Electronic Supplementary Material (ESI) for Analyst
This journal is © The Royal Society of Chemistry 2012

Rapidly Quantifying Drug Sensitivity of Dispersed and Clumped Breast Cancer Cells by Mass Profiling

Jennifer Chun[1], Thomas A. Zangle[2], Theodora Kolarova[3], Richard S. Finn[3], Michael A. Teitell,[2,4] and Jason Reed[5].

[1]Bioengineering Interdepartmental Program, [2]Department of Pathology and Laboratory Medicine, [3]Department of Medicine, Division of Hematology/ Oncology, [4]Broad Stem Cell Research Center, Jonsson Comprehensive Cancer Center, and Molecular Biology Institute, David Geffen School of Medicine, Los Angeles, California, USA.

[5]Department of Physics, Virginia Commonwealth University, Richmond, VA, USA.

Corresponding Authors:

Jason Reed
Department of Physics
Virginia Commonwealth University
701 W. Grace Street
Richmond, VA 23284
Tel: (310) 206-6227
E-mail: jreed@cnsi.ucla.edu

Michael A. Teitell
Department of Pathology and Laboratory Medicine
David Geffen School of Medicine at UCLA
675 Charles Young Drive South, 4-762 MRL
Los Angeles, CA 90095
Tel: (310) 206-6754

E-mail: mteitell@ucla.edu

Supplementary Information

This document contains the Materials and Methods.

Electronic Supplementary Material (ESI) for Analyst
This journal is © The Royal Society of Chemistry 2012

Supplementary Methods

Cell lines and Culture

BT-474, SK-BR-3, MDA-MB-231, and MCF-7 breast cancer cell lines were obtained from American Type Culture Collection (Rockville, MD). All lines were maintained in RPMI 1640 (Cellgro; Manassas, VA) growth media supplemented with 10% fetal bovine serum (Omega; Tarzana, CA) and 1% penicillin, streptomycin, and L-glutamine.

Drug Treatment

Clinical grade trastuzumab (Herceptin) (Genentech; South San Francisco, CA) was used at 20ug/ml.

Proliferation Assays

5×10^4 cells were seeded into 12-well plates and allowed to adhere and grow for 2 days before beginning treatment. On days 0, 3, 5, and 7 of treatment with 20ug/ml Herceptin, cells were trypsinized and counted. To calculate the fold change, the doubling time was determined ($DT = t*[\log(2)/\log(Nt/N0)]$) for control and drug treated samples and the fold change taken as DTdrug/DTctrl. DT = doubling time, **t** = time, Nt = number of cells at time **t**, N0 = number of cells at time **t** = 0.

Confocal Fluorescent Imaging

Cells were seeded onto chambered coverglass and allowed to adhere overnight. Cells were fixed with 3.7% formaldehyde in 1 x PBS, pH 7.4, and permeablized in 0.1% Triton-X. Samples were then incubated with Alexa 568-Phalloidin actin stain (Invitrogen; Grand Island, NY) and DAPI. Confocal images were taken with a Zeiss LSM 780 CCD camera using Zen 2010 software.

This journal is © The Royal Society of Chemistry 2012

Interferometer

The live cell interferometer has been described previously. (1-3) The system consists of a modified Bruker NT9300 optical profiler (Bruker; Tucson, AZ) with a 20X 0.28NA Michelson interference objective. The Michelson interferometer contains a beam splitter, reference mirror, and compensating fluid cell to account for the optical path differences induced by the fluid surrounding the sample. The phase shifting (PSI) method was used to capture phase images of the cell samples. To enable multi-sample imaging, LCI employs a small motor to adjust the interferometer reference mirror for small differences in cover glass optical path length at each sample well.

In our experiments, the assay time of ~6h was much shorter than the average time to division (24 hr+) and only a few cell divisions were observed per sample per run. However, in principle all of the cells tracked from time zero in the assay can be observed up to and through division, and their resulting daughter cells tracked in kind, allowing both total mass accumulation and total cell number to be measured in longer duration assays.

Data Analysis

Image analysis was performed using a custom, multi-step program written in Matlab (Mathworks Inc., Natick, MA). The first step was a phase-unwrapping step to remove phase-errors (integer wavelength errors due to the ambiguity inherent in quantitative phase imaging) which remained after processing by the Goldstein phase unwrapping algorithm employed by Bruker Vision software (Bruker Nano Inc., Tuscon, AZ). This algorithm uses multiple random walks away from each pixel to remove integer wavelength jumps and non-physical excursions below background level. The second step is to segment each image into cell or colony objects using a combination of a local adaptive median filter and a watershed transform. Finally, objects identified by image segmentation were tracked using the particle tracking code adapted for Matlab by Daniel Blair and Eric Dufresne, based on the IDL particle tracking code developed by (2).

Electronic Supplementary Material (ESI) for Analyst
This journal is © The Royal Society of Chemistry 2012

Supplementary Refs

1. J. Reed, M. Frank, J. Troke, J. Schmit, S. Han, MA. Teitell, et al. *Nanotechnology*. 2008, **19**.

2. J. Reed, J. Troke, J. Schmit, S. Han, MA. Teitell, JK. Gimzewski. *ACS Nano*. 2008, **2**, 841-6.

3. J. Reed, J. Chun, T. Zangle, S. Kalim, J. Hong, S. Pefley, et al. *Biophys J*. 2011, **101**,1025-31.

4. JC. Crocker, DG. Grier. *Journal of Colloid and Interface Science*. 1996, **179**, 298-310.