

Flow Managers Meeting

Standardisation in Flow Pt 2

The Ideal Standardisation Protocol

Need to Measure?

- Machine Alignment
- Sensitivity
- Linearity
- Spectral Response
- Flow Rate
- Anything Else?

Flow Managers Meeting

Standardisation in Flow Pt 2

Machine Alignment

Simplest to define

The Instrument is properly aligned when the laser(s) is (are) accurately pointed at the interrogation point(s) and the interrogation point is properly focussed on the detectors

Measured using small (often $3\mu\text{m}$) highly fluorescent particles

Normally set up by Engineer (Not always perfectly)

Flow Managers Meeting

Standardisation in Flow Pt 2

Sensitivity

Bit More Difficult

Should be measured as an MESF value for the lowest detectable intensity for a given PMT Voltage

Effected by Alignment, emission filter, laser power, flow rate, temperature, detector, amplifier, day of the week, whether there's a Y in the month etc

Most often measured by "whether we can see all 8 (or 6 or 5) peaks in the bead set"

Flow Managers Meeting

Standardisation in Flow Pt 2

Linearity

Hmm

For a given detector there tends to be a range of drive voltages over which it's response can be said to be linear.

Outside of that range things get curvy.

Often measured using either "normal" multi peak beads or with beads designed to be used with a linear amp where the relative intensities are known accurately.

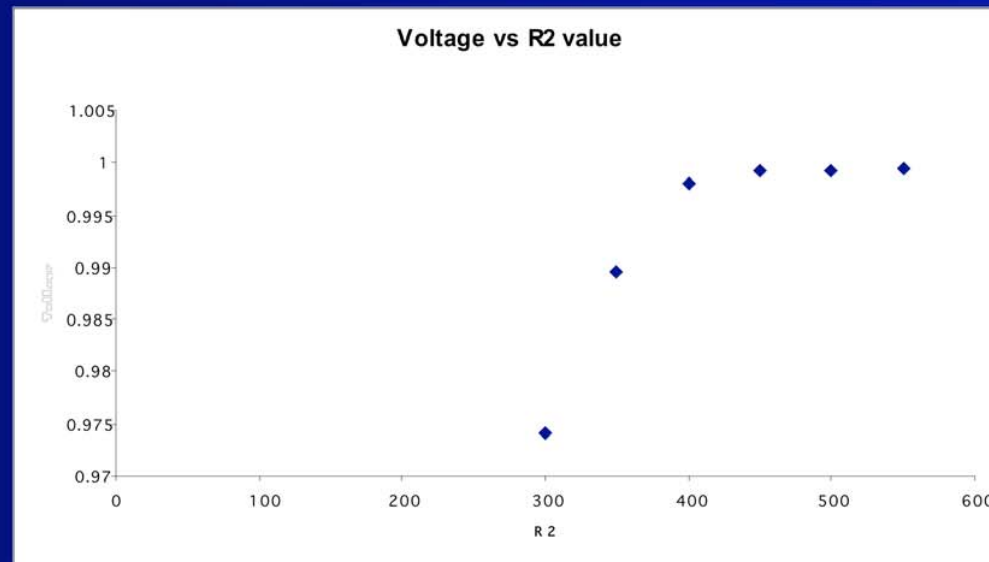
Q. When the linear range of a detector is determined how often does it change. Is it enough to measure once and then recommend avoiding the non linear range

Flow Managers Meeting

Standardisation in Flow Pt 2

Linearity

Sample Output



Flow Managers Meeting

Standardisation in Flow Pt 2

Spectral Response

Depends

Comes down to a combination of the detector sensitivity curve and the filter combination

For the detector, PMTs are supplied on the whole as "standard" or "red sensitive"

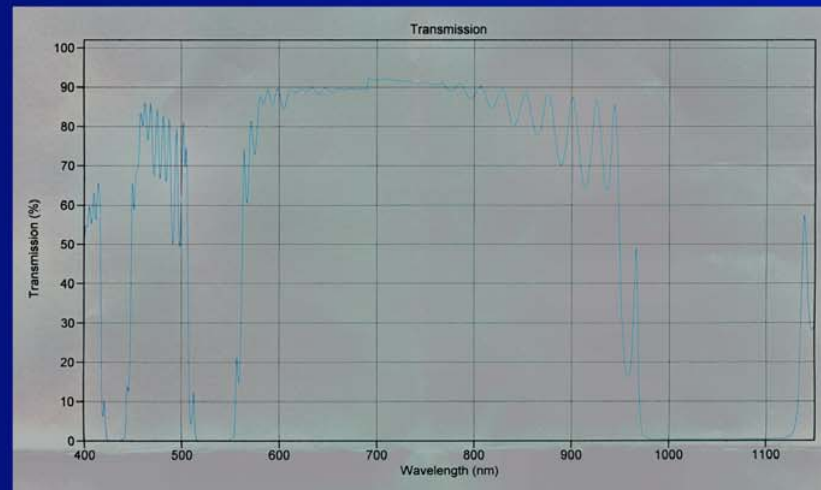
Filters should come with a response curve. If they don't you can always find a friendly Spectrophotometer operator, you may be surprised.

Are filters tested at the correct angle of incidence?

Flow Managers Meeting

Standardisation in Flow Pt 2

Spectral Response



Flow Managers Meeting

Standardisation in Flow Pt 2

Flow Rate

Easy

Will effect CV through increased/decreased turbulence.

Can be checked very simply with calibrated counting beads

Presumably even easier on syringe based systems (but I ain't got any of them)

Flow Managers Meeting

Standardisation in Flow Pt 2

Stream in Air Sorters

Optical set up done when used

May be different set up for different users

May use multiple lasers in weird orders

Any thoughts?

Flow Managers Meeting

Standardisation in Flow Pt 2

The Questions

1. Are these all the criteria we need to approach
2. Do we need all of them
3. What about multi laser machines
4. What about machines with configurable outputs
5. What about the effect of new amplifier technologies
6. What is the minimum monitoring needed to ensure they all stay in spec
7. What is spec