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RESEARCH ARTICLE

ON THE NATURE OF LASER INDUCED FLUORESCENCE, SEM AND IRIDESCENT PATTERNS OF MOLLUSCAN SHELLS

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Abstract

In the present work we report the Laser Induced Fluorescence (LIF), SEM and Iridescent Patterns of molluscan Shells. The intensity distribution patterns of different iridescent patterns are worked out with the help of software (Image J). The iridescent colours of shells are ascribed to diffraction and also due to laminated structure. Laser induced fluorescence spectra are recorded for a specimen in a organic solvent, using blue, green and red diode laser. Nine fluorescence peaks at 470, 450-600, 680, 718, 720, 734, 756, 820 and 840 nm are observed and their assignments have been proposed. The SEM patterns of a powered specimen are analysed.

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Introduction:-

Molluscan shells have been the subject of study among research workers during last many decades for interesting physical phenomena exhibited by these objects. The materials of the shells present different appearances in different cases. It is also well known that the molluscan shells have high environmental significance and provide information on physiological events such as the duration of spawning period, growth etc. Moreover they are clearly related to taxonomy and phylogeny. In an extensive study of Molluscan shell structures Boggild [1] described and classified the main categories from mineralogical, crystallographic and micro-structural characters. The most widespread structure was the aragonite crossed lamellar layer. However, distinctive micro structural and mineralogical features were noticed in different families. In subsequent periods studies were mainly directed towards bivalve shells [2-5]. Several decades earlier Raman and coworkers devoted much attention to the iridescent substances called the mother-of-pearl and investigated experimentally various optical properties including iridescent patterns [6-10]. It is generally estimated that the shell of a molluscan built out of calcium carbonate has 3000 times higher fracture resistance than crystal of calcium carbonate. In the present work we would like to critically examine Raman's work specifically in connection with the iridescent patterns exhibited by the shells. Recently, Konwar [10] and Konwar et.al. [11] have investigated experimentally various optical and spectroscopic properties of few molluscan shells. They have also recorded laser induced fluorescence of few shells in their natural form in a classical spectrograph using green Ar ion laser (60mW). The spectra recorded by the workers consist of a diffuse and broad band in the red yellow sector of the spectrum. It is worthy of remarks here that, in general, a sample of Molluscan shell contains calcium carbonate or calcite. Calcite is by far the more common in nature as compared to aragonite. These two forms, calcite and aragonite are chemically the same but physically different. Mother-of-pearl consists of chalk in the rarer form of aragonite, but as numerous crystals. These are imbedded in a horny substance forming layers approximately parallel to the surface of the shell. According to Raman [9] there may be eight thousand to twelve

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thousand such layers per centimeter of thickness of the mother-of-pearl. We note here that the fluorescence band recorded earlier [11] on a classical spectrograph has its origin in the organic components in the sample of Molluscan shells (using organic solvents) have been estimated [12, 13] and it was seen that the chief components are the calcium carbonate (95 – 99.9%) and organic materials (5 – 0.1%). In the present work we report the laser induced fluorescence spectra of organic solvent extracts of molluscan shells excited with the help of blue, green and red diode lasers. The spectra have been recorded with the help of a sophisticated mini spectrometer. The fluorescence is presumably originating from the organic components in the molluscan shells. As may be inferred from the spectra described in section 3, many new features are present in the laser induced fluorescence spectra not reported in any earlier works. In spite of the extensive works done during several decades on various aspects of molluscan shells, the emergence of the field of nanoscience has greatly influenced the field of biomineralization. In the present work we have also reported the scanning electron microscopic (SEM) patterns for a molluscan shell sample in a powdered form in order to find a possible correlation with the iridescence colours.

Iridescence patterns of molluscan shells: What they are?

