Video Article

Comparison of Three Different Methods for Determining Cell Proliferation in Breast Cancer Cell Lines

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Abstract

Measuring cell proliferation can be performed by a number of different methods, each with varying levels of sensitivity, reproducibility and compatibility with high-throughput formatting. This protocol describes the use of three different methods for measuring cell proliferation *in vitro* including conventional hemocytometer counting chamber, a luminescence-based assay that utilizes the change in the metabolic activity of viable cells as a measure of the relative number of cells, and a multi-mode cell imager that measures cell number using a counting algorithm. Each method presents its own advantages and disadvantages for the measurement of cell proliferation, including time, cost and high-throughput compatibility. This protocol demonstrates that each method could accurately measure cell proliferation over time, and was sensitive to detect growth at differing cellular densities. Additionally, measurement of cell proliferation using a cell imager was able to provide further information such as morphology, confluence and allowed for a continual monitoring of cell proliferation over time. In conclusion, each method is capable of measuring cell proliferation, but the chosen method is user-dependent.

Video Link

The video component of this article can be found at https://www.jove.com/video/54350/

Introduction

The tumor suppressor gene, p53, is an essential regulator of a number of cellular processes, including cell cycle arrest, apoptosis and senescence[1]. It is responsible for maintaining genomic stability, and is therefore crucial for maintaining the balance of cell death and cell growth. Mutations in p53 are common in cancer and are the major cause of p53 inactivation leading to uncontrolled cancer cell proliferation[2]. Interestingly, mutations in p53 only account for approximately 25% of breast cancers[3], suggesting that other mechanisms are responsible for the loss of p53 function. The recently discovered p53 isoforms have been shown to be overexpressed in a number of human cancers, and can modulate p53 function[4,5]. We have previously shown that the p53 isoform, Δ40p53, is the most highly expressed isoform in breast cancer, and is significantly upregulated in breast cancer cells, when compared to normal adjacent tissue[6]. Following this, we stably transduced the human breast cancer cell line MCF-7 to overexpress Δ40p53 using the LeGO-iG2-puro+ vector (GFP+)[7]. These cells were used to investigate if high Δ40p53 expression increases cell proliferation rates in breast cancer cells.

There are many direct and indirect methods of measuring cell proliferation of cultured cells *in vitro*[8,9]. These can be performed either as continuous measurements over time, or as endpoint assays[10]. Conventional methods are still useful, such as cell counting using a hemocytometer. This assay is a low cost and direct measure of the cell number, but it does rely on large cell counts and highly skilled training to minimize error and large standard deviations from the counts. The need to perform measurements compatible with high-throughput formats has led to the development of multiwell-plate assays. These luminescence-based assays measure cell numbers based on a luminescent signal that is proportional to the metabolic activity of the cell[11,12]. More recently, the introduction of high content imaging platforms has allowed for new tools which monitor cell proliferation while providing quantitative and qualitative phenotypic data collection, and includes a variety of systems[13]. All of these methods provide avenues to measure cell growth, either by continuous measurement or endpoint assays, and each possess a range of advantages and disadvantages with regards to sensitivity, throughput of sample numbers, and cell information, all of which can be weighed accordingly depending on the research question.

This protocol describes three different methods for measuring cell proliferation *in vitro*, with each method utilizing different ranges of sensitivity, reproducibility and multi-well plate formats. This protocol aimed to compare the use of a hemocytometer counting chamber, a luminescence-based cell viability assay, and cell imager, in the measurement of cell proliferation over a 96 hour time course. To do this, the growth of vector-transduced cells (MCF-7-LeGO) was compared to cells transduced to overexpress Δ40p53 (MCF-7-Δ40p53), using three different cell densities. Cell proliferation was measured every 24 hr for up to 96 hr. Each method was found to have its own advantages and disadvantages, and depending on the aim of the experiment, each still is a valuable method for providing information on the rate of proliferation.

Protocol

1. Preparing Cells for Proliferation Assays

Note: Prepare the two cell lines in the same manner and seed in the same format for each method to be analyzed.

