A Good Practice Towards Effective UV Imaging of a Crystallization Experiment

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Abstract

Intrinsic UV fluorescence of protein has been used to detect protein crystals and to differentiate them from non-protein crystals such as a salt.[1] This technique has been further implemented into automated UV fluorescence microscopic imagers, such as Rigaku's Minstrel DT and HT UV, that have been proved to boost the productivity of protein crystallization. The quality of the UV image of a crystallization drop is crucial for getting reliable information out of the experiment. UV images of lower quality could result in false positives and false negatives. Therefore, it becomes necessary to understand the UV image quality so as to make the best usage of the technology. Among several attributes of image quality, the system resolution, the optical contrast, and the signal-to-noise ratio are at the top of the list. The optical resolution and contrast are system dependent characteristics based on the design of the optical train. The signal-to-noise ratio, on the other hand, depends largely on the crystallization drop and media. The protein sequence and its tertiary structure, as well as the chemical environment in the crystallization solution directly affect the level of UV fluorescence signals. However, a significant amount of background noise is contributed by the consumables with which the crystallization experiment is carried out. Our experiments have indicated that UV absorption and non-specific fluorescence associated with the consumables are the major sources of noise in a UV imaging experiment. We will describe the methods that we used to quantify the effect of the consumables to the UV imaging quality and will provide Rigaku recommendations for consumable selection. Examples will be provided.

Introduction

Ultraviolet (UV) fluorescence has become an increasingly useful method in the past decade for detecting protein crystals.[2] Coupled with the emergence of automated imaging technology, scanning large numbers of protein crystallization experiments with UV light in a high throughput manner has become a reality. In UV imaging, fluorescing amino acids (including tryptophan) absorb photons at an excitation wavelength and emit UV light at longer wavelengths. The emitted light is captured by a UV-capable camera, allowing a scientist to easily distinguish protein crystals from crystals which do not contain amino acids (such as salt crystals). The quality of a UV image can be judged by two major factors: 1) the UV signal intensity from the crystal and 2) the signal-to-noise ratio comparing the intensity of the crystal to the UV background noise. Consumables such as sealing films and crystallization plates used in crystallization experiments play an important role in achieving good UV image quality.

When a UV image is taken, UV light passes through the seal twice: once while light at the excitation wavelength travels from the UV light source to the drop and once while fluorescence light emitted from protein in the drop travels to the camera (Fig. 1). As a result, absorption of UV light by the seal directly affects UV signal from the crystal recorded by the camera. The absorbance spectrum of sealing media, such as films and cover slips, can be determined using a spectrophotometer. Light with a wavelength in the region of 280 nm – 295 nm is of most importance due to the absorption of photons in this region by tryptophan.

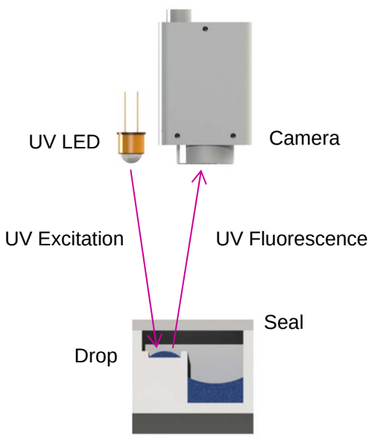

Figure 1. Schematic drawing of UV light from a light source through a protein sample drop to a camera.

The plastics in many sitting drop plates exhibit nonspecific UV fluorescence in the same range as the emission spectrum of protein crystals. This background fluorescence reduces the signal-to-noise ratio of UV signal intensity from protein crystals to the background signal of the drop sample. A high signal-to-noise ratio allows for easy detection of protein crystals in UV images.

Images of crystallization drops in direct contact with a plate are affected by the UV background. Therefore, a low UV background plate is preferred for the sitting drop experiments but not for the hanging drop experiments. Nevertheless, the UV absorption of the sealing media affects both crystallization experiments and a low UV absorption seal is preferred. We have studied the UV properties of different sealing films and crystallization plates. In this poster, we present methods that evaluate the UV characteristics of plates and seals as well as examples of how they affect the resulting UV images.

