EXTENDING THE LINEAR READING RANGE OF A LUMINOMETER

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BACKGROUND

Chemiluminescent assays currently apply complicated math models to define calibration curves and QC fit to these calibration curves varies by lot.

Photo-detectors employ a conventional approach of photon counting, which is hampered at high count rates due to non-linearity at the upper limit. This non-linearity is a result of pulse saturation. As light levels increase, multiple pulses begin to be counted as a single pulse.

To avoid this counting saturation, an alternate approach is to use analog voltage measurements to approximate light intensity. This alternative is a better method at high light levels but cannot be used alone since it is not as precise at low levels.

An approach to combine the traditional photon counting method with the analog measurement method was developed for an automated immunoassay analyzer.

PROBLEM STATEMENT

Non-linearity of the relationship between sample light output and photon counts or relative light unit (RLU) can lead to imprecision of concentration results. Therefore, some assay calibrations require complicated math models to describe this relationship.

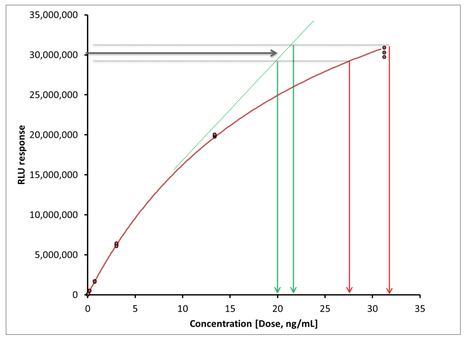


Figure 1 Linearity effects on precision with the same RLU response.

The goal is to develop a method that extends the linear reading range of the luminometer.

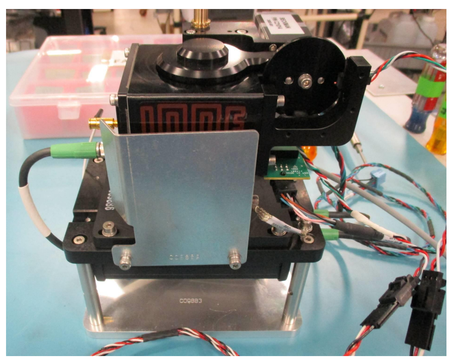


Figure 2 Luminometer subsystem

This product is in development and is not available for sale. Pending achievement of CE compliance; not yet available for in vitro diagnostic use. Pending clearance by the United States Food and Drug Administration; not yet available for in vitro diagnostic use in the US. For Investigational Use Only.

PRINCIPLE OF SIGNAL SATURATION

PMT stands for photo-multiplier tube, which is used to create signals from very low light input. The photons (i.e. light input) hit the photocathode which then emits electrons. These electrons are then reflected off the dynodes effectively multiplying them. These secondary electrons at the last dynode are finally collected by the anode.

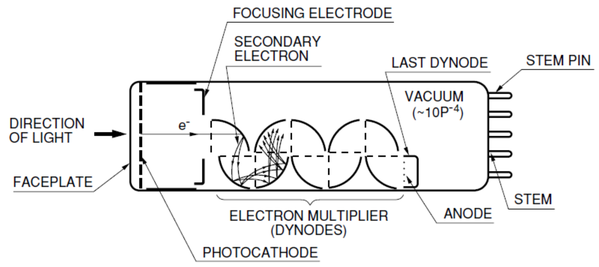


Figure 3 Construction of a PMT[1]

There is a limit on pulse-pair resolution, which is defined as the minimum time interval at which each individual pulse can be discriminated from another.

When incoming light is low, the emitted electrons converted to pulses can be discriminated correctly.

When high light levels yield photons inundating the PMT, the emitted electrons converted to pulses are received at such a high frequency the counting circuit can no longer distinguish between each individual pulse and may count many pulses as a single pulse.