1. Grow MCF-7-LeGO and MCF-7-Δ40p53 [7] cells to 75-80% confluence in T 75 cm^2 tissue culture flasks using phenol-red free Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 200 mM L-glutamine, 2 µg/ml insulin and 1 µg/ml Puromycin at 37 °C with 5% CO_2. Handle cells in a sterile biosafety cabinet class II.
   Note: The amount of time it takes to grow the cells to confluence varies depending on the seeding dilution. In preparation for plating the cells for the proliferation assays, seed the cells at a 1:3 dilution in supplemented DMEM and maintain in normal growth conditions for 3 days until 75-80% confluency.
2. To remove the cells from the flask, pour off the media into a waste container. Immediately wash the cell layer with 2 ml of pre-warmed 2x trypsin. Aspirate trypsin. Add a further 2 ml of pre-warmed trypsin to the cell layer and place in an incubator for 5 min at 37 °C with 5% CO_2.
   1. Once the cells detach, wash the cells with 10 ml warmed fresh supplemented DMEM. Transfer the cell suspension to a sterile 15 ml tube and centrifuge at 491 x g for 5 min at RT. Carefully aspirate and dispose of the supernatant.
3. Resuspend the cell pellet in 5 ml of fresh supplemented DMEM. Perform a cell count to determine the correct density to seed the cells in 96-well plates.
   1. Dilute 100 µl of the cell suspension in 900 µl 1x Dulbecco's Phosphate Buffered Saline (DPBS). Place a new 60 µm sensor onto the automated cell counter. Hold down the plunger and submerge the sensor in the diluted cell suspension. Slowly release the plunger on the cell counter. Remove the sensor from the cell counter when completed.
      Note: The cell count will be displayed on the cell counter in cells/ml.
4. Seed cells at a final volume of 100 µl (in DMEM) in a 96-well plate at ~20% confluency to allow room for cell growth and measurement of proliferation over 3-5 days (see **Table 1**). Seed all cell lines in triplicate.
   Note: For this experiment, three different cell densities were assessed to determine the optimal cell density. The required cell numbers are summarized in **Table 1**. Seed cells at a lower density if measurement of more long-term growth is required.
   1. For the hemocytometer and luminescence-based assays, prepare one plate per time point (*i.e.* 4 plates per assay). For the cell imager assay, prepare only one plate for the duration of the experiment.
5. Maintain cells at 37 °C with 5% CO_2 for 24 hr.

2. Determining Cell Count Using a Hemocytometer

1. Pre-warm both medium and trypsin to 37 °C in a cell culture incubator, oven or water bath. Aspirate media from cells into a waste container, wash once with 30 µl of 2x trypsin, and aspirate the trypsin.
2. Wash cells again with 30 µl of 2x trypsin, and incubate for 5 minutes at 37 °C. Gently tap edge of plate to dislodge cells from the plate. Add 50 µl of supplemented DMEM and mix cells by pipetting until the cells form a single cell suspension.
3. Prepare hemocytometer by cleaning surface and glass cover with 70% ethanol.
4. Place the glass cover-slip over counting chambers and affix until 'Newton's refraction rings' can be seen between the cover-slip edges and the hemocytometer.
   Note: These indicate that the cover-slip has correctly adhered to the hemocytometer.
5. Gently pipette 20 µl of cell suspension under the cover-slip, filling the counting chamber by capillary motion. Place hemocytometer under 10x magnification of a microscope and visualize the counting chambers in the grid layout.
6. Count cells in the 4 outer squares of the grid layout. To improve accuracy, repeat counts of additional outer squares if required, or until the count has reached 70 - 100 cells[14,15].
   1. Calculate cell concentration per ml as follows:Average cell count per square x dilution factor (if used) x 10^4
7. Repeat steps 2.1-2.8 every 24 hr for 96 hr post-seeding.

3. Determining Cell Proliferation Using a Luminescence-based Assay

Note: This is an endpoint measurement. Once the reagent is added to the cells, the plate can only be quantified once.

1. Thaw luminescence reagent in a 22 °C water bath for 30 min.
2. Gently mix reagent by inverting the bottle to obtain a homogenous mix.
3. Equilibrate one plate of seeded cells (from step 1.5) at RT for 30 min.
4. Add 100 µl of luminescence reagent to each well. Mix contents on an orbital shaker for 2 min. Allow plate to incubate for 10 min at RT.
5. Setting up a luminescence experiment using the multi-mode plate reader software.
   1. Turn on multi-mode plate reader and open the software. In the 'task manager', select 'experiments' and 'create new'.Select 'file' from side toolbar, and under 'protocol' tab, select 'procedure'.
   2. Select 'Grenier 96 flat bottom' plate type, and check the 'use lid' box. Select 'read' action under 'read method' box. Select 'luminescence' detection method. Click 'OK'.
   3. In the read step box, select the wells to be scanned under the 'full plate' tab, and select wells to act as blank wells.

4. Set the filter set to empty filter (*e.g.*, plug, Em: hole, mirror: none). Set the gain to 135, with an integration time of 0.5 sec per well and a read height of 6.5 mm. Click 'OK' to save settings for the luminescence read.

6. Performing a Luminescent Read
    1. Eject plate holder by selecting 'instrument control' tab and click on 'plate out'.Place 96-well plate onto the plate holder, ensuring the lid is on. Close the plate holder by clicking on 'plate in' under 'instrument control' tab. Click on 'read now' from toolbar to perform luminescent read.
    2. Export the relative luminescent unit (RLU) measurements for each well to a spreadsheet for further analysis.

