

UV Fluorescence Imaging Guide

By Dr. Ellen Gualtieri, Formulatrix

This guide was compiled in order to help you understand the factors that contribute to satisfactory and unsatisfactory UV images in order to analyze your own image results, and to provide you with tips for generating better UV images.

I. BACKGROUND

UV imaging relies on the intrinsic fluorescence from three aromatic amino acids found in protein samples: tryptophan, tyrosine and phenylalanine. Tyrosine fluorescence is usually quenched in native proteins, resulting in decreased fluorescence emission, and phenylalanine fluorescence is very weak, making tryptophan the main source of UV fluorescence.

In general, fluorescence intensity depends on the concentration of tryptophan in the sample. However, quenching from either the protein itself or other additives can negate fluorescence from protein crystals and result in a false negative when imaged with UV, even if the crystals have a relatively high concentration of tryptophan.

False positives in UV imaging can be caused by ligands, small molecules and/or salts that fluoresce in UV. Protein can also adhere to non-protein crystals resulting in what appears to be a UV fluorescent protein crystal. Phase separations and spherulites also yield images with contrast similar to protein crystals, making it difficult to determine if protein crystals are present in these conditions.

Crystallization plates and seals can also affect UV imaging due to absorption of the excitation UV or fluorescence from the plastics. Please see the [Formulatrix UV Plate Compatibility Guide](#) for more information.

Sections II and III of this guide summarize typical causes of false UV positives and negatives to aid in the interpretation of UV fluorescent images. A more comprehensive study of UV imaging for protein crystallography can be found by Sebastien Desbois, Shane A., Seabrook and Janet Newman in *Acta Cryst.* (2013). F69, 201–208.

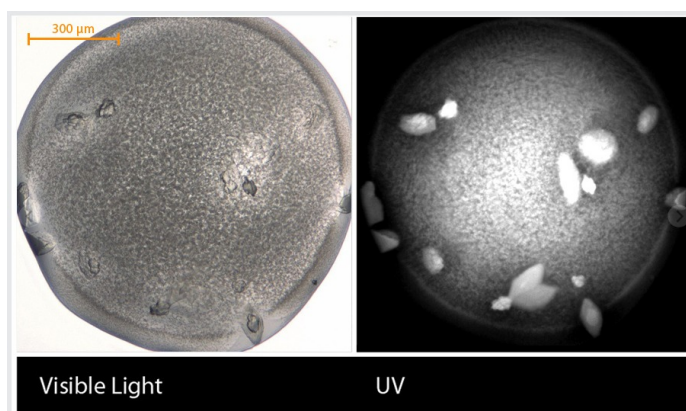
II. TYPICAL CAUSES OF FALSE NEGATIVE AND FALSE POSITIVE UV IMAGING RESULTS

False negative results from UV imaging are commonly caused by one of the following reasons:

- Proteins with low tryptophan concentration.
- Spectral shifts due to ligand binding or protein-protein interactions.
- Absorption of UV from the protein, e.g. the heme group in catalase absorbs strongly at the emission wavelength of tryptophan.
- Quenching from screen solutions, e.g. nitrate and cobalt quenching (see Example 1).
- Quenching from imidazole that may be present in protein after purification with hexahistidine tags.

False positive results are commonly due to one of the following reasons:

- Phase separation.
- Protein aggregation.
- Absorption of protein on non-protein crystals.
- Fluorescent small molecules and salt crystals, e.g. Primuline Yellow.



III. SAMPLE UV IMAGES AND DISCUSSION

Example 1: Effect of Quenching on UV images

Figure 1 depicts UV fluorescent images of glucose isomerase screened against SaltRX screen. Row D shows significantly reduced fluorescence due to quenching from nitrates caused by ammonium and sodium nitrate present in that particular row of the screen, even though protein crystals are present in those drops.