Materials

Crystallization seals
- Greiner VIEWseal transparent sealing film (676070)
- Hampton Research hanging drop siliconized glass circle cover slides, 18 mm diameter (HR3-239)
- Hampton Research Crystal Clear sealing film (HR3-609)
- Hampton Research Crystal Clear sealing tape (HR4-506)
- Molecular Dimensions LCP glass cover slide (MD11-52)

Crystallization reagents and protein
- Lysozyme (20 mg/ml) Hampton Research Crystallization Kit HR7-108
- Lysozyme Crystallization Buffer: Sodium Chloride 1.4 M, Sodium Acetate 0.1 M, pH 4.2

Crystallization plates
- Rigaku UV+ Low Profile Microplate 206899
- Greiner CrystalQuick low profile, polypropylene 609171
- Greiner CrystalQuick low profile , cycloolefin 609871

Lab instruments and software.
- Rigaku Minstrel DT UV Imager (Fig. 2)
- Art Robbins Phoenix Liquid Handler
- Molecular Devices SpectraMax M2 Spectrophotometer
- ImageJ Image Analysis Software

Figure 2. Rigaku Minstrel DT UV

Experimental Procedure

Three experiments were performed to evaluate different consumables and their effects on UV image quality characteristics: (1) absorbance spectroscopic experiments of different sealing films, (2) UV imaging experiments using different sealing films and different crystallization plates, (3) quantitative image analysis.

Absorbance spectroscopic experiments with crystallization seals. Each seal listed in the Materials section was attached to an SBS format frame and loaded into the Molecular Devices SpectraMax M2 spectrophotometer. The absorption spectrum, a measurement of optical density (OD), was then gathered and graphed for each seal over a wavelength range of 220 nm to 400 nm and with a stepping resolution size of 2 nm. An absorbance spectrum without a seal was also gathered to establish a baseline.

Imaging experiments. All images (both visible and UV) were gathered using the Rigaku Minstrel DT UV. UV images were captured at an exposure time of 600 ms using a long pass filter. Well-formed lysozyme crystals were selected for UV imaging. Five UV images were taken of the same crystal using five different seals on the Rigaku UV+ plate. Crystals from the Greiner 609171 and 609871 plates were imaged using the same sealing film (Hampton Research HR-506) with both visible and UV light. Blank wells from the Greiner plates with no protein drops or seals were also imaged in UV to measure the UV background fluorescence.

Image analysis. ImageJ was used to quantitatively determine the background UV intensity of each plate by calculating the average UV intensity of the blank plate images. The signal-to-noise ratios of the UV images of crystals from the Greiner 609171 and 609871 plates were also determined by finding the average intensity of the crystal from each plate and dividing it by the average intensity of the well.

Results

Absorbance spectra

The sealing films, Greiner 676070, Hampton Research HR3-239, and Hampton Research HR4-506, gave clear UV images and had an absorption that was less than 0.16 OD at 290 nm, which corresponds to a 14.8% of absorption of UV excitation light. Both Hampton Research HR3-609 and Molecular Dimensions MD11-52 sealing films gave washed out UV images and had an absorption that was greater than OD of 2.0 (Fig. 4), which corresponds to a 86.5% of absorption of UV excitation light. Table 1 lists the UV absorption of each seal at the excitation wavelength.