7. Repeat steps 3.1-3.6 to record proliferation every 24 hr up to 96 hr after seeding cells.

4. Determining Cell Count Using a Cell Imager

1. Setting up a cell imaging experiment using the multi-mode cell imager software
    1. Turn on multi-mode cell imager and open the software.
    2. In the 'task manager', select 'experiments' tab and 'create new'.On the 'file' toolbar, click on 'protocol' tab and open 'procedure' tab.Select 'set temperature' action. Set incubator to 'on' and set temperature to 37 °C and check 'preheat' before continuing with next step' box. Click 'OK' to save settings.
    3. Select 'read' action. Click on 'image' detection method, select 'endpoint/kinetic' read type and 'filters' optics type. Click 'OK'.Click on 'full plate' tab and select wells on the plate to be imaged. Click 'OK' to save the settings.
    4. Select the 2.5X objective from the drop down 'objectives' options. Under 'channels' tab, select two channels: GFP 469, 525 and Bright Field. Check 'auto' exposure for both channels and select the auto-exposure well.
    5. To determine auto focus settings, select 'auto-focus' and click on 'options'. For the auto-focus options, select the 'scan and then auto focus' method. Click 'OK' to save settings.
    6. Set the horizontal and vertical offset from center of well (µm) to 0.Select to scan multiple images per well in a 3 x 2 montage, with horizontal spacing (µm) = 2,881 and vertical spacing (µm) = 2,127. Click 'OK' to save procedure settings.
    Note: See **Table 2** for read parameters.

2. Performing Read Experiment on Selected Plate
    1. Click on the 'instrument control' tab and select the 'plate out' function.Place 96-well plate in plate holder, ensuring the lid is on. Under the 'instrument control' tab, select the 'plate in' function. Select 'read now' from plate tab. Repeat read every 24 hr up to 96 hr post-seeding.

3. Analysis of Cell Count Using the Cell Imager Software.
    1. To analyze an image, click on the 'data' tab on the imaged plate, select 'picture [GFP 469, 525 + Bright field]'. Double click on an imaged well.
    2. Click on a loaded image. Select 'analyze' and select a single image of the montage to be analyzed. Click 'OK'.Check the 'GFP' channel only, and set parameters as outlined in **Table 3**. Click 'START' to apply parameters to the imaged cells.
    3. Observe the cell counting mask placed over the imaged cells, which is used to determine the cell count per image. Click 'apply changes' and maintain these settings for each plate imaged throughout the experiment.
    4. Once back at the imaged plate, click on the 'data' drop down menu and select 'cell count'. This will generate the total cell count per well.
    5. Export all data to a spreadsheet by clicking on the 'export' tab for further analysis of the cell counts per well.

Representative Results

To study different methods of measuring the proliferation of cultured cells, the cell proliferation of MCF-7-Δ40p53 transduced cells was compared to the non-transduced MCF-7-LeGO breast cancer cell line. The three methods that were compared – the conventional hemocytometer method, cell viability luminescence assay, and cell imaging analysis- are outlined in the schematic diagram (**Figure 1**). Each method has advantages and disadvantages to accurately measure cell counting over time, and the most effective method depends on the endpoint requirements of the experiment and the information that can be acquired for a cell population.

The growth of MCF-7-LeGO and MCF-7- Δ40p53 cells were measured after 24 hr for 4 days. As shown in **Figure 1**, the two cell lines were seeded at three different cell densities (1.5 x 10^3; 3 x 10^3; 5 x 10^3 cells/well) and cell counting was performed 24, 48, 72 and 96 hr after seeding. Each method demonstrated increased cell proliferation, using a hemocytometer, a luminescence-based assay, and a cell imager (**Figures 2a, b** and **c**, respectively) over 96 hr. The greatest increase in cell proliferation was seen at the highest cell density (5 x 10^3 cells), and also showed the most reproducible results at each time point, suggesting this to be the optimum cell density for measuring proliferation in these cells. There was no significant difference in cell proliferation between the cell lines.

The measurement of cell proliferation between the different methods was compared by a linear regression analysis[6] (**Figure 3; Table 4**). There was a significant correlation between each of the different methods tested, when comparing cell proliferation at all of the cell densities in both cell lines (**Figure 3a** and **b**). The strongest correlation was observed between the comparison of the luminescence-based assay, and the cell imager (R^2 = 0.8899, p ≤0.0001; R^2 = 0.9805, p ≤0.0001, **Figure 3a** and **b**, respectively).

A visual representation of cell proliferation using the cell imager is shown (**Figure 4**). As this method uses continual measurements of a single plate, the cells can be imaged every 24 hr until the cells reach close to 100% confluence. As shown in **Figure 2**, the growth rates of the MCF-7-LeGO and MCF-7-Δ40p53 cells were comparable across all time points. This method provides useful cellular information, including being able to visually monitor cell growth across multiple days, and compare cell size and cell morphology between different cell lines.

