Analyst

View Online / Journal Homepage
Dynamic Article Links

Cite this: DOI: 10.1039/c2an36058f

www.rsc.org/analyst

COMMUNICATION

Rapidly quantifying drug sensitivity of dispersed and clumped breast cancer cells by mass profiling†

Jennifer Chun,[a] Thomas A. Zangle,[b] Theodora Kolarova,[c] Richard S. Finn,[c] Michael A. Teitell*[bd] and Jason Reed*[e]

Received 2nd August 2012, Accepted 2nd October 2012
DOI: 10.1039/c2an36058f

Live cell mass profiling is a promising new approach for rapidly quantifying responses to therapeutic agents through picogram-scale changes in cell mass over time. A significant barrier in mass profiling is the inability of existing methods to handle pleomorphic cellular clusters and clumps, which are more commonly present in patient-derived samples or tissue cultures than are isolated single cells. Here we demonstrate automated Live Cell Interferometry (LCI) as a rapid and accurate quantifier of the sensitivity of single cell and colony-forming human breast cancer cell lines to the HER2-directed monoclonal antibody, trastuzumab (Herceptin). The relative sensitivities of small samples (<500 cells) of four breast cancer cell lines were determined tens-to-hundreds of times faster than is possible with traditional proliferation assays. These LCI advances in clustered sample assessment and speed open up the possibility for therapeutic response testing of patient-derived solid tumor samples, which are viable only for short periods *ex vivo* and likely to be in the form of cell aggregates and clusters.

Last year (2011) in the United States, 230 480 women were diagnosed with breast cancer and 39 520 women died from the disease.[1] The clinical course and outcome for this common malignancy remains variable despite therapies that are usually guided by a combination of clinical assessments, including tumor subtype, clinical grade and stage, and the expression of estrogen (ER), progesterone (PR), and amplified HER2 cell surface receptors.[2,3] Unfortunately, breast cancers expressing ER, PR, and/or amplified HER2 surface receptors do not always respond to therapies that target these receptor-linked pathways, making the analysis of expression of these biomarkers alone insufficient for treatment decisions. For example, breast cancers with amplified HER2 expression frequently do not respond to the humanized monoclonal antibody trastuzumab (Herceptin).[4] Furthermore, initially responsive, receptor positive tumors may become refractory to targeted therapies over time, which occurs for HER2-amplified breast cancers[5] and many other types of cancer as well.

A common feature, and failure, of current biomarker approaches in breast and other cancers is their typically static, snapshot-in-time surrogate nature that does not directly evaluate tumor cell responses to particular agents for specific patients. A superior approach, if one was available, could be to rapidly determine by real-time monitoring how a tumor responds to a battery of candidate therapies and then to pick the agent(s) that are most efficacious for that particular patient's disease. Real-time mass profiling of living cells is a new and reproducible biophysical measurement modality that may provide a superior approach. Live cell mass profiling is accomplished primarily using optical methods[6–9] or micro-fabricated sensors[10,11] and can yield rapid, continuous quantification of single-cell dry mass changes in a population of cells exposed to a changing external environment, including the detection of cellular responses to growth-inhibiting or cytotoxic agents.[6] Unfortunately, due to technical limitations, live cell mass profiling has been constrained to cell types that exist as spatially isolated single cells, such as bacteria, yeasts, and lymphocytes. This is a substantial roadblock to the effective use of mass profiling for solid tumor therapeutic response testing, such as in breast cancer. In general, dissected solid tumor samples, even when mechanically disaggregated, exist as a combination of small and large multi-cellular clumps, sheets, or spheres, rather than as purely single cells. Also, the agitation required to separate solid tumors into single cells may damage the cells and disrupt the cell–cell and cell–matrix interactions which may be crucial to maintenance of the malignant phenotype and required to assess agent responsiveness.[12,13]

Using a mass profiling approach termed automated Live Cell Interferometry (LCI) we have overcome this inhibitory barrier. With LCI we profiled the therapeutic response kinetics of breast cancers that grow in culture as both single cells and as large colonies or clusters. These organized colonies were up to 50 cells in size and in principle much larger colonies could be accurately measured. We