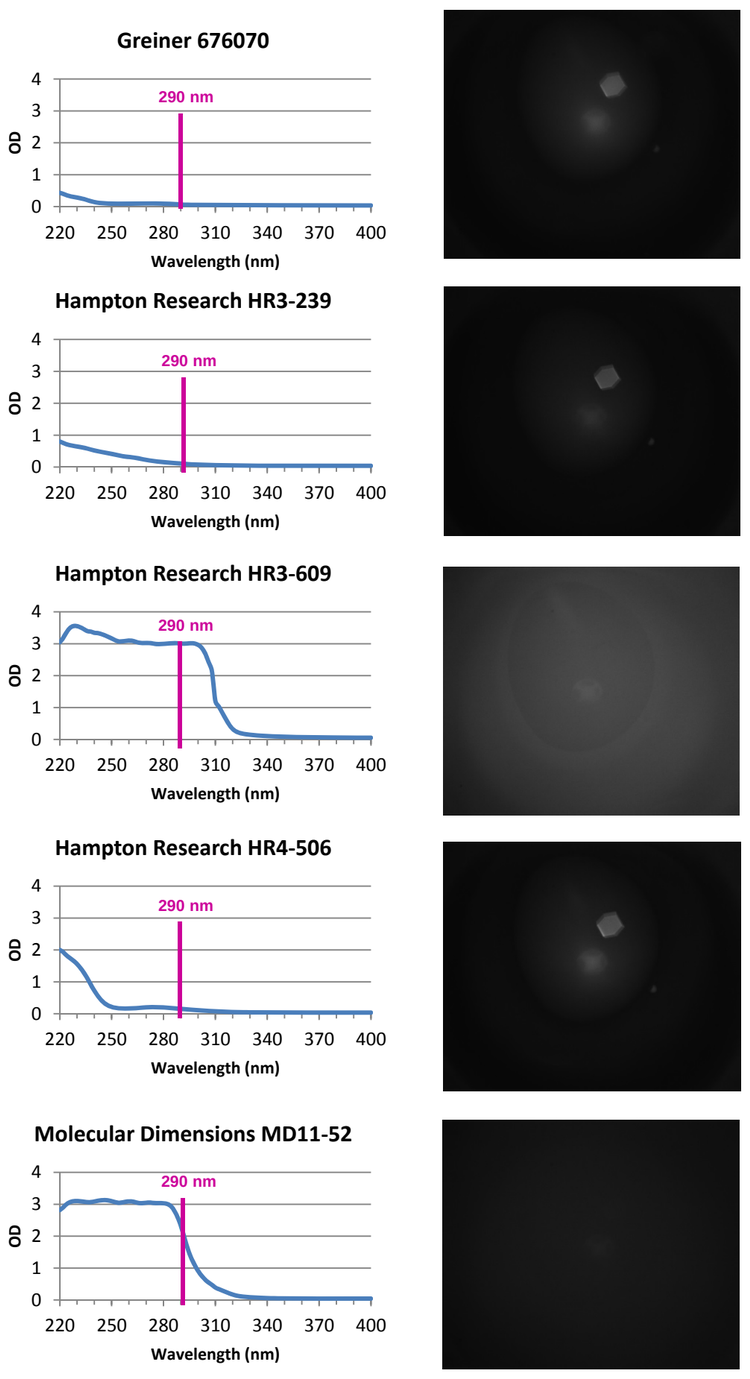

Figure 4. Absorption spectra and UV images of lysozyme crystals using common seals.

Table 1. Absorption of seals at 290nm

Seal	Absorbance (OD)
Greiner 676060	0.066
Hampton Research HR3-239	0.112
Hampton Research HR3-609	3.010
Hampton Research HR4-506	0.153
Molecular Dimensions MD11-52	2.329

Plate UV background

The Greiner 609171 plate made of a polypropylene material showed washed out UV images (Fig. 5a) with a signal-to-noise ratio of 1.1 (Table 2) due to high UV background fluorescence from the plate. The Greiner 609871 plate made of a cycloolefin material gave clear UV images (Fig. 5b) with a signal-to-noise ratio of 3.9 (Table 2) due to low UV background fluorescence, which provides an easily detectable UV signal in high throughput applications. Fig. 6 shows the comparison of UV images of empty wells without drops of two different plates, Greiner 609171 and 609871, indicating the different level of background fluorescence measured by signal-to-noise ratio (Table 3) due to different materials.

