PC3 EC_{50} was 2.21 μM. (**D**) LNCaP EC_{50} was 0.99 μM, and PC-3 EC_{50} was 2.21 μM. (**E**) LNCaP EC_{50} was 1.87 μM, and PC3 EC_{50} was 11.14 μM. (**F**) A composite graph indicating the event count observed when the cells were exposed to 3, 10, and 30 μM of the 5 compounds depicted in the graphs.

Cell cycle

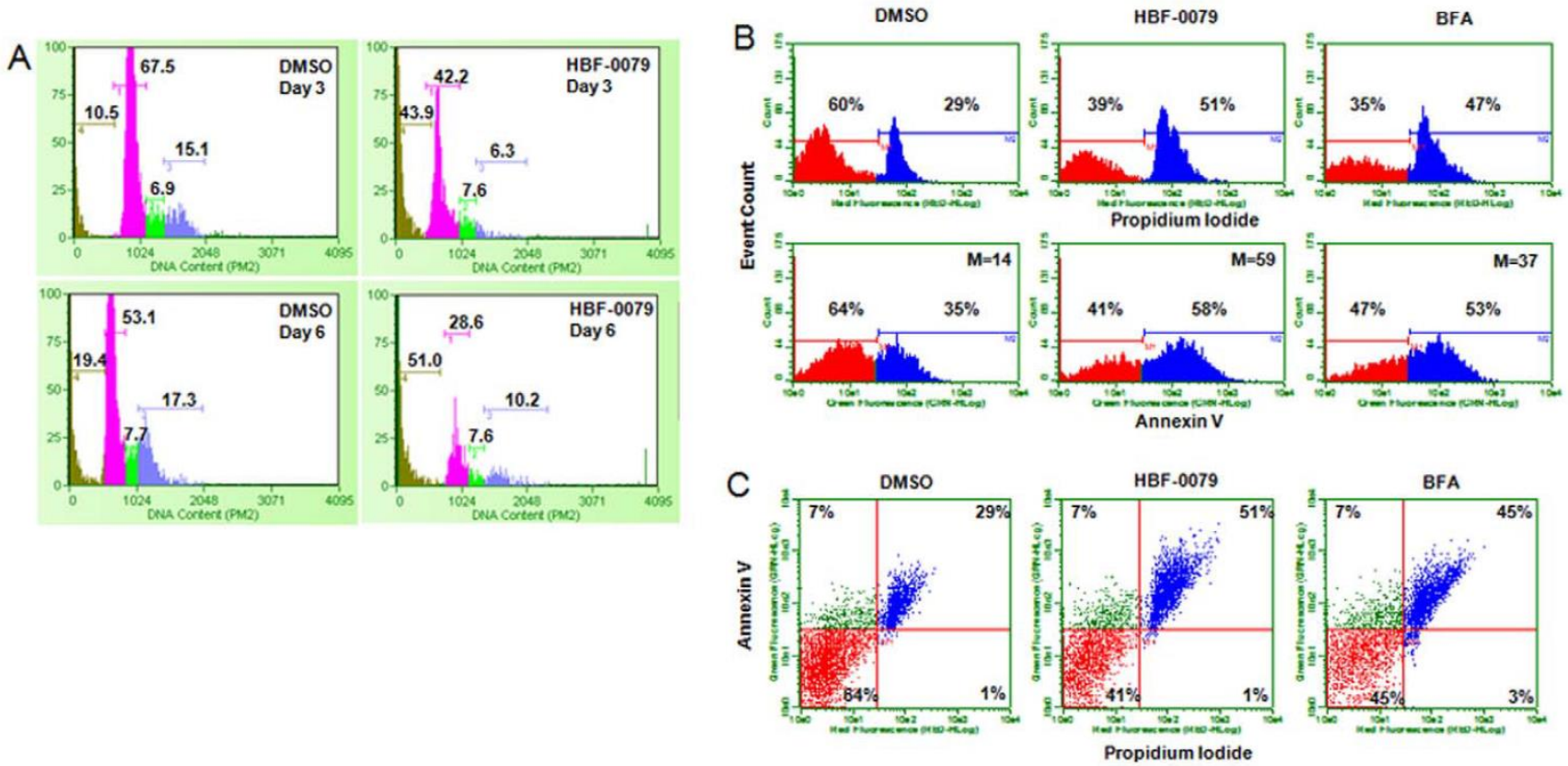


Figure 4. HBF-0079 induces cell cycle arrest and apoptosis in HCC cells. (A) Log-phase Huh7 cells were incubated in the absence or presence of 10 μM HBF-0079 or 0.5% DMSO for up to 6 days. Cell cycle phase distribution was determined at days 3 and 6 by PI labeling of total DNA content flow cytometry, and analysis through the Guava Cell Cycle Assay software module. From left to right, colored areas indicate $SubG_0/G_1$, G_0/G_1, S, G_2/M phases. Cell population percentages in each phase are indicated above peaks. (B) PI vs annexin V staining of Huh7 cells treated with DMSO, HBF-0079, or BFA for 6 days. Top panels depict histograms of staining intensity vs cell/event count; bracketed areas indicate percentage of cells positive for strong vs weak staining with PI or Annexin V as indicated by the X axis. Shift in toward stronger Annexin V staining is indicated by median value (M) calculated from a linear axis (not shown). (C) Dot plot analysis of PI vs Annexin V co-staining of data set from (B), with percentage of cells in each quadrant indicated. Cells in upper right quadrant are positive for both PI and Annexin V, indicating onset of apoptosis.
doi:10.1371/journal.pone.0054595.g004

Cuconati A, Mills C, Goddard C, Zhang X, Yu W, et al. *PLoS ONE* **2013**, *8*, e54595.