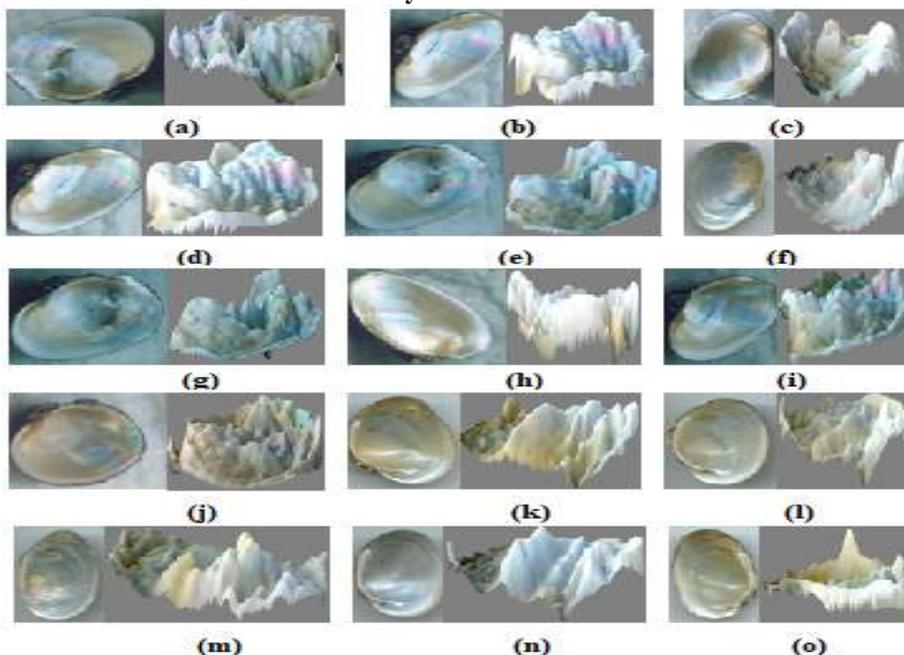


Fig: 1 Iridescence pattern of molluscan shells and their three dimensional intensity distribution patterns.

In some molluscan shells the coloured patterns, known as the iridescence patterns, are usually seen under normal condition of illumination. The iridescence colours of the shells have been usually ascribed to either interference or diffraction. Though the diffraction component is well established to account for the multicoloured iridescent patterns in shells the origin of the change in body colour in the case of pearl has not been fully established. Lord Rayleigh (following up some of his father's work) also was interested in iridescent colours of and optical structure producing it and one of his papers is devoted to the iridescent of beetles [14]. Similarly Ramdas [15] had investigated feeble iridescence from potassium chloride crystal. Potassium chloride belongs to the monoclinic class of crystal and in its natural occurrence takes the form of flat plates containing many twins. When the crystal plate is held so as to reflect light obliquely and is turned around in its own plane, colours alternately appear and disappear twice in its complete revolution. The spectral characteristic of the reflected light also varies with the angle of incidence. All the effects are associated with stratified media. Raman picked up the topic of stratified media and studied various specimens of natural origin for study. His study of the plumage of birds is the first of a series of publication, soon followed by two papers on iridescent shells [6,7]. In a recent work one author has described [16] how butterfly wings involve nano particles and systems containing photonic band gap. The work described by the author apparently needed more evidences to establish these facts.

We reproduce some examples of the molluscan shells under illumination by a broad band light of a scanner connected to a computer, as shown in Fig: 1(a, b, c,). We have also used the software (image J) to measure the three dimensional intensity patterns of the iridescent molluscan shells. It may be noted that the 3-D intensity distribution patterns are actually the manifestations of the optical properties associated with the alternate layers of conchin and aragonite crystal layers in different specimens. It is worthy of remark here that conchins are organic substances which are primarily proteins. Inner layers of calcium carbonate interface with a network of conchin and are impregnated with a variety of mineral salts. The calcium is usually in the form of calcite crystals in marine species and aragonite in terrestrial species but mixture of crystal types do occur. A close examination of the intensity distribution patterns show several peaks on the background and they are actually related to the conchin layers intersected with the surface of the shell and their configuration depends upon the curvature of the intersecting surfaces and the angle at which they meet. We thus find that method of three dimensional intensity patterns give qualitative information about the nature of iridescent pattern.

Laser induced fluorescence spectra of molluscan shells:

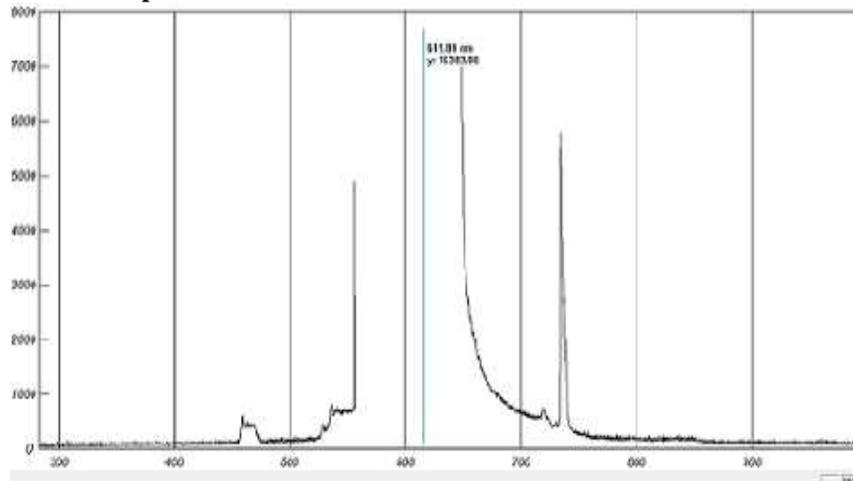


Fig: 4 Laser induced fluorescence spectrum of molluscan shell excited with the help of a red diode laser (~650nm, 20mW)

LIF of different molluscan shells are not in order and one of the LIF has no captions.

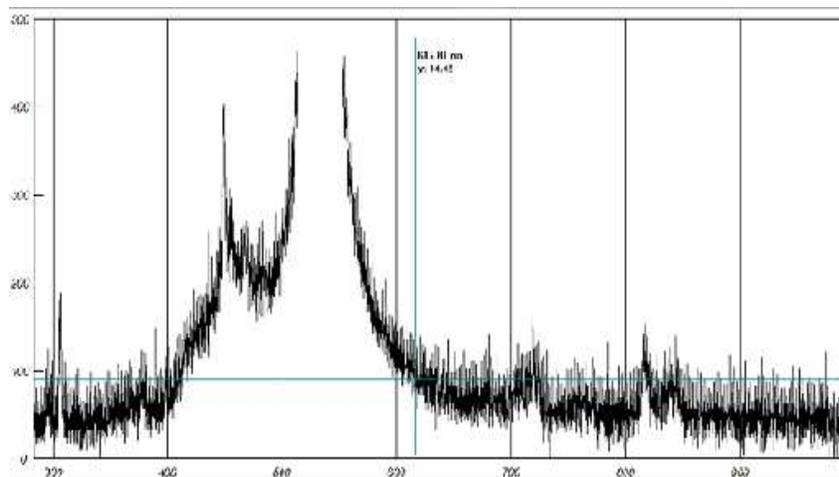


Fig: 3 Laser induced fluorescence spectrum of the liquid extract of molluscan shell excited with the help of green diode laser (~540nm, 30mW)

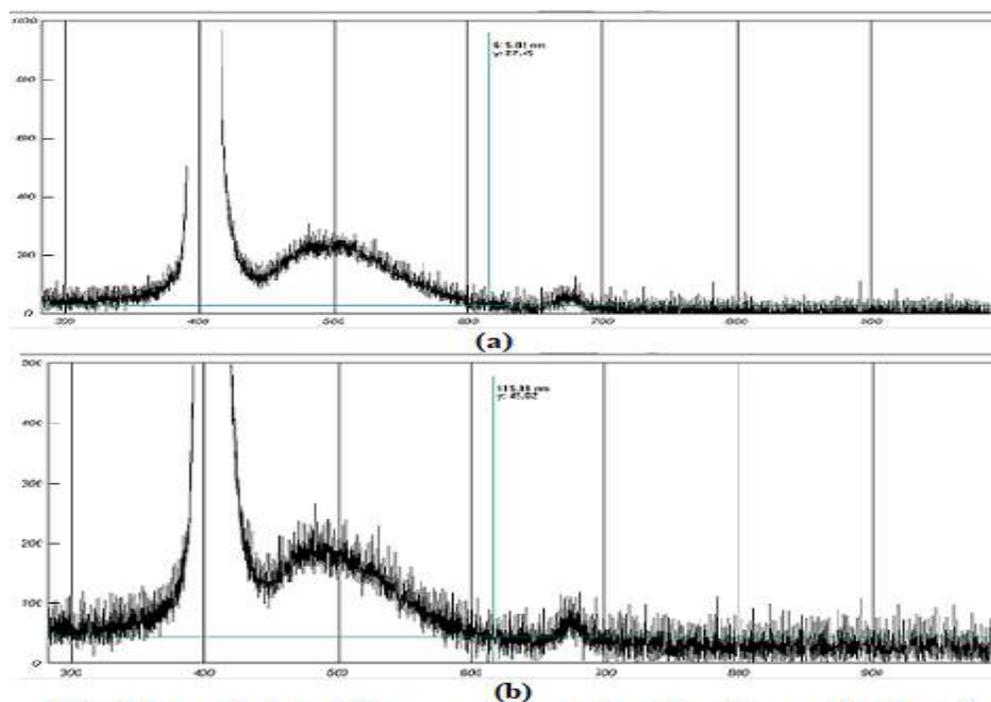


Fig: 2 Laser induced fluorescence spectra of molluscan shell excited by blue diode laser (~450nm, 10mW)

