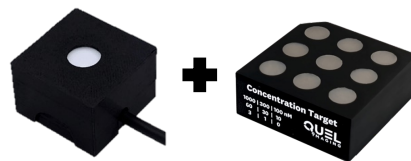


Summary: QUEL Imaging provides a suite of reference targets to measure performance characteristics of fluorescence imaging systems. The radiometric emitter target (RET) provides a stable calibrated radiance output that mimics fluorophore-specific spectral emission. This target is the ideal solution to monitor the performance of fluorescence imaging systems, reference target photobleaching, and provides a pathway to quantitative imaging.



Using the radiometric target as a long-term stable radiance source

The radiometric emitter target (RET) is a calibrated solid-state emitter that provides adjustable radiance output with spectrum within fluorophore-specific emission bands [Fig 1]. Standard configurations include ICG and OTL38 specific products with the availability of custom spectral emission from the visible to the short infra-red. The adjustable radiance ($\sim 0.01 - 100 \mu\text{W}/\text{cm}^2/\text{str}$) accounts for a wide range of system-specific fluorescence sensitivities. This is important because the same concentration of fluorophore will generate different detected emission intensities as a function of the system optics and excitation irradiance, which generally varies between systems and with source-to-imager distance. The RET output radiance is controlled through an integrated power source which provides 20 discrete levels or through an external linear DC power supply.

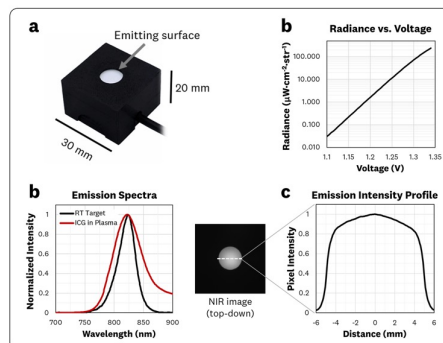


Figure 1 Radiometric emitter target alongside characterization of radiance, spectrum, and emission.

Using the RET at a fixed radiance output enables its use as a long-term stable reference

to monitor imaging system performance and the entire line of QUEL Imaging's fluorophore-specific reference targets. Using both the RET and fluorophore-based reference targets decouples the excitation irradiation and imaging signal detection when measuring a system's fluorescence response. The full monitoring of fluorescence imaging system performance requires a 1) Concentration sensitivity target to monitor fluorescence response [Fig 2a-b], 2) Radiometric emitter target (RET) to monitor the imaging signal detection and account for reference target photobleaching [Fig 2c-d], and 3) Calibrated optical power meter for measuring the excitation irradiance. An example of a system monitoring measurement protocol is found below [Fig 2], which establishes a baseline monitoring response for the imaging system and the fluorescence reference target.

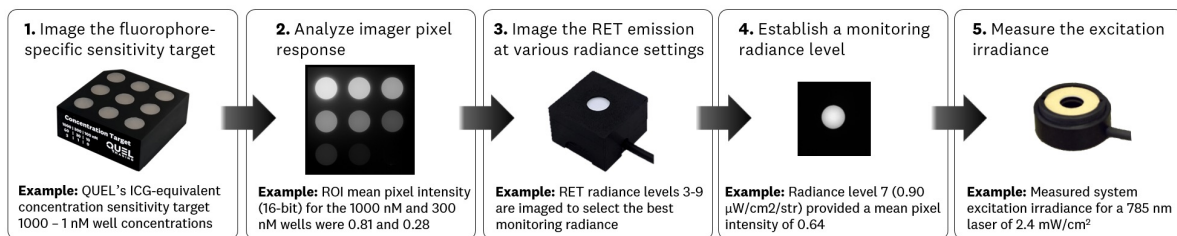


Figure 2 Example protocol for establishing a baseline system response alongside a monitoring radiance level that serves as a long-term reference to account for fluorescence target photobleaching, imaging parameter changes, and excitation irradiance degradation.

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 [Fig 2] 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 [Fig 3]. The measurement of the corrected ROI response S'_c , monitoring radiance pixel response I_r , 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 $d > 0.4$.

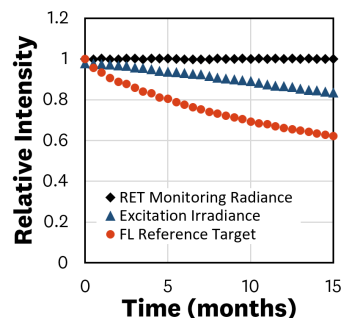


Figure 3 The long-term stable RET monitoring radiance allows decoupling of the reference target signal and excitation irradiance decay