7.1 Microspectrophotometer

7.1.1 Function Verification ("Calibration") of a Microspectrophotometer

Verification with NIST Traceable Standards shall be performed each day samples are run, prior to use of the instrument for any casework. Within the software and manuals for this instrument, the manufacturer refers to the following function verification as a "calibration". This is not actually a calibration, as it is merely measuring the function of the instrument, not changing or adjusting its settings.

7.1.1.1

Equipment Required:

- NIST Traceable Calibration Standards

7.1.1.2

The NIST Traceable Filter Set contains:

- 1 Calibration Certificate with date of certification
- 1 Clear Reference Filter
- 1 Holmium oxide Filter
- 1 Didymium Filter
- 1 Neutral Density Filter (OD = 0.1)
- 1 Neutral Density Filter (OD = 0.5)
- 1 Neutral Density Filter (OD = 1.0)
- 1 wooden box for filter storage

The appropriate filters are re-certified every two years by CRAIC Technologies (instrument company). The certificates are maintained with the instruments, and are marked with an expiration date. Contact CRAIC for instructions on submitting the filters to them for recertification. Contact information is with the instrument manuals.

7.1.1.3

Background of Wavelength Calibration using Holmium Oxide & Didymium Filters:

The spectra of Holmium oxide and Didymium filters show peaks at well-documented wavelengths. The Holmium oxide filter has peaks in the UV and visible wavelength regions, and can be used to calibrate the wavelength scale of a microspectrophotometer from 200 nm to 750 nm.

The Didymium filter has peaks in the visible and near IR wavelength regions, and can be used to calibrate wavelength from 400 nm to 950 nm.

How to Check the Wavelength Calibration of the microspectrophometer:

- Measurement of Wavelength Accuracy and Precision

- Power on the instrument. Wait 30 minutes for the lamps to stabilize.
- Follow the calibration directions provided with the instrument.

If the wavelength values of peaks are not within expected QC values, the software will indicate this with an error message. If this occurs, check the settings and parameters and repeat the measurement. If the result is reproducible, remove the instrument from service and contact the Forensic Science Division equipment technicians and/or the instrument manufacturer for further instructions/repair. The instrument cannot be utilized for casework if this instrument verification/calibration is not successful.

7.1.1.4

Detecting trends:

Wavelength and photometric verification checks are run each day of use, and the reports are saved on the microspectrophotometer computer. If the wavelength or absorbance values are not within expected QC values, the software will indicate this with an error message and the instrument shall not be used. The reports are available for examination to detect trends. The filters used for verification checks are recertified every two years. Instrument issues, servicing instances and/or repairs are recorded in a logbook kept with the instrument.

7.1.2 Operation of a Microspectrophotometer:

The Microspectrophotometer may be used on any sample in which measurement of ultraviolet, visible or fluorescence characteristics of a sample is desired. Samples should be mounted on slides with cover slips for measurement in transmittance or fluorescence modes. Samples may be mounted directly for measurement in reflectance modes. Quartz slides and coverslips with glycerol mounting media should be utilized for measurement of samples in the UV region of the spectrum. Visible region measurement can be accomplished on samples mounted on glass slides in a wide variety of mounting media including Cargille oils and adhesives.

7.1.2.1 Starting the Instrument

In order to turn on the system properly, please follow these steps:

- Turn on any lamp housing power supplies first (one by one, as these items draw the most power)
- Turn on the microscope/microspectrophotometer (See hardware diagram for power switch locations, and specific instrument manual)
- Turn on computer
- Let the system stabilize for about 30 minutes

7.1.2.2 Calibration

The calibration of the instrument shall be checked on each day samples are run on the instrument (see 7.1.1 above). Wavelength measurements and calibration reports of these standards are kept in a calibration file on the instrument's computer.

7.1.2.3 System Alignment

Before taking any measurements, it is important to ensure the system is as aligned as possible, in order to achieve the best spectral results. Alignment procedures will differ depending on the type of measurement (transmission, reflectance, etc.), and the illumination source used. In general, all measurements will require alignment in sample focus and aperture/field stop diaphragms.

Sample Focus: The working distance of the objective is a fixed value and varies with each objective. This distance represents the length from objective to sample. Once the sample is visible in the microscope eyepieces (if available - instrument specific) or digital camera, the sample is focused.

Aperture (A) and Field (F) Diaphragm: Both transmission and reflectance light paths contain two diaphragm types, field and aperture. Both diaphragms are labeled correspondingly.

In order to ensure A and F diaphragm alignment, please follow these steps or see the instrument manual:

- With a sample in focus (reflectance sample must be used to align reflectance; transmission sample must be used for transmission).
- Close down the Field (F) diaphragm until it appears on the computer screen, or in the microscope eyepieces, and bring it into focus utilizing the condenser focusing knob.
- Using the corresponding adjustment screws center the Field diaphragm in the image.
- Use condenser screws for transmission and adjust set screws above and below the Field diaphragm for reflectance.

7.1.3 Running of Samples

Samples may be run in transmittance, reflectance or fluorescence modes in the UV and/or visible regions of the spectrum. Plotting in absorbance is recommended. Refer to the instrument manual for specific

information on procedures to run samples in each mode. Dark scans and reference scans must be run whenever collection modes or spectral region parameters are changed.

See sub-discipline specific procedure manuals for further requirements or recommendations concerning recommended sampling modes and/or number of items or areas to be sampled.

7.1.4 Interpretation of Spectra

It is normal that some variation may occur between two samples, especially in the intensity of absorbance values. Some variation in the wavelength of absorbance values may also occur. The analyst must determine the inter-sample variation of wavelength when variation of wavelength of absorbance values occurs between questioned and known samples.