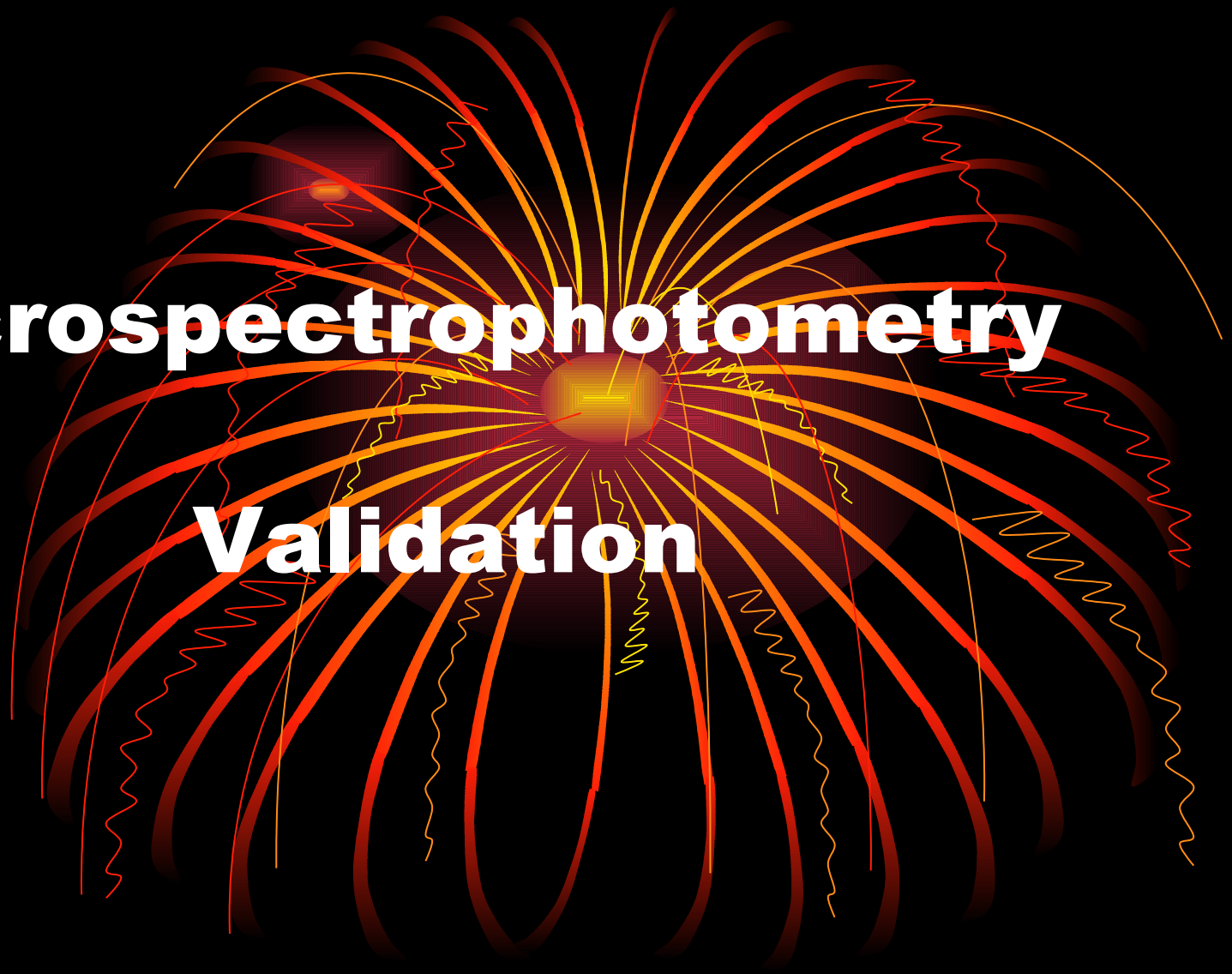
Microspectrophotometry Validation



Reasons for Changing Instruments

- Reduced reliability.
- Limited efficiency.
- Limited availability and cost of replacement parts.
- Problems with Service contract.

