CHAPTER 2

PART A: DESIGN CONSIDERATION OF THE SET-UP FOR INVESTIGATIONS IN SCANNING LASER MICROSCOPY

PART B: MICROPROCESSOR BASED RECORDING AND MEASUREMENT OF OPTOELECTRONIC SIGNAL IN SCANNING MICROSCOPY
2A.1 INTRODUCTION

Scanning laser microscopy has, of late, become a powerful method of microscopic investigations. We here report the design of a set-up for investigating certain properties in scanning laser microscopy. Electronic signal has a special advantage as regards processing and storage. Electronic form of image signal can, however, be obtained by using even a video camera to record light variations in the image plane of a conventional microscope. However, the geometry of a conventional microscope confines its resolution to within Rayleigh limit and attractive point by point image processing offered by a scanning system is absent in this case. This is why we have chosen to design a set-up for laser microscopy using scanning mechanism in stead.
2A.2 OVER-ALL DESCRIPTION OF THE SET-UP

Here a laser beam has been used as a source as it provides possibilities of investigation in coherent, incoherent and partially coherent imaging depending on the arrangement for illumination as well as detection[1,2]. Besides, the use of high power laser makes it possible to focus an intense beam down to a diffraction limited spot-size and thus explore and process optically nonlinear image signal. In our set-up we have used a He-Ne laser yielding an output of 1 mW at 6328 Å. In this set-up the spatial transmittance of an object is converted into a series of time varying signals by the photodetector. As in fig.2A.2.1, a beam of laser light is focused by an objective onto the object specimen such that the specimen is mechanically scanned in the focal (X,Y) plane. The object specimen is constrained to move only in the focal plane through the use of taut piano wires. The signal collected by the photodetector via the collector lens may be led to a CRT through a signal processing unit. These electrical signals may then be displayed on the CRT by leading the electrical signal from the photodetector to the Z-modulation of the CRT and by locking its X,Y movement to the X,Y movement of the mechanically scanned raster for the object specimen. Although we could easily switch over to this mode, we have concentrated on the investigations of one dimensional scan signal as regards various investigations (fig.2A.2.2).
Fig. 2A.2.1 Scanning Optical Microscope geometry
Fig. 2A.2.2 Scanning Optical Microscope (SOM) geometry for line scanning
Photograph P-2A.2.1: Designed set-up for investigations in Scanning Optical Microscopy

Photograph P-2A.3.1: A close-up view of the scanning system in the designed set-up for investigations in Scanning Optical Microscopy
The objective and collector lenses used are standard microscope objectives. The system thus developed by us is shown in the photograph P-2A.2.1.

2A.3 THE SCANNING MECHANISM

The arrangement of mechanical scanning with the fixed beam focused to a spot is specially suited for achieving uniformly good resolution at all scan positions though not very suitable for fast scanning. An arrangement of scanning with moving light beam, however, provides nonuniform resolution over the focal plane because of change in the angle of illumination though a faster rate of scanning may be achieved in this case. The movement of the object specimen in X,Y direction has been achieved by using moving coil vibrators. The vibrators thus used can provide a smooth motion of 1 mm travel at frequencies upto 5 KHz. The vibrators are connected to the object holder at two corners 90 degree apart via flexible connection links and object holder now moves the object placed between the objective lens and the condenser lens. Thus the scanning achieved is linear in one direction and discrete in direction transverse to it. It has further been found that when both directional scans are used, the system gives a smooth motion until a frequency of around 3 KHz discrete in direction transverse to it. A close view of the scanning mechanism is shown in photograph P-2A.3.1.
2A.4 THE DETECTOR AND AMPLIFIER SYSTEM

The detector used is a photodiode whose response is reasonably fast and can respond up to 0.1 Gigahertz of frequency as per specification. The output from the photodiode is fed through an amplifier system capable of achieving a maximum linear gain of 80 and having a bandwidth of 20 KHz frequency (Fig. 2A.4.1). The output from the amplifier may be fed into a CRT as Z-modulation while the CRT raster is synchronized with the vibrators. But for one dimensional signal scanning, the output signal is displayed as vertical signal variation on the screen.