In the present section we discuss the laser induced fluorescence (LIF) of molluscan shell sample in an organic solvent. In the usual procedure immersion of the finely grinded powder of the shells in a glass vessel containing a suitable organic solvent (ethyl alcohol) enables the organic components of the molluscan shell responsible for their fluorescence to be quickly extracted. The extract may then be transferred to an observation cuvette of suitable length which is held against a brilliant radiation originating from a diode laser source. In the present case we have used diode lasers of three different colours red (~650nm, 20mW), green (~540nm, 30mW) and blue (~450nm, 10mW). The cuvette with the liquid sample is held in front of the opening end of the optical fiber connected to a mini USB spectrometer is connected to a laptop where the necessary software is installed for recording the spectra. Fig:2 (a, b) shows the spectra of the liquid extract of molluscan shell excited by blue laser. Fig:3 shows the laser induced fluorescence spectrum of the liquid extract of molluscan shell excited with the help of a green diode laser (~540 nm, 30mW) It is worthy of remark here that the fluorescence peaks lying below 500nm are spurious peaks present in the source itself. Similarly Fig: 4 exhibits the laser induced fluorescence spectrum of molluscan shell excited with the help of a red diode laser (~650nm, 20mW).

We now proceed to sum up the results which have emerged in this section. We first show the LIF peaks of the molluscan shell excited by three different lasers in the visible sector of the spectrum in Table: 1. The molluscan shells have been procured from the Maguri Bill (Wet land, saturated area of land) near Dibru Saikhowa National Park of Tinsukia district, Assam (India). It is quite significant to note that this area of land contains molluscan shells in abundance. We may also add here that such wetlands cover 5—10% of Earth's terrestrial surface [17]. Thus the present work on LIF of molluscan shell samples presumably bears significance in connection with any environment.

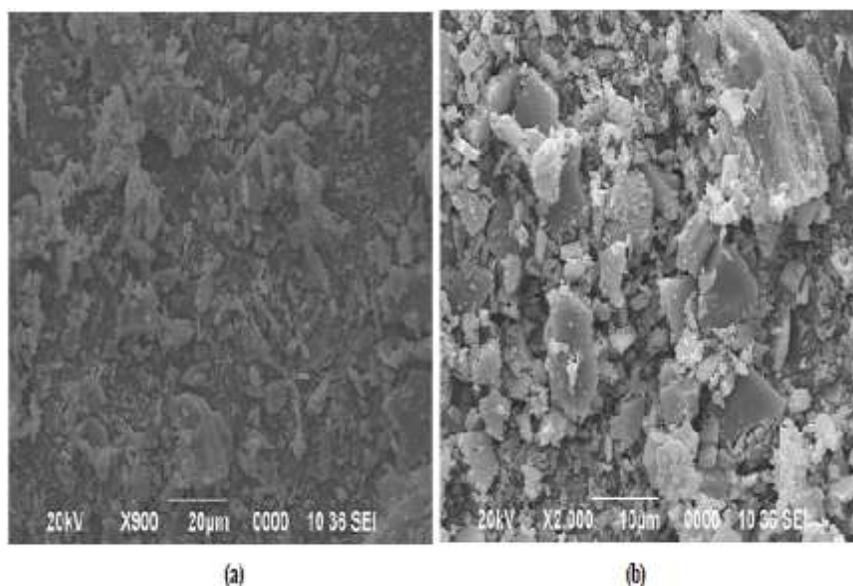
Table 1:- Laser induced fluorescence spectra of molluscan shells in the range 450- 1000 nm excited by blue, green and red diode lasers.

