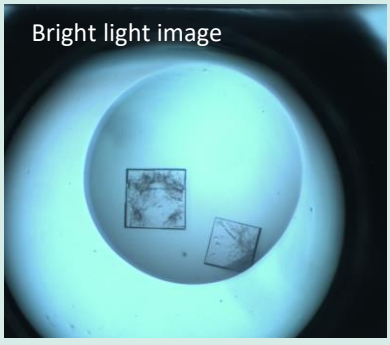
Trace Fluorescence Imaging for Immediate Crystal Identification

Trace fluorescence imaging (TFI) can be understood as an alternative to intrinsic fluorescence imaging. In contrast to the latter, TFI requires a covalently bound fluorescence dye which was Carboxyrhodamine in all shown examples. However low energy excitation light is sufficient for fluorescence imaging.



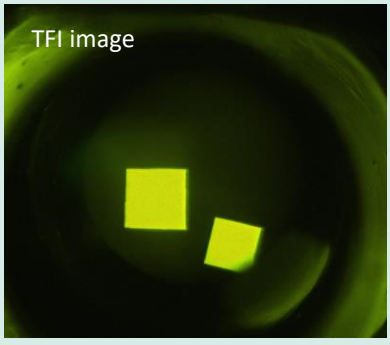
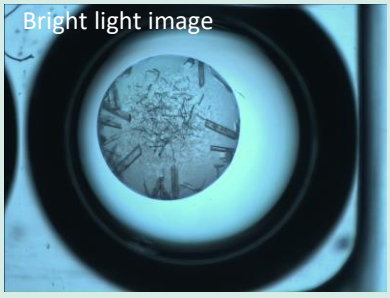


Figure 1.1
Bright light and trace fluorescence images of covalently labelled crystals. Here the epifluorescence optics of SpectroLight 610 has been used. A standard crystallization procedure was applied to utilize a 96-well plate.



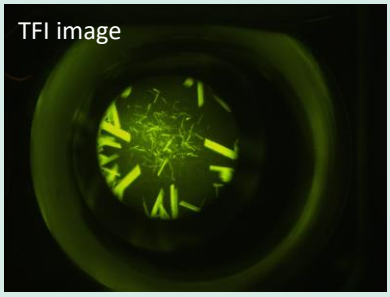


Figure 1.2
Although the average level of stained proteins is about 5%, the fluorescence light intensity as well as the low background is remarkable. In contrast to intrinsic fluorescence, where the fluorescence properties of the amino acid tryptophan were used when excited with UV light, here low energy green light is sufficient for exciting fluorescence. Photochemical reactions are reduced to a minimum even with long exposure times.

SpectroLight 600 can be optional equipped for applying TFI. All components can be integrated into the standard set-up.

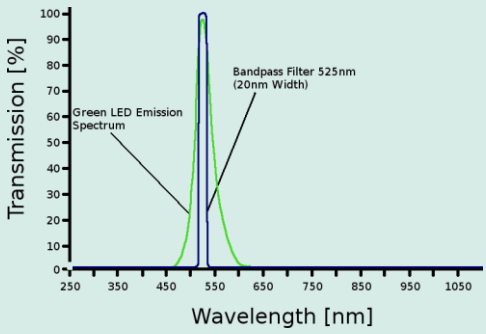
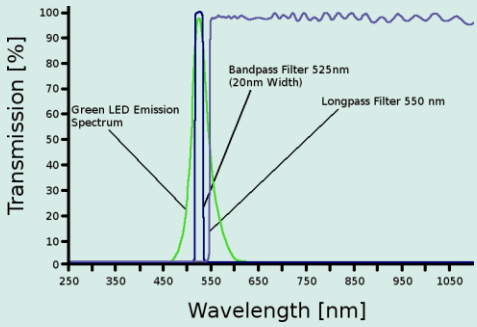
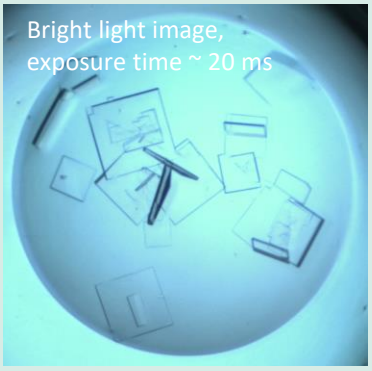


Figure 1.3: Excitation
For the trace fluorescence excitation, filter and light source match precisely, otherwise the required level of sensitivity and suppression of background fluorescence can't be achieved.





