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Use Guide: Radiometric-Emitter Targets

1. Description

Radiometric-emitter targets (RETs) are designed to mimic fluorescence by emitting light of a known wavelength and adjustable radiance. They provide a stable reference against which to compare fluorescence signals and track system performance or the photobleaching of fluorescence phantoms and reference targets.[1] RETs decouple the fluorescence signal capture from laser excitation, allowing for unique system optimization and quantitative characterization workflows. They can also be used to map the spatial light collection efficiency (sometimes referred to as relative illumination) of an imaging system by moving the target across the imaging field of view and capturing the intensity of the RET at each location. This provides an assessment of the imaging system's location-dependent fluorescence capture efficiency, assuming uniform illumination.

There are two versions of the target. The powered version, which is more commonly used, consists of a solid-state emitter housed in a 30 mm x 30 mm x 20 mm box connected to an adjustable power supply. The power supply provides 20 fixed voltage levels to drive the emitter. Use of this version is detailed in **Section 4. Imaging the target**. In the wired version, there is no attached power supply. Instead, the box containing the emitter is connected to electronic leads to allow more control over the driving voltage supplied to the emitter. For additional notes on operating the wired version, see **Appendix 1**. **Operating wired radiometric-emitter targets**.

QUEL Imaging currently manufactures RETs with wavelengths centered around 720 nm, 800 nm, and 825 nm. Targets can also be manufactured for custom fluorescence applications. Please email sales@quelimaging.com for more information.

2. Intended use

Radiometric-emitter targets are intended for the following use cases:

- They mimic fluorescence emission without requiring laser excitation, so they can be used to optimize fluorescence capture of an imaging system.
- They provide a stable reference to compare fluorescent phantoms over time to detect/track photobleaching.
- They provide a stable reference to track system performance over time.
- Given their characterized radiance output, these targets can serve as a reference in fluorescence imaging experiments to determine the radiance level of a fluorescent signal.
- They can be used to characterize the spatial light collection efficiency (relative illumination) of an imaging system by placing the target at multiple positions around the field of view.

- They can be used in longitudinal fluorescence imaging studies with systems that have an auto-gain feature to constrain the gain setting.
- Together with a concentration target, they can be used to map out the linear range of a fluorescence imaging system (contact us for more information at info@quelimaging.com)

3. Use considerations

When using the radiometric-emitter target, it is important to:

- Allow the RET time to stabilize its radiance output per the product documentation after turning it on and whenever changing the power level prior to imaging it.
- If intending to image the RET in the presence of external illumination (e.g. imaging next to a fluorescent object that requires excitation), consider that this light may alter the stability of the RET (see **Section 6. Limitations)**. QUEL Imaging recommends performing an additional output stability assessment with the imaging system intended for use.
- Acquire images using the same settings intended for normal use of the imaging system (i.e., camera exposure time, camera gain, working distance, ambient lighting conditions, etc.) if applicable.
- If the imaging system performs an overlay of the fluorescence image over a "white light" image, perform analysis on just the fluorescence image and not the overlay image.

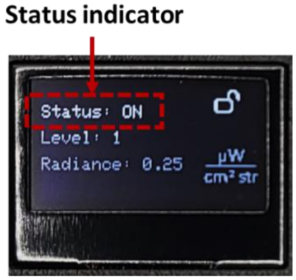
4. Imaging the target

4.1. Adjustable power supply user interface:

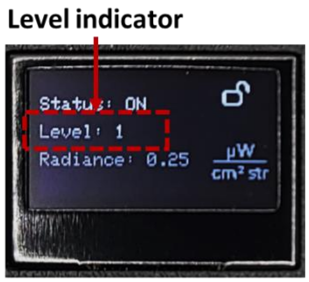
Operating the powered RET is done from the adjustable power supply (shown below). The interface has two controls to interact with – the On/Off button and the Level (Lock) knob:

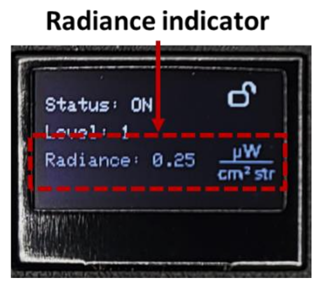
- On/Off button – Press this button to turn on/off power to the emitter (the power supply must be connected to an electrical outlet). The status indicator on the screen will indicate whether power is currently on or off.