[a]*Bioengineering Interdepartmental Program, Los Angeles, California, USA*
[b]*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, USA. E-mail: mteitell@ucla.edu; Tel: +1 310 206-6754*
[c]*Department of Medicine, Division of Hematology/Oncology, Los Angeles, California, USA*
[d]*Broad Stem Cell Research Center, Jonsson Comprehensive Cancer Center, and Molecular Biology Institute, David Geffen School of Medicine, Los Angeles, California, USA*
[e]*Department of Physics, Virginia Commonwealth University, 701 W. Grace Street, Richmond, VA 23284, USA. E-mail: jcreed@vcu.edu; Tel: +1 310 206-6227*
† Electronic supplementary information (ESI) available. See DOI: 10.1039/c2an36058f

quantified the median growth rates of small populations of cells/colonies (<500 cells) from four breast cancer cell lines exposed to trastuzumab over the course of six hours, which allowed us to rapidly differentiate trastuzumab-sensitive from trastuzumab-resistant samples. In our study, LCI was performed without prior knowledge of which breast cancer lines expressed amplified HER2 surface receptor or at what level. Notably, LCI identified sensitive and resistant samples about a log-order more quickly than possible using traditional techniques, such as cell proliferation assays.[14] This improvement in speed and sensitivity opens the way for assessing sensitive *versus* refractory HER2-amplified breast cancer responses. It also provides a potential route for translation to the clinic, where often fragile, patient-derived cells are viable for short periods only and samples are most likely to be in the form of a heterogeneous mixture of single cells and aggregated clumps for many if not all solid tumor types.

Mass response profiling of four human breast cancer cell lines was performed over six hours using LCI with co-incubation of 20 μg ml^{-1} clinical grade trastuzumab. For each cell type, a control well containing only culture media and a treated well containing trastuzumab were measured simultaneously. Two of the lines are known to express HER2 at high levels (BT-474 and SK-BT-3), while the other two (MDA-MB-231 and MCF-7) exhibit near-normal, low-level expression of HER2.[14] Importantly, these cell lines grow with very different morphologies. MDA-MB-231 and SK-BR-3 lines grow as single-cells or in loose disaggregated clusters, whereas MCF-7 and BT-474 lines grow as dense multi-cellular colonies (Fig. 1). The relative scale between a single MCF-7 cell (mass ~5×10^2 pg) and a large colony (~22×10^3 pg) covers a 44-fold difference in mass (Fig. 2).

The mean normalized mass accumulation rates for each of the four samples are plotted at 30 min intervals in Fig. 3a. Little or no growth inhibitory effect was observed in the low HER2-expressing lines MDA-MB-231 and MCF-7 over the course of the experiment. In contrast, the growth rates of treated and control samples for HER2 amplified high-expressing lines, BT-474 and SK-BR-3, began to diverge at ~4 h of treatment and became highly significant by six