2A.5 ELECTRONIC CONTRAST VARIATION

The contrast of a microscopic specimen is a fundamental problem specially for biological specimens. In animal tissues for example, it is often desired to image cells which exhibit little detail to naked eye. However suitable level of amplification of optoelectronic signal coupled with the electronic arrangement for black level reduction make scanning optical microscope (SOM) s specially useful for studying such specimens. The system we have developed is capable of measurement of contrast that may be achieved in the electronic image to be displayed.
Fig. 2A.4.1 Amplifier system for detection of the signal.
2A.6 TRANSFER FUNCTION OF THE SYSTEM

Whereas the resolution that may be achieved in such set-up equals that of a conventional microscope, better transfer functions may be achieved through special imaging geometry[1]. These are considered in detail in chapter 3.

2A.7 AREAS OF APPLICATION OF THE SET-UP DEVELOPED

The set-up thus developed is specially useful for studying response of the optoelectronic part of SOM consisting of the objective, collector, scanning and detection sub-systems that are parts of the total system designed. Thus response of SOM to inputs like straight edge, bar pattern, weak phase signals may be conveniently studied under various scanning geometries. The system may also be used for super-resolution studies, i.e., studies regarding resolution beyond Rayleigh limit. Availability of high power laser source would enable even nonlinear optical phenomena to be studied.

Besides the system is also easily adaptable to the so-called photovoltaic mode(fig.2A.7.1a) in which the objective lens focuses light directly onto the semiconductor material. This method has been used by Distefano and Cuomo[3] for investigating grain boundaries in polycrystalline semiconductor.
Fig. 2A.7.1 (a) Photo-diode in the photo-voltaic mode

Fig. 2A.7.1 (b) Photo-diode in the photo-conducting mode
The set-up thus developed can also be used for monitoring electronic devices that depend on photo-induced effects[4]. In the photo current mode (illustrated in fig.2A.2.7.1b), the current through the reverse biased semiconductor junction may be monitored and corresponding micrograph be utilised for presence of defects like dislocations[5]. The line scan of these images may also be suitably employed for such studies.

Measurement of life-time of excess carriers[6] gives an important insight into the capability of of a semiconductor specimen for responding to quickly changing light signals. The SOM geometry may be slightly altered to determine life-time of excess carriers in semiconductors. One such method is based on direct measurement of diffusion length \( L_D \) so that it leads to the determination of carrier life-time through the equation

\[
L_D = \sqrt{D \tau}
\]

\[ \text{eqn.2A.7.1} \]

where the diffusion constant \( D = (kT/e)\mu \), \( \mu \) is being the carrier mobility, \( T \) the temperature, \( e \) the electronic charge and \( \tau \) the carrier life-time. Here an effective illuminated slit may be focused on the semiconductor surface by the arrangement of a fast scan in a SOM geometry when electron-hole pairs are produced in the semiconductor. The excess carrier density, a function of carrier life-time, decreases with distance from the illuminated line and is measured by a reverse-biased collector electrode. The current is proportional to the density of the
**Fig. 2A.7.2a** Illustration of the principle of excess carrier life-time measurement

**Fig. 2A.7.2b** Experimental arrangement for excess carrier life-time measurement
carriers at each point thus explored. Therefore the diffusion length would be the distance of the explored point at which it decays to $1/e$ of the initial value.

Alternatively (fig. 2A.7.2a, b) the light from the laser source in the SOM geometry is pulsed by a revolving opaque screen in which there is a small opening. The parallel light emerging from the collector lens is allowed to pass through a large area slit on the semiconductor specimen. The on-off light signal thus reaching the semiconductor specimen induces a voltage across the resistance $R$ due to conductance changes in the semiconductor specimen. From the decay portion of the photoconductance trace thus obtained in an oscilloscope, excess carrier life-time may be obtained by investigating the time taken by the signal to decay to $1/e$ of the maximum voltage once the decay starts.

Thus the designed set-up may be used for a variety of applications.
2B.1 INTRODUCTION

Microprocessor, since its first appearance [7,8], has been widely used in measurement, monitoring, data acquisition, control and display of relevant information regarding both standard and random signals. Various expensive systems adapted for recording and processing of signals have been developed to meet the demand for faster rate of processing of more and more voluminous signals in physical systems used in industrial automation and sophisticated research and development efforts. We here report the design of a less expensive microprocessor based system for recording and measurement of changes in amplitudes of optoelectronic signals of scanning laser microscopy and consequently of finding the peak of a random signal. Though the system is designed primarily for use with
reference to line scan signals in laser scanning microscopy, the system, in principle, can be adapted to measure any parameter provided a suitable transducer is found to sense it and then convert it to the corresponding electrical signal.