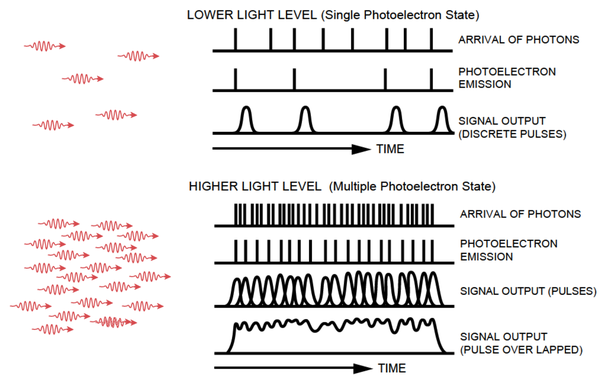


Figure 4 Saturation of pulses at high light levels over time.[2]

SOLUTION: COMBINED COUNTING METHODS

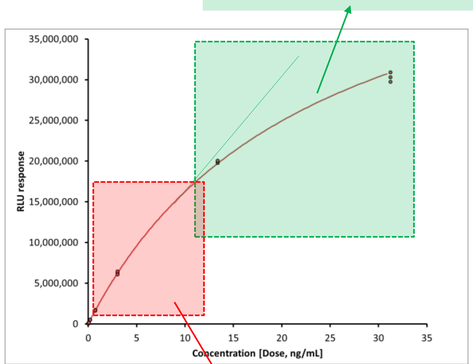
Signal saturation can be avoided by selectively using both photon counting and analog voltage measurement modes based on light level presented to the PMT in the luminometer subsystem.

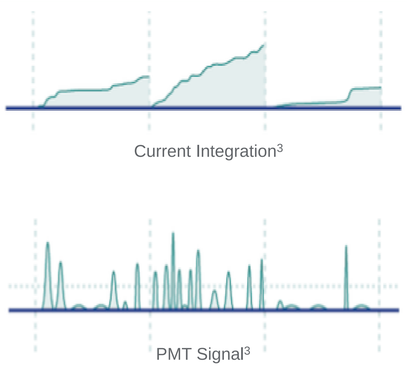
The photon-counting method is better for low light levels, as it can distinguish individual photons as long as they are far enough apart. The analog voltage measurement is better for high light levels and can be used to approximate photon counts. The crossover point when the counting method transitions from photon-counting to analog voltage measurement should be well before signal saturation (with some safety margin).

This solution can be accomplished by establishing a relationship between photon counts and analog voltage at lower light levels (i.e. before pulse saturation).

When light level is high → measure via **Analog Voltage**

Analog voltage measurements are not subject to pulse saturation and therefore can be used at higher light levels. However, this method cannot be used alone as dark counts and low light level samples would suffer from imprecision.





When light level is low → measure via **Photon-counting**

The process of counting photons has been a widely utilized and accepted method in immunoassay testing. However, due to pulse saturation it can be limiting at high light levels. Pulse saturation causes non-linearity in the RLU to concentration relationship which require more complicated math models to describe the relationship between light and concentration.

Figure 5 Combination of photon-counting and analog voltage measurement methods

COMPARISON TEST METHOD

An experiment was designed to test existing chemiluminescence immunoassays to compare the traditional photon counting method and the combined counting method. The following steps were conducted on a new prototype immunoassay analyzer. Four different assays, with varying assay formats, were evaluated.

1. Run Access immunoassay calibration material with known concentrations
2. Record resulting RLU of each concentration
3. Plot RLU vs. concentration for both counting methods

Both RLUs and linearity of the calibration curves were compared. The new combined method used 12 million RLU as the crossover point. The crossover point is the RLU at which all photon counts below this RLU are reported as photon counts and all above this RLU are approximated photon counts based on analog voltage.

RESULTS

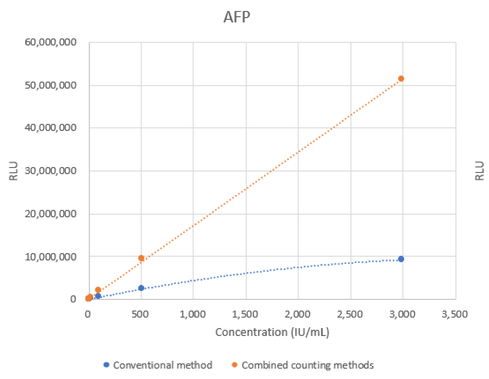


Figure 6 Access AFP assay comparing conventional (photon-counting only) vs. new combined counting method

