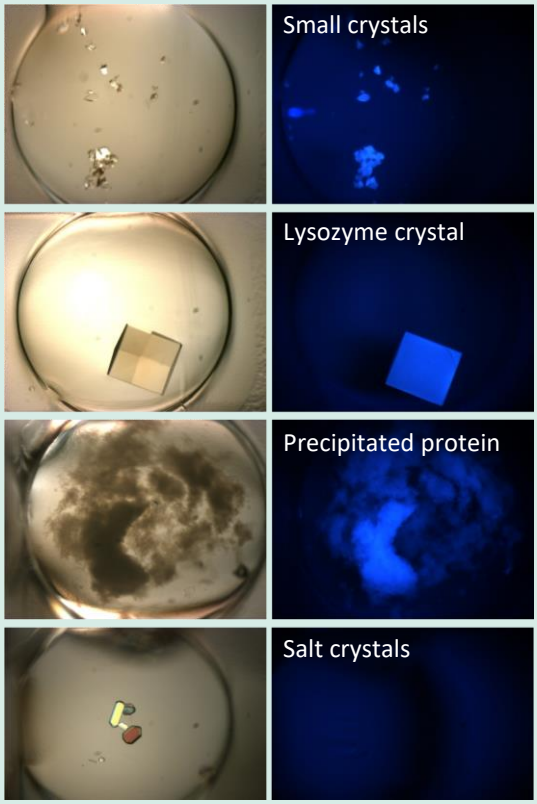
Intrinsic Fluorescence Imaging

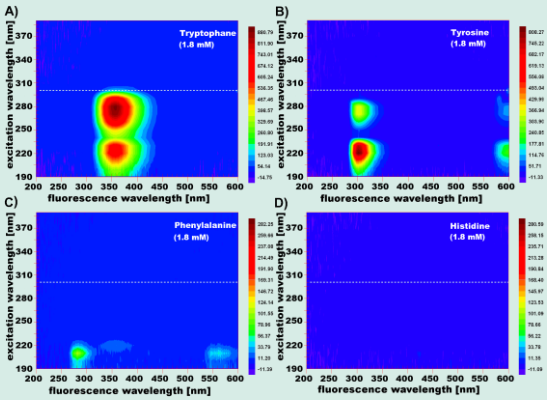
In protein crystallization, a widely used application for UV light is intrinsic fluorescence imaging. The fluorescence of proteins is particularly generated by the UV-fluorescence properties of tryptophane. Excited with ultraviolet light, tryptophane shows a characteristic blue fluorescence of about 360 nm. The main application of intrinsic fluorescence imaging is an in situ check of whether a crystal consists of salt or protein.

96 well plates are available in low background fluorescence polymer types. Therefore, intrinsic fluorescence imaging is a widely used method for quick and reliable protein crystal identification without the necessity to open the crystallization containment.

Also, precipitated proteins show intrinsic fluorescence. In contrast, salt crystals don't show fluorescence at all, when illuminated with the same UV-light spectrum. Even when they show birefringence, making it difficult to distinguish them from protein crystals when illuminated in bright light. However, when UV-light illumination was applied the lack of fluorescence makes distinguishing from protein crystals easy.

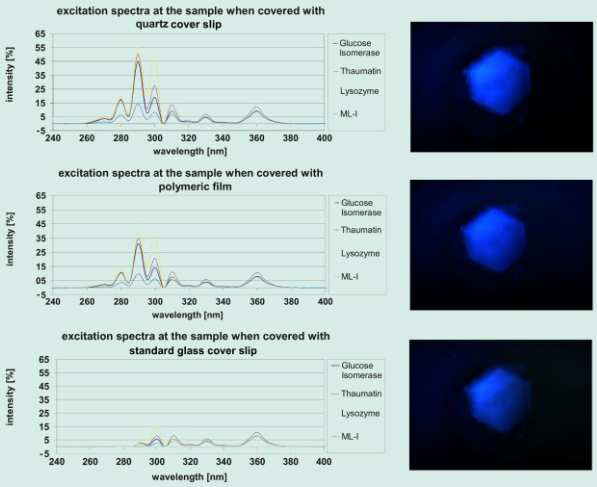


Fluorescence Excitation can be achieved on all proteinogenic aromatic amino acids. However, the intrinsic fluorescence intensity in the visible or near UV range of the light spectrum is dominated by the amino acid tryptophan. On average 93% of all proteins have at least one tryptophane in their sequences. Thus, intrinsic fluorescence excitation can be used in almost all projects.



