

Use Guide: Uniformity and Distortion Targets

1. Description

Reference Uniformity and Distortion (RUD) targets enable the simultaneous characterization of two important aspects of a fluorescence imaging system's performance – (1) fluorescence signal uniformity and (2) image distortion. The RUD target consists of a grid of equally spaced wells filled with luminescent material, embedded within a matrix of non-fluorescent, light-absorbing material. Illuminating the target with the appropriate excitation wavelength causes fluorescence to be emitted from the wells. The standard target dimensions are 110 mm x 110 mm x 20 mm. The wells are 1 mm in diameter and spaced at intervals of 2 mm, covering an area of 100 mm x 100 mm.



Both uniformity and distortion characterization are essential for ensuring consistent performance across fluorescence-guided surgery systems, as outlined in the AAPM Task Group 311 guidance.¹ Fluorescence signal uniformity of an imaging system is crucial to assess when making decisions based on fluorescence intensity. For example, in a surgical procedure involving ICG perfusion, a blood vessel may appear well-perfused in the center of the image but poorly perfused in one corner. In such cases, the observed difference in fluorescence intensity does not reflect true biological function but rather stems from non-uniformities in the illumination or collection efficiency of the imaging system. Understanding this can guide users or manufacturers to rely on quantitative data from only a portion of the imaging field of view. Image distortion, referring to spatially dependent variations in magnification across the imaging area, is equally important to characterize, as it can cause inaccuracies when estimating the size and shape of anatomical structures or their positions relative to each other.^{1,2}

QUEL Imaging currently manufactures uniformity and distortion targets for a wide use of NIR fluorescence applications. Note that the RUD target utilizes a photostable luminescent compound (S800-01) that has a broad excitation spectrum from 400 - 790 nm, with a broad emission peak at ~820 nm. Custom targets can be manufactured to meet the needs of specific fluorescence applications. Please contact sales@quelimaging.com for more information.

2. Intended use

Uniformity and distortion targets are intended for the following use cases:

- Characterizing the fluorescence signal uniformity of a fluorescence imaging system.
- Characterizing the spatial distortion of a fluorescence imaging system.

3. Use considerations

When using the uniformity and distortion 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, etc.).
- If the field of view is larger than the target, translate the target across the field of view and capture multiple images using the same acquisition parameters to achieve full characterization.
- If the imaging system overlays the fluorescence image with a “white light” image, perform the analysis on the fluorescence image only, not the overlay.
- The luminescent material of the RUD target is excited by a wide range of wavelengths, from visible to near-infrared. Therefore, if the imaging system uses both white light and fluorescence excitation, ensure the white light is turned off before imaging the target to accurately assess fluorescence uniformity.

4. Imaging the target

For best results, the following is recommended when imaging the uniformity and distortion target:

- 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 uniformity assessment. Tilting could also introduce keystone distortion that may not otherwise be present in the imaging system.
- Focus the imaging system on the top surface of the phantom.



- For signal uniformity assessment, ensure none of the wells are saturated in the fluorescence images. Saturated pixels may falsely indicate higher uniformity than is accurate. If normal imaging parameters cause saturation, adjust the camera exposure/gain to avoid it.
- If the target is smaller than the field of view and characterization is needed across the entire view, acquire multiple images using the same acquisition parameters with the target in different positions spanning the field of view.
- Avoid imaging the target alongside other fluorescent objects or light sources, as these could contaminate the signal and skew results. In some cases (e.g. systems with auto-gain), a RET may

