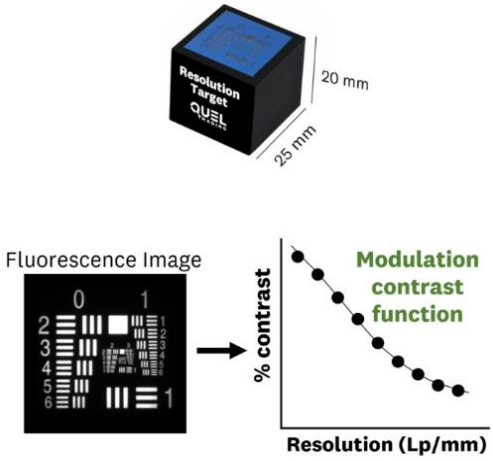
QUEL Imaging, LLC
85 N. Main St. Suite 142
White River Junction, VT 05001

Use Guide: Fluorescence Resolution Targets



1. Description

Fluorescence resolution targets provide characterization of the spatial fluorescence resolution of an imaging system. They consist of a block of fluorescent material overlaid with a negative USAF 1951 low-reflective glass resolution target. This allows excitation and emission light to penetrate through the resolution target, resulting in fluorescent resolution features. Determining the smallest resolvable group element provides an estimate of the smallest feature size that can be resolved by the fluorescence imaging system. This determination is commonly made by analyzing an image of the target and finding the group and element numbers (and hence spatial resolution) at which the contrast between the lines reaches 26.4%, known as the Rayleigh criterion.[1,2]

QUEL Imaging manufactures resolution targets that are available for the following fluorophore applications: ICG (ICG-01 targets), Pafolacianine and IRDye® 800CW (Q800-01 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

Fluorescence resolution targets are intended to be used for:

- Evaluation of an imaging system's fluorescence spatial resolution capability.
- Measurement of the depth of field (DoF) by positioning the target at varying heights while maintaining a fixed focus, thereby determining the range over which the system maintains adequate focus (see **Appendix 1**).

3. Use considerations

When using a fluorescence resolution target, it is important to:

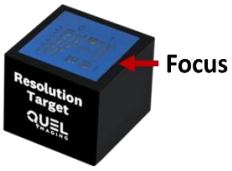
- Acquire images using the same settings intended for normal use of the imaging system (e.g., camera exposure time, camera gain, working distance, ambient lighting conditions, et c.).
- If the system has multiple fluorescence modes (e.g. different camera gain settings), characterization should be performed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

- If the imaging system 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.

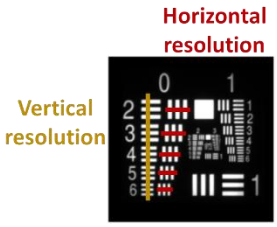
4. Imaging the target

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

- Make sure to focus the imaging system on the top surface of the target – any defocus will reduce the contrast that is calculated and underestimate the resolving power of the fluorescence imaging system. If the imaging system also performs white light imaging, it may be helpful to use this mode for focusing since the lines will be sharper than they appear in fluorescence.



- Position the target within the imaging field of view such that it is as close to aligned with the axes of the image as possible – this way, sets of vertical and horizontal lines can be analyzed to provide horizontal and vertical resolution, respectively, if desired.



- Position the target such that the face is orthogonal to the imaging axis – this is important since a tilt may artificially reduce or increase contrast for some line pairs due to different working distances, leading to inaccurate results.
- 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 radiometric emitter target (RET) may be needed to constrain the gain setting. Furthermore, a RET can be used to monitor the photobleaching of our fluorescence targets (see ‘Use Guide: Radiometric-Emitter Targets’ at Downloads and Resources Page for more information).