Status indicator

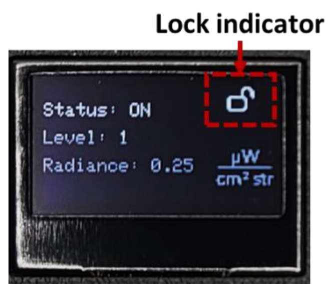


- Level (Lock) knob – Rotate this knob to adjust power to the emitter. The power supply provides 20 discrete power levels, which have been selected with the radiance output characterization of the individual RET. Turning the knob also changes the level indicator on the screen to the current power level and radiance indicator to the current light output of the emitter.





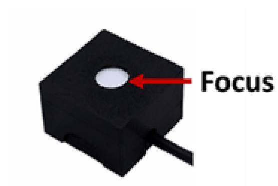
- Level (Lock) knob – Depress the knob to lock in the current power setting. After doing so, rotating the knob will not change the power level until it is pressed again. The lock indicator in the top right of the screen shows the current lock setting.



4.2. *Imaging recommendations*:

For best results, the following is recommended when imaging the radiometric-emitter target:

- Allow the RET time to stabilize per the product documentation prior to imaging it.
- Focus the imaging system on the emitting surface of the target.



Additional recommendation when using the RET for any form of comparison imaging (e.g. tracking photobleaching of a phantom or monitoring imaging system performance over time):

- As much as possible, position the RET in the same location within the field of view (a jig may be helpful for this). This will remove any variation due to spatial non-uniformity of the imaging system.

- See pages **Appendix 2** for more information on using the RET to monitor system response and target photobleaching over time.

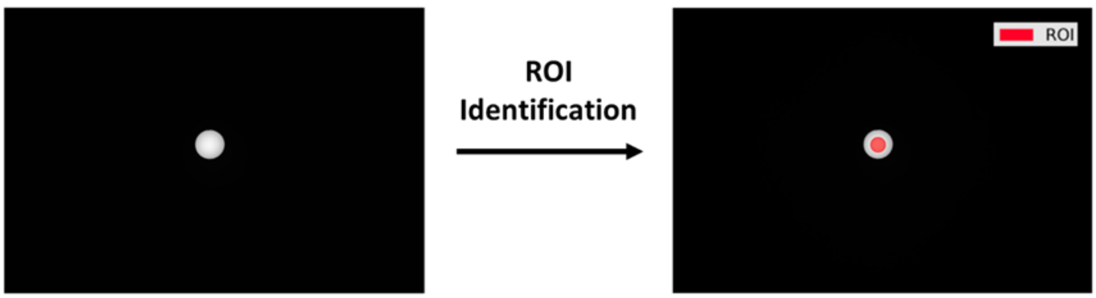
Additional recommendation for creating relative illumination maps:

- Turn off or block all sources of light from the imaging system and capture multiple images of the RET across the field of view.

5. How to analyze images

5.1. *Analyzing individual images*:

Calculate the RET output intensity as an average over an ROI centered on the emitting surface. QUEL Imaging recommends an ROI radius one half that of the emitting surface.



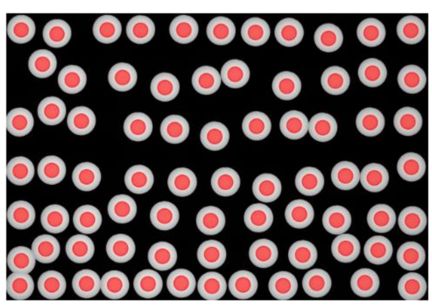
5.2. *Monitoring reference target photobleaching*:

See **Appendix 2** for more information on using the RET to monitor system response and target photobleaching over time.