Cell cycle

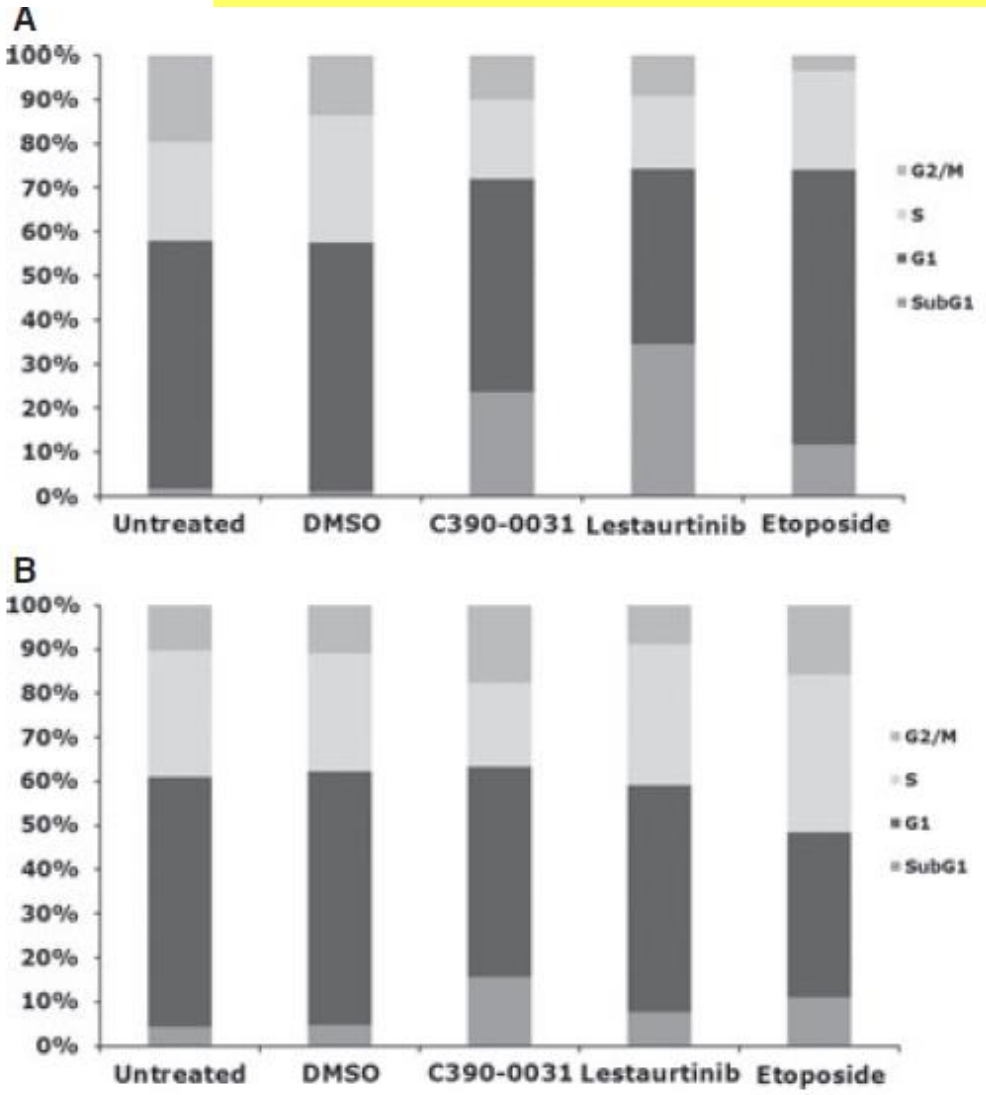


Figure 5: Flow cytometry data for SY5Y TrkA (A) and TrkB G7 (B) cells.

Leitão, A.; Schramm, A.; Eggert, A. *Chem. Biol. Drug Des.* **2013**, *82*, 233-241.

