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Design and Development of Advanced Spectrophotometer

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ABSTRACT

A spectrophotometer is an optical instrument for measuring the intensity of light relative versus wavelength. A simple way of describing this would be that the spectrophotometer measures the intensity of each color of light. The system works when electromagnetic energy, called light in visible region, is collected from the sample and enters the monochromator through an aperture (the input light is shown by the yellow line). There it is separated into its component wavelengths by an optical grating and the separated light is then focused onto a CCD array detector. This paper represents in a micro spectrophotometer, such as those made by CRAIC Technologies, the 508 PV™ Microscope Spectrophotometer adds uv vis spectroscopy of microscopic sample areas, color imaging, thin film thickness measurement and colorimetry capabilities to your optical microscope. It helps with diverse applications such as colorimetry of pixels on flat panel displays, reflectometry of vitrinite coal and thin film thickness measurements. The CCD is made up of thousands of pixels (or individual light detectors) where the intensity of each wavelength is then measured. The CCD is then read-off to a computer and the result is a spectrum which displays the intensity of each wavelength of light.

Keywords:- CCD, Spectrophotometer, Microspectrophotometer, Monochromator

I. INTRODUCTION

This guide provides some simple and easy to use design guidelines and formulas for designing, evaluating and comparing various diode array, diffraction grating based spectrometers designs The input to the design process is the **wavelength range** you want to cover and the **optical resolution** by which you need to resolve the various structures in your spectrum (often peaks). In the visible region, we perceive such electromagnetic energy as different colors of light. The system works when when white light enters the monochromator and is separated into a rainbow featuring each color. As shown in fig.1. this rainbow, with blue light on one end and red on the other, would be focused on to the CCD[1]. Each pixel of the CCD then measures the intensity of a color. The results are a spectrum such as the one shown below. As shown, the blue pixels emit blue light, the green pixels emit in the green portion of the spectrum and the red pixels emit red light.

II. DESIGN

Individually, the microscope is an optical instrument that uses lenses and mirrors to produce magnified images of microscopic objects or microscopic areas of larger objects as shown in fig.1. The sample is illuminated in one several ways:

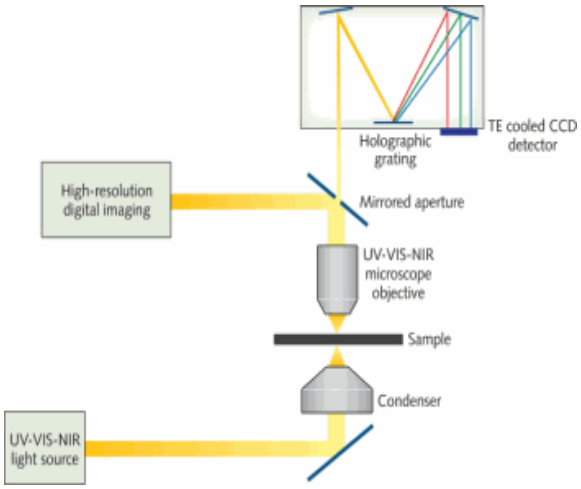

Fig. 1. Microspectrophotometer design

A schematic shows the generalized optical path for a microspectrophotometer configured for absorbance micro spectroscopy and imaging. Transmission illumination where light is focused onto the sample by the condenser[2,3]. The portion collected by the microscope objective has been transmitted through the sample Incident or reflectance illumination where light is again focused onto the sample but through the objective. As shown in fig.2 the reflected light from the sample is collected by the same objective. Reflectance can be further subdivided into specular and diffuse incident illumination.