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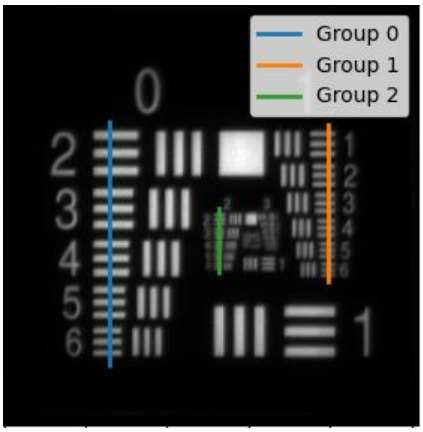
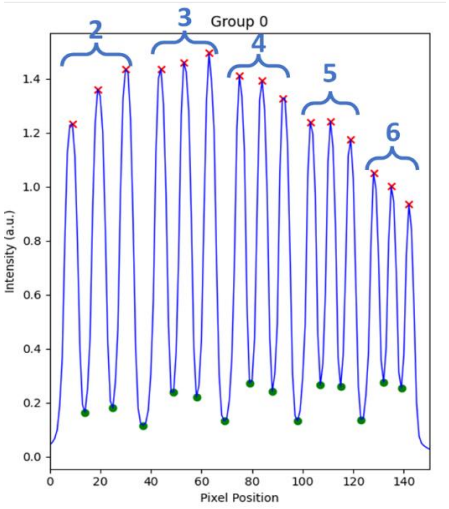
5. How to analyze images

Fluorescence resolution targets enable the user to assess the optical resolution of a fluorescence imaging system. To analyze images:

- Identify groups and elements to assess. In the example below, the horizontal elements in groups 0, 1 and 2 are selected for analysis.



- The image below shows the intensity profile down the blue line from the example above. Each element has three peaks.



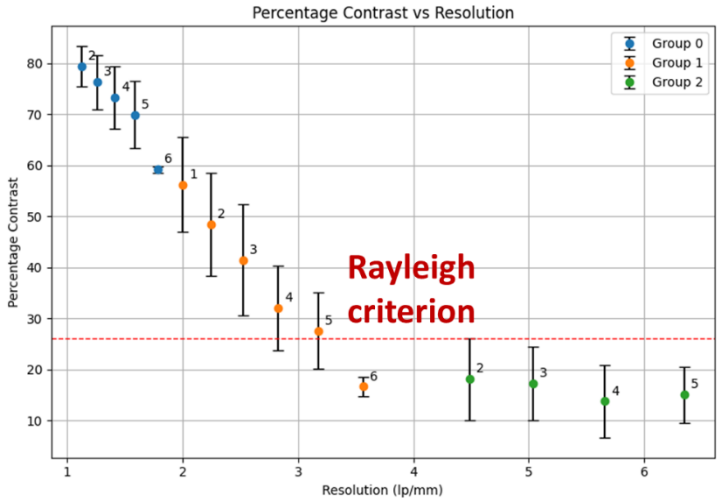
- For each peak in each element, calculate contrast using the equation below, and take an average for that element.

$$C = \frac{I_{max} - I_{min}}{I_{max} + I_{min}}$$

- Determine the spatial frequencies of the elements for which contrast was calculated. QUEL Imaging's fluorescence resolution targets use groups 0 to 7 of the USAF 1951 resolution chart, ranging in spatial frequency from 1.12 lp/mm to 288 lp/mm. Spatial frequency can be calculated from the group (G_n) and element (E_n) numbers by:

$$f = 2^{\left[G_n + \frac{E_n - 1}{6}\right]}$$

- Plot contrast against spatial frequency. The Rayleigh criterion can be used to provide a metric for resolvability – this is where the contrast reaches 26.4%. In the example used here, the smallest resolvable element is group 1 element 5, which has a spatial frequency of 3.17 lp/mm.



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 in GitHub. For more information on the analysis portal or repository contact info@quelimaging.com.

6. Limitations

- In order to characterize the spatial resolution across the imaging system's field of view, the target may need to be imaged and analyzed in multiple locations.
- 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' at Downloads and Resources Page for more information on photobleaching monitoring). Data on the photostability of these phantoms is available here in 'Directions for Use: Reference Target Product Family' at Downloads and Resources Page.

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.

APPENDIX 1: Determining Depth of Field (DoF)

To measure the DoF, the target can be positioned at varying heights relative to the fixed focus of the imaging system. By analyzing the fluorescence signal at different heights, one can determine the range over which the system maintains adequate focus, thus characterizing the DoF.[2] To determine the depth of field using the fluorescence resolution target:

- Position the target at the imaging system's fixed focus height and capture an image.
- Move the target incrementally to different heights above and below the initial focus plane.
- Capture images at each recorded height to observe changes in fluorescence signal and resolution.
- Analyze the images as per **Section 5. How to analyze images** to determine the height range over which the system maintains adequate focus, identifying the DoF.