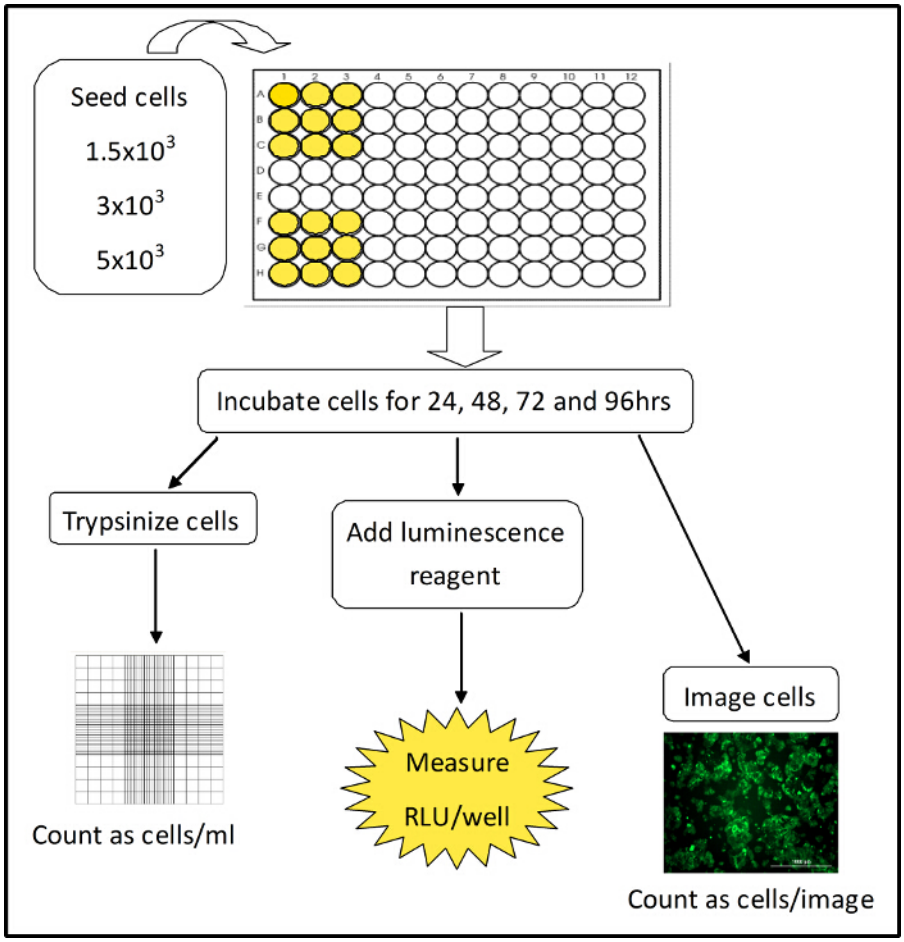

Figure 1: A Schematic Diagram of the Three Different Methods Measuring Cell Proliferation. Cells were seeded at three different cell densities into multiple 96-well plates and incubated at 37 °C with 5% CO_2 for up to 96 hr. Every 24 hr, cell proliferation was measured by either using a hemocytometer, a luminescence-based assay or cell imaging. Please click here to view a larger version of this figure.