Cell death

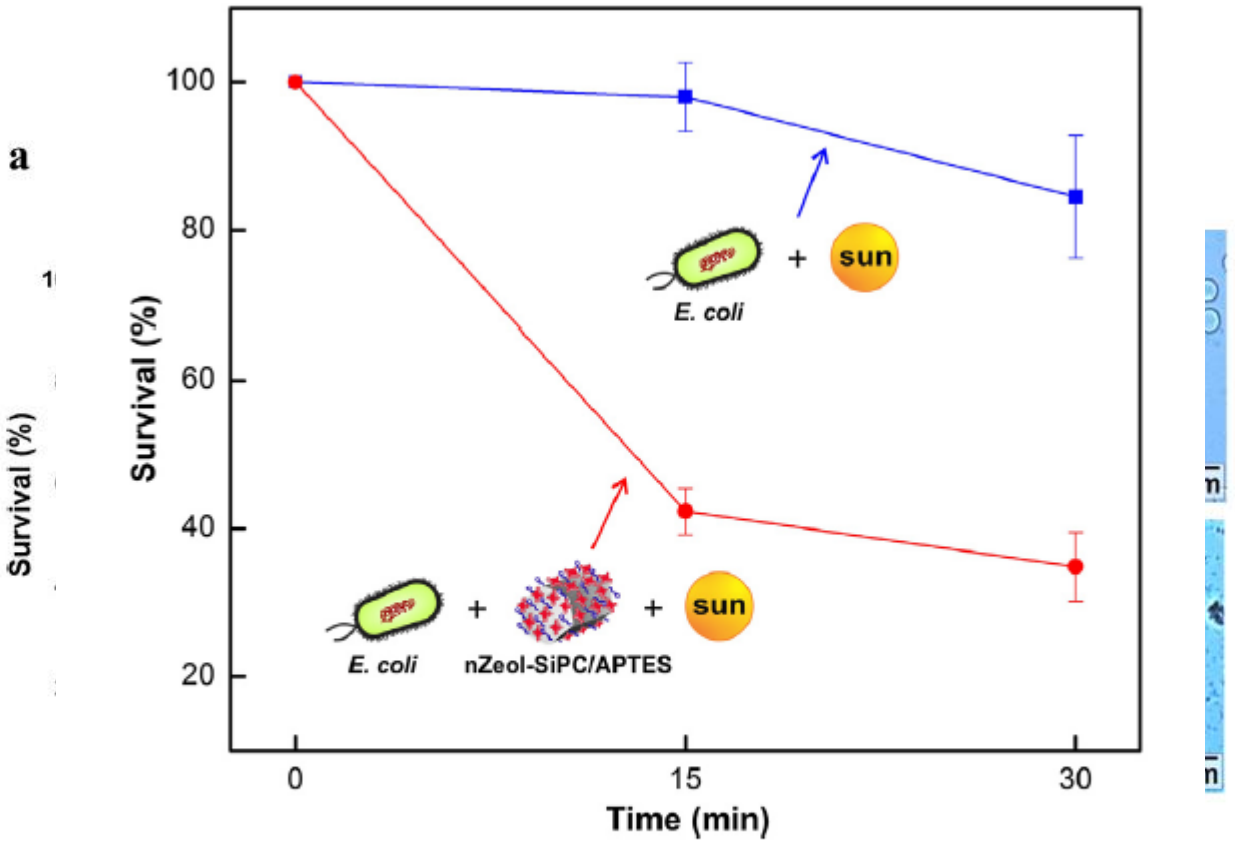


Fig. 2. (a) Cell viability assays after (c) 30 min irradiation in

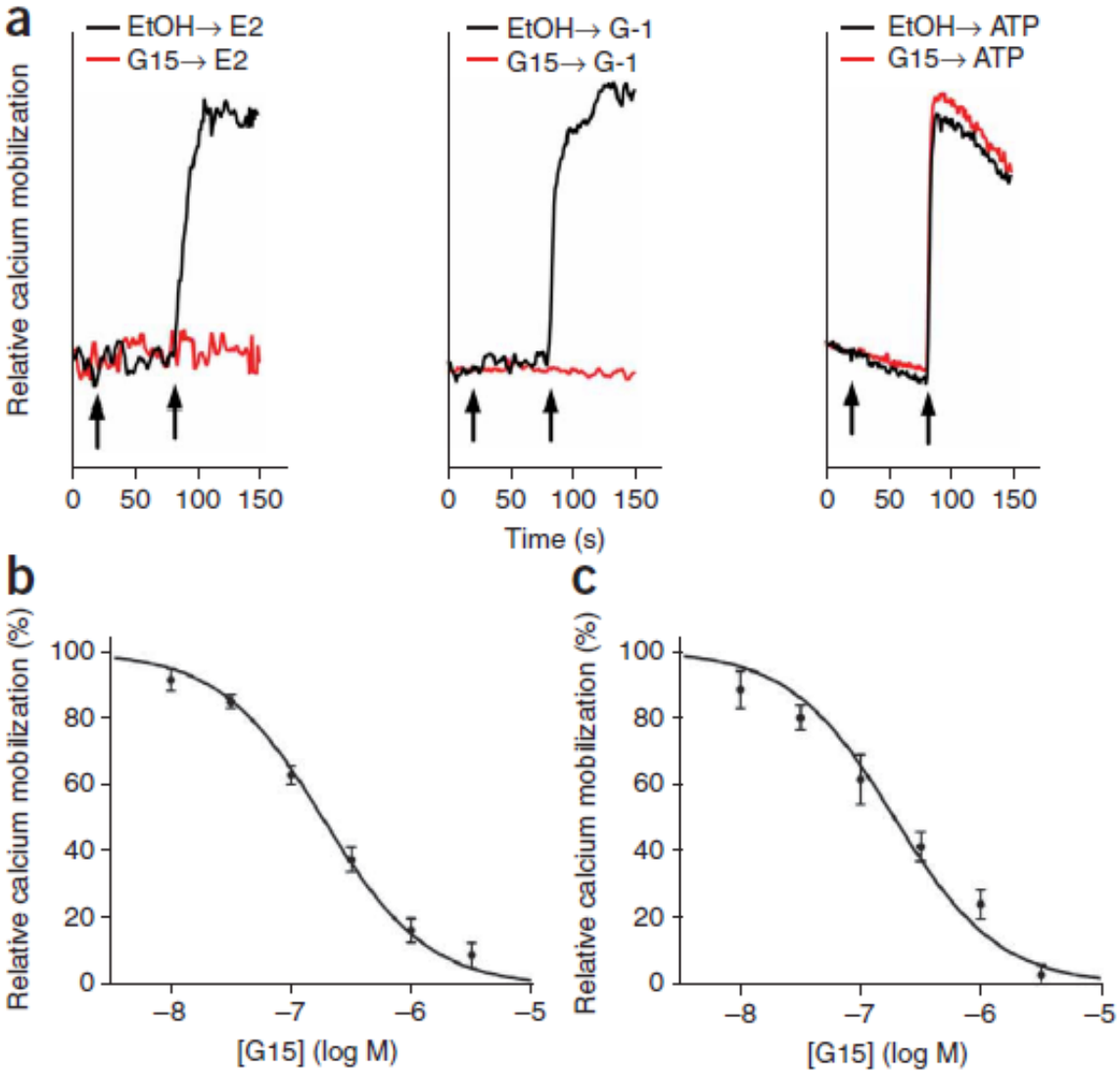
Fig. 4. Influence of the nanomaterial on the viability of *E. coli* cells in the first minutes of sunlight irradiation (1000 W m^{-2}). Experiments were done in triplicate in two different days.