However, the use of microprocessor requires that the signal be converted into digital one before it can be processed by a microprocessor. This can easily be done with a standard ADC chip. We have, however, used the cheaper option of channeling analog signals through a Voltage Controlled Oscillator in the shape of a square wave signals of different frequencies such that the output frequency corresponds to the magnitude of the input analog signal.

2B.2 HARDWARE

The hardware part consists of a (i) VCO (Voltage Controlled Oscillator) and (ii) the switching transistor (2N2219) circuit. The scheme is as shown in Fig. 2B.2.1. Here the analog input voltage drives the VCO. The output of the VCO is a rectangular wave whose period is directly proportional to the input analog voltage. Thus voltage variation is effectively converted into frequency variation by the VCO. To monitor the corresponding changes in frequency, synchronization is essential. For this purpose the RST 7.5 facility of microprocessor 8085 is used. The unknown square wave whose frequency is to be monitored is
Fig. 2B.2.1 Scheme for monitoring analog signals
fed to RST 7.5 of microprocessor via the transistor 2N2219 used as a switch. Now, RST 7.5 is a hardware restart and it responds to positive edge triggering only. It can be triggered by a pulse and when the pin is active, the internal circuits of the 8085 produce a hardware call (also called vector interrupt) to a pre-determined vector location where the starting address of a service routine is stored.

2B.3 VCO

A VCO produces an output whose frequency is dependent on the amplitude of an input voltage. The most desired characteristics of a VCO is its stability and reproducibility. Stability means that the output frequency should remain constant when the control voltage applied to the VCO is constant. Further reproducibility means that a given output frequency should correspond to only one control voltage, irrespective of supply voltage deviation. In other words, the output frequency should be an image of the control voltage, however magnified or demagnified the image may be. In actuality, this condition is hard to attain. So success lies in bringing the frequency as close to the ideal condition as possible.

A good tracking ability is another natural corollary of VCO merits. Good tracking means that the VCO must follow the
prescribed characteristics as closely as possible, whether these are linear or exponential or any other. Reliability of the VCO refers to the ability to retain the initially tested conditions and specifications during the course of its operation in varying environments.

We have designed the VCO with 555 timer. Input is given to pin number 5 and output is taken from the pin number 3 in the circuit of the VCO. The Voltage controlled oscillator has been working satisfactorily in the desired range of 1 mV to 1.8 V linearly.

2B.4 SOFTWARE TO GENERATE AND STORE COUNT CORRESPONDING TO A FULL WAVE OF THE SOURCE

The software is designed to generate counts corresponding to a full wave of the source and consequently stores this count in the memory. It is designed in such a way that count program should execute when the start of the pulse appears and should stop when the end of that pulse occurs.

The count should be stored in memory whenever there is a change in the frequency. The CY flag status is used here in the program to branching to count program as well as for display. We now present the flow chart of the program for monitoring the frequency of the unknown source.
FLOW CHART FOR MONITORING FREQUENCY OF UNKNOWN SOURCE

START

SET INTERRUPT CIRCUIT

INTERRUPT HARDWARE

COUNT = 00

DELAY

INTERRUPT HARDWARE

COUNT = COUNT + 1

DISPLAY

NO

YES

YES

NO
PROGRAM

MAIN PROGRAM:

FLOW CHART

START

SET UP INDEX FOR STORING DATA

INITIALIZE STACK POINTER

USE CARRY FLAG STATUS

ENABLE INTERRUPT CIRCUIT

STOP
SUBROUTINES: A number of subroutines are also prepared as stated below.

1. COUNT SUBROUTINE

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**Flowchart:**

- **Decision:** Is CY = 1?
  - **Yes:** Proceed to DATA STORING PROGRAM
  - **No:**
    - ENABLE INTERRUPT CIRCUIT
    - SET THE CARRY FLAG
    - SET UP DE AS COUNTER
    - LOAD DELAY REGISTER
    - DECREMENT DELAY REGISTER
    - UPDATE COUNT
2. SUBROUTINE FOR STORING DATA

FLOW CHART

1. DISABLE INTERRUPT
   GET THE LOWER BYTE COUNT
   DECREASE THE MEMORY INDEX

   \( P_c = N_c \)
   \( Z = 1 \)

