QUEL Imaging, LLC
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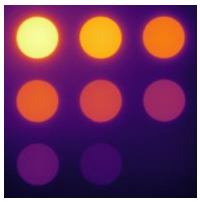
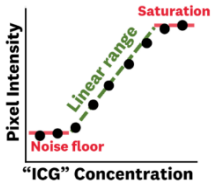
Use Guide: Concentration Targets

1. Description

Concentration targets provide characterization of the fluorescence sensitivity of fluorescence imaging systems. They can be thought of as shelf-stable serial dilutions of the fluorophore of interest, allowing for repeatable evaluation of the i

maging system or routine quality checks without the need for liquid phantoms. Unlike liquid phantoms, which need to be made fresh for each use, are not easily transportable, and can be subject to human error in their creation, our concentration targets provide consistent, reliable performance.[1,2] This document covers both standard and mini-sized concentration targets.





QUEL Imaging manufactures concentration targets that mimic fluorescence in the presence of tissue optical properties. ICG (ICG-01 targets), OTL-38/pafolacianine (O38-01 targets), and 700 nm channel fluorophores such as SGM-101 (Q700-01 targets). Custom targets can be manufactured to meet the needs of specific fluorescence applications. Please contact sales@quelimaging.com for more information.

2. Intended use

Concentration targets are intended for the following use cases:

- Assessment of the fluorescence sensitivity of an imaging system.
- Characterization of the imaging system's range of linearity, where an increase in fluorophore concentration is matched by a proportional increase in fluorescence intensity.
- Identification of the noise floor of a fluorescence imaging system.
- Identification of the saturation point of a fluorescence imaging system.

3. Use considerations

When using a concentration target, it is important to:

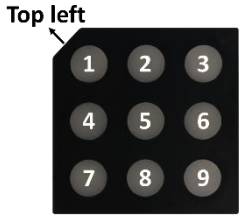
- Acquire images using the same settings intended for normal use of the imaging system (i.e., camera exposure time, camera gain, working distance, ambient lighting conditions, etc.).
- If the system has multiple fluorescence modes (e.g. different camera gain settings), analysis should be performed and reported for each mode.

- If the imaging system has an auto-gain feature, consider placing a radiometric-emitter target (RET) in the field of view to constrain the gain setting (see the Use Guide: Radiometric-Emitter Targets for more information).
- If the imaging system compresses images prior to displaying on a monitor and the displayed images are used in decision-making, perform the same compression prior to analyzing images of the concentration target.
- If the imaging system performs an overlay of the fluorescence image over a "white light" image, perform the analysis on just the fluorescence image and not the overlay image.

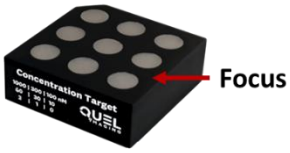
4. Imaging the target

For best results, the following is recommended when imaging the concentration target:

- Position the target within the imaging field of view such that the chamfered corner points to the top left. This will arrange the wells from highest (well #1) to lowest fluorophore concentration (well #9) going first across the columns then down the rows (see image below).



- Position the target such that the faces of the wells are orthogonal to the imaging axis – this is important since a tilt may artificially reduce or increase the fluorescence intensity of some wells due to different working distances, leading to inaccurate results.
- Focus the imaging system on the top surface of the phantom.

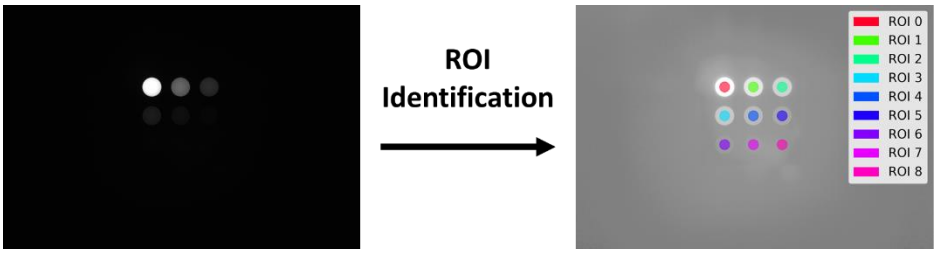


- If it can be avoided, do not image the target together with other fluorescent objects or light sources since these could contaminate the signal from the target and skew results. In some cases (e.g. systems with auto-gain), a RET may be needed to lock down the gain setting. Furthermore, a RET can be used to monitor the photobleaching of our fluorescence targets (see the Use Guide: Radiometric-Emitter Targets for more information).

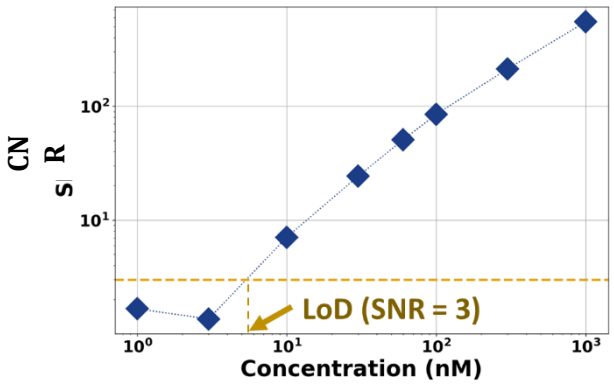
5. How to analyze images

Concentration targets enable the user to determine an imaging system's range of linearity as well as an estimate for the noise floor, limit of detection (LoD), and saturation point. To analyze images:

- Calculate well intensities as an average over an ROI centered on each well. QUEL Imaging recommends an ROI radius one half that of the well (i.e., a 5 mm ROI for a 10 mm diameter well). It may be easier to identify wells if the fluorescence image is viewed on a log scale (see images below).