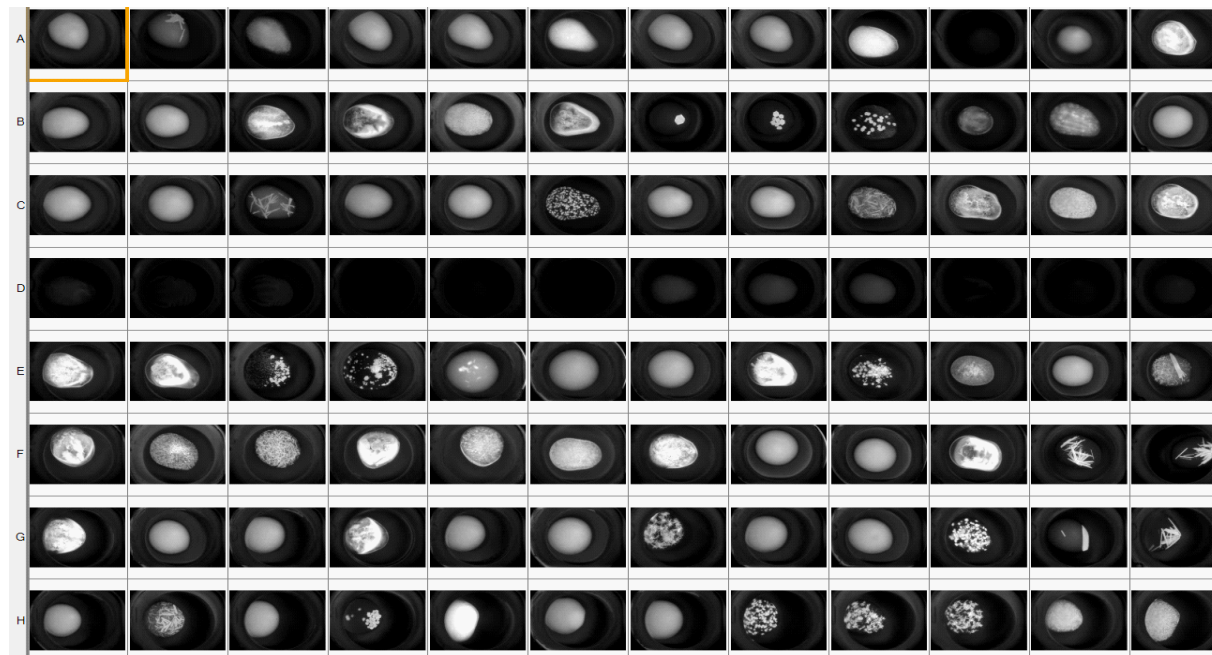


Figure 1. Glucose isomerase sample and SaltRX screen imaged with UV. Row D contains ammonium nitrate and sodium nitrate that quenches UV fluorescence, resulting in false negatives.

Example 2: Effect of Plate Fluorescence on UV Images

Figure 2a depicts a drop imaged on a SWISSCI MRC2 crystallization plate that has minimal fluorescence. The crystallization drop is easily imaged in UV and not obscured by background fluorescence. Image 2b depicts a drop imaged on a highly fluorescent plate, obscuring contrast in the protein drop.

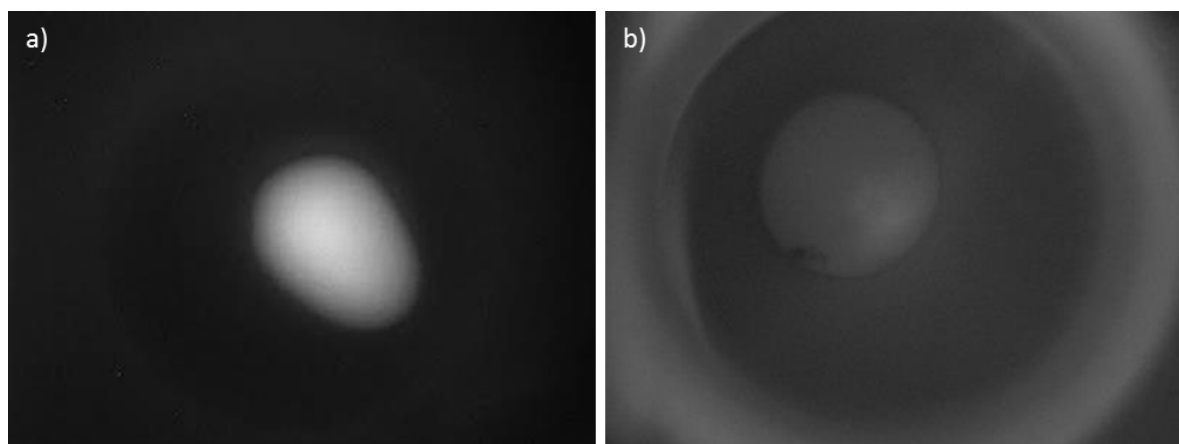


Figure 2. Drops imaged on crystallization plates with varying fluorescence properties.