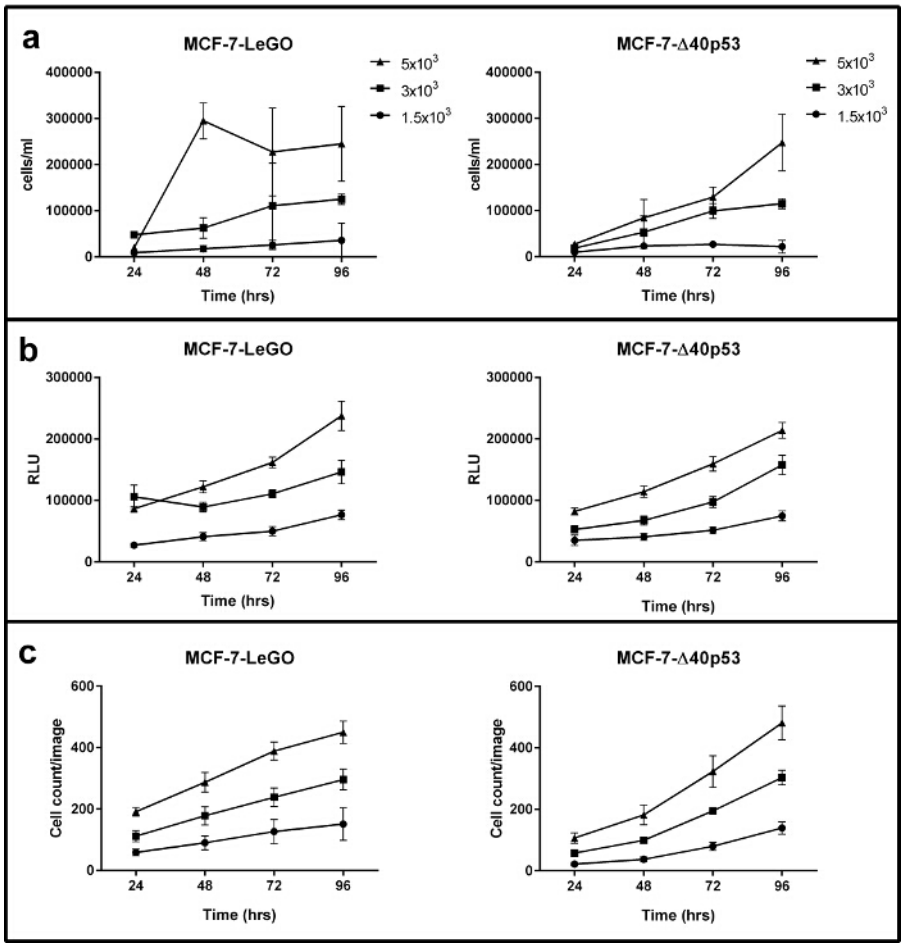

Figure 2: Cell Proliferation Measurements after 96 hr. Cell counts were measured every 24 hr for 96 hr in MCF-7-LeGO and MCF-7-Δ40p53 cells seeded at three different cell densities. Cell counts were measured using a (**a**) hemocytometer in cells/ml, (**b**) using a luminescence-based assay in relative luminescent units (RLU), or (**c**) a continuous cell count using a cell imager in cells/image. Conditions for the cell imager are shown in Table 2. All experiments represent the mean of three independent experiments, ± the S.D. Please click here to view a larger version of this figure.

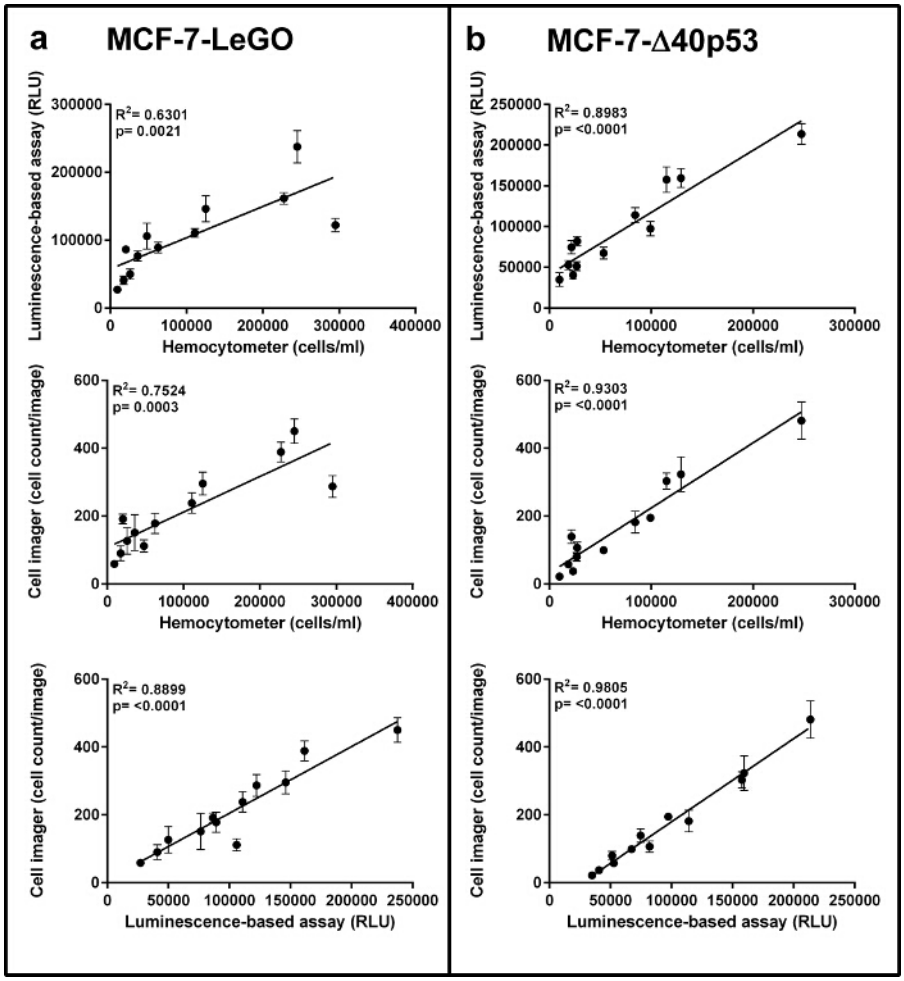

Figure 3: Comparison of Three Different Methods Measuring Cell Proliferation in Breast Cancer Cell Lines. A linear regression analysis was performed to compare the correlative relationships between the different methods examined for measuring cell proliferation in (**a**) MCF-7-LeGO cells and (**b**) MCF-7-Δ40p53 cells. A Pearson's correlation coefficient was calculated and the significance was determined ($p < 0.05$) between the different methods measuring cell proliferation. All results represent the mean ± the S.D of three independent experiments. Please click here to view a larger version of this figure.