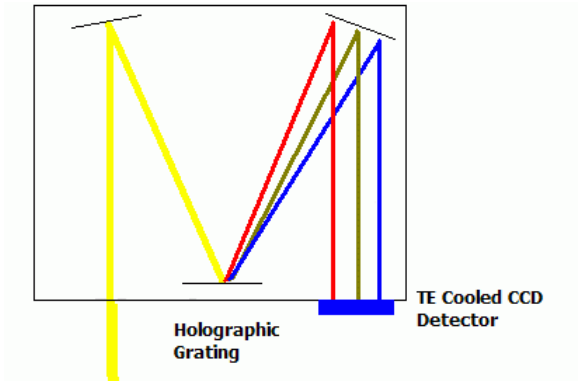

Fig.2 Reflected light

III. SPECTROMETER WORKS

Basically, a spectrometer is an optical system consisting of two lenses/mirrors that produces an image of the input slit on the detector. In between the lenses/mirrors is placed a diffraction grating which disperses different wavelengths in different angles as shown in fig3. This causes different wavelengths of light entering the input slit to be imaged to different position on the detector array[4].

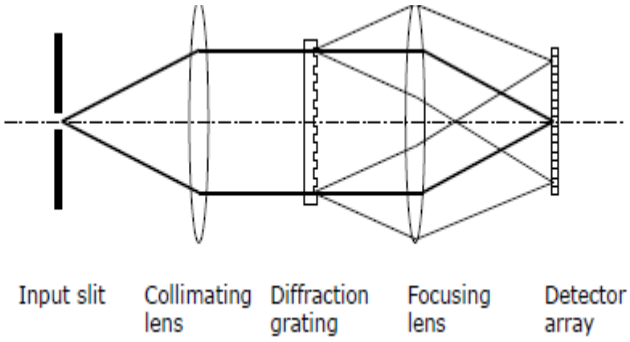

Fig.3. working of spectrometer

The UV-visible-NIR microspectrophotometer is designed as shown in fig.3. to measure the spectrum of microscopic areas[5] or microscopic samples[6]. It can be configured to measure the transmittance, absorbance, reflectance, polarization and fluorescence of sample areas as smaller than a micron.

IV. MICROSPECTROPHOTOMETER COMPONENTS

The microspectrophotometer is sometimes called a microscope spectrophotometer as it combines an optical microscope and a highly sensitive spectrophotometer[7]. In actuality, the microspectrophotometer is a fully integrated, purpose built instrument while the microscope spectrophotometer is an add-on component for a standard microscope[8]. As such, add-on

components have certain performance limitations due to the microscope itself. An integrated instrument avoids those limitations as it is designed specifically for microspectroscopy[9].

4.1. Emission: where the sample emits light after the sample has been stimulated in some manner. One example is fluorescence where a fluorophore is excited with one wavelength of light and emits light of a different wavelength. The emitted light is collected by the microscope objective. On the following pages are shown two common spectrometer geometries; the transmission grating based and the crossed Czerny-Turner. Also, the figure4 defines the key design parameters of a spectrometer.