Wavelength (nm)	Relative intensity	Exciting Radiation (nm)	Assignments'
470	s	430	$v_1(N_2 2^{nd} +ve)$
450-600	s (broad)	430	v_2
680	s	430	$v_3(N_2 1^{nd} +ve)$
718	vw	650	$v_4(CaO)$
720	w	540	$v_4(CaO)$

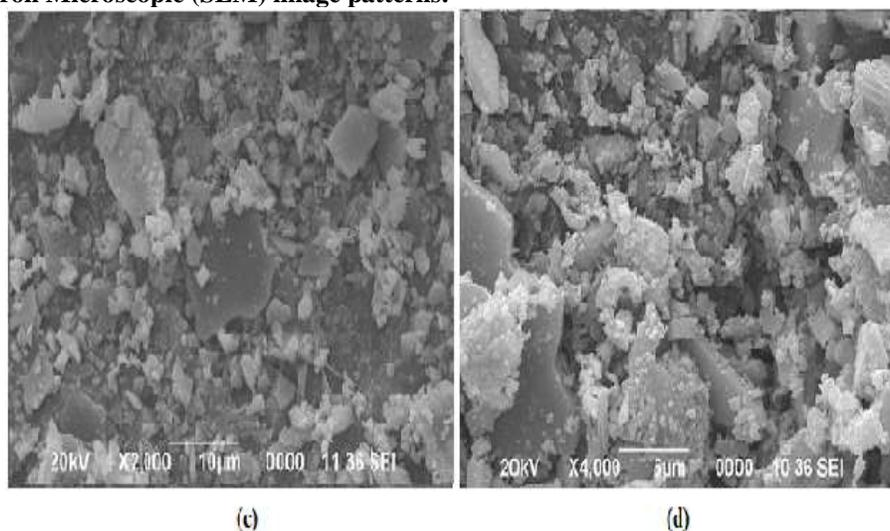
734	vvs	650	$v_5(\text{CaO})$
756	vw	540	v_6
820	ms	540	$v_7(\text{CaO})$
840	ms	540	v_8

s = strong, ms = medium strong, vvs = very very strong, w = weak and vw =very weak

As may be inferred from Table:1, we have identified eight fundamental band systems in the LIF spectra of the molluscan samples. The bands are not excited by a single laser. As regards the proper identification of the LIF peaks we believe that some of them may originate from the dense protein component (conchins) (0.1—5%) as we have used an organic solvent to separate the organic part. We emphasize here that the diffuse band system in the orange sector reported by Konwar et. Al. [11] in a molluscan shell sample in its natural form is due to the orange band system of CaO which is supported by the fact that this band system is excited in carbon arc when CaCO_3 is placed in the gap [18]. In Table:1 the most intense line at 734 nm is probably of diatomic origin, preferably the CaO radical in the extreme red system with transition $A' \Sigma \rightarrow X' \Sigma$ [18]. Similarly we identify the peak at 720 and 718 nm as persistent line originating from CaO [18]. The medium strong band at 820 and 840 nm are also due to the diatomic radical CaO.



Scanning Electron Microscopic (SEM) image patterns:



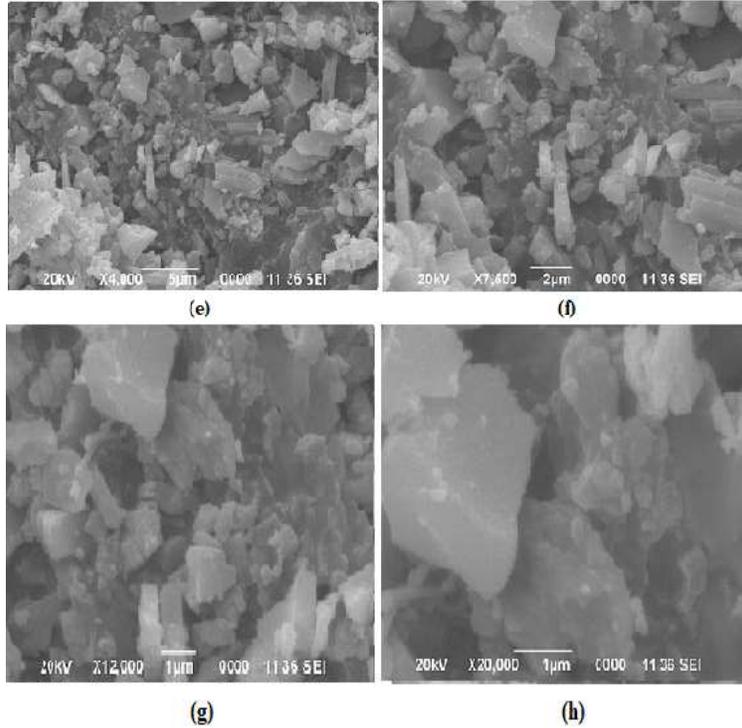


Fig: 5 (a, b,..... h) SEM patterns of the powdered specimen of molluscan shell at different magnification