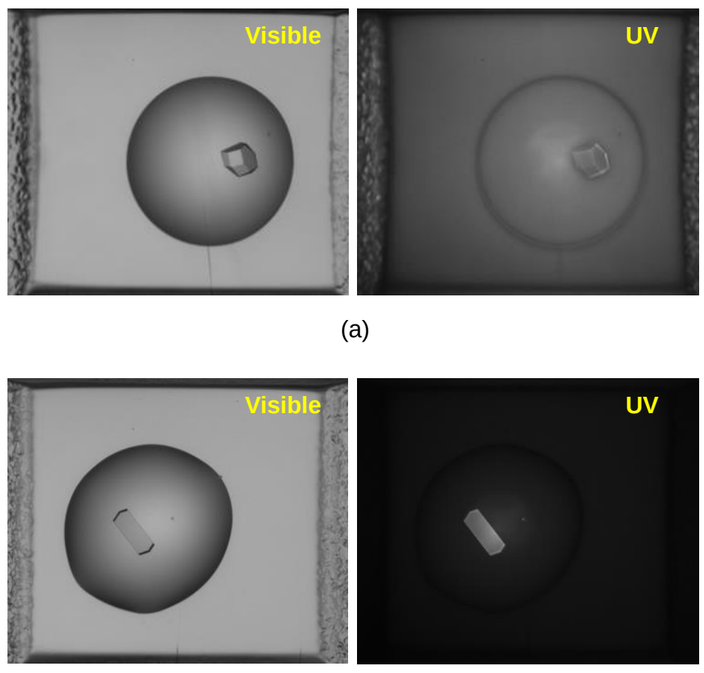

Figure 5. Visible and UV images of lysozyme crystals comparing UV plate background. (a) Greiner 609171; (b) Greiner 609871

Table 2. Signal-to-noise ratio from UV images of different plates

Plate	Signal-to-noise Ratio
Greiner 609171	1.1
Greiner 609871	3.9

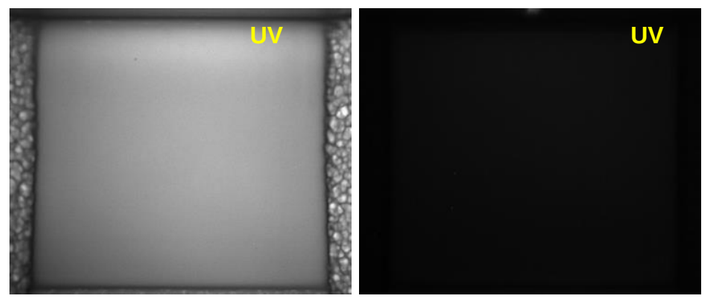

Figure 6. UV images of plates with no protein drop or seal. (a) Greiner 609171; (b) Greiner 609871

Table 3. Average background UV intensity of plates expressed as a percent of maximum intensity

Plate	Background UV Intensity
Greiner 609171	51%
Greiner 609871	4%

Discussion

The absorbance spectra and corresponding UV image of each seal clearly indicate the impact of choice of seal on the resulting UV image quality. The Greiner 676070 had an absorption of 0.0661 OD at 290 nm and yielded clear and bright UV images, while the Hampton Research HR3-609 had an absorption of 3.0097 and yielded dark UV images. Good UV image quality is highly correlated with experiments that use a low UV absorption seal. Although a longer exposure time can increase the intensity of UV images of protein crystals, it can also amplify the UV background noises as well as cause potential damage to crystals. Rigaku recommends that seals used for UV imaging have an absorption OD of less than 0.16 at 290 nm in order to achieve optimal UV image quality.

The background UV fluorescence of the plate affects the signal-to-noise ratio of crystal UV images. The Greiner 609171 and 609871 are two good examples, where a large difference in noise intensity was observed, 51% and 4%, respectively. The low background Greiner 609871 resulted in a clear UV image of a crystal while the high background Greiner 609171 yielded a washed out UV image of a crystal that is more difficult to detect. The method of taking a UV image of a blank plate with no protein and determining the UV background intensity provides a metric that can be used to compare the UV background a plate contributes to the final image. A low UV background is correlated with a high signal-to-noise ratio and vise versa. Cycloolefin (also known as cyclic olefin copolymer) used in Greiner 676070 is a plastic which in general produces low background fluorescence of crystallization plates. The primary contribution of nonspecific fluorescence from a plate is typically from its plastic. Rigaku recommends that plates used for UV imaging have a plate background average intensity of less than 12% of maximum intensity when exposed at 600 ms.

[1] Jian Xu, Max Petersen, and Michael Willis, ACA 2010 poster presentation, Chicago, USA
[2] Jian Xu, ACA 2009 oral presentation, Toronto, Canada