Chapter 2

Theory and Instrumentations of the Methods used

2.1 Introduction

Spectroscopy is the study of matter and their properties by investigating light or particle that are emitted, absorbed or scattered by the matter. It is also defined as an experimental charting of the energy-level structures of physical systems, or normally means analysis of various types of radiation— electromagnetic or particle emission [1]. It can be considered as a method of prime interest in adding one’s knowledge about the structure of matter, and to provide a basis for quantum physics, relativistic physics and quantum electrodynamics. It has several forms; for example, the mass spectroscopy, in which various isotopes of a chemical element is separated, and the acoustical spectroscopy, which deals with the analysis of acoustical wave trains [2]. Similarly, in nuclear spectroscopy the study of resonance is associated with bombarding particles of various energies, and in recent times a new spectroscopy is defined, which is related with the plots of energy-level diagrams for mesons and baryons [2].
Most of the knowledge about the structure of atoms and molecules is primarily based on spectroscopic investigations, and spectroscopy contributes much to the present state of atomic and molecular physics so that information on molecular structures and the interaction of molecules with surroundings can be simply derived in various ways from absorption or emission spectra [3]. However, although the final analysis of a spectrum involves the fitting of line or band positions and relative intensities with a computer program, one may gain much qualitative information simply through an inspection to a spectrum [4].

Each region of spectroscopy from radiofrequency to gamma rays contain enormous amount of information that can be successfully used for investigative purpose. With the advancement of laser sources and modern equipments, the applications of spectroscopy have become more widespread, and spectroscopy becomes the most useful tool for the experimental physicists, the chemist, the biologist, the environmentalist, the archaeologist, and so on. In this chapter, a detailed view of the basic theoretical aspects and instrumentation techniques of different spectroscopic and optical methods used in the investigation, namely visible emission, steady-state and time-resolved spectroscopy are presented. Principles of photomultiplier tube, interference and diffraction grating experiments used in this thesis work are also included.

2.2 Steady-state and Time-resolved spectroscopic measurements

A detailed aspect on the phenomena of steady-state and time-resolved spectroscopy is widely described in ‘Principles of Fluorescence Spectroscopy’ by J. R. Lakowicz [5], where the steady-state measurements are considered as the most common type that performed with constant illumination and observation. In this typical measurement, the sample is first illuminated with a continuous beam of light to create continuous excited states which is then followed by recording intensity of the emission spectrum. The steady-state is reached almost immediately as soon as the sample is exposed to light. Most of the fluorephores have a
timescale of the order of nanoseconds, as such most measurements are considered as steady-state measurements. Steady-state spectroscopy can be best applied for measurement of both absorption and emission spectra, and it is possible to present both kinds of spectra in a wavelength or in a wavenumber scale.

Time-resolved measurement, on the other hand, is used for measuring intensity decay or anisotropy decay in which after a little exposure of the sample to a pulse of light the intensity decay is recorded with a high speed detection system. In this measurement, the pulse width of the light should be shorter than decay time of the sample, as well as the detection system should also permit to record the intensity decay at least in a nanosecond timescale [5]. It may be considered significant that though the time-resolved measurements are applied to fluorophores having a lifetime of nano seconds, there is much qualitative information that can be gained by monitoring the intensity patterns of various luminophores in the time-domain, whose emission rates are slow from milliseconds to seconds.

The steady-state observation is generally considered to be an average of the time-resolved phenomena over the intensity decay of the sample. This can be shown by considering a fluorophore displaying a single decay time ($\tau$). The intensity decay is

$$I(t) = I_0 \exp(-t / \tau)$$

(1)

where $I_0$ is the intensity at $t = 0$. The steady-state intensity ($I_{SS}$) is related to decay time as

$$I_{SS} = \int_0^\infty I_0 \exp(-t / \tau) dt = I_0 \tau$$

(2)

where the parameter $I_0$ depends on fluorophore concentration and instrumental parameters.

From equation (2), it is clear that the steady-state intensity is proportional to the lifetime [5].

Time-resolved spectroscopy (TRS) is a primary research tool in biochemistry and biophysics. Through this, one can easily measure the temporal dynamics, or may be provided
with the kinetic information of photophysical processes at the expense of lower sensitivity as compared to that steady-state spectroscopy [6, 7]. As an example, time-resolved spectroscopy provides information on how an absorption band or the fluorescence emission of a material decays over time [7]. Although time-resolved spectroscopy is well established among chemists or physicists, there is a lot of interest among biologists too, as time-resolved spectroscopy and time-resolved imaging serve as the pathways for understanding the biological processes, which are revealed by looking onto a photon or other emissions as a function of time [7]. The time-resolved measurement always presents more information about the molecular environment of fluorophores as compared to the steady-state ones. In time-domain fluorometry, the decay time ($\tau$) is calculated from the slope of a plot of log $I(t)$ versus $t$, or from the time at which the intensity decreases to $1/e$ (normally 37 %) of the intensity at $t = 0$ [5].