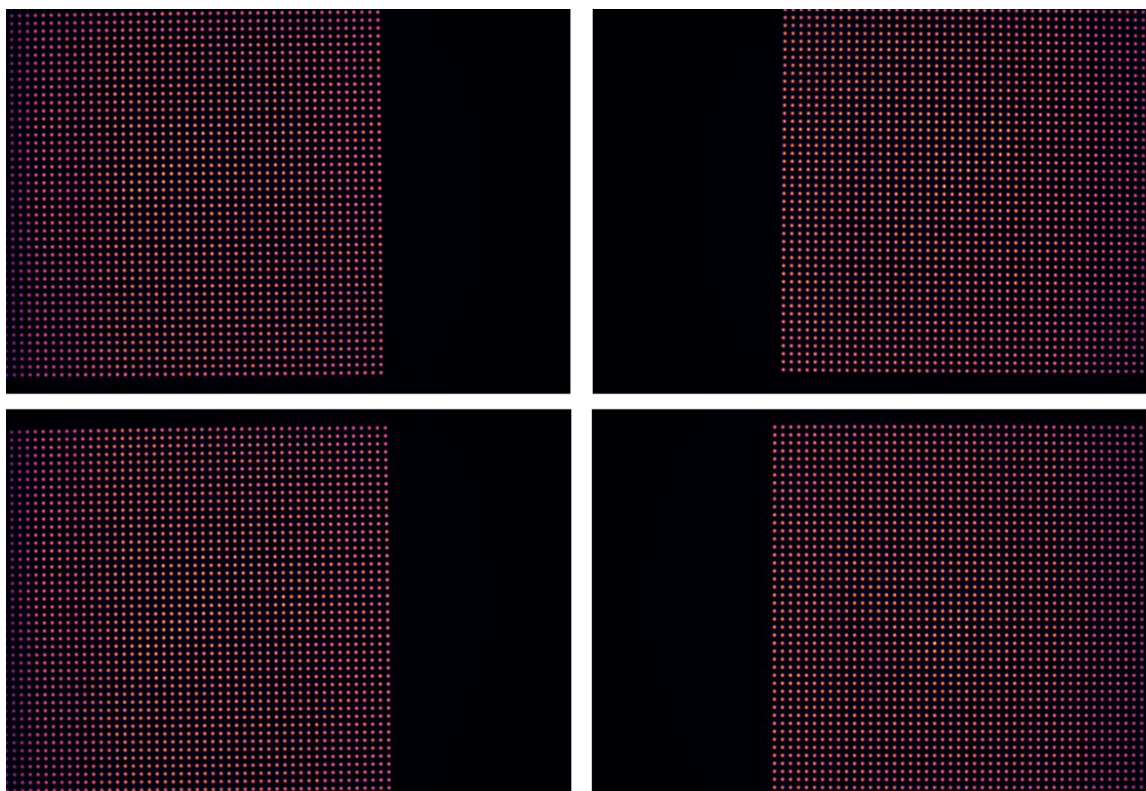
be needed to lock down the gain setting (see the [Radiometric Emitter Targets Use Guide](#) for more information).

5. How to analyze images

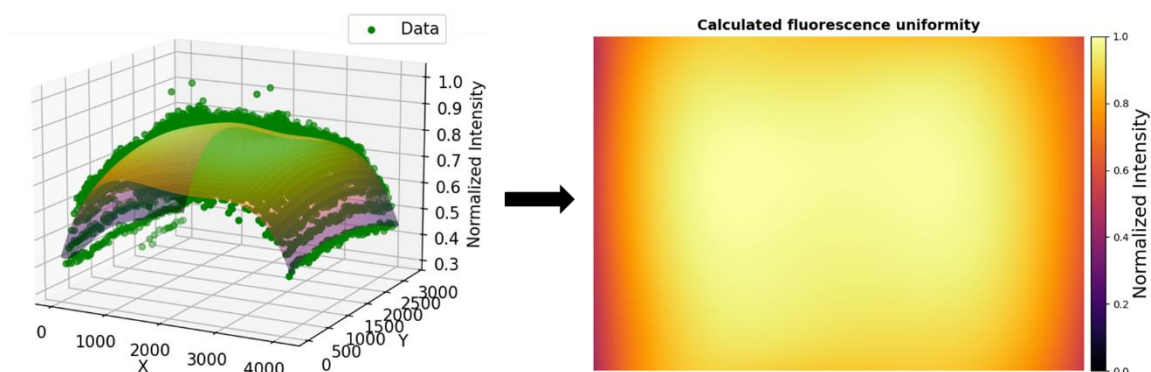
The following sections describe the recommended RUD target image analysis for assessing uniformity and distortion. QUEL Imaging has developed analysis libraries to assist customers in processing the acquired images of the RUD target. The analysis code is available via [QUEL-QAL Repository on GitHub](#). Detailed explanations of the analysis code can be found in the [corresponding RUD Wiki](#).

