

# In vivo bioluminescence imaging in *Galleria mellonella* to non-invasively quantify fungal infection over time

**Commonly used acronym:** Greater Wax Moth, BLI, caterpillar

Created on: 28-05-2025 - Last modified on: 28-05-2025

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**Specific Research Group or Service** Biomedical MRI

**Country** Belgium

**Geographical Area** Flemish Region

## SCOPE OF THE METHOD

<b>The Method relates to</b>	Animal health, Human health
<b>The Method is situated in</b>	Basic Research, Translational - Applied Research
<b>Type of method</b>	In vivo
<b>Used species</b>	<i>Galleria mellonella</i> larvae (Greater wax moth)

<b>Targeted organ system or type of research</b>	Drug screening, virulence assays, fungal infection modeling
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## DESCRIPTION

### Method keywords

Galleria mellonella

in vivo imaging

In Vivo Imaging System (IVIS)

Bioluminescence

fungal infection

longitudinal

non-invasive

drug screening

Virulence assays

### Scientific area keywords

antimicrobial resistance

antifungals

aspergillosis

cryptococcosis

fungal infections

Aspergillus

Cryptococcus

### Method description

Longitudinal imaging of bioluminescent fungi in *Galleria mellonella* can complement standard survival and health scoring data by quantifying the fungal burden over time and monitoring early infection before clinical symptoms arise, and by more sensitively and more early detection of treatment effects. By using bioluminescence flux as a readout of fungal burden instead of relying only on secondary health effects, drug doses can be linked to a quantitative reduction in fungal burden which is valuable towards further experimenting in e.g. mice. It also provides a more standardized readout that is less prone to inter-lab variability in e.g. rearing of larvae, possibly leading to intrinsic differences in health. We validated and published this method for aspergillosis (azole-resistant and susceptible) and cryptococcosis, and have shown translatability of this method to *Galleria mellonella* models of *Candida*, *Fusarium* and *Mucorales* spp. In short, larvae are injected with the bioluminescent fungus of interest, and after injection of 40 µg/g D-luciferin, they are placed individually in a black 12-well plate inside of the IVIS Spectrum (or other detection system) and photon flux from each individual larva can be quantified. Daily imaging was performed. Longitudinal follow-up has been tested up to 1 week, depending on the virulence and inoculum size of the infectious agent. Treatment was injected daily starting from 1h post-infection. Between imaging timepoints, larvae were kept individually in 12-well plates at 37°C in the dark with food during the whole experiment.

## **Lab equipment**

- Hamilton syringe;
- Incubator 37°C;
- Luciferase-producing fungus;
- D-luciferin;
- IVIS Spectrum or other Bioluminescence Imaging equipment (e.g. plate reader).

## **Method status**

Internally validated

Published in peer reviewed journal

## **PROS, CONS & FUTURE POTENTIAL**

### **Advantages**

- Quantitative, non-invasive readout of fungal burden over time,
- Sensitive detection of treatment efficacy over time,
- More informative results compared to only health and survival readouts,
- Cheap, easy and time-efficient,
- No ethical requirements,
- Can be kept at human temperature, 37°C,
- Innate immune system present,
- Easy and cheap to rear the larvae in-house.

## **Challenges**

- Luciferase-producing fungal strain is required,
- No adaptive immune system,
- *In vivo* model, so prone to variability,
- Limited follow-up time due to life cycle of caterpillar (pupating),
- No commercially available research-grade *Galleria mellonella* larvae in Europe.

## **Modifications**

We developed this method using an IVIS Spectrum imaging device, but other detectors of bioluminescence flux could also work (e.g. plate reader) given the possibility to keep the larvae in a 12-well plate.

## **Future & Other applications**

This method can also be translated to bacterial infections (luciferase-producing species or presence of lux-operon).

## **REFERENCES, ASSOCIATED DOCUMENTS AND OTHER INFORMATION**

### **References**

Vanhoffelen E, Michiels L, Brock M, Lagrou K, Reséndiz-Sharpe A, Vande Velde G. Powerful and Real-Time Quantification of Antifungal Efficacy against Triazole-Resistant and -Susceptible *Aspergillus fumigatus* Infections in *Galleria mellonella* by Longitudinal Bioluminescence Imaging. *Microbiol Spectr*. 2023 Aug 17;11(4):e0082523. doi: 10.1128/spectrum.00825-23. Epub 2023 Jul 19. PMID: 37466453; PMCID: PMC10433797.

Vanhoffelen E, Vermoesen L, Michiels L, Lagrou K, Reséndiz-Sharpe A, Vande Velde G. Sensitive bioluminescence imaging of cryptococcosis in *Galleria mellonella* improves antifungal screening under in vivo conditions. *Virulence*. 2024 Dec;15(1):2327883. doi: 10.1080/21505594.2024.2327883. Epub 2024 Mar 13. PMID: 38465639; PMCID: PMC10939141.

Vanhoffelen E, Van Win T, Van Braeckel E, Reséndiz-Sharpe A, Cammue BPA, Lagrou K, Thevissen K, Vande Velde G. Combinations of posaconazole and tacrolimus are effective against infections with azole-resistant *Aspergillus fumigatus*. *Front Cell Infect Microbiol*. 2025 Apr 25;15:1550457. doi: 10.3389/fcimb.2025.1550457. PMID: 40353221; PMCID: PMC12062170.

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*Financed by*