Since emission is a random event and each excited fluorophore has a same probability of emitting in a given period of time therefore the excited state population decays exponentially. The fluorescence intensity is always proportional to number of excited molecules and the lifetime is the inverse of the total decay rates. The lifetime of a fluorophore is generally taken to be the average time that it remains in the excited state. As the fluorophores emit randomly throughout the decay, the lifetime may also be defined as a statistical average [5].

2.3 Emission spectroscopy

Emission spectrum is produced whenever substance atoms are excited by a high temperature. The emission spectroscopy is therefore concerned with characteristic radiation, which is generated when atoms or molecules are introduced into thermal or electrical sources. After excitation, the atoms or molecules when return to different levels of ground state from excited states emit radiations in the form of discrete wavelength of light, known as spectral lines or
bands, respectively. The wavelength of a spectral line is inversely proportional to the energy difference between the initial and final energy levels involving in the transition. As no two elements have identical energy levels, no two elements have identical spectra.

In emission spectroscopy, the emitted light is analysed by a spectrometer, which sorts out and records spectral lines or bands according to their wavelength. For an atomic or molecular spectrum, which is the characteristic of that atomic or molecular species, the positions where these lines or bands occur in the electromagnetic spectrum can be used for qualitative analysis, and their interpretations also, can be performed by using two methods. In the first method, the spectral lines or bands are compared with the standard table of known wavelength of emission lines for the individual lines. The second method involves a direct comparison with the spectrum of a single element or a standard mixture of metals, produced on the same photographic plate or film. In either case, at least three predominant lines must be matched for an element to be identified. Emission spectroscopy is considered to be advantageous because the sample requires less or no preliminary treatment. The quantitative analysis is based on the fact that the intensity of a spectral line or band emitted by an atom or molecule is a function of concentration of that atom or molecule in the emitting source. Though the emission spectroscopy has a widespread use nowadays, its quantitative applications are limited because of the difficulties encountered in reproducing the radiation intensities, and with great care its relative errors can be reduced to only 1 or 2 percent. The instruments used in emission spectroscopy go by several familiar names, which must be carefully distinguished.

2.3.1 Spectrometer and spectrograph

A spectroscope is defined to be an instrument that measures the spectrum of light. The earlier versions of spectroscope consist of a slit, a prism and a screen with markings to indicate various wavelengths or frequencies, while the latter versions are calibrated with electronic
detectors [8]. Quite similar to a spectroscope, the spectrometer, which uses prism and diffraction grating as dispersive elements measures spectral distribution or quantity by simply dispersing the image of a slit with the spectral bandwidth of the detector that defined by an exit slit, and the aperture time in spectrometer is normally of some small fraction of the measurement time interval [9]. Spectrometers are classified to the dispersive element used, the optical mounting of the dispersive element, and the manner through which the spectra recorded. Photographic or photoelectric are the most common methods for recording spectra in spectrometers. The basic instrument consists of a slit, collimating mirrors, a grating, focusing mirror, a detector collecting lens and a CCD detector. The experimental set up of a spectrometer for recording emission spectra in the present thesis work is shown in figure 1.

The spectrograph is another instrument which measures spectral distribution or quantity by dispersing the image of a slit onto a detector, which is usually a photographic film, evaluating the whole spectral profile simultaneously by using a multitude of discrete detection positions or a continuous but spatially resolved detection surface; and the basic advantage of a spectrograph is its aperture time, which can approach the measurement time [9]. This prism instruments can take either glass or quartz as refracting surfaces.