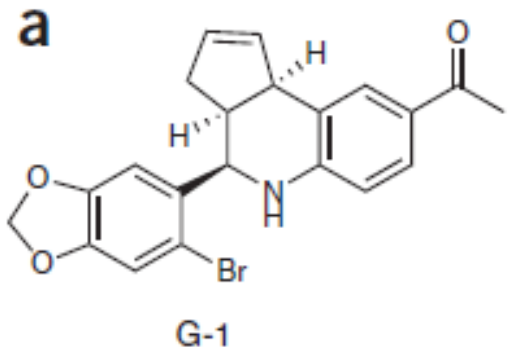
BALB/c cells before (b) and

s.

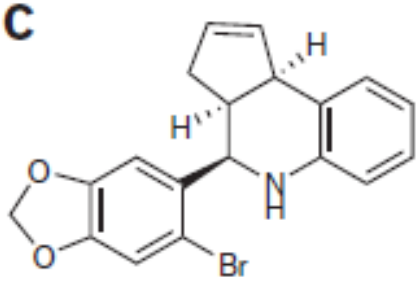
Leitao, R.C.F.; Trujillo, L.N.; Leitao, A.; Albuquerque, R.Q.
Solar Energy **2015**, *122*, 1117-1122.

Cell-signalling assay





G-1



G15

Dennis, M.K.; et al. *Nat. Chem. Biol.* **2009**, *5*, 421-427.

Cell-signalling assay

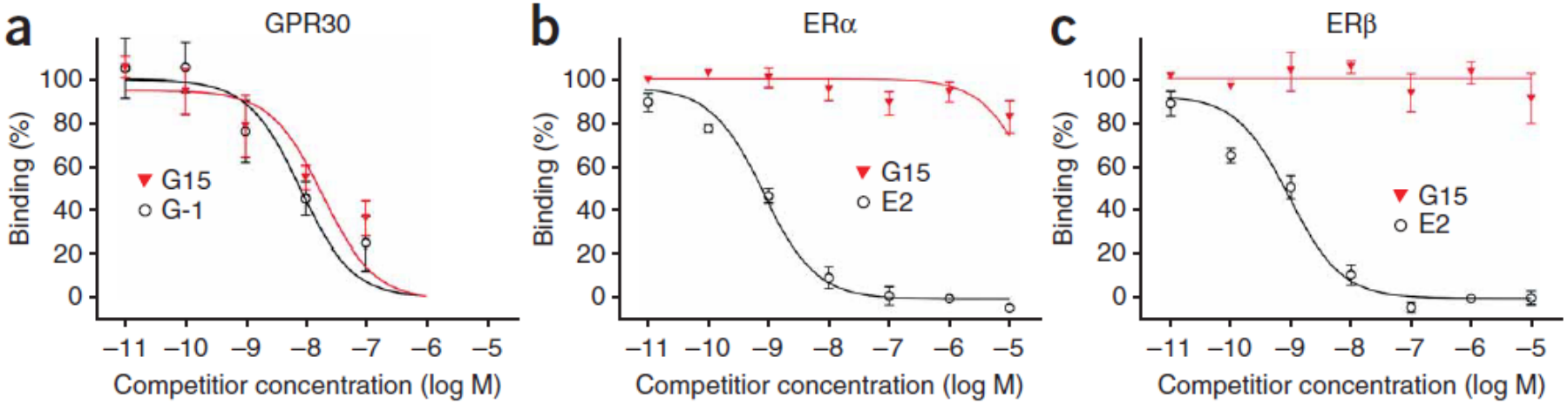


Figure 2 Ligand binding properties of G15. Ligand binding affinities of 17β-estradiol, G-1 and G15 for GPR30, ERα and ERβ. (**a**) For GPR30, Hec50 cells, which endogenously express GPR30 but do not express ERα or ERβ, were incubated with trace quantities of an iodinated G-1 derivative and the indicated concentration of either G-1 or G15 as competitor. (**b**,**c**) For ERα and ERβ, COS7 cells were transfected with either ERα-GFP (**b**) or ERβ-GFP (**c**). Competitive ligand binding assays were performed using 10 nM E2-Alexa633 and the indicated concentration of either 17β-estradiol (E2) or G15. Data indicate the mean ± s.e.m. of at least three separate experiments.

Dennis, M.K.; et al. *Nat. Chem. Biol.* **2009**, *5*, 421-427.