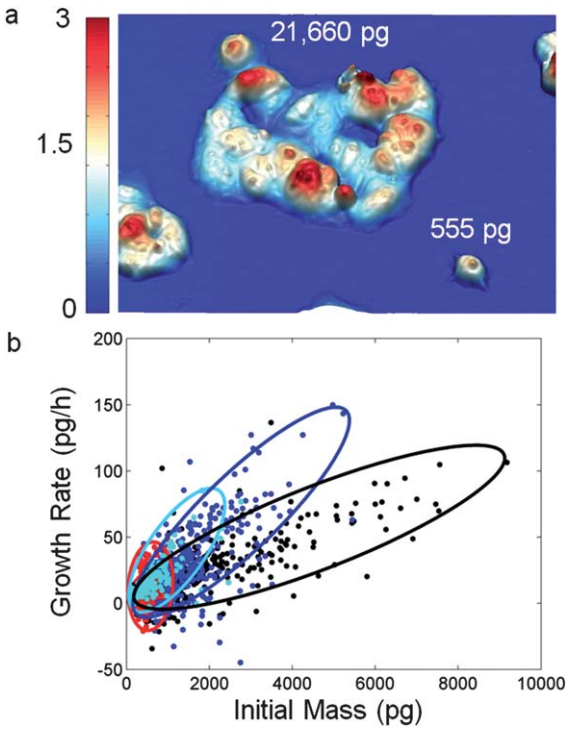

Fig. 2 The LCI enables simultaneous imaging of single cells and large colonies. (a) The LCI image shows a single MCF-7 cell (555 pg) alongside a multi-cellular MCF-7 colony (21 660 pg) consisting of approximately 52 cells. Color scale denotes mass density in pg μm^{-2}. (b) Composite scatter plot of growth rate *vs.* initial mass for all cell lines. MDA-MB-231 (red), MCF-7 (blue), SK-BR-3 (cyan), and BT-474 (black) are overlaid to show the range of cell and colony sizes and growth rates of the different lines. Colored ellipses indicate range for each population. Colony forming lines (MCF-7 and BT-474) span the range from a single cell to large, multi-cellular colonies. The 21 660 pg-mass colony in (a) is an outlier, included to illustrate the dynamic range of the LCI technique.

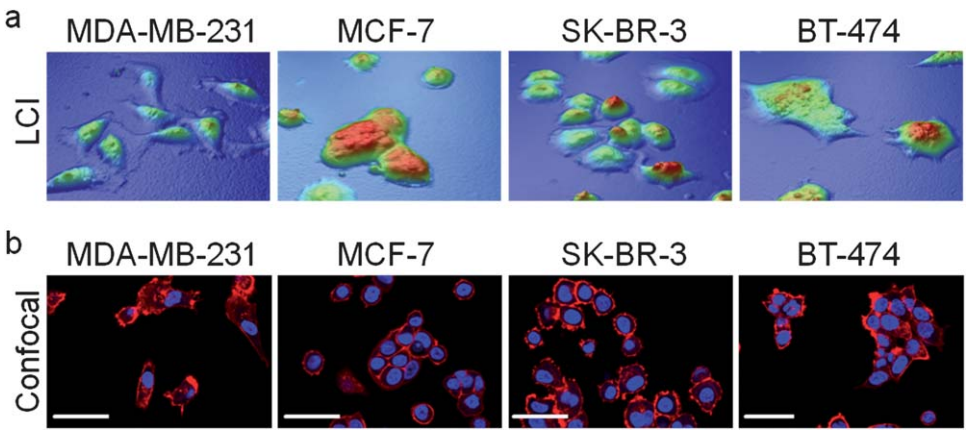

Fig. 1 Single cells, cell clusters, and dense colonies can be imaged and mass quantified by LCI. (a) Representative LCI images for each cell line. Single cells, loose clusters, and dense colonies are reproducibly quantified in real-time with LCI. Scale bars are 50 μm. (b) Representative confocal fluorescent images breast cancer cell lines. SK-BR-3 and MDA-MB-231 cell lines grow as single cells or in loose clusters, whereas the BT-474 and MCF-7 cell lines grow as dense multi-cellular colonies. Red is Alexa 568 phalloidin actin stain and blue is a DAPI nuclear stain. Note that images in panels (a) and (b) are not from the same sample.