Quartz spectrographs normally have greater optical range (2000 Å — 10000 Å) than the glass spectrographs (3700 Å — 8000 Å). Also, the spectrum length in the quartz instrument is 22 cm while it is only 14 cm in the glass spectrograph. But as compared to the quartz, the glass spectrograph has a greater angular dispersion, giving rise to a smaller reciprocal linear dispersion (Å/mm). The working principle of a simple prism spectrograph is shown in figure 2. It basically consists of a slit, a collimator lens, prism, a compound camera lens and a photographic plate. The light through the slit passes to the spectrograph and finally falls on the photographic film. A photograph of a Hilger & Watts glass spectrograph is shown in figure 3.
The assignment of wavelengths in a spectrum, produced in the photographic film can be performed by using Hartmann formula, which is given in the following form

\[ \lambda = \lambda_0 + \frac{c}{(d_0 - d)} \]  \hspace{1cm} (3)

where \( d_0 \) is a constant, \( d \) is the measured distance from an arbitrary point in the spectrum of the film to the line of unknown wavelength. The arbitrary point in the spectrum may be located on one of the three lines of known wavelength. The constant ‘\( d_0 \)’ depends on the position of fiducial mark ‘\( c \)’ on the selected region of the spectrograph, while ‘\( \lambda_0 \)’ is a constant of

**Figure 2.1** Experimental set up of the HR2000 spectrometer for recording emission spectra.

**Figure 2.2** Schematic diagram of a simple prism spectrograph. The light after slit is parallelized by a collimator lens, diffracted by the prism and then imaged on to a photographic detector (film) by camera optics.
Figure 2.3 The Hilger & Watts glass spectrograph at the Photonics laboratory, Department of Physics, Gauhati University.

spectrograph and should always have a same value for a given instrument [10]. The distances of the arbitrary points can be measured from the lines of known and unknown wavelengths. Finally, the unknown constants $\lambda_0$, $d_0$ and $c$ are determined by substituting the three known wavelengths $\lambda_1$, $\lambda_2$ and $\lambda_3$ for $\lambda$ and the separations $d_1$, $d_2$ and $d_3$ for $d$ in the relation 3, given above [11].

2.3.2 Photomultiplier tube

A photomultiplier tube capable of detecting individual photons is generally referred to be a vacuum tube that can be used to detect low energy photons in the ultra-violet to visible range, high energy photons such as X-rays and gamma-rays, and ionizing particles using scintillators. A photomultiplier tube is mostly used in time-resolved measurements. The remarkable features like large sensing area, ultra-fast response and excellent timing performance, and high gain and low noise make PM tubes superior to any light detecting devices [12].

A PM tube is best known as a current source in which the current is proportional to light intensity. It responds to individual photons, and the pulses are detected as average signal
or counted as individual photons [5]. It essentially consists of an input window, a photocathode, focusing electrodes, an electron multiplier and an anode. The anode is usually sealed into an evacuated glass tube. A small number of electrons (smaller than the number of incident photons) is released at the photocathode, then multiplied, and focused by a series of electrodes called dynodes. The dynodes are connected to a voltage chain that produced by a high-voltage supply and a series of voltage dividers. The typical potential difference applied between adjacent dynodes is about 100 V so that the electrons strike the dynodes with about 100 eV of energy [13]. Finally, the anode collected the multiplied secondary electrons emitted from the last dynode.

Both time and spectral response are regarded as the most important factors for smooth functioning of a photomultiplier tube. For steady-state measurements though the time response of a PMT is not important, it is very essential in time-resolved measurements. There are generally three main timing characteristics of a PMT— the transit time, the rise time and the transit time spread, and among all these the transit time spread is the most important specification for time-resolved measurements, which is emanating from different geometric paths taken by electrons from photocathode to anode. The transit time refers to the time interval between arrival of a photon at cathode and the arrival of amplified pulse at anode [5]. Rise time is the time that is required for PMT anode signal to rise from approximately 10% to 90% of its final level and it is determined by the transit time variation in PMT. The spectral response, on the other hand, is determined by the type of transparent material that is used in the window, as well as chemical composition of the photocathode. As the incident wavelength generates photocurrent in a PMT, the input windows are transparent to the desired wavelengths [5]. The experimental set-up used for our time-resolved measurements is shown in figure 4.
A photomultiplier tube module is a similar device to PM tubes whose uses have become more widespread in recent days. It is a compact form of conventional photomultiplier tube, which is assembled with an inbuilt PM tube, a voltage-divider circuit and a high-voltage power supply circuit. In addition, various functions such as signal conversion circuit, photon counting circuit and cooling devices are also all integrated into a single package. Thus, a PMT module eliminates troublesome wiring for high voltages and allows easy handling as this operates from a low external voltage of only 15 V. Additionally, the anode in a PMT module need not to be made ground as it is already assembled within the package. With great advantage, both PM tubes and modules are nowadays extensively used in several fields, for example, in medical diagnosis, chemical analysis, scientific research and industrial measurements [14].