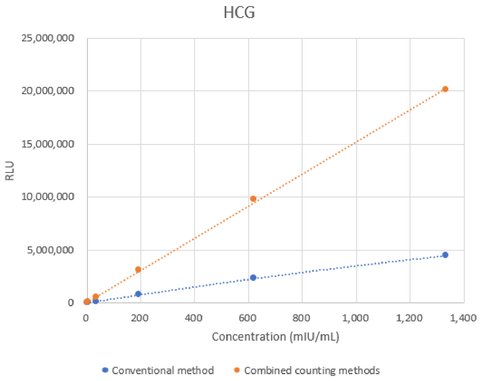


Figure 9 Access Total Beta-hCG (5th IS) assay comparing conventional (photon-counting only) vs. new combined counting method

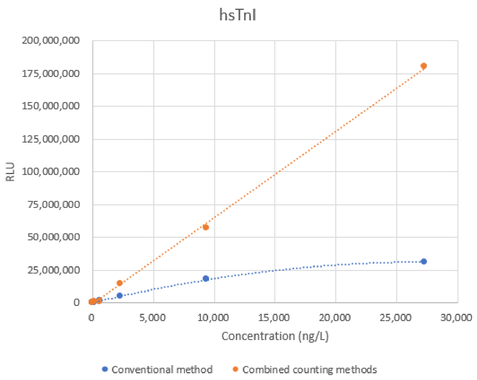


Figure 7 Access hsTnI assay comparing conventional (photon-counting only) vs. new combined counting method

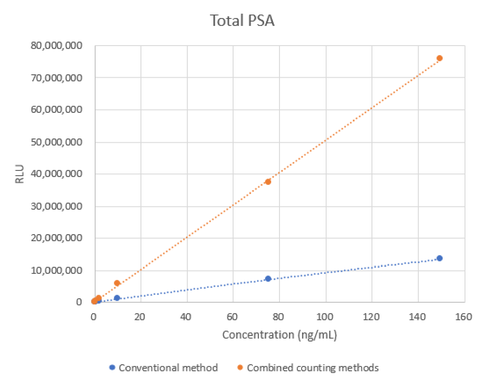


Figure 10 Access Hybritech PSA assay comparing conventional (photon-counting only) vs. new combined counting method

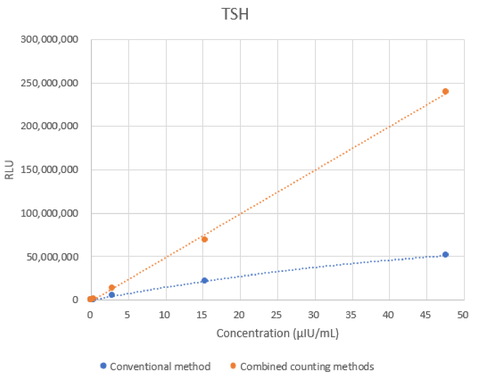


Figure 8 Access TSH (3rd IS) assay comparing conventional (photon-counting only) vs. new combined counting method

CONCLUSION

The new combined method and hardware successfully extended the linear range of RLUs by ~10 fold, from up to 40 million RLU to at least 400 million RLU as the upper limit before signal saturation. Linearity is maintained within the entire range of RLUs. The extended linear measuring range of the new combined method allows for less complicated curve fitting math models to be applied for calibration curves.

References

1 Hamamatsu Photonics K.K., "Chapter 2 Basic Principles of Photomultiplier Tubes", *Photomultiplier Tubes: Basics and Applications* (February 2006)
2 Hamamatsu Photonics K.K., "Analog Mode and Digital Mode (Photon Counting Mode)", *Photon Counting: Using Photomultiplier Tubes* (April 2001)
3 Benninger, Richard K.P and Piston, David W. "Single-Photon Detectors: Fluorescence microscopy benefits from advances in single-photon detectors". *LaserFocusWorld.* Endeavor Business Media, L.L.C., 01 June 2009. Web. 23 July 2019.