MethodsX 4 (2017) 413–422

Contents lists available at ScienceDirect

MethodsX

journal homepage: www.elsevier.com/locate/mex

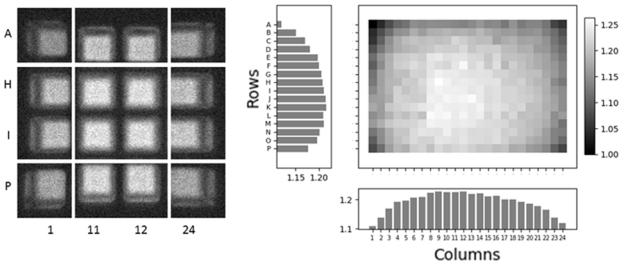
Method Article

Improved high-throughput quantification of luminescent microplate assays using a common Western-blot imaging system

Liam J. Hawkins, Kenneth B. Storey*

Institute of Biochemistry and Department of Biology, Carleton University, 1125 Colonel By Drive, Ottawa, Ontario K1S 5B6, Canada

GRAPHICAL ABSTRACT



ABSTRACT

Common Western-blot imaging systems have previously been adapted to measure signals from luminescent microplate assays. This can be a cost saving measure as Western-blot imaging systems are common laboratory equipment and could substitute a dedicated luminometer if one is not otherwise available. One previously unrecognized limitation is that the signals captured by the cameras in these systems are not equal for all wells. Signals are dependent on the angle of incidence to the camera, and thus the location of the well on the microplate. Here we show that:

- The position of a well on a microplate significantly affects the signal captured by a common Western-blot imaging system from a luminescent assay.
- The effect of well position can easily be corrected for.
- This method can be applied to commercially available luminescent assays, allowing for high-throughput quantification of a wide range of biological processes and biochemical reactions.

* Corresponding author.
E-mail addresses: liam.hawkins@carleton.ca (L.J. Hawkins), kenneth.storey@carleton.ca (K.B. Storey).

https://doi.org/10.1016/j.mex.2017.10.006

ARTICLE INFO
Method name: Improved luminescent assay quantification using a Western-blot imaging system
Keywords: Luminescent assay quantification, HDAC activity, Epigenetic assay
Article history: Received 30 May 2017; Accepted 17 October 2017; Available online xxx

Method details

Background information

Luminescent assays offer a simple, sensitive, and high-throughput method for measuring a vast range of biological processes. Since the emitted light from luminescent assays is the result of a chemical or biochemical reaction, it is uniquely less complex than other assay detection modalities such as absorbance or fluorescence. These methods require equipment that at a minimum contains a light source and sensor, while luminescent detection requires just a sensor. Whether a luciferase based reporter assay is used to study gene expression, or chemiluminescent assay for measuring biochemical reactions, it is generally thought that a spectrophotometer capable of detecting the luminescence signal is required. Previous reports have demonstrated that common inexpensive devices, such as smartphones, can replace expensive conventional equipment [1,2]. The simplicity of measuring luminescence can be taken advantage of since other types of equipment, such as CCD cameras, can convert light emitted from a luminescent reaction to a digital signal for quantification. Previously it was shown that a Western-blot imaging system can be adapted to reliably detect and quantify luminescent signals [3]. Expanding the use of a common piece of laboratory equipment such as this can be preferable to purchasing a dedicated luminometer, if one is not already present. Although this technique has comparable performance to a dedicated luminometer, an additional source of error is introduced when measuring a high number of microplate wells, or if the measured wells are far apart. As demonstrated in Fig. 1, the appearance to the camera of a microplate well is dependent on the angle of incidence to the camera itself. Wells located close to the optical axis of the lens show a complete view of the contents of the well, whereas the contents of wells further from the optical axis are partially obstructed on one side of the well and signal is reflected off the wall of the opposite side. This perspective error, which we refer to as well position effect, is due to the entocentric lens used in conventional cameras. This well position effect could be eliminated optically by replacing the entocentric lens with a telecentric lens. Unlike entocentric lenses, with telecentric lenses the chief rays from an object are parallel to the optical axis of the lens, meaning magnification and perspective is consistent regardless of the objects perpendicular distance to the optical axis. Telecentric lenses have been used for biological imaging and sensing applications previously [4,5], however, they are significantly more complex and expensive than entocentric lenses, and thus would mitigate any of the cost savings generated by using a Western-blot imaging system instead of purchasing a luminometer. Here we present an improved method of luminescence assay quantification using a common, unmodified, Western-blot imaging system that corrects for this well position effect, increasing precision of measurements across a microplate.

Equipment setup

A 96- or 384-well Costar opaque black microplate was placed on the platform of a western-blot imaging system (G:BOX, Syngene, Frederick, MD). Using the live acquisition software, the microplate was aligned to the center of the view of the camera so all wells were visible (Fig. 2A). Masking tape was placed around one corner of the microplate to act as an alignment guide for subsequent readings (Fig. 2B). Aperture size, zoom, and focus settings from the acquisition software were recorded and kept constant for all experiments.