In this section we describe the SEM results obtained from a grinded powered sample of a molluscan specimen. The objective of this work is not to work out a crystallographic texture of the nacreous layer. This is primarily aimed at observing and estimating the size of the grinded material particles along with finding a possible correlation with the iridescent pattern. Fig: 5(a, b, c, d,..... h) show the SEM patterns of the powered specimen of molluscan sample at different magnifications. It will be seen from the SEM patterns exhibited in Fig: 5 that the characteristic feature of the material particles in a molluscan bivalve shell are visualized at successive stages of magnification. It is worthy of remarks here that the SEM images of the grinded powered sample in the present work are highly varied in shapes and sizes. This is expected as we have already mutilated the mollusk shell, by grinding it. Even then one can clearly observe the regular brick wall-like structure which are generally arranged in continuous parallel laminae around $0.5 \mu\text{m}$ thick (Fig:5 d) separated by sheets of inter lamellar organic matrix [19- 21]. The salient feature of the SEM image at highest magnification (X20,000) (Fig: 5h) shows undoubtedly the existence of numerous microstructures ($\sim 0.01 \mu\text{m}$).

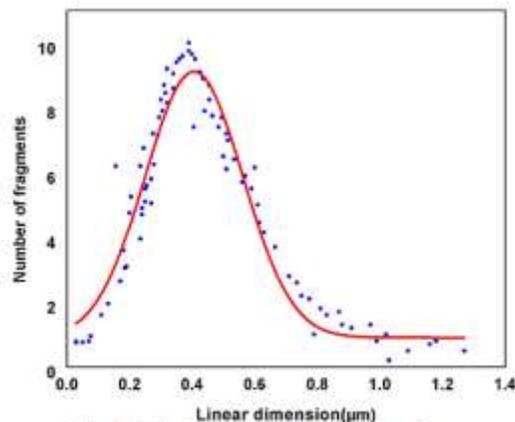


Fig: 6 The distribution pattern of the number of fragments against their linear dimensions

At this stage we find it worthwhile to work out the out the distribution pattern of sizes of various grinded particles which appear in a particular SEM pattern (Fig: 5 c for example) against their number. For this purpose about hundred fragments of different linear dimensions (in μm) against their number have been selected. The distribution pattern is exhibited in Fig:6. The pattern is on a sufficiently small scale to be suitable for exact measurements being made on it with a millimeter scale. Nevertheless a Gaussian pattern emerges as may be inferred from the Fig: 6. the graph is asymmetric in nature. The result is not unexpected. Such types of graphs may be generated in numerous other cases. One may naturally ask, what are the applications of such graphs? One may reasonably believe that they are helpful in characterizing the particular shell.

Summary and Conclusions:-

From what have been described in the earlier sections we sum up the results and draw appropriate conclusions. The foregoing studies are primarily concerned with the iridescence of molluscan shells, laser induced fluorescence of a molluscan shell and scanning electron microscope (SEM) image patterns of a powered specimen. The SEM patterns indicate the presence of numerous gaps between the micro particles and nano structures. A significant fact of observation in iridescence is the patterns of fluctuating luminosity seen and recorded in the Image J intensity distribution patterns. Earlier Raman [9] was of the opinion that the iridescence of mother-of-pearl was a partly diffraction effect that would occur not only when light is incident externally at the surface, but also when it emerges at the surface after suffering reflection at the internal laminations. The optical effects are due to both the surface corrugation and the internal laminations. The present work on the intensity distribution pattern worked out by the software (Image J) and the SEM image patterns presumably supports Raman's view on iridescence of molluscan shells.

The laser induced fluorescence spectra excited by three different lasers having wavelengths at three different sectors of the spectrum namely blue, green and red, yield interesting results. We infer from Fig: 4 that the spectra consist of two broad and categories. The first category exhibits broad and diffuse bands and the second category exhibits well defined bands. As analyzed in Table: 1, the strong band in the region 460- 600 nm is presumably originates from organic components in the molluscan shell and has not been properly identified yet. Other well defined bands are identified by as belonging to either some persistent lines belonging to CaO or N_2 (1st and 2nd positive). The analysis presented in Table:1 has been made for the first time and it is quite reasonable to believe that it will throw much needed light on the material science associated with the formation of molluscan shells. The distribution pattern of the fragments of the grinded specimen as exhibited in Fig: 6 may also be used to understand the buildup process in a molluscan specimen.

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The SEM images are recorded at the Department of Physics of Tezpur University.

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