5.1. Fluorescence signal uniformity assessment:

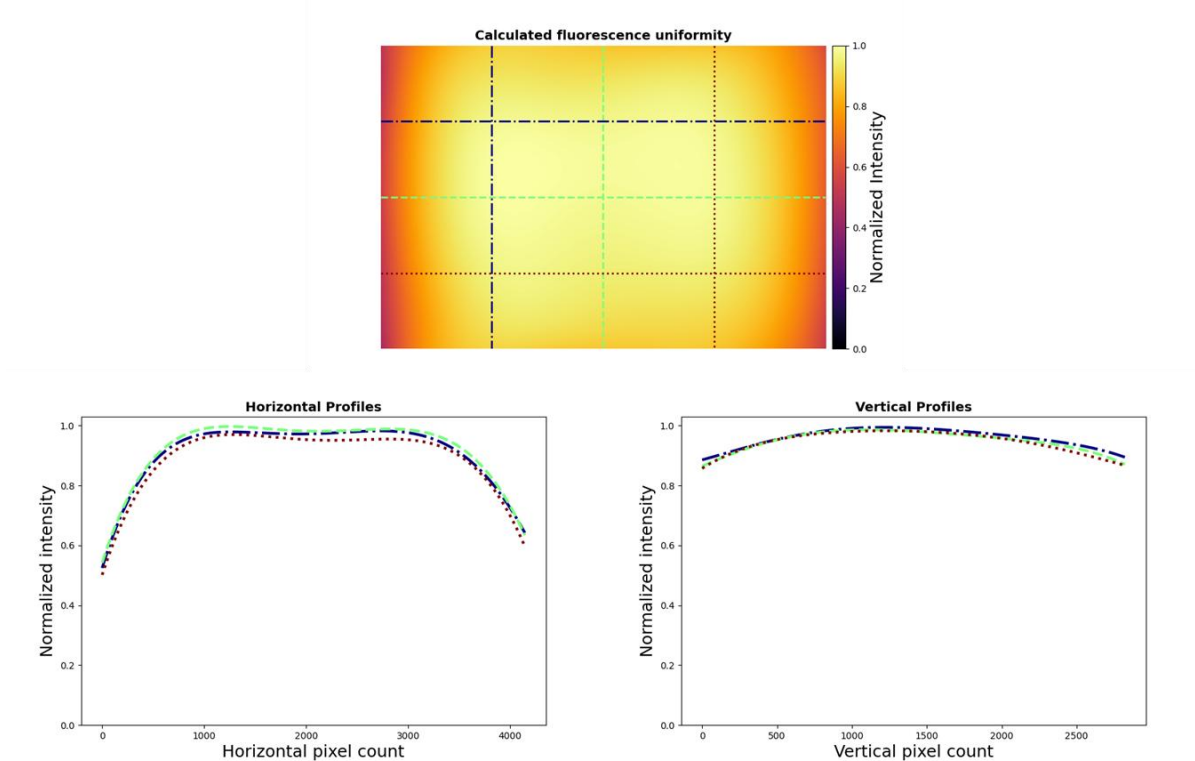
You may need to analyze one or multiple images, depending on the size of the field of view relative to the target. In the example below, four images were taken to cover the entire field of view:



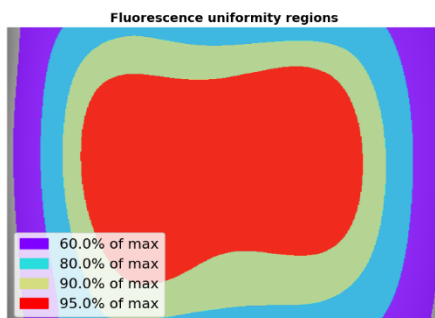
For each image, identify the center locations of the wells and their mean intensities. QUEL Imaging recommends calculating the mean intensity within circular ROIs that are half the radius of the wells, centered on each well. Using interpolation or fitting techniques, generate a map of fluorescence intensity across the field of view. In the example below, the map was created using bivariate B-spline interpolation:



This map represents the spatial uniformity of fluorescence capture across the field of view. The map can be further analyzed to produce line profiles:



Additionally, the map can be analyzed to generate iso-maps (regions where the fluorescence intensity is uniform within some percentage). For this example, a central rectangular region that covers about 40% of the field of view is uniform within 95% of the maximum intensity:

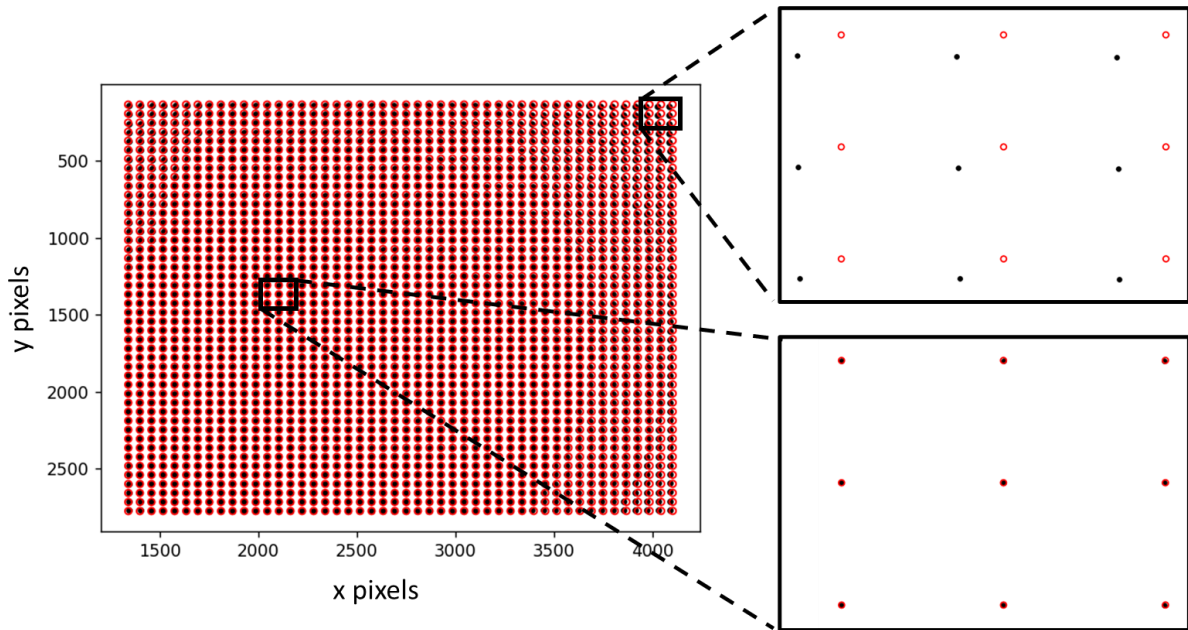


In the example used here, we can see that fluorescence will be dimmer by about 40-50% on the left and right edges of the field of view. A device manufacturer could use this information to crop the field of view that is displayed to end-users, or an end-user could use this information to determine the best object positioning within the field of view for optimal results.

5.2. Distortion assessment:

Distortion includes radial distortion (barrel and pincushion) and keystone distortion.³ Keystone distortion is usually due to a tilt of the object or image plane with respect to the optical axis. While the RUD target is also capable of highlighting keystone distortion, the analysis presented here only quantifies radial distortion. If a significant amount of keystone distortion is present in images, this should be corrected, if possible (e.g., make sure the object plane is perpendicular to the imaging axis), before analyzing for radial distortion. This guide follows the principles of ISO 17850:2015 for assessing local geometric distortion but does not strictly adhere to all its provisions, particularly regarding the imaging requirements and limitations specific to fluorescence imaging. For full quantification in accordance with ISO 17850:2015, please refer to the standard.

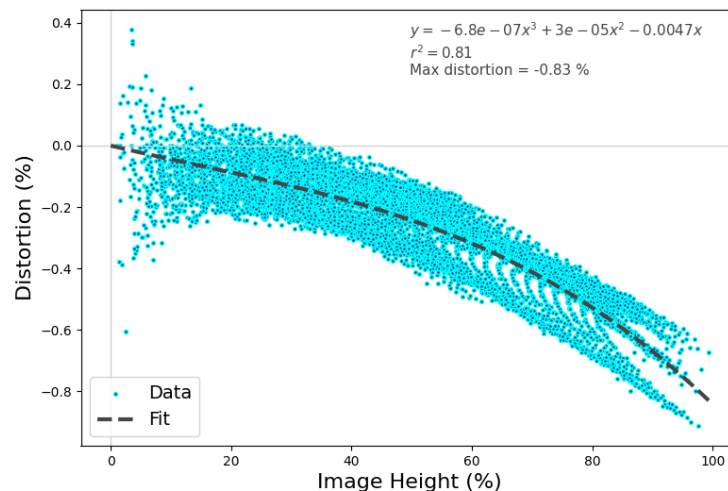
When quantifying radial distortion with the uniformity and distortion target, there may be one or multiple images to analyze, depending on the size of the field of view in relation to the target. For each image, determine the center locations of the fluorescent wells. Under the assumption that the center of the image has no distortion, find the average spacing between wells around the image center. Use this to create a grid of equally-spaced points oriented to align with the wells in the image center – this will serve as the expected positions of wells. In the example below, the black dots represent the identified well centers, while the red circles represent the grid of expected well positions. For the example shown, some barrel distortion is present (observable in the upper right corner where the black dots representing actual well positions are closer to the image center than the red circles representing expected well positions):



