Seeing is Believing: Enabling Functional Imaging of NanoLuc® Technologies with Bioluminescence Microscopy

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1. Introduction

Research for new therapeutics often use cell-based assays with microplate readers to generate relative light unit (RLU) and relative fluorescence unit (RFU) values for cellular responses. However, it is becoming increasingly desirable for researchers to want to view their cell models to correlate image results with traditional microplate reader results. This is particularly important for assays that measure protein translocation, internalization, degradation of target proteins and cellular organelles, as well as cellular processes like cell migration, cell proliferation and apoptosis.

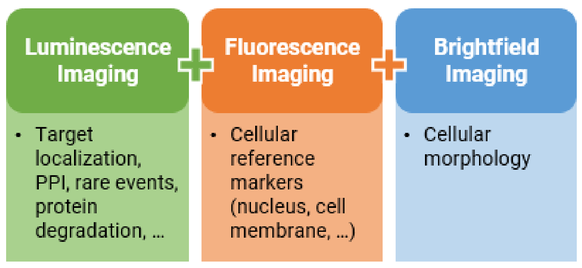
Using imaging technologies, researchers can validate appropriate expression and localization, identify rare cellular events, differentiate responders versus non-responders, perform outlier removal, and analyze mixed cell populations. Today, fluorescence microscopy is widely used as a tool to accomplish these tasks. However, the use of fluorescence comes with several limitations, including 1) limited sensitivity, 2) high background, 3) fluorophore photo-toxicity, and 4) photo-bleaching.

Here we introduce an affordable, benchtop microscope capable of luminescence, fluorescence and brightfield microscopy to enable imaging of Nano luciferase-based assays, including NanoLuc®, NanoBRET™ and NanoBiT®.

2. GloMax® Galaxy Bioluminescence Imager

- Study protein dynamics and cellular physiology
- Living & fixed cells & tissues
- Use NanoLuc® technologies for rare events, assay validation, analysis of mixed cell populations

Figure 1. GloMax® Galaxy Bioluminescence Imager with PC and monitor.



3. Imaging Low Abundance Endogenous Proteins

A. Binary Complementation of NanoBiT® Enzyme

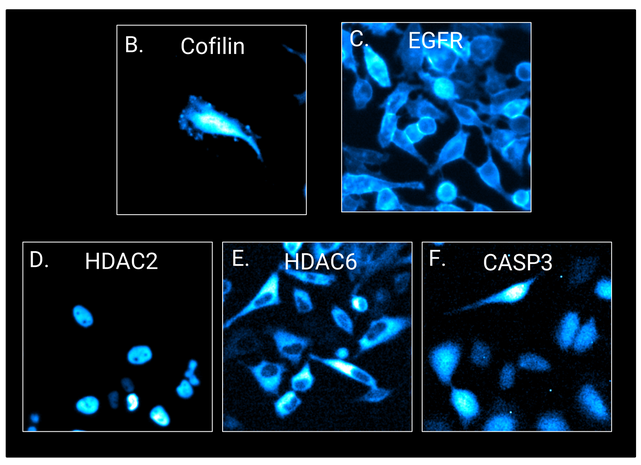
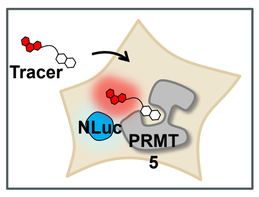


Figure 2. To assess the capability of GloMax® Galaxy to resolve luminescence from low abundant endogenous proteins, a HiBiT / LgBiT complementation assay was used (Panel A). Endogenous protein targets were HiBiT-tagged using CRISPR Cas9 in HeLa cells. LgBiT was then expressed ectopically in each cell model. Bioluminescence images were captured using 1 minute exposure times for Cofilin (Panel B) EGFR (Panel C) and HDAC2 (Panel D). HDAC6 and CASP3 represented very low expressing proteins and they were exposed for 3 minutes (Panel E) and 5 minutes (Panel F) respectively.

4. Detecting Protein:Small Molecule Interactions with NanoBRET®

A.



B.

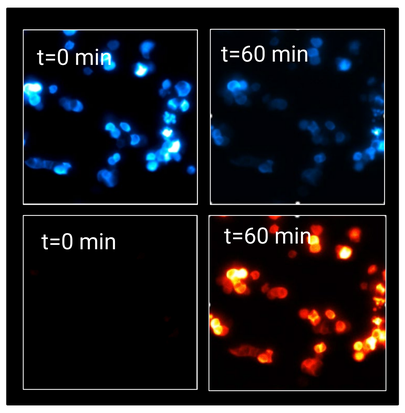
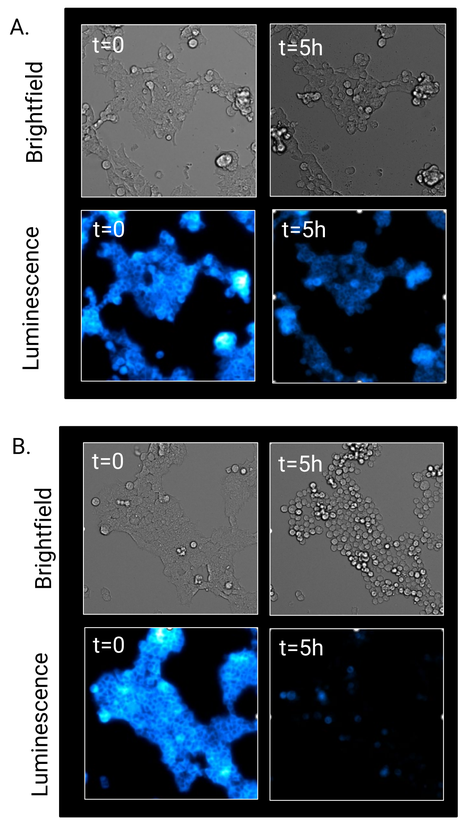


