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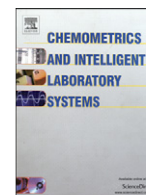

# Label free quantification of time evolving morphologies using time-lapse video microscopy enables identity control of cell lines and discovery of chemically induced differential activity in iso-genic cell line pairs

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ABSTRACT

Label free time-lapse video microscopy based monitoring of time evolving cell population morphology has potential to offer a simple and cost effective method for identity control of cell lines. Such morphology monitoring also has potential to offer discovery of chemically induced differential changes between pairs of cell lines of interest, for example where one in a pair of cell lines is normal/sensitive and the other malignant/resistant. A new simple algorithm, pixel histogram hierarchy comparison (PHHC), for comparison of time evolving morphologies (TEM) in phase contrast time-lapse microscopy movies was applied to a set of 10 different cell lines and three different iso-genic colon cancer cell line pairs, each pair being genetically identical except for a single mutation. PHHC quantifies differences in morphology by comparing pixel histogram intensities at six different resolutions. Unsupervised clustering and machine learning based classification methods were found to accurately identify cell lines, including their respective iso-genic variants, through time-evolving morphology. Using this experimental setting, drugs with differential activity in iso-genic cell line pairs were likewise identified. Thus, this is a cost effective and expedient alternative to conventional molecular profiling techniques and might be useful as part of the quality control in research incorporating cell line models, e.g. in any cell/tumor biology or toxicology project involving drug/agent differential activity in pairs of cell line models.

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## 1. Introduction

In cancer research cell lines are widely used as models for studying key phenomena such as cell proliferation, development or death. Similarly, in toxicology research cell line models are used to study whether or how various chemical compounds in isolation or chemical mixtures induce detrimental perturbations in vitro. Recently there has been a surge in the number of available cell lines and the range of cancer types they represent. However, cell line cross-contamination, genetic drift, microbial infection and manual human mistakes are well known factors behind poor reproducibility of findings from cell line models. Therefore there is an increasing demand for fast and simple assays that afford expedient characterization of any unknown cell line to one of among a set of possible alternatives (authentication). Confirmation of cell line authenticity prior to embarking on a project one can prevent the generation of invalid and thus misleading scientific information, thereby safeguarding time and funding. Indeed there are several cases of retracted publications owing to cross-contamination of cells [1,2]. Because of this history many journals now request cell line validation prior to publication [3].

A good cell culturing practice is instrumental to ensuring consistent and reproducible results within cell biology broadly. For instance, long term culturing should be avoided because over-passaged cells have the tendency to undergo genetic drift, leading to phenotypic and genotypic changes [4–6]. Microbial infection, which can severely impact cell phenotype, is another important issue. It has been estimated that 15–30% of cell cultures currently used are infected with mycoplasma [7]. Several methods for the detection of intra- and interspecies contaminations are reported, all relying on molecular techniques [8–10]. These methods are very accurate but often protracted, elaborate and expensive so there is room for label free methods for cell line authentication, which are sufficiently fast, simple and inexpensive to be used on a daily basis in any cell biology laboratory or any cancer or toxicology project involving cell culturing.

*Abbreviations:* TEM, time evolving morphologies; PHHC, pixel histogram hierarchy comparison; OMB-DHC, Omnibus multi-branching divisive hierarchical clustering

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One example of such a novel label free technology was recently introduced by Irelan et al., enabling rapid and quantitative assessment of cell line quality, identity, and functionality [11]. This method exploits changes in electrical impedance in the cell population studied as measured by a dedicated instrument with ability to record subtle changes in electrical impedance using tailor made microtiter plates [11]. This is an attractive technique, but relatively expensive since the accurate measurement of changes in electrical impedance needs a dedicated instrument and tailored microtiter plates. Label free quantification of TEM differences between in vitro cell line models, using a generic time lapse video microscope combined with properly designed image/signal processing algorithms, might provide many advantages and deserves further exploration.

Substantial morphological divergence, as a function of time between two supposedly identical cell populations, should be considered as a strong warning that one of the populations either is contaminated, subject to a genetic drift, infected by a microbial agent or simply mislabeled. Similarly, it would be of pharmacological and/or toxicological interest to identify induced change(s) in morphology in response to a chemical cue (relative to unexposed cells of the same kind), which differ from that of another similarly treated cell line.