Example 3: Effect of Exposure Times on UV Images

In the following series of images, the same drop was imaged with various exposure times. Image 3a is underexposed at an exposure time of 50 ms, and detail is lost in the image. Image 3b is correctly exposed at an exposure time of 200 ms, resulting in ideal drop content detail. Figure 3c is overexposed at an exposure time of 500 ms, causing a loss of detail in particular areas when compared to image 3b.

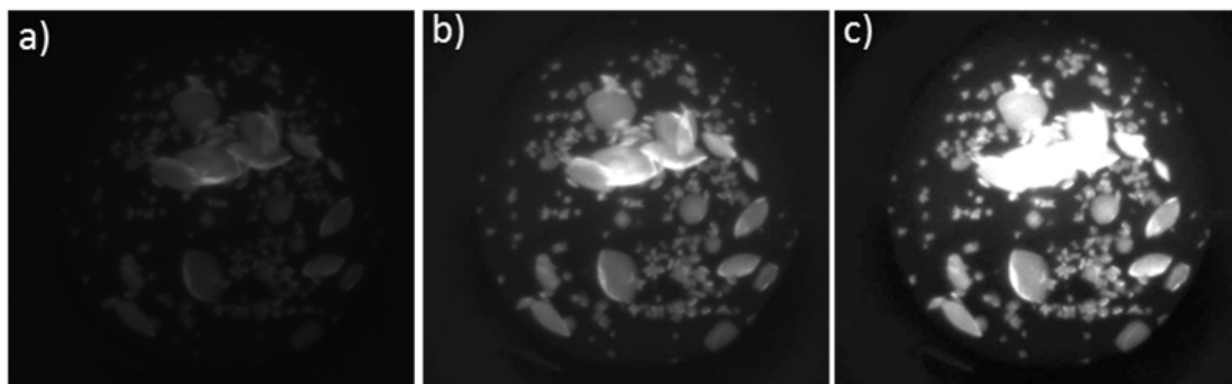


Figure 3. Protein crystals imaged with UV at different exposure times.

Example 4: Effect of Leveling on UV Images

The series of images depicted in Figure 4 shows the same drop before and after the brightness and contrast was adjusted using the Image Tuning tool in Rock Maker. Image 4a is dark, and shows little drop content detail when compared to image 4b, which has been leveled appropriately in Rock Maker and shows more of the drop's contents.