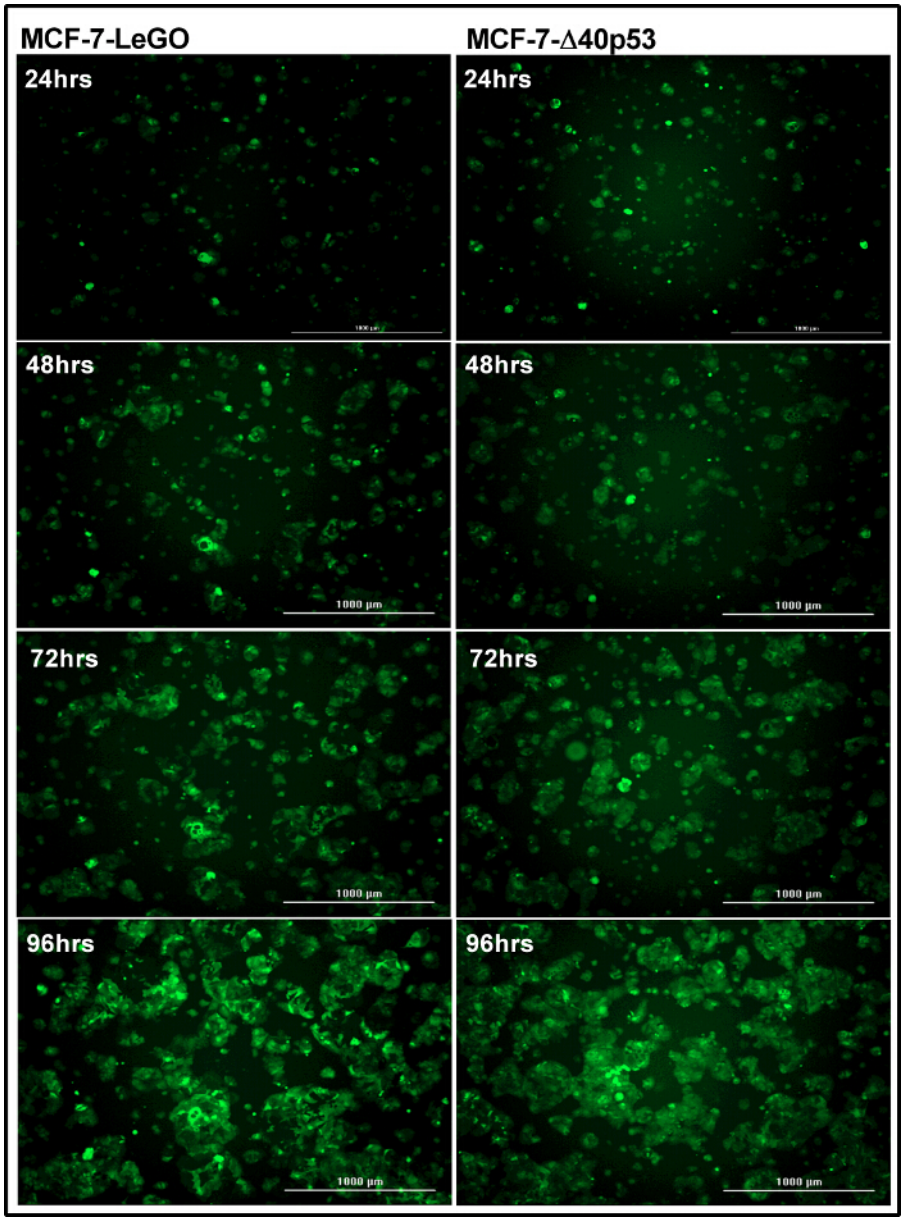

Figure 4: Images of GFP-positive MCF-7-LeGO and MCF-7-Δ40p53 Breast Cancer Cells. Representative images of cells from 5 x 10^3 seeded cells captured using a cell imager every 24 hr. The scale bar represents 1,000 µm. Parameters for cell imaging are summarized in Table 2. Please click here to view a larger version of this figure.

	Cell density (cells/well)		
Cell type	1.5 x 10^3	3.0 x 10^3	5 x 10^3
MCF-7-LeGO	76 µl in 8.4 ml media	152 µl in 8.3 ml media	254 µl in 8.2 ml media
MCF-7-Δ40p53	93 µl in 8.4 ml media	185 µl in 8.3 ml media	308 µl in 8.2 ml media

Table 1: Cell Seeding Volumes for 96 well plates.