For example, it is of potential interest to identify chemical agents that increase the fraction of elongated cells in one among a pair of indicator cell lines, while at the same time decreasing the number of cells containing visible vesicles exclusively in the other counterpart. Assuming that one cell line of a matched pair represents normal/healthy phenotype and the other line a diseased state, a TEM disparity between accordingly induced changes would suggest selective test agent bioactivity and thus revealing a potential inroad to drug development or, alternatively, a hazardous chemical with detrimental action on normal/healthy cells or accelerating a disease. Chemical agents that induce cellular phenotypes along different morphological directions are presumably also modulating (patho-)physiology of those cells along distinct routes that might either provide therapeutic opportunities or disclose undesirable effects. Cellular models of various mammalian tissues are another inroad to TEM-based characterization of differential response to drugs or agents; tissue/organ specific actions arguably carry high potential pharmacological and toxicological relevance. In order to demonstrate the potential application of such morphological analyses, in this work we present results from a simple image processing method denoted pixel hierarchy histogram comparison (PHHC) to 10 different cancer cell lines as well as to three iso-genic cell line pairs. Thus the potential of PHHC for use in quality control procedures as well as in heterogeneity analyses of iso-genic cell lines for identification of chemicals (drugs) with differential activity was explored.

First, a set of 10 cancer cell lines of different origins was monitored using a phase contrast video-microscopy system (IncuCyte HD) and their TEMs were extracted and compared using our new PHHC computational method. Then, unsupervised clustering and supervised machine learning based classification methods were applied to the assignment of identity, based on TEM recording. The results were encouraging, suggesting that the TEM profiles are sufficiently information rich for basic identity control. In the second analysis three iso-genic cell line pairs, differing pairwise with respect to a single allele variant, were compared to identify differences within such pairs using their respective TEMs. Finally, the iso-genic cell lines were exposed to a library of 1266 pharmacologically active compounds. Here, the objective was to identify compounds with an ability to induce substantially different time-evolving morphologies between the iso-genic cell line pairs. Such compounds are of potential pharmacological interest because their differential effects would be related to a specific single allele against an otherwise identical genetic background. Several compounds that induced different morphological changes in pairs of iso-genic cell lines were identified in this screening campaign.

## 2. Materials and methods

### 2.1. Cell cultures for quality control

Cells of 10 different cell lines together with authentication were purchased from American Type Culture collection (ATCC, Rockville, MD, USA) (Table 1). Cell culture reagents were purchased from Sigma-Aldrich, unless otherwise stated. In the first analysis cells were seeded in 384 well plates at a density of 2500 cells per well and the experiment was performed three times (N = 3). Each cell line was seeded in 8 separate wells on the same plate. In the second analysis (used for supervised machine learning) each cell line was seeded in 80 wells on a separate microtiter plate.

### 2.2. Iso-genic cell line pair

Three pairs of iso-genic cell lines together with authentication were obtained from Horizon Discovery Ltd., United Kingdom. The pairs employed are human colon carcinoma cell line HCT116 and its variant HCT116KRASwt/− with a KRAS allele knocked out, human colorectal adenocarcinoma cell line DLD-1 and its variant DLD-1KRASwt/− with KRAS allele knocked out and human rectal carcinoma cell line RKO and its variant RKOBRAFwt/− with BRAF V600E allele knocked out. All cells were grown in McCoy's 5A medium (cat# M8403) supplemented with 10% heat inactivated fetal calf serum (cat# F6131), 2 mM glutamine (cat# G7513), 100 μg/ml streptomycin and 100 U/ml penicillin (cat# P0781) at 37 °C in humidified air containing 5% $CO_2$. A flow chart displaying the methodology of comparing perturbed iso-genic cell lines is shown in Fig. 1.

### 2.3. Time lapse video microscopy based monitoring

The plates were incubated and monitored at 37 °C for 72 h in our IncuCyte HD (Essen BioScience Inc., Ann Arbor, MI, USA) which is an incubator equipped with a fully automated phase contrast microscope and images were taken every 6 h thus resulting in 12 images per movie. The microscope is equipped with a 20× objective with the ability of imaging high definition and high quality phase contrast images (1024 ∗ 1280 pixels) that provide morphological information not found with fluorescent-only imaging. HD imaging is insensitive to focus and illumination aberrations caused by fluid meniscus. For the video microscopy screening the 3 iso-genic cell line pairs were seeded separately at a density of 2500 cells per well, using a pipetting robot Precision 2000 (BioTek Instruments Inc., Winooski, VT) in 384-well microtiter plates (Nunc). Four 384 well plates per cell line were used with each plate having three columns without drugs (columns 12, 13 and 24) that served as controls (48 wells) and 1 column with a culture medium only (column 1) that served as blank (16 wells). Four plates were imaged simultaneously with the ability of the IncuCyte to hold 6 plates at the same time. For a 384 well plate only one field per well can be

**Table 1**
Cell lines used in the study of quality control.

| Cell line | Origin |
|---|---|
| MCF-7 | Breast cancer |
| hTERT/TERT1 | Renal cortex |
| CRL 1676 | Melanoma |
| HTB-63 | Melanoma |
| DF mel | Melanoma |
| MEL-JUSO | Melanoma |
| HCT 116 | Colon carcinoma |
| DLD1 | Colorectal adenocarcinoma |
| RKO | Rectal carcinoma |
| RPTEC | Kidney |

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