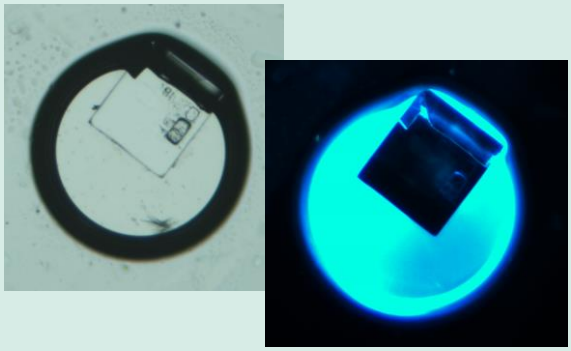
Transmission properties of the most abundant plate sealing materials are quite different when it comes to the UV-light regime of the light spectrum.

Application of a broad excitation spectrum: Particularly shorter wavelengths (λ < 300 nm) were absorbed by standard covering materials. When incident illumination is applied for UV-light fluorescence such properties matter. However, if a UV-excitation spectrum is applied instead of a monochromatic or a narrow band spectrum, the usage of a variety of covering materials is possible. On the right, examples of covering materials and their transmission spectra are shown. Significant absorption is observed at wavelengths between 260 - 300 nm by the sealing material. Nevertheless, fluorescence is still remarkable intense due to excitation by remaining wavelengths > 300 nm.

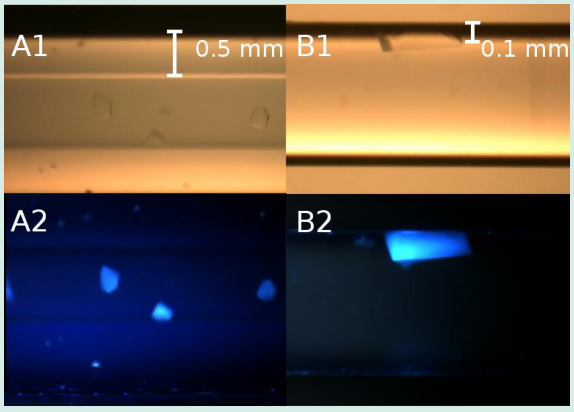


Intrinsic protein fluorescence in 96 well plates is the standard application for crystal identification. If a plate and sealing film with low background fluorescence properties are used, intrinsic fluorescence can be as intense as in this example.

Intrinsic fluorescence Imaging in LCP can be applied as well, even when the sample is covered in a sandwich arrangement by applying glass sheets. In this particular example, the protein is still located in the LCP, while the crystal is not proteinogenic due to its lack of intrinsic fluorescence.



Intrinsic fluorescence in capillaries: Crystallization in capillaries is of some use in liquid-to-liquid counter diffusion experiments. Although quartz capillaries are available, mostly common glass capillaries are used due to cost efficiency. Capillaries might significantly differ in their wall thicknesses depending on the type of experiments and required mechanical stability. On the right, we have tested thin-walled and quite thick-walled capillaries. When a broad UV spectrum was applied, intrinsic fluorescence imaging was possible even with 0.5 mm thick glass walls.



Salt crystal fluorescence and background: Inorganic salts or PEGs are commonly used as precipitants in protein crystallization; such salt crystals don't have aromatic groups or conjugated π-electron systems at all. Therefore, the intrinsic fluorescence of salt crystals is highly unlikely. This allows using intrinsic fluorescence imaging as a cost and time efficient *in situ* protein crystal identification method.