- Sort the well intensities according to the concentrations printed on the phantom, from 0 (control) to highest. Baseline the data by subtracting the intensity of the 0 (control) concentration well from each.
    - Further divide the baselined data by the standard deviation of the 0 (control) concentration well to get the contrast-to-noise ratio (CNR). The limit of detection can be estimated as the concentration at which the CNR reaches 3.[1,2] Example data shown below on log-log plot).



- Convert the background-subtracted intensities and concentrations to log scale in order to fit an equation of the form:

$$(intensity) = m(concentration) + C$$

    - Plot the data and find the region over which a fit of the data to the equation above produces a slope (m) ~equal to 1. This is the range of linearity. The noise floor can be estimated as the average of the points below the limit of detection.

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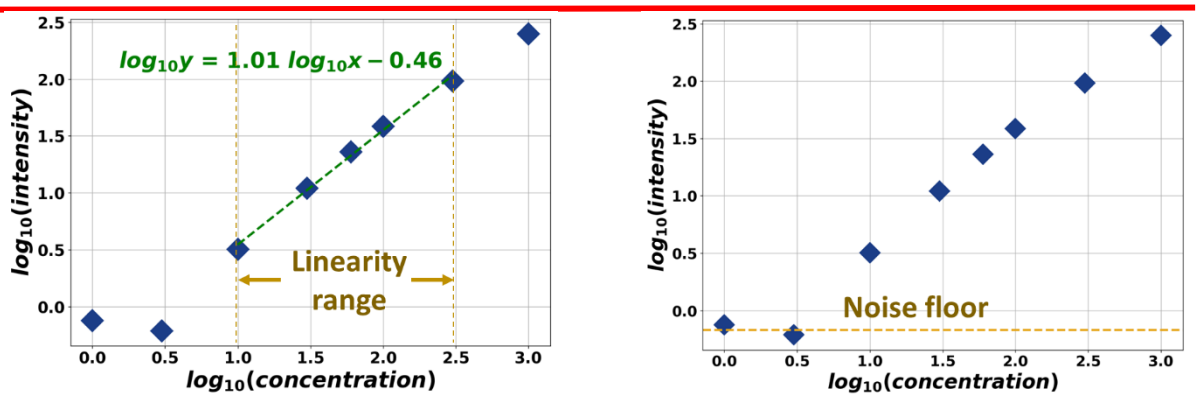

- The saturation point (if present) can be estimated as the concentration above which there is no longer an appreciable increase in fluorescence intensity.

QUEL Imaging provides image analysis through a web-portal at FGS.QUELimaging.com. Furthermore, sample analysis code is available through our Reference Target Analysis (RTA) repository on GitHub. For more information on the analysis portal or repository, contact info@quelimaging.com.

6. Limitations

- The standard 9-well concentration targets provide 9 data points with which to evaluate an imaging system's fluorescence sensitivity. For this reason, analysis can only provide estimates of the LoD, noise floor, saturation point, and linearity range, not precise concentration values at which they occur.
  - Please contact QUEL Imaging (sales@quelimaging.com) to inquire about custom phantoms with more specific fluorophore concentration ranges tailored to your application's requirements.
- The QUEL Imaging Reference Target Product Family is designed for use in research, evaluation, and demonstration environments – at no time should these products be used in clinical care.
- To mimic in-vivo fluorescence, QUEL Imaging uses organic fluorophores in our phantoms. As a result, these phantoms are susceptible to photobleaching. It is important to monitor the targets for photobleaching (see Use Guide: Radiometric-Emitter Targets for more information on photobleaching monitoring). Data on the photostability of these phantoms is available here in the Directions for Use: Reference Target Product Family.

7. Handling and care

- Handle the targets with gloves to avoid getting dirt and oils on the imaging surfaces. If cleaning is needed, use isopropyl alcohol and soft lint-free material. Avoid prolonged skin contact.

- To prolong shelf life, these targets should be stored at room temperature (20 - 25°C) and unexposed to light when not in use. QUEL Imaging recommends keeping the targets in their original shipping packaging for storage.
- Avoid exposing targets to direct sunlight.

8. References

1. Pogue B.W., Zhu T.M., Ntziachristos V., Wilson B.C., Paulsen K.D., Gioux S., Nordstrom R., Pfefer T.J., Tromberg B.J., Wabnitz H., Yodh A., Chen Y., Litorja M. AAPM Task Group Report 311: Guidance for performance evaluation of fluorescence-guided surgery systems. *Medical Physics*, 51:740-771. 2024.
2. 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.