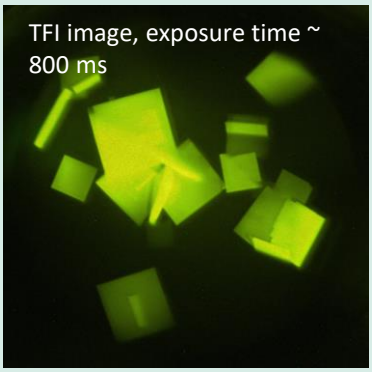
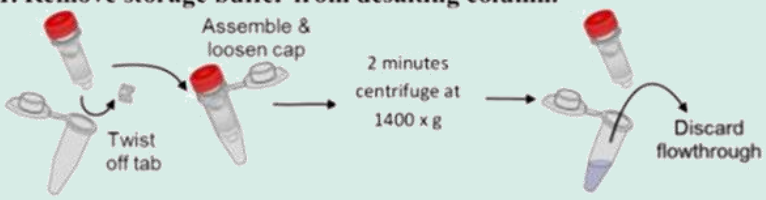


Figure 1.4: Emission
Excluding excitation light is as important as the fluorescence excitation itself. Usability of trace fluorescence based on an optimal combination of excitation but also emission detection optics.

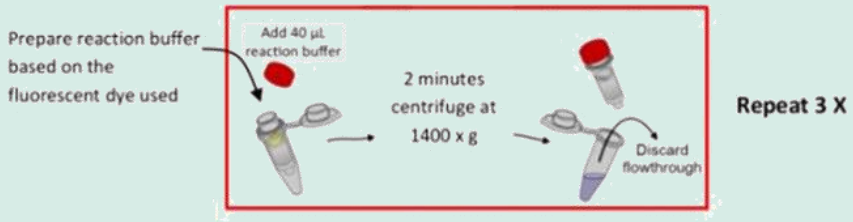
Preparatory:

Start with a stock protein solution of as low as 80 µL at the concentration of 12.5 mg/mL in user's preferred crystallization buffer. To prepare one of the provided desalting columns in reaction buffer follow the illustration below:

Prep 1. Remove storage buffer from desalting column.

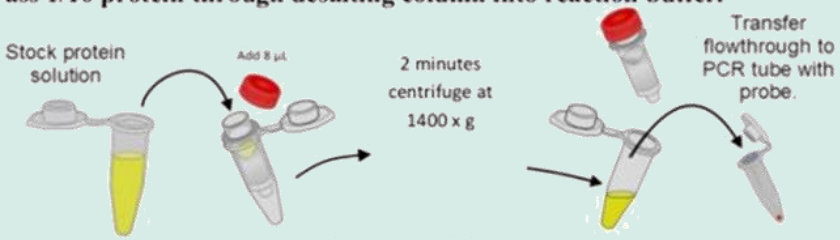


Prep 2. Equilibrate with trace labeling reaction buffer.



Trace Labeling:

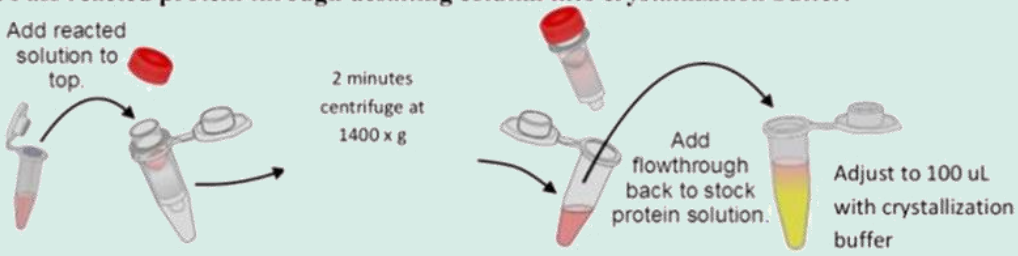
Step 1. Pass 1/10 protein through desalting column into reaction buffer.



Step 2. Equilibrate column in crystallization buffer while reaction occurs.

Set aside the PCR tube with protein and probe and allow it to react while equilibrating the same desalting column in user's crystallization buffer. See Prep 2 above for equilibration instructions.

Step 3. Pass reacted protein through desalting column into crystallization buffer.



Crystallization plates can now be set up using standard procedures. The plates can be viewed using transmission microscopy as normal. To visualize under fluorescent conditions, use an epifluorescence microscope at low power (4X objective) in a darkened room. Set the excitation and emission wavelengths depending on the fluorescent dye used. For maximum viewing ease and efficiency, use the automated Crystal X-2 to rapidly collect photographs of the crystal wells in visible or fluorescent light.