Validation Plan

- Once a new instrument has been purchased it is necessary to produce a validation plan.
- The plan will include the parameters to be tested.
- Each parameter will, where possible, have a standard and target:
  - Standard – criteria that ***must*** be met for the system to be considered acceptable.
  - Target – performance criteria that are desirable but not essential.

Measurement Parameters

- Before beginning the validation of the instrument measurement parameters must be decided.
- Use the parameters recommended by the manufacturer or those which proved to be most acceptable when preliminary measurements are carried out during installation or initial testing.
- Check the settings using calibration standards or samples run on other validated instruments.

Validation Plan Parameters

- **The Instrument:**
  - **Measurement Window – optimum size and boundary accuracy.**
  - **Wavelength Registration – accuracy of peak position.**
  - **Photometric Measurement – check intensity at each wavelength.**
  - **Noise Level – check 100% line.**
  - **Second Order Effects – this is where the measurement at a given wavelength includes some component of light from multiples of that wavelength.**

Validation Plan Parameters continued

- **The Instrument:**
  - **Spectral Distortion – Caused by system overload i.e. excess light reaching the detector.**
  - **Focus Level – Is the level of focus critical to spectral shape and peak position?**
  - **Limit of Detection – The system should be able to generate acceptable results from fibres over an acceptable absorbance range e.g. 0.1 – 1.4AU**
  - **Fluorescence – The system should be able to measure fluorescent samples.**
  - **Time – Is the system faster, with respect to producing spectra, than your previous system?**

Validation Plan Parameters continued

- **The Instrument:**
  - **Operator Effects – Do different operators generate identical data?**
  - **Bleaching Effects – If fibres are measured at the same point during Validation will they bleach?**
  - **External Interference – Does the laboratory environment influence the results e.g. lighting, heating, vibrations.**
  - **Polarisation Effects – Does polarisation affect the results?**

Validation Plan Parameters continued

- **Software Issues: (both data capture and analysis).**

The areas to be validated in this section will to some degree depend on the software used and the needs of the laboratory.

- **Algorithms:**
  - **Are the algorithms used to calculate absorbance values accurate?**
  - **The peak finding algorithm should contain no logical flaws and the software must implement it correctly.**
  - **Colour Calculations – If complementary chromaticy co-ordinates are going to be generated they must be accurate.**

Validation Plan Parameters continued

- **Software issues:**
  - **Output Spectrum – does the output spectrum accurately reflect the data in both intensity and absorbance plots?**
  - **Input of Case Data e.g. case number etc.– Is this possible and to an acceptable level?**

Validation Plan Parameters continued

- **Other:**
  - **Consumable Variables e.g. glycerol, slides.**

Validation Plan – An Example

- **Wavelength Registration.**

The Standards and Targets were set as follows:

Standard (1) – The wavelength must be accurate to +/- 2.5nm.

Target (1) – The wavelength must be accurate to +/- 1.0nm

Standard (2) – The adjusted root mean square standard deviation of the mean of each peak must be less than 1.5nm.

Target (2) – The adjusted root mean square standard deviation of the mean of each peak must be less than 1.0nm.

Validation Plan - An Example continued

- **Wavelength Registration.**

Standard (3) – There must be less than 0.5nm drift in the mean position over 24 hours.

Target (3) – There must be no shift in peak position throughout the validation study.

A Holmium Oxide sample was measured on 8 occasions (10 measurements each time) over a 16 day period. Where necessary the results were compared with published peak positions for Holmium Oxide.

Validation Plan – An Example continued

- **Wavelength Registration.**

Conclusions were as follows:

- ***The wavelength accuracy was within 1nm (the Target).***
- ***The wavelength precision fell within the acceptable limits of 2.5nm; all bar one peak was within the target range of 1.0nm.***
- ***There was no evidence of a shift in peak position throughout the study.***
- ***All results demonstrated that they were reproducible.***