The output from a PM tube is stored in an oscilloscope. A digital storage oscilloscope (DSO) is a device that contains advanced features including better trigger, storage and display capacity as compared to the conventional ones. It stores and analyses the signal digitally, and
simultaneously gives the numerical and visual display of the waveforms. A digital storage oscilloscope is generally used to study the two dimensional plot of constant varying voltage versus time. From construction point of view, an oscilloscope contains an in built horizontal sweep as well as vertical deflection plate so that whenever a signal reaches vertical deflection plate, depending on whether it is positive or negative, causes a glowing dot to move up or down, respectively. The horizontal sweep controls the movement of the dot across the screen horizontally. The graph of a signal on the display, which is to be measured, can be obtained from the combine action of the sweep and the deflection plate.

2.4 Interference

Interference is an optical phenomenon, arising as a result of superposition of two coherent waves [3]. Optical interference is defined as an interaction of two or more light beams, producing a resultant irradiance, which deviates from the sum of the component irradiances [15]. It is primarily based on the distribution of intensities of the superposed beams. The widely held view of this phenomenon is found in many textbooks, where light from a source is divided by suitable apparatus into two beams before getting superimposed. The intensity in the region of superposition is varied from point to point between maxima, which exceed the sum of the intensities in the beam, and the minima may be zero.

In general, two methods can be employed for obtaining beams from a single beam. The first one is called division of wave-front in which a beam of light is divided by passage through apertures placed side by side. Wavefront splitting is useful only with sufficiently small sources. In the other one, called division of amplitude, the beam is divided at one or more partially reflecting surfaces, at each of which part of the light is reflected and the remaining part is transmitted, and can be realised with extended sources [16]. In all, to have interference, the two beams must have very nearly the same frequency, as any considerable difference in frequency
would cause in a rapidly varying and time dependent phase difference, which, in turn, would result the interference term to average to zero during the detection interval [15]. To observe fringes the two sources need not to be in phase; a shifted but identical interference pattern would also occur if there is some initial phase difference in between the two as long as it remains constant. Such sources may be or may not be in step, but are always coherent. In the context of the present thesis, in addition to the Young double hole and the Michelson interference experiments, a brief description on how the contrast or visibility of the fringes changes with increasing optical path difference have also been discussed in this chapter.

2.4.1 Young’s double hole experiment for spatial coherence

It is a matter of common practice that the determination of spatial coherence of a light source can be best realised from the Young’s double hole experiment. The experimental arrangement as described by K. Thyagarajan and A. Ghatak in ‘Lasers: Fundamentals and Applications’ [17] is shown in figure 5, where S represents a point source with $S_1$ and $S_2$ as two holes. Light coming from the point source S illuminates the two holes coherently with spherical waves. For equal optical path lengths $S_1O$ and $S_2O$, interference fringes formed near observation point O will be of good contrast. Now, let us consider $S'$ as another point source placed near S such that waves coming out from those have no phase relationship. As such, the interference pattern produced on the screen is the superposition of intensity distributions of the patterns, which is formed due to both S and $S'$. On moving $S'$ slowly away from S, the contrast of the interference pattern on the plane of observation P will become poorer, which is due to the fact that the pattern formed by $S'$ is slightly displaced in relation to the one produced by S, and for a particular separation interference maximum produced by S falls on interference minimum produced by $S'$ and vice versa. In such a case, the interference fringes produced on screen is
washed away. For a particular position of $S$ where the path difference between $S_2S'$ and $S_1S'$ becomes $\lambda/2$ ($\lambda$, wavelength of light source), $S'$ produces interference minimum at $O$, and two fringe patterns would be out of step [17].

Considering $SS' = l, S_1S_2 = d$, and the distance in between $S$ and plane of holes is $D$.

Assuming $D >> l, d$, the condition for disappearance of interference fringes is,

$$S'S_2 - S'S_1 = \frac{\lambda}{2} - \frac{ld}{D}$$  \hspace{0.5cm} (4)

or

$$l = \frac{\lambda D}{2d}$$  \hspace{0.5cm} (5)

For an extended source, interference fringes will be observable as long as,

$$l \ll \frac{\lambda D}{d}$$  \hspace{0.5cm} (6)

and for a source of width $l$, interference fringes of good visibility will be observed from interference from two sources $S_1$ and $S_2$, separated by a distance $d$ as long as,

$$d \ll \frac{\lambda D}{l}$$  \hspace{0.5cm} (7)

If $\theta$ be the angle subtended by the source at the holes/slits, then

$$d = \frac{\lambda}{\theta}$$  \hspace{0.5cm} (8)
where the distance \( l_w = \frac{\lambda}{\theta} \) is called the transverse or lateral coherence length of the source S [17].