Read parameters	
Plate type	96 well
Mode	Image
Objective	2.5X
Color	GFP (469, 525), Bright Field
Exposure	Auto
Focus	Auto (Scan then auto focus)

Table 2: Read Parameters for Cell Imaging Using the Cell Imager.

Imaging parameters	
Threshold	5000
Minimum object size (µm)	30
Maximum object size (µm)	300

Table 3: Imaging Parameters for Cell Counting Analysis.

MCF-7-LeGO		
	R square	**p-value**
Hemocytometer *vs.* luminescence-based assay	0.6301	0.0021
Cell imager *vs.* hemocytometer	0.7524	0.0003
Luminescence-based assay *vs.* cell imager	0.8899	< 0.0001
MCF-7-Δ40p53		
	R square	**p-value**
Hemocytometer *vs.* luminescence-based assay	0.8983	< 0.0001
Cell imager *vs.* hemocytometer	0.9303	< 0.0001
Luminescence-based assay *vs.* cell imager	0.9805	< 0.0001

Table 4: Linear Regression Analysis of the Three Different Proliferation Methods Tested.

Method	Advantages	Disadvantages	Technical notes	Final output
Hemocytometer	Low cost	High human error	Pipette multiple times to prepare single cell suspension	Cells/ml
	Requires minimal equipment	Requires single-cell suspension	Perform multiple counts to achieve accuracy	
	Direct cell count	High number of cells required for accurate assessment of cell count		
		Endpoint		
Luminescence-based assay	Use with multiwell-plate formats	Expensive reagents	Protect from light	Relative Luminescent Units (RLU)/well
	Easy to perform	Requires luminescent plate reader	Include control wells to determine background luminescence	
	Fast assay	Temperature-sensitive		
	Provides cell viability information	Variable depending on metabolic activity of cells		
		Indirect measurement		
		Endpoint		
Cell imager	Continuous measurement	Expensive imager	Ensure cell imager is set to 37 °C	Cells/image
	Temperature control	Skill intensive	Avoid unnecessary shaking or disruption of cells	
	Provides cellular information	Variable depending on confluence of cells		
	Cost-effective (if you have the imager)	Relative count		
	Direct measurement			
	Automated imaging of multiwell-plate format			

Table 5: Comparison of the Advantages and Disadvantages of the Different Cell Counting Methods.

Discussion

In this protocol three different methods of measuring cell proliferation in cultured cells were examined. Each method was capable of reproducible and accurate measurements of cell proliferation over 96 hr, and the results were comparable between each of the methods tested (**Figure 2 and 3**). Both the luminescence-based assay and cell imaging method produced the most robust results, showing linear increases in cell proliferation after 96 hr (**Figure 2b**, **c**). Additionally, cell imaging over time depicted no significant difference in the growth rates between the transduced and non-transduced cell lines (**Figure 4**).

There are many advantages and disadvantages for each method examined in this protocol, see **Table 5** for a summary. The conventional cell counting method using a hemocytometer is a low cost method that requires very little additional reagents or effort to prepare and run. Furthermore, this method quantitates an absolute cell count in cells/ml[14]. However, there are serious disadvantages, which include the time consuming nature of the cell counting, high error rates that results in large standard deviations between counts, and the fact that a high range of cell numbers are necessary for accurate cell counts. This can be seen in **Figure 2a,** where cell counting using the hemocytometer showed variable results at the low cell densities, and large standard deviations at the later time points. These disadvantages make this method useful for cell counting of small sample sizes, and inadequate for larger high throughput measurements where smaller plate sizes and seeding densities are required. These limitations could be alleviated if the cell density was increased, such that the minimum number of cells counted began at a threshold of greater than 100 cells. The more diluted the cell suspension, or lower the cell density, the greater chance of counting less than 100 cells and therefore increasing the variability between replicates[15]. However, this method is unsuitable for a 96 well plate, due to the low cell surface area, and hence, the insufficient number of cells that can be used in the analysis. This highlights the lack of high throughput capabilities of this method and a clear disadvantage for users who require this capability.

The luminescence-based assay determines cell viability by measuring the amount of ATP, which is a measure of the presence of metabolically active cells[16]. This assay is designed for high throughput screening of multiple samples in a 96 well plate format to determine cell proliferation. This simple method quantitates cell proliferation as a relative luminescence unit (RLU) using a plate reader, which is proportional to the ATP present in the metabolically active cells. However, the major disadvantage of this method is the cost of the reagent, and the dependence of the measurement on the metabolic activity of the cells. Various cell culture conditions, such as temperature or cell cycle time points, can readily affect the amount of ATP produced by the cells, which is directly proportional to the luminescent signal generated by the assay[17]. Therefore, it

is important to empirically determine that the conditions of the experiment do not interfere with the metabolic activity of the cells and potentially impede the relative luminescent signal generated by the cells.

