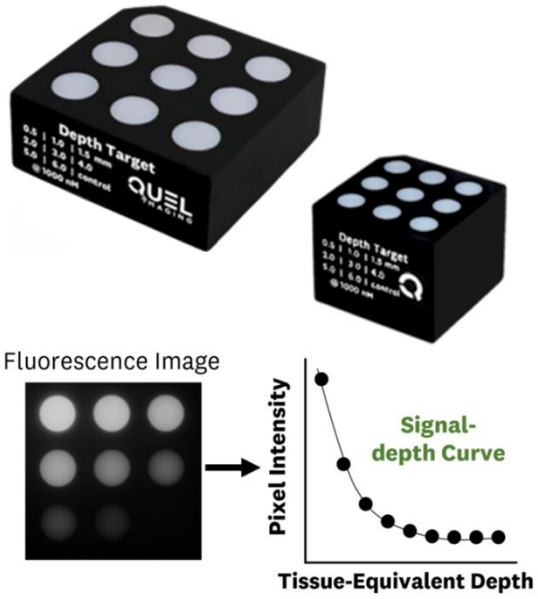
QUEL Imaging, LLC
85 N. Main St. Suite 142
White River Junction, VT 05001

Use Guide: Depth Signal Targets



1. Description

Depth signal targets provide characterization of the dependence of fluorescence signal on depth for fluorescence imaging systems. They are useful for understanding how a fluorescence signal from a fixed concentration of fluorophore changes when buried beneath different thicknesses of tissue-mimicking material. These targets offer a reliable and shelf-stable solution for this analysis, addressing the variability and inconvenience associated with methods such as layering slices of biological material (e.g., bacon) over a fluorescent phantom.[1,2] This document covers both standard and mini-sized depth targets.

These targets contain 9 wells. The first 8 wells contain a fixed concentration of fluorophore with tissue-equivalent optical properties, buried beneath increasing thicknesses of tissue-equivalent non-fluorescent material. The final well contains just the tissue-equivalent material, which serves as a control.

QUEL Imaging manufactures depth signal targets that mimic fluorescence in the presence of tissue optical properties. Our targets are available for the following fluorophore applications: 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

Depth signal targets are intended to be used for:

- Characterization of a fixed fluorescence signal's relationship to depth through material of specific optical properties.

3. Use considerations

When using a depth signal target, it is important to:

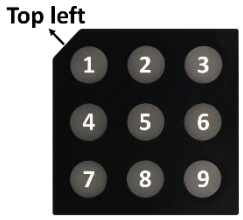
- Acquire images using the same settings intended for normal use of the imaging system, (e.g., camera exposure time, camera gain, working distance, ambient lighting conditions, et c.).

- If the system has multiple fluorescence modes (e.g., different camera gain settings), characterization 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 depth signal 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.

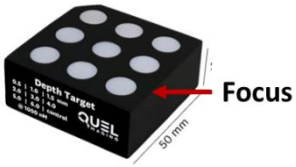
4. Imaging the target

For best results, the following is recommended when imaging the depth signal 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 shallowest to deepest fluorescent inclusion going first across the columns then down the rows, with the control well last (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.

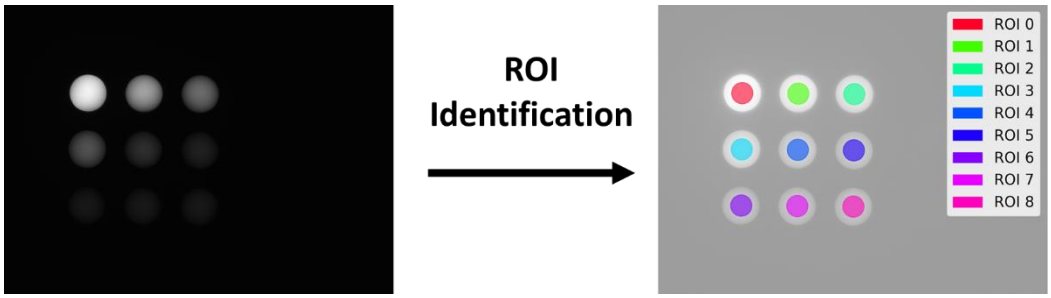


- If it can be avoided, do not image the target together with other fluorescent objects or light sources since these could contaminate the signal from the target and skew results. In some cases (e.g. systems with auto-gain), a RET may be needed to constrain the gain setting. Furthermore, a RET can be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

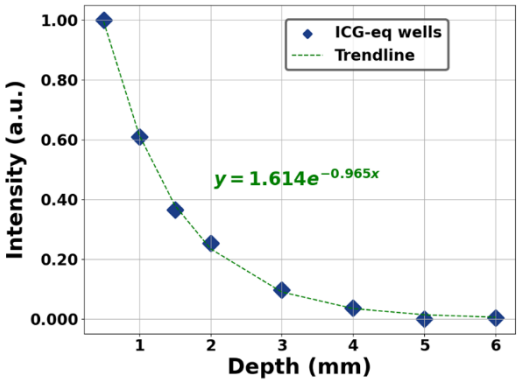
5. How to analyze images

Depth signal targets enable the user to evaluate how a fluorescence signal measured by an imaging system changes with tissue-equivalent depths. 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 depths printed on the phantom, from shallowest to deepest, then control last.
- Subtract the intensity of the control well from the other 8 wells and plot the background-subtracted intensities against depth to observe how the fluorescence signal decreases with depth.
- The plot shows fluorescence intensity at discrete depths. If desired, fit the data to an exponential equation in order to estimate fluorescence signal at any depth.



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

- These depth signal targets provide a means to evaluate change in fluorescence intensity with depth. However, they do not provide an assessment of the spread or blur of the signal with depth.
    - Consider using QUEL Imaging's depth resolution targets to obtain both intensity drop-off and spread information. Contact sales@quelimaging.com for more information.
- The characterization results are specific to the optical properties and geometries of the respective target imaged and should not be generalized to other optical properties or geometries.
- 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.