2.4.2 Michelson interferometer experiment for temporal coherence

In the Michelson interferometer as illustrated in ‘Fundamentals of Optics’ [18], the two beams that obtained by division of amplitude are sent in different directions against plane mirrors and again brought together to form interference fringes. The schematic diagram of the Michelson interferometer experiment is shown in figure 6. It consists mainly of two highly polished plane mirrors \( M_1 \) and \( M_2 \), placed exactly perpendicular to each other and two plane parallel glass plates \( G_1 \) (rear side partially silvered) and \( G_2 \) (compensating plate). Light coming from an extended source S is divided into two beams—a reflected and a transmitted of equal intensity. The beam reflected normally from \( M_1 \) passes through \( G_1 \) a third time and reaches observer’s eye or detector. Similarly, the beam reflected from \( M_2 \) passes through \( G_2 \) for the second time, and after reflecting from the surface of \( G_1 \) reaches into eye/detector [18]. The two reflected beams interfere and produce interference fringes at eye (E) or at a detector.

It is well known that interference fringes will be formed only if a time delay \( \Delta t \) is introduced in between two beams such that,

\[
\Delta t \Delta \nu \leq 1 \tag{9}
\]

where \( \Delta \nu \) is the bandwidth of the light source. The time delay,

\[
\Delta t \approx \frac{1}{\Delta \nu} \tag{10}
\]

is called the coherence time of the light, and the corresponding path difference,

\[
\Delta l = c \Delta t = \frac{c}{\Delta \nu} \tag{11}
\]

is known as the longitudinal or temporal coherence length of the light [19].
For equal optical path lengths of the two beams or when both the mirrors are equidistant from $G_1$, that is, the time taken for two waves in traversing two different paths is same then the contrast of the interference fringes formed is always good. The fringe contrast at zero path difference of the mirrors is also good for a source which has a band of frequencies as the different wavelength components produce interference patterns superimposed on one another [17]. For an extended source, say, sodium lamp, if the movable mirror ($M_1$) is slightly moved away from beam splitter ($G_1$) then the contrast in the fringes goes on decreasing, and for a difference of a few millimeters in path lengths the fringes are no longer visible [17]. A rough understanding of this phenomenon can be explained as follows, where the fringes are considered to arise from the addition of spatially periodic distributions, each of which formed by a frequency component present in the light spectrum. The periodic distributions have different spatial periodicities, and with increasing time delay in between the two beams, their addition leads to a less and less well-defined fringe patterns, because of the fact that the

\[ \text{Figure 2.6 The Michelson interferometer.} \]
maxima of various monochromatic contributions get more and more out of step. For a sufficiently long time delay, the periodic intensity distributions will get so much out of step that the superposed pattern will no longer exhibit any pronounced maxima and minima, i.e., no fringes at all [19]. For a source, with increasing time delay the interference pattern disappears according to the relation 10.

2.4.3 Diffraction grating

A diffraction grating is an arrangement that imposes on an incident wave a periodic variation of amplitude or phase or both, and is characterized by its transmission function [16]. It is a very powerful optical instrument for the study of spectra, consisting of a number of parallel equidistant slits of the same widths [18], separated by equal opaque spaces. From construction point of view, it consists of a series of parallel grooves that are ruled on a hard glassy or metallic material. These grooves are extremely closely spaced, which is of the order of 1 \( \mu \text{m} \). Usually gratings are coated on ruled surfaces with a reflecting material such as aluminium so that they may also act as mirrors [20].

In a grating, with very large number of slits, the principal interference maximum becomes extremely sharp narrow lines, while the intensity in the secondary maxima becomes negligibly small. The other colours appear as per the grating equation,

\[
(e + b)\sin \theta = \pm n\lambda
\]  

where \((e + b)\) is the grating element, and \(\theta\) is the angle of diffraction.

For a given order \(n\), the angle of diffraction depends on \(\lambda\), and for a source having many wavelengths, say, \(\lambda_1, \lambda_2, \lambda_3,\ldots\), as many lines will appear in that order (except for \(n = 0\)). Thus, a line spectrum is formed in each order. But, for the central image \((n = 0)\), as the path difference is zero for every wavelength, the central maxima of different wavelengths coincide forming the
central image. Hence, the central image becomes of the same colour as that of the primary source of light. On the other side of the central image a similar set of spectra are formed, and in each order the spectrum line corresponding to the shortest wavelength is formed on the side towards the central image [21].
References