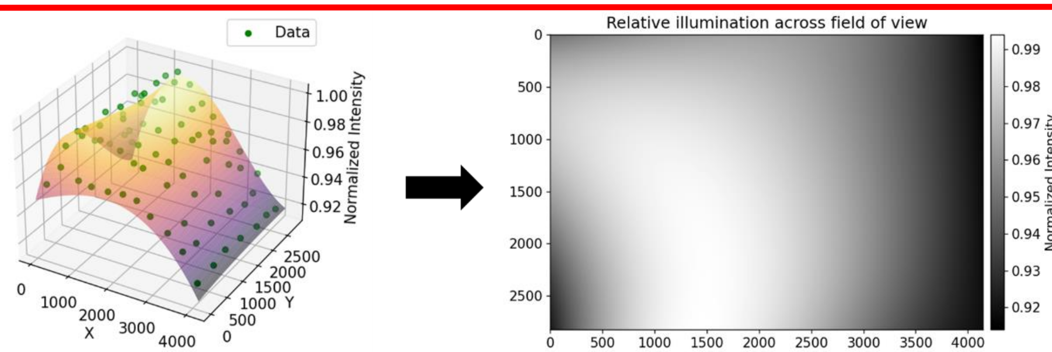
5.3. *Generating relative illumination maps*:

To generate relative illumination maps from multiple images spanning the field of view:

- Locate the centroid of the RET in each image, and calculate the average intensity as described above (example below shows composite image with identified ROIs).



- Use interpolation or fitting techniques to generate the relative illumination map from the extracted data (example shown below uses bivariate B-spline interpolation to generate a representation of the surface).



6. Limitations

- The radiance output of these targets may vary beyond the specified stability if imaged while being illuminated, particularly at high irradiance. This depends on the power and wavelength of the illumination source. QUEL Imaging recommends the customer perform an output stability evaluation prior to using the target in an environment where it is being illuminated at high irradiances for prolonged periods. For details, please contact us at info@quelimaging.com and inquire about the respective RET testing protocol.
- The target is designed for use in research, evaluation, and demonstration environments – at no time should these products be used in clinical care.

7. Handling and care

- Handle the target with gloves to avoid getting dirt and oils on the imaging surfaces.
- Remove dust from the emitting surface using gentle air (e.g., an air bulb) – DO NOT clean surface with isopropyl alcohol or other liquids.
- There are no user-serviceable parts – DO NOT open the LED housing or the attached power supply. Warranty will be void if opened.
- We recommend annual re-characterization of RET radiance output. Email sales@quelimaging.com to inquire about re-characterization services.

8. References

1. Ochoa M.I., Ruiz A., LaRochelle E., Reed M., Berber E., Poultsides G., Pogue B.W. Assessment of open-field fluorescence guided surgery systems: implementing a standardized method for characterization and comparison. *Journal of Biomedical Optics*, 28(9):096007. 2023.

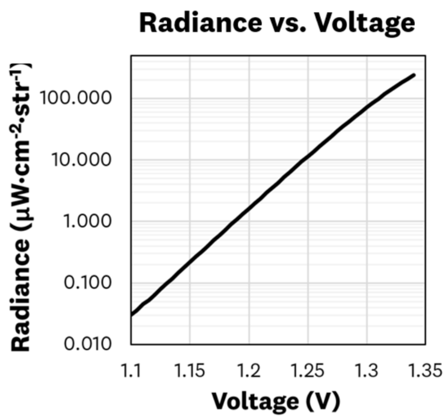
Appendix 1: Operating wired radiometric-emitter targets

Figure A1-1: Radiometric emitter target alongside banana-plug connectors.

The wired version of the RET consists of the emitter housed in a 30 mm x 30 mm x 20 mm box attached to banana plug leads (**Figure A1-1**). To operate, connect the banana plugs to a voltage source. Consult the specification sheet of the specific RET to find the limits of the driving voltage for that RET. Do not drive the RET beyond the voltage range listed.

Operating the radiometric emitter target: The targets are operated in a constant voltage mode where the current limit and voltage limit should be set to the reported max voltage and current values provided in the characterization data. To control the output of the radiometric target, the use of linear DC power supplies with at least 1 mV resolution and voltage accuracy of 0.1% is recommended.