   GET THE NEW UPPER BYTE COUNT
   DECREASE \( H_r \) register

   COMPARE U-BYTE COUNT WITH PREVIOUS U-BYTE COUNT
   \( P_c = N_c \)
   \( Z = 0 \)

   UPDATE \( H_r \) by 2

   COMPARE L-BYTE COUNT WITH PREVIOUS L-BYTE COUNT

2. INCREASE \( H_r \) REGISTER
   STORE U-BYTE COUNT IN MEMORY
   INCREASE \( H_r \) REGISTER
   STORE THE L-BYTE COUNT
   UPDATE \( H_r \) FOR STORING NEXT DATA

   SAVE THE CONTENTS OF \( H_r \) REGISTER

   (2) GO TO DISPLAY

UPDATE \( H_r \) BY 2

(1)
3. SUBROUTINE FOR DISPLAY FREQUENCY IN TERMS OF COUNT

FLOW CHART

(2)

TRANSFER COUNT FROM DE TO HL REGISTER

DISPLAY PROGRAM

INTRODUCE DELAY

RETRIEVE Hrp

GO TO THE MAIN PROGRAM
Fig. 28.4.1 Hertz vs Volt graph of the VCO arrangement
Fig. 2B.4.2 Frequency versus count curve
Fig. 2.4.3. Count vs Volt as achieved in microprocessor software
Further two standard subroutines[7] are used to (i) displaying unknown frequency using look up table and (ii) flow chart to flash signals denoting that higher pre-selected frequency has been crossed. Otherwise the frequency versus count graph could be referred to for monitoring the unknown frequency. In the graph 2B.4.2. we have shown the reading starting from 50 Hz upto 100 Hz. Though the frequency-count graph is nonlinear beyond 75 Hz, the desired range of analog signal voltage can be accommodated in the linear range of the frequency-count graph. The software using look up table is developed in such a way that it should give display frequency ranging within a pre-selected range. When the unknown frequency is greater than a certain pre-selected frequency, then it may be made to flash a signal 20 times signifying that if the frequency exceeds that pre-selected frequency, it will go to the main programme. The voltage versus frequency curve is shown in fig.2B.4.1, the frequency versus count curve achieved is shown in fig.2B.4.2 and finally the analog voltage versus count achieved is shown in fig.2B.4.3.

2B.5 MONITORING THE PEAK VALUE OF A WAVE:
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It is assumed that the peak values for a wave is always above a reference value Vr. With this assumption the hardware required for this purpose can be simplified(Fig 2B.5.1).
Fig. 28.5.1 Scheme for monitoring peak value of a wave.
In this scheme, the count program which is installed in microprocessor 8085 starts whenever the signal-value exceeds the reference value \( V_r \) and count program is synchronized using facility \( \text{RST 7.5} \) of the \( \mu \text{P} \). The output of the comparator is connected to the port-A, pin-\( P_{A0} \) of the microprocessor. The software is designed to reject the count value which is less than the output value. Then the count value corresponding to the peak value is stored.

The software can also be developed to scan their count values stored in memory location to select the highest peak value of the given wave and the software flow chart is detailed under the heading 'program to monitor the highest peak'. The software is designed in such a way that the highest peak is detected from 20 no. of peaks and the operation will be repeated. The program to monitor the highest peak is also incorporated in the software. The highest peak can thus be evaluated using software.
FLOW CHART FOR MONITORING PEAK VALUES OF A WAVE

START

SET UP PORT A OF 8255 AS INPUT PORT

IS $P_{Ao} = 1$ ?

SET INTERRUPT CIRCUIT

INTERRUPT HARDWARE

COUNT = 00

HARDWARE INTERRUPT

IS $P_{Ao} = 1$ ?

YES

ISOLATION PROGRAM

DISPLAY

DELAY

NO

COUNT = COUNT + 1

YES

NO
It is clear that by this effort of finding the peak values of a wave and then the highest peak can also be utilised to study the minima values of a wave and correspondingly the minimum of a wave by inverting the waveform. We have thus been able to design a system which can store the line scan wave and determine its peak or the dip values. It is however clear that since the sampling frequency is around 4 KHz, the line scan frequency should be sufficiently low so that around 300 picture points may be sampled. This creates no problem for our set-up where laser source of 1 mW is used. However, for nonlinear studies in SOM geometry, line scan at low frequencies would heat up the specimen and thus may lead to its damage.
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