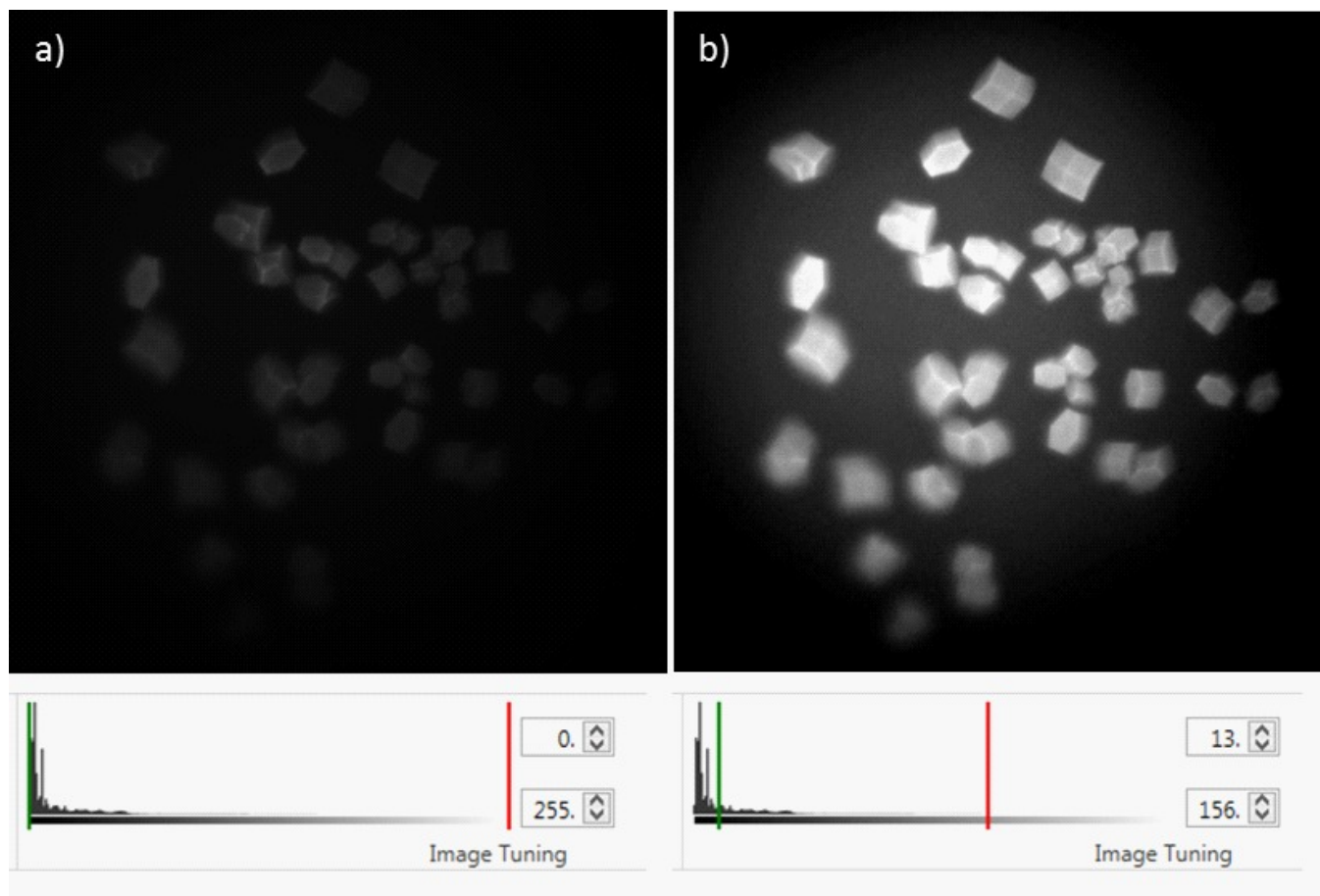


Figure 4. Protein samples imaged with UV before and after leveling has been applied.

Example 5: Effect of Focus on UV Images

In Figure 5 below, image 5a depicts a poorly focused extended focus image (EFI), resulting in loss of detail. This was caused by the start and stop z range being below the focal plane of the crystals in the drop. By adjusting the z range to start at the bottom of the drop and then finish at the top, an appropriately focused image was obtained as shown in image 5b.

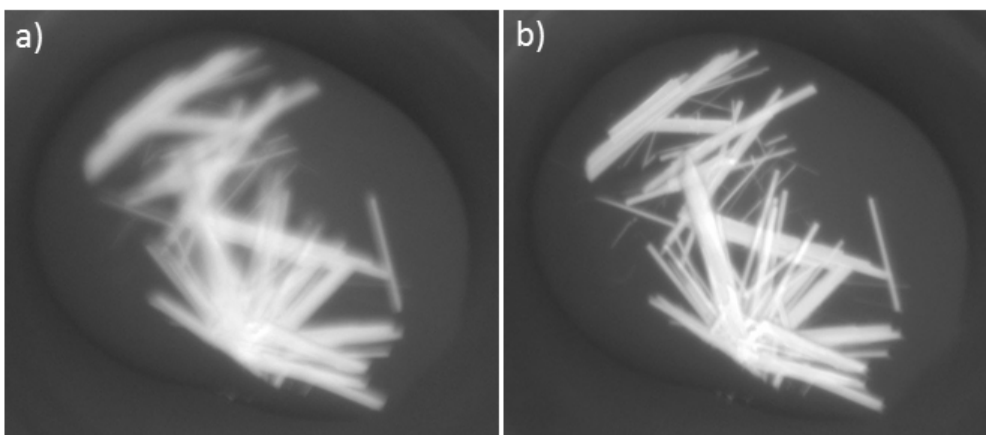


Figure 5. Protein crystals imaged with UV at varying focus.

IV. QUICK REFERENCE FOR BETTER UV IMAGING

- Be sure to use non-fluorescent plates and seals (see [Formulatrix UV Plate Compatibility Guide](#) for more information).
- Increasing exposure time yields higher-contrast images resulting in easier object identification.
- Adjusting image contrast in Rock Maker with the Image Tuning tool often reveals more image detail.
- Make sure that the drop is being imaged in Z in its entirety: Use the F11/F12 keys in Rock Maker to look through the "slices" that contribute to the EFI. If the first or last several slices are blank, the top or bottom of the drop might be outside the z-range captured during imaging. The z-range can be adjusted via Rock Imager's Plate Type Definition file for that particular plate type.
- Check that plate seals do not block UV light.