Automated Method for Determination of Infectious Dose (TCID$_{50}$) using Celigo Imaging Cytometer

WHITE PAPER
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Introduction

Qualitative assessment of pathogenic infections, as well as the protective effects of candidate therapeutics, often relies on the observation of specific pathological changes within the host cell. These phenotypic changes, such as cell rounding, swelling/shrinking, granularity, etc., are known as a Cytopathic Effect (CPE) and can be visualized via light microscopy [1-3]. As the magnitude and localization of the CPE may vary considerably, careful examination of replicate samples at various titers is required for reliable, qualitative results. This subjective approach, which is specific to the infectious agent as well as the host cell, is tedious, time consuming and low throughput, requiring manual well-by-well examination by highly skilled personnel.

Nexcelom’s Celigo® imaging cytometer has been applied to provide automated, rapid assessment of viral infectivity in a range of plate formats [4]. Using f-theta optics, Celigo provides high quality, whole-well images using bright field and/or fluorescent illumination. Automated segmentation and analysis provide quantitative and objective output of CPE based on characteristic changes to the host cell monolayer.

Celigo provides several key benefits:

- Objective segmentation and quantitative output of magnitude of infection
- Automated sample analysis reduces time, labor, and variability
- High throughput and scalable – less than 5 min for 96- or 384-well plate
- Captures high resolution, whole-well images for documentation or manual assessment of CPF
- Supports related fluorescent based functional assays relevant to infection (e.g. expression analysis, apoptosis)
Materials and Methods

TCID$_{50}$ Assay Protocol

**Figure 1.**

- Count and plate host cells into microtiter plate (96, 384-well) and allow cells to adhere
- Density should allow for cells to remain viable in absence of infection

**Preparation of Cells**

- Prepare serial dilutions of virus with appropriate diluent
- Overlay virus onto cells and allow to absorb at least 2 hours
- Remove inoculum or leave virus on cells

**Preparation of virus stock**

- Incubate for a pre-determined amount of time to allow for virus infection and cytopathic effects (days, weeks)

**Monitor plates**

- Monitor CPE over days or week depending on the cultural characteristic of virus

**Record results**

- Record the # of wells positive for CPE and the # wells negative for CPE
- Calculate TCID$_{50}$

**Bright Field Imaging**

- BVDV (Bovine Viral Diarrhea Virus) Infection of BK6 (Bovine kidney) cells
- 96-well plates were scanned on the Celigo using the Cell Counting Application
- A dark focus was selected to better separate the healthy monolayer from the infected cells

**Figure 2.**
Determining Best Analysis Method to Score CPE

- Each cell/virus pair will have distinct morphological changes
- Analysis setting can be created and saved for each pair
- CPE was scored + based on the appearance of dark, rounded cells
- In this example, the reduction in confluence (a) and the increase in sick/dying cells (b) scored equally well

Figure 3A and B.
Results Tab Displays Plate and Well Level

- Bovine Viral Diarrhea (BVD) infection of Bovine Turbinate cells (BT2)
- Threshold function indicates red wells (infected) vs. green wells (uninfected)
- Breakpoint of infection is clearly visible at plate level
- Well level data are exported as CSV
- Celigo results correlated 100% with assessment via light microscope

Figure 4.
**MOI by GFP-Indicator Virus**

- Known concentrations of viral stock (ng/mL) are used to infect primary fibroblasts. Infectious titer is calculated by measuring the % of infected cells/dose.
- Viability and overall cell health can also be determined by staining with propidium iodide for sick/dying cells (not shown)
- The Celigo can scan and report up to 4 separate channels at one time (Bright field, red, green, blue)

*Figure 5.*
**Fluorescent Titers from IFA**

- Celigo allows for multiple analyses/plate to be carried out in one experiment. (a) Resulting thumbnails following scan are displayed. Zoomed-in images of infection by BVD1 (b) and BVD2 (c) demonstrate the varied morphologies of infected cells as well as the localization of the viral antigen.

- Antibodies directed against viral antigen are often used as indicators of infection. Unlike TCID\textsubscript{50}, where a single event of infection is scored as a positive, these assays measure % infection. Intensity of FL signal may also be used to indicate strength of infection.

**Figure 6A-C.**
**Calculation of TCID\textsubscript{50}**

**Definition:** 50% tissue culture infectious dose of a virus

- Determination of TCID\textsubscript{50}, MOI, and neutralization assays are easily calculated by exporting the well-level data as CSV into a number of online calculators.

**Figure 7.**

Spearman-Karber formula:

\[
M = x_k + d [0.5 - (1/n)r]
\]

- \(x_k\) = dose of the highest dilution.
- \(r\) = sum of the number of “-” responses.
- \(d\) = spacing between dilutions.
- \(n\) = wells per dilution.

**Conclusions**

The Celigo \textit{S} Imaging Cytometer is a benchtop \textit{in situ} cellular analysis system that rapidly provides high integrity whole-well images for routine bright field and fluorescent cellular analysis with a simple workflow.

- Settings for each cell type and virus can be saved and re-used to assure objective results.
- Additional information such as cell health and cell cycle status can be determined from the same plates using Nexclom-designed applications.
- Time course studies can be carried out by scanning plates over several days/weeks allowing for additional information than may be generated by carrying out end-point assays alone.
- User-friendly and intuitive software allows even those with little imaging experience to generate valuable data.

**References**