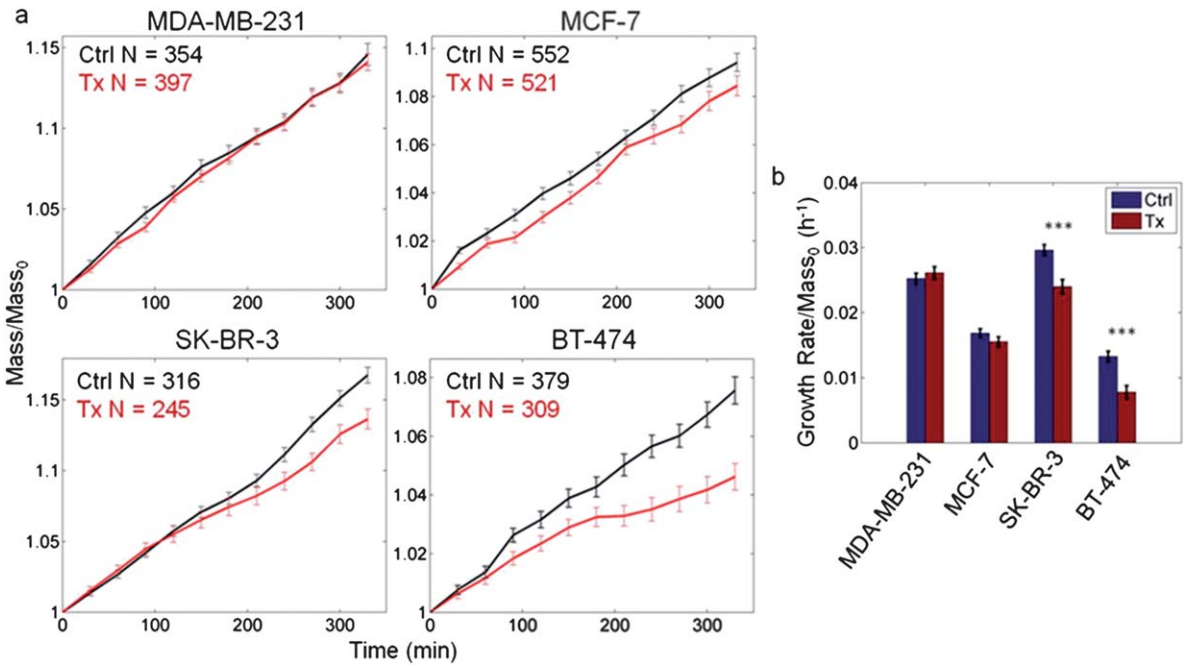

Fig. 3 LCI reproducibly quantifies breast cancer cell growth inhibition due to trastuzumab within 3–5 h. (a) Population mean normalized mass *versus* time plots for each cell line with 20 μg ml^{-1} trastuzumab treatment (error bars indicate Standard Error of the Mean, SEM). (b) Growth inhibition due to trastuzumab treatment becomes highly significant ($p < 0.001$) for the SK-BR-3 and BT-474 lines by 6 h. Hourly growth rates are calculated from a linear fit to mass accumulation data.

hours ($p < 0.001$) (Fig. 3b). Of the two sensitive lines, SK-BR-3 exists primarily as isolated single cells whereas BT-474 grows in small colonies, indicating that colony formation is not predictive or required for trastuzumab sensitivity or resistance.

We also compared the LCI-measured response to trastuzumab to that determined with traditional multi-day, cell counting growth-inhibition assays (see ESI†). In all four cases, the trastuzumab sensitivity measured by LCI over 6 h was concordant with that measured by cell counting over 3–7 days (Table 1), including cell lines' relative sensitivities to trastuzumab: BT-474 > SK-BR-3 > MCF7 > MDA-MB-231.

These results show that live cell mass quantification *via* LCI can rapidly and sensitively detect the biologic response to trastuzumab in breast cancer regardless of the physical configuration or association of the cells being examined. Other recently developed live cell mass profiling methods, such as MEMS micro-resonators, can measure the mass of single cells instantaneously with high accuracy, depending on the configuration of the micro-resonator.[10,11] A drawback to this approach is that in order to achieve sufficient sensitivity, the active area of the resonator must be on the order of microns or smaller, which makes the continuous measurement of mixtures of single cells and larger multi-cellular colonies, as occurs for most solid tumor types, very difficult.

In general, quantitative phase optical microscopy approaches, including LCI, possesses mass measurement precision and accuracy on par with MEMS-based approaches,[6] and their application to the study of cell clusters and clumps has been limited by practical difficulties associated with high throughput phase imaging, rather than by fundamental physical limitations. Converting a raw phase image into mass information can be computationally challenging, particularly in the case of clusters of cells and objects with complex internal structures and optical thicknesses that are large compared to the illumination wavelength.[15] In the work reported here we have overcome this barrier. We have developed a unique, automated algorithm to correct 'unwrapping' errors inherent in phase images of large objects, which is designed specifically to correct the type of errors generated in phase images of single cells and cell clusters (see ESI†). In LCI, this feature is combined with an automated cell tracking algorithm which identifies individual cells and colonies in the corrected image and tracks their masses through a time series stack of hundreds of frames. In practical terms, these improvements allow us to achieve high throughput and retain high mass resolution for multi-well, multi-hour time series experiments, where tens of thousands of images must be collected and analyzed, yielding thousands of mass *versus* time data points per run. Larger multi-well sample plates (*e.g.* 48 well format) can be used with the existing LCI hardware directly when the computational infrastructure used to analyze images in a timely fashion is increased proportionally (for example, by using a