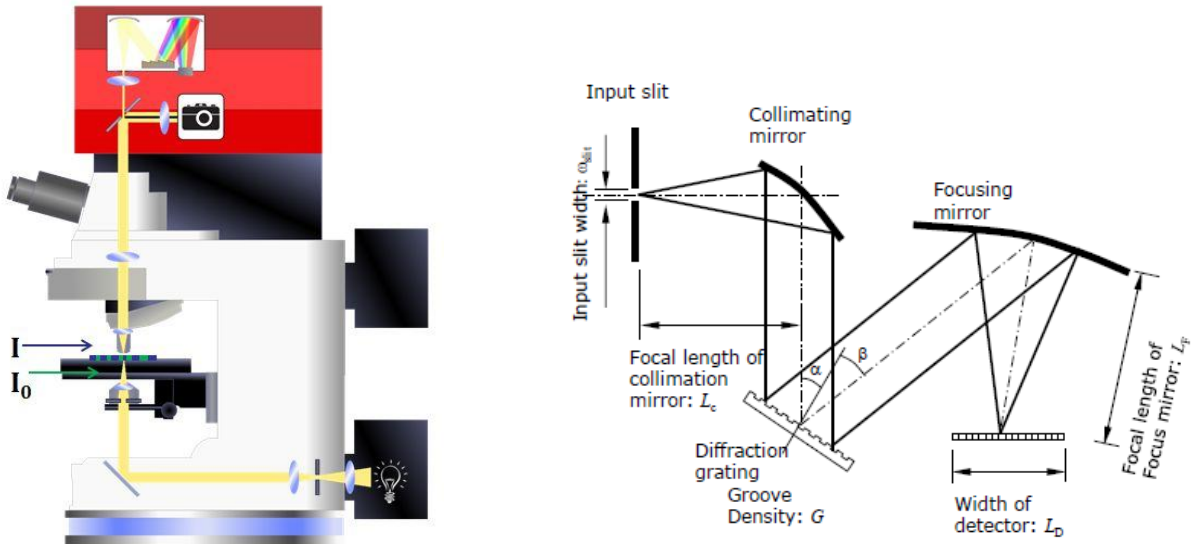

Fig.4. Design of Microspectrophotometer

V. ALGORITHMS USED FOR MICROSPECTROSCOPY

Transmittance

Transmittance calculates a transmission spectrum from a sample and reference single-beam spectra. From Two Single-Beam Spectra a transmission spectrum using the following formula:

$$\%T = \frac{S - D}{R - D} \times 100$$

where R is the single beam spectrum of the reference material, S is the single beam spectrum of the sample and D is the dark counts of the system[10].

From Absorbance Data a transmission spectrum can be generated using the following formula:

$$\%T = antilog(2\text{-}A)$$

where A is the absorbance value. This is a calculation from data that has already been collected[11].

Absorbance

Absorbance calculates an absorption spectrum from a sample and reference single-beam spectra or from a transmission spectrum.

From Two Single-Beam Spectra an absorption spectrum can be generated using the following formula:

$$A = -\log \frac{S - D}{R - D}$$

where R is the single beam spectrum of the reference material, S is the single beam spectrum of the sample and D is the dark counts of the system.

From Transmission Data an absorption spectrum can be generated using the following formula:

$$A = 2 - \log \%T$$

where %T is the percent transmittance value. This is a calculation from data that has already been collected.

Reflectance

Reflectance calculates a reflectance spectrum from a sample and reference single-beam spectra or from an log 1/R spectrum.

From Two Single-Beam Spectra a reflectance spectrum can be generated using the following formula:

$$\%R = 100 \times \frac{S - D}{R - D}$$

where R is the single beam spectrum of the reference material, S is the single beam spectrum of the sample and D is the dark counts of the system[11,12].

5.1. Choose geometry

The first step is to choose among the Czerny-Turner or LGL type geometries. For the Czerny Turner a typical value for F is around 30o wheras transmission gratings are generally used in the Littrow configuration and –1st order where a

$$\alpha = -\beta \Rightarrow \Phi = 0^{0}.$$

5.2. Choose grating

The second step is to choose a diffraction grating. Most grating vendors have an on-line catalogue where you can find one or more grating options to try in your design. You should choose a grating that has high diffraction efficiency in your wavelength[13]. The important parameter that you shall use for the design in the next steps is the groove density *G*.

5.3. Choose magnification

As mentioned earlier, the spectrometer is imaging the input slit to the detector and we generally want to have the slit as wide as possible to collect as much light through the input slit as possible. Therefore, the magnification in

the system *M* should preferably be close to 1 which means that the width of the input slit ideally is imaged 1:1 onto the detector array[14].

5.4. Calculate focal length of colli lens

As in any imaging system the magnification is determined by the ratio between the focal lengths of the two lenses in the system. For a spectrometer this ratio has to be slightly modified due to the defelction along the beam path in the grating[15]. However, once the magnification is chosen the focal length of the collimation mirror/lens can easliy be calculated.

VI. RESULTS

Spectrometry is the analysis of the light spectrum. It has an enormous range of applications. Every organic and inorganic substance has its own unique ‘footprint‘ in terms of light absorption and reflection[16]. Thus it can be recognized by spectrometry. But precise spectrometers are bulky and costly since they split up the light into different colors (frequencies), which are then measured separately as shown in fig.5.