Figure 3. To demonstrate utility for bioluminescence imaging using GloMax® Galaxy to study protein target engagement, a NanoBRET® assay was developed for methyltransferase PRMT-5 , (Panel A). HEK116 cells expressing a PRMT5-NanoLuc fusion were transfected with a small fluorescent tracer molecule and assayed for 60 minutes. Prior to tracer addition, 3-minute exposure captures were collected over a 15-minute period. After tracer addition, 3-minute exposure captures were collected over a 60-minute period (Panel B). Binding of the fluorescent tracer to PRMT5 was detected by the increase in signal in the acceptor channel, indicative of BRET.

5. NanoBiT® PPI Targeted Protein Degradation of Endogenous $GSPT_1$

Figure 4. To demonstrate utility for bioluminescence imaging using GloMax® Galaxy to study protein degradation, HEK293 cells expressing endogenous Nano-Glo® HiBiT-tagged $GSPT_1$ and stably expressing Nano-Glo® LgBiT were treated with DMSO control (Panel A), or CC-885 molecular glue degrader which also induces cell death (Panel B). Cells were assayed with Nano-Glo® Vivazine™ Live Cell Substrate and imaged over a 5-hour period using an integrated stagetop incubator.



6. Instrument Control and Image Acquisition Interface

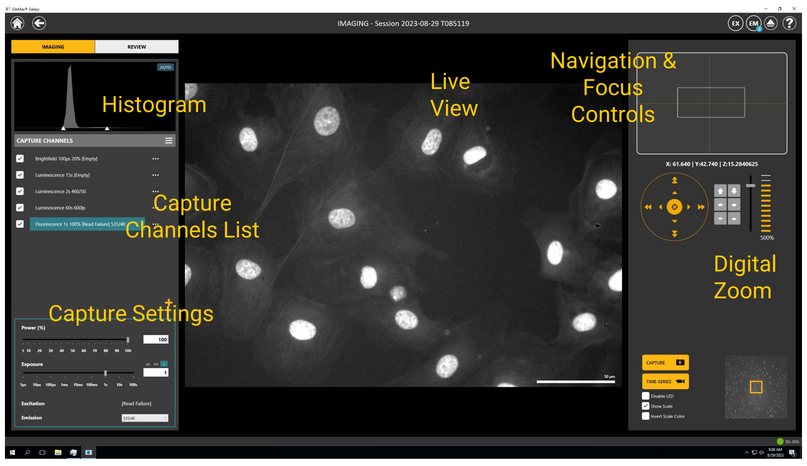
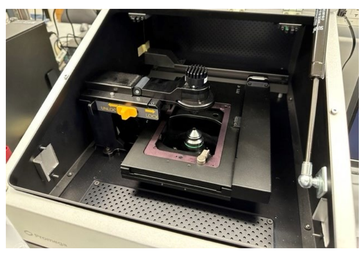


Figure 5. GloMax® Galaxy control and acquisition software interface.

7. Interior View and Accessories

A.



B.

Figure 6. GloMax® Galaxy interior view.

C.

Figure 6. GloMax® Galaxy interior view (Panel A), fluorescence excitation modules (Panel B), and stagetop incubator accessory (Panel C). Pre-built excitation/emissions as well as customizable modules will be available. A stage top incubator and gas controller for long-term kinetic imaging (Panel B) will be available as an accessory.

8. System Specifications

SPECIFICATIONS	
Dimension (W x H x D)	14.7 in x 18.8 in x 21.0 in (37.3cm x 47.7cm x 53.3cm)
Weight	62 lb (28 kg)
Sample Vessels	Slide, microchamber, 35mm dish, 6, 12, 24, 48, and 96-well plates
Capture Modes	Brightfield, Fluorescence, Luminescence, and Filtered Luminescence
Excitation Source	LED, transillumination
Objective	Nikon 20X Plan APO Lambda D, 0.75 NA, 1mm WD
System Magnification	10.4X
Sensor and Pixel Size	CMOS, 3200x2200 pixels, 4.5µm x 4.5µm pixel size
Maximum Field of View	1.4mm x 0.95mm
Resolution Limit	1.3 to 2.0µm
Environment Control	Optional: Stagetop chamber and Controller with built-in gas mixer

9. Conclusions

Expand your research capabilities and use of NanoLuc® technologies with an affordable bioluminescence imaging system.

Available late 2024.

For more information, contact your sales representative or Promega Technical Service at esp_techserv@promega.com

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