Table 1 Trastuzumab (Herceptin) sensitivity for four breast cancer cell lines as determined by a 7 day proliferation assay (column 3), and by growth inhibition measured by LCI at six hours (column 4). Growth inhibition at 6 h is defined as: (pct. growth Ctrl − pct. growth Tx)/pct. growth Ctrl. Calculation of doubling time is described in ESI†

Cell line	HER2 status[a]	7 day doubling time Tx *vs.* Ctrl ± SEM	6 h growth inhibition Tx *vs.* Ctrl ± SEM
MDA-MB-231	HER2 low	0.99 ± 0.05-fold	3.2 ± 5.8%
MCF-7	HER2 low	1.13 ± 0.05-fold	10.3 ± 5.9%
SK-BR-3	HER2 high	1.45 ± 0.06-fold	18.5 ± 5.4%
BT-474	HER2 high	5.55 ± 0.99-fold	39.0 ± 8.5%

[a] HER2 status (column 2) was determined by O'Brien *et al.*[14]

workstation with more processor cores). The increased speed of analysis and quantification of therapeutic responses for aggregated cell clumps, sheets, and spheres provides exciting new opportunities for agent selection and prognosis in solid tumor therapy.

Acknowledgements

We thank K. Niazi and S. Rabizadeh (NantWorks and CNSI, UCLA) for helpful discussions. This project is supported by the Whitcome Training Program (to J.C.), NIH training grants (T32CA009120 and CA157940 to T.A.Z.), a UC Discovery Biotechnology Award (Bio07-10663 to M.A.T.), the NIH Roadmap for Medical Research Nanomedicine Initiative (PN2EY018228 to M.A.T.) and by NIGMS (R01GM094388 to J.R.).

References

1 R. Siegel, E. Ward, O. Brawley and A. Jemal, *Ca-Cancer J. Clin.*, 2011, **61**, 212–236.
2 M. Ignatiadis, C. Desmedt, C. Sotiriou, E. de Azambuja and M. Piccart, *Clin Cancer Res.*, 2009, **15**, 1848–1852.
3 M. Ignatiadis and C. Sotiriou, *Nat. Rev. Clin. Oncol.*, 2012, **9**, 12–14.
4 J. A. Wilken and N. J. Maihle, Primary Trastuzumab Resistance: New Tricks for an Old Drug, in *Toward Personalized Medicine for Cancer*, ed. D. Braaten, 2010, pp. 53–65.
5 R. Nahta and F. J. Esteva, *Breast Cancer Res.*, 2006, **8**, 215.
6 J. Reed, J. Chun, T. Zangle, S. Kalim, J. Hong and S. Pefley, *et al.*, *Biophys. J.*, 2011, **101**, 1025–1031.
7 J. Reed, J. Troke, J. Schmit, S. Han, M. A. Teitell and J. K. Gimzewski, *ACS Nano*, 2008, **2**, 841–846.
8 J. Haldar, Z. Wang, G. Popescu and Z. Liang, *IEEE Trans. Biomed. Eng.*, 2011, **58**, 2489.
9 B. Rappaz, E. Cano, T. Colomb, J. Kuhn and C. Depeursinge, *et al.*, *J. Biomed. Opt.*, 2009, **14**, 034049.
10 M. Godin, A. K. Bryan, T. P. Burg, K. Babcock and S. R. Manalis, *Appl. Phys. Lett.*, 2007, **91**, 123121.
11 K. Park, L. Millet, N. Kim, H. Li, X. Jin and G. Popescu, *et al.*, *Proc. Natl. Acad. Sci. U. S. A.*, 2010, **107**, 20691–20696.
12 B. E. Miller, F. R. Miller and G. H. Heppner, *Cancer Res.*, 1981, **41**, 4378–4381.
13 M. S. Wicha, G. Lowrie, E. Kohn, P. Bagavandoss and T. Mahn, *Proc. Natl. Acad. Sci. U. S. A.*, 1982, **79**, 3213–3217.
14 N. A. O'Brien, B. C. Browne, L. Chow, Y. H. Wang, C. Ginther and J. Arboleda, *et al.*, *Mol. Cancer Ther.*, 2010, **9**, 1489–1502.
15 D. Ghiglia and M. Pritt, *Two-Dimensional Phase Unwrapping: Theory, Algorithms, and Software*, John Wiley & Sons, 1998.