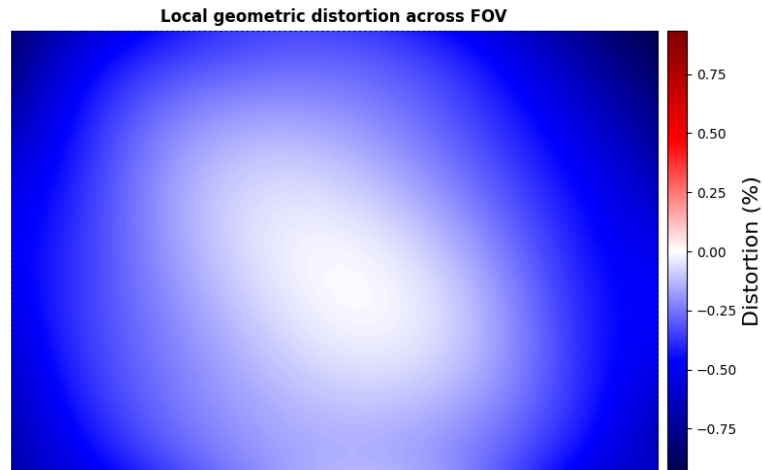
Calculate distortion as:

$$\text{distortion}(\%) = \frac{\text{actual distance} - \text{expected distance}}{\text{expected distance}} \times 100$$

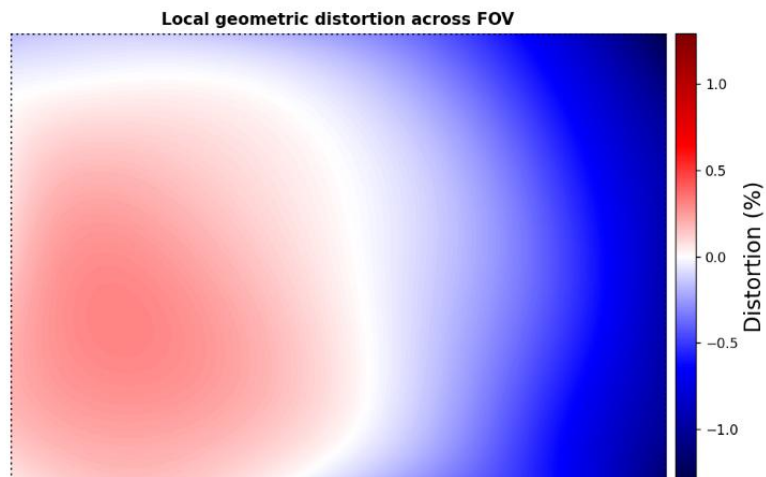
where *actual distance* and *expected distance* are measured from the image center. For this example, there is a little bit of barrel (or negative) distortion but overall, it is less than 1%. Closer to the image center, results will be more influenced by the accuracy of the well-finding method (small errors from finding well centers may be amplified), leading to noisier data. Fitting of the results (e.g., to a polynomial going through the origin) can be performed to provide a cleaner representation of the data. In the example below, the distortion data is fitted to a 3rd-degree polynomial, as distortion is a 3rd-order aberration with respect to image height:



A spatial map of the distortion can also be generated by fitting the data points as a function of their location in the image, rather than distance from the image center. This can help highlight the presence of keystone distortion – in the absence of keystone distortion, the map should ideally be radially symmetric.



Below is an example of the distortion map generated from an imaging system with keystone distortion. There is positive distortion on the left side of the image, and negative on the right. In a case like this, the fitted radial distortion curve would be a very inaccurate representation. Ensure that the object plane is perpendicular to the imaging axis, then reimage the target and reanalyze.



6. Limitations

- In order to characterize fluorescence uniformity or distortion across the field of view of an imaging system, the target may need to be translated to multiple positions and multiple images acquired.
- The RUD targets utilize broadband absorbing luminescent compounds, such that fluorescence uniformity assessment should be performed only under illumination exclusively from the designed excitation sources of the imaging system.
- 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.

7. Handling and care

- Handle the targets with gloves to avoid getting dirt and oils on the imaging surfaces. If cleaning is needed, use distilled water and soft lint-free material. Avoid prolonged skin contact.
- DO NOT USE SOLVENTS (such as alcohol or other chemicals) to clean the RUD target. Doing so can damage polymer content and alter the optical properties and surfaces.
- 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.
3. Hollows G., James N. Distortion. *Imaging Resource Guide*, Section 3.3. Edmund Optics. Available at: <https://www.edmundoptics.com/knowledge-center/application-notes/imaging/distortion/>