Adjusting the emitted output: The radiometric target output is adjusted by varying the driving voltage with the power supply. The voltage utilized should be within the range of the provided characterization data and should not exceed the max voltage to prevent damage to the RET. The provided output characterization (radiance, voltage, and current) is normally provided in 0.01V steps for twenty different levels. A sample radiance vs. voltage curve is provided in **Figure A1-2**.



Recommended power supply manufacturers:
Tektronix/Keithley, Keysight, Agilent

Example power supply: Keithley 2231A-30-3.

Appendix 2: Using the RET to monitor system response and target photobleaching

The full monitoring of fluorescence imaging system performance requires: 1) a concentration sensitivity target to monitor fluorescence response, 2) a RET to monitor the imaging signal detection and account for reference target photobleaching, and 3) a calibrated optical power meter for measuring the excitation irradiance. An example of a system monitoring measurement protocol is found in **Figure A2-1**, which establishes a baseline monitoring response for the imaging system and the fluorescence reference target.

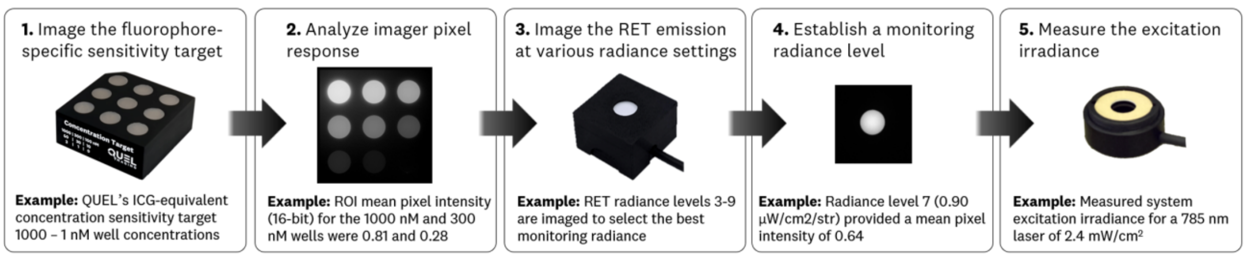


Figure A2-1: Example protocol for establishing a baseline system response alongside a monitoring radiance level that serves as a long-term reference to account for fluorescence target photobleaching, imaging parameter changes, and excitation irradiance degradation.

Establishing a system-specific RET monitoring radiance level and baseline response allows for the subsequent monitoring of imaging system and reference target performance. The recommended monitoring method generates a normalized response $S_c = I_c \cdot I_r / E$, for each sensitivity target ROI, where I_c is the pixel response of each concentration ROI, I_r is the monitoring radiance pixel intensity response, and E is the measured excitation irradiance at the imaging plane. The initial characterization (**Figure A2-1**) is used to generate a baseline system response for each ROI of the concentration sensitivity target (S_{c_b}, i.e. $S_{1000nM\_b} = 0.216$ using the Fig 2 data). Subsequent system response measurements for each ROI (S_c) are corrected using their respective baseline response such that $S_c' = S_c \cdot S_{c\_b} / S_s$. This corrected response accounts for changes in the system irradiance and reference target photobleaching (**Figure A2-2**). The measurement of the corrected ROI response S_c', monitoring radiance pixel response Ir, and measured excitation irradiance E are sufficient to monitor a system's long-term performance. Note that, the photobleaching decay (d) of the reference target is quantitatively defined as $d = I_c / I_{c\_b}$ where a new reference target and baseline measurement should be used if $d > 0.4$.

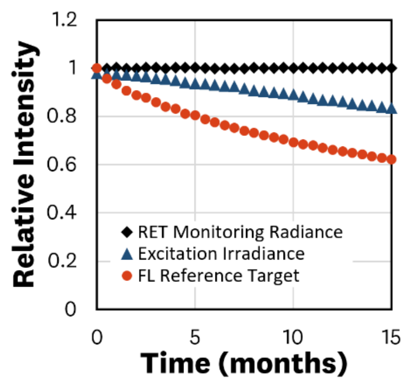


Figure A2-2: The long-term stable RET monitoring radiance allows decoupling of the reference target signal and excitation irradiance decay