Fluorescence Cuvette Cleaning

Important:

- Make sure that any sample/solution you might have left in your cuvette will not react violently with whatever solution/liquid you will use for cleaning!
- **Do <u>not</u> use** strong bases such as potassium or sodium hydroxide. They can damage the cuvettes.

A. **Low Molecular Weight Water Soluble Samples**

1) Rinse with purified water.
2) Then, rinse with acetone or ethanol.
3) Blow dry with clean, dry, compressed air or nitrogen.
4) Clean the exterior of the cuvette by wiping gently with lens cleaning paper wetted with acetone or ethanol.

Make sure that you do not build up pressure in the cuvette. Don't use house air without having a filter installed. This is often sufficient to clean the interior of the cuvette.

You can also use a cuvette washer for this if you have one. I do not recommend cuvette centrifuges for drying.

B. **Biomacromolecules**

Particularly denatured proteins can be quite sticky and their removal from quartz surfaces often needs more aggressive cleaning methods. Three methods are listed below. You may need to try more than one of them before your cuvette is clean.

I. Starna and Hellma supply their own brands of cuvette cleaning agents, usually solutions of anionic and non-ionic detergents in the pH range 9.5 to 12.

Their websites give guidance on cuvette cleaning and their recommended protocols are good. However, they are not always enough to remove protein from cuvette surfaces.

II. "Nano Strip" (from Cyantek)

Wear lab coat, goggles, gloves, closed-toed shoes and long pants, all while working in a fume hood! To use Nano Strip follow the Nitric Acid protocol below using Nano Strip instead. <u>Be Careful, plastic transfer pipettes or flat gel loading tips will not hold up to Nano Strip</u>.

III. Nitric Acid

Wear lab coat, goggles, gloves, closed-toed shoes and long pants, all while working in a fume hood!

- After use, take the cuvette from the holder and remove as much of the sample as possible using a fine-tipped disposable transfer pipette or a gel loading tip for micro-cuvettes.
- Rinse four or five times with either buffer or purified water (a buffer rinse is used for samples with proteins that precipitate in purified water). If a buffer rinse is used, it should be followed by further rinsing with purified water.
- Rinse several times with acetone or ethanol, then blow dry thoroughly with clean dry compressed air or nitrogen.

Note:
You can also use a cuvette washer for this if you have one. I do not recommend cuvette centrifuges for drying.

It is particularly important that the cuvette is completely dry before the next step. If it is not, heat from mixing acid with water or ethanol might damage the cuvette!
Continue with either the clear cuvette or black antireflective coating cuvette steps below.

Clear Cuvettes (no black coating on the outside)

- Immerse in concentrated nitric acid, making sure that the air in the cuvette is fully displaced with the acid.

Caution:
Make sure that the vessel containing the acid is clearly marked with its contents and is stored in a safe place. Make sure that the cuvette does not get damaged while moving the vessel around.

- Allow cuvette to soak for 10 minutes to 30 min (or overnight with 2 M nitric acid or if using Nano-Strip soak 30 min).
- Over a drip tray, carefully remove the cuvette from the acid using stainless steel tweezers, gripping the cuvette lightly by the neck, not by the optical surfaces.
- Using a fine-tipped disposable transfer pipette or gel loading tips, carefully remove as much of the acid as possible from the cuvette. (Again Nano-strip is not compatible with plastic pipettes)
- Carefully rinse the cuvette with purified water several times.
- After at least 7 rinses, measure a fluorescence spectrum of the water filled cuvette to prove that it is clean using the parameters you would use for measuring the sample from which the contaminations came from.
- Rinse the clean cuvette with acetone or ethanol and dry it.
- Clean the exterior of the cuvette by wiping gently with lens cleaning paper wetted with acetone or ethanol. Check that the cuvette is clean by holding it up to the light. There should be no visible contamination or smears.
- If it is not needed immediately, store the cuvette in a clean, dry environment.

Cuvette with Black Antireflective Coating on the Outside

Do not immerse coated cuvettes in nitric acid. Strong acids (and certain solvents) will degrade the coating.

- fill the cuvette with acid making sure not to drip any on the outside surface using a transfer pipette or pipet tip. Avoid metal syringe needles or glass since they might scratch the cuvette.
- Making sure that the air in the cuvette is fully displaced with the acid.

Caution:
Make sure that the vessel containing the acid is clearly marked with its contents and is stored in a safe place. Make sure that the cuvette does not get damaged while moving the vessel around.

- Allow to soak for 10 minutes to 30 min (or overnight with 2 M nitric acid), (with Nano-Strip 30 min should be enough).
- Using a fine-tipped disposable transfer pipette or gel loading tips, carefully remove as much of the acid as possible from the cuvette. (Again Nano-strip is not compatible with plastic pipettes)
- Carefully rinse the cuvette with purified water several times.
- After at least 7 rinses, measure a fluorescence spectrum of the water filled cuvette to prove that it is clean using the parameters you would use for measuring the sample from which the contaminations came from.
- Rinse the clean cuvette with acetone or ethanol and dry it.
- Gently clean the outside of the cuvette with acetone or ethanol using a soft lint free tissue. Check that the cuvette is clean by holding it up to the light. There should be no visible contamination or smears.
- If it is not needed immediately, store the cuvette in a clean, dry environment.