The third method examined for determining cell proliferation was a live cell imager and software to perform a relative cell count. The cell imager has high-throughput capabilities as it can be automated to capture multiple images for each well, in real-time along with temperature control, using the same cells over the duration of the assay. As shown in **Figure 4**, cell proliferation can be monitored in the same plate over a time course, which can provide additional information about the cell population along with cell counting analysis. This is very advantageous as it eliminates cross-plate variability and cell seeding error, which is commonplace in 96 well plate formats. The software accompanying the cell imager allows for the quantitation of cell counts using the parameters outlined in **Table 2** and **3**. It is therefore useful in a high-throughput setting and can easily be multiplexed to other assays to measure cellular functions such as apoptosis or cytotoxicity. A major disadvantage of the system is the software, which is limited in its ability to split touching objects. While it can perform this function in lower confluence cells, it is a major limitation of the assay and therefore requires cell-type specific optimization. Also, while individual experiments using an imager are very low cost on a plate-by-plate scale, this method would only be cost effective if the instrument is already set up in a laboratory. Therefore, this provides a novel method for measuring cell proliferation of cultured cells, and has the major advantage of requiring no additional reagents, and is capable of 96 well plate automated counting, making this method appropriate for high-throughput analysis. This is a significant improvement on the conventional hemocytometer cell counting method, and is a more cost effective option to the luminescence-based assay.

The critical step when using the cell imager is during the analysis of the captured images and subsequent cell count per image. These images can be processed using a number of cell imaging platforms. It is therefore paramount to determine the most appropriate software for the cell type under investigation. It would be useful to validate the cell counts from the images acquired by the cell imager using different imaging software. This protocol could be applied to any cultured cells, however the analysis parameters would have to be defined for each cell line. This can be time consuming at first, but once the parameters have been set, the method can be automated to image whole plates at a time.

It is important to note that these assays are in fact an indirect measure of proliferation, as they measure cell number (hemocytometer, cell imager), or metabolic activity (luminescence-based assay), over a period of time. These methods can be easily amended to measure other important factors that can influence proliferation. For example, the trypan blue exclusion method can easily be incorporated into either the hemocytometer or cell imager method to exclude dead cells and hence determine cell viability[13]. The luminescence-based assay can be multiplexed with a cytotoxicity assay to provide additional information about cell health, and the metabolic activity measured during this assay is also a measurement of cell viability[12]. Therefore, the flexibility of these assays to be multiplexed with other assays to provide additional cellular information is a strong advantage of each of these methods.

This protocol used the human breast cancer cell line MCF-7 which have been stably transduced with the LeGO-iG2-puro+ vector containing the GFP gene. This allows the use of the GFP optics filter to image the cells, without the need to add any dye agents into the culture medium. This method can easily be modified to image GFP-negative cells or even primary cell lines, either by imaging the cells using the bright field optics type, or by labelling the cells with a proliferation marker. The methods compared in this protocol are robust enough to be used in a wide variety of different cell lines, however the optimal protocol settings for each individual cell line should be determined first. Imaging of cells using the bright field channel represents the best option for primary cells or those that are slow growing, due to the long-term nature of this method. End-point assays, such as the luminescence-based assay, are not well-suited for the analysis of long-term cell growth.

This protocol has described three different methods for measuring cell proliferation *in vitro*. Each method can be routinely performed in the laboratory to measure cell growth, and most laboratories would possess the necessary skills to perform one of these methods. However, there are also a range of other assays available for measuring dividing cells. These can include methods that measure DNA synthesis, metabolic activity, associated antigens of proliferation or ATP concentration[9]. For example, a number of assays have been developed to measure the rate of DNA synthesis of cells by labelling cells with a radioactive substance such as 3H-thymidine. A drawback of this method is the obvious use of radioactive substances, and their disposal. Other methods which are routinely used for measuring cell proliferation include the use of BrdU labelling of newly formed DNA, which can be measured using flow cytometry[18]. Flow cytometry can be used to analyze both cell number as well as cell cycle information. This may be an advantageous outcome for particular experiments, however the disadvantages of this method include the additional time and steps, expensive reagents and increased user-trained skills in order to operate the flow cytometer and analyze the resulting data[18]. Therefore, there are many different assays available to scientists to evaluate the growth of cells, and the chosen method is largely dependent on the cell type used, the user and their skill level, and the type of data required at the endpoint of the experiment.

In conclusion, three different methods of measuring cell proliferation were compared using breast cancer cells. Each method has many advantages and disadvantages, and is therefore user-dependent and based on their requirements for each experiment, in terms of determining the most appropriate assay to perform cell counting.

Disclosures

The authors declare that they have no competing financial interests.

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