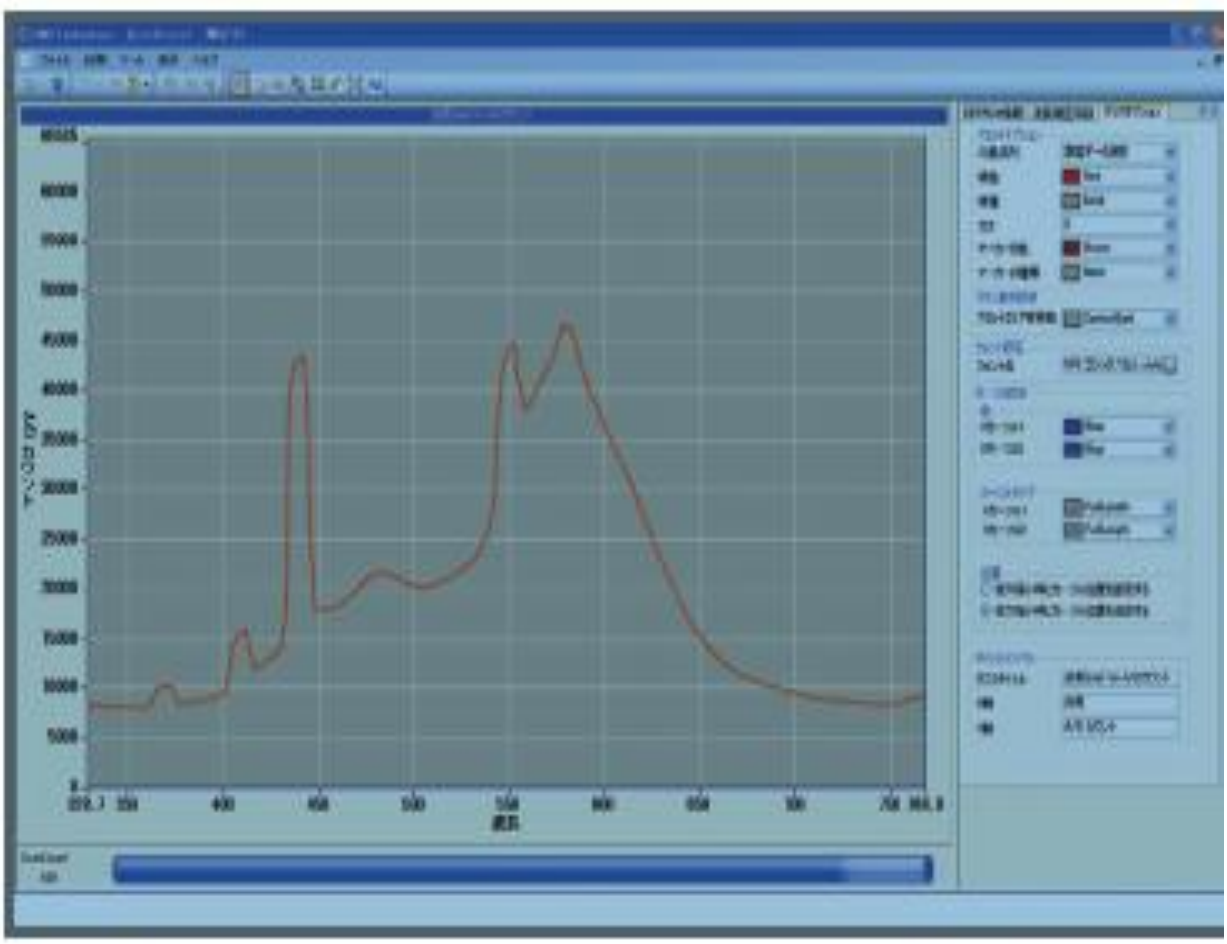

Fig.5 light frequency

The sensor can measure only a narrow range of light frequencies. To increase the frequency range, the researchers placed two of these membranes above each other closely. The two membranes affect each other.

Changing the separation gap between them by a tiny amount also changes the light frequency that the sensor recognizes.

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