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Imaging the surface ultrafine structure of red blood cells in physiological condition with scanning probe microscopy

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Enough results in surface structure of mitochondria with optical microscope (i.e. Fluoresce microscope) and Electron microscope (i.e. TEM) have reported in the last 50years; however, it's a pity that nearly all of these methods can not get the real physiological resources for the equipments' limitation. As the effective and excellent tool for biologic surface imaging and manipulation, scanning probe microscope (i.e. AFM) could not only reach high resolution, but also fit to physiological environmental condition. It's the proper technique for biologic samples special in physiologic specimen. However, the author and his workgroup haven't encountered any correlating reports with AFM. Aims: Get ultrafine surface structure of the mitochondria in air and physiologic buffer at room temperature with high resolution AFM, and found the proper process and application for AFM in the newest fields. Methods: Mitochondria have been isolated as the improved method of Walker JE. Following the exact witness for the mitochondria in inner conformation with TEM, high resolution tapping mode/contact mode AFM have been applied to the mitochondria in air or different buffer such as PBS, sucrose solution and physiologic sodium/potassium solution at room temperature respectively. Furthermore, author have investigated the influence of ion gradients to the conformation of mitochondria, and got some evidences on the samples' physical and physiologic properties in which liquid cell and electrochemical cell of the AFM appendixes have been sufficiently utilized. Results: Exact and clear inner mitochondrial properties can get from TEM imaging which have seriously proved the effectivity and feasibility of Walker JE method. Then, 50 μl 0.25M sucrose-60 mg/ml mitochondria solution was deposited onto the fresh cleaved mica or directly injected into the liquid cell respectively for further AFM. Both lead us to get the beautiful and satisfying images. Discussion: Through improved the Walker JE's method and treated the sample properly, authors have founded programmed process for the AFM observation of mitochondria. Further studies have led us to the ultrafine surface structure and the relation between conformation and ion gradients or solution concentration. All of these new scopes may provide us fresh understanding into supramolecular structure and chemical process of mitochondria.

***Keywords*:** atomic force microscopy; mitochondria; topography; nano-structure; nano-behavior

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Sample preparation techniques for the observation of native biological specimens with atomic force microscope

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The excellent sample preparation and an appropriate imaging environment are the key to successful atomic force microscopy (AFM) of native biologic structures. The most basic requirement of the sample preparation is to anchor the specimens firmly to a supporting surface so that the position of the probe with respect to the specimen can be defined with high precision during image process. The immobilization is generally based on adsorption or covalent bonding of the specimen on a solid and flat support. Using these methods, one can investigate the specimen under physiological conditions. This is an important advantage since most biological objects require an aqueous buffer solution to preserve their structure and function. However, there is an important class of surface-active macromolecular structures that occurs naturally at the interface of an aqueous subphase and the air. These structures can be prepared at the air-water interface in a Langmuir trough and then transferred onto a solid support using the LB technique prior to investigation by AFM, Lipid bilayers prepared on a mica surface using the LB technique can also serve as a support for the reconstitution of purified membrane proteins. Furthermore, they can be used for the immobilization of membrane-bound biomolecules.The monitoring of reversible conformational changes were only possible as a result of improved immobilization methods, and underline the potential of the AFM to image dynamic biological processes in which we discuss the adsorption and chemisorptions techniques for the attachment of native biological specimens, and the LB technique for preparing surface active films for the AFM investigation.Glass coverslips are widely used as a transparent specimen support. The amorphous surface can be chemically or physically modified to manipulate its adsorption or chemisorptions properties. They are best suited for all experiments in which visible light is transmitted across the sample, as in SNOM or in combined light and scanning probe microscopy. Organic contaminants, dust and other particles are removed

by washing once with concentrated HCl/HNO_3 (3:1) and five times for 1 min with SDW water in an ultrasonic bath. This process makes the coverslips clean and smooth i.e. R.M.S. roughness be about 1 nm. As the most commonly used support for imaging biological specimens in the AFM, mica minerals are characterized by their layered crystal structure. They are chemically relatively inert. The perfect basal cleavage provides atomic flat surfaces over several hundreds of square micrometers. The net negative charge of the basal oxygen between these double layers is balanced by a layer of hexagonally coordinated cations. This layer is disrupted by standard cleavage procedures for example by means of scotch tape. The resultant basal plane is negatively charged.

***Keywords*:** sample preparation; biological specimens; atomic force microscope; ultrafine stucture; techniques

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The study of gene amplification by laser capture microdissection and real-time quantitative PCR

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To explore the method by laser capture microdissection (LCM) and real-time quantitative PCR for testing gene amplification. The Formalin-Fixed and Paraffin-Embedded (FFPE) blocks of esophageal squamous cell carcinoma (ESCC) tissues were used as the experiment material. The uniform tumor cell group and normal squamous epithelia cells next carcinoma were separated by laser capture microdissection (LCM), and DNA was extracted. Afterward, CCND1 gene amplification was detected respectively by common PCR and Real-time quantitative PCR. LCM could separate tumor cell and normal cell of ESCC effectively. The results of common PCR showed that there were difference of CCND1 gene amplification between tumor cells and normal cells, but the quantity of CCND1 gene amplification difference could not be ascertained. Real-time quantitative PCR analysis proved that CCND1 gene in the tumor cell is 4 folds than in normal cell. The combination of LCM and real-time quantitative PCR is a sensitive and exact method for testing gene amplification.

***Keywords*:** laser capture microdissection; real-time quantitative PCR; gene amplification; ESCC

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Breakthrough technology for large-scale protein production with transient transfection using CHO suspension cultures

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Large-scale transient transfection in mammalian cells for recombinant protein (r-protein) production provides a useful tool for functional, structural and therapeutic studies as well as drug discovery. CHO cells are currently the predominant mammalian cells for protein production. Invitrogen has developed FreeStyleTM MAX CHO Expression System, a complete animal origin free, large-scale transient transfection system that produces high quantity r-protein in suspension CHO cells. The system includes a CHO cell line adapted for suspension culture, serum-free culture medium and a transfection reagent. Cell manipulations or changes of growth medium post transfection are not required. The system is easily scalable, ranging from small shake flask to bioreactor-scale cultures. It provides high transfection efficiency and high protein yields. Our results demonstrated that we were able to achieve over 60 % transfection efficiency by GFP expression and over 50 mg/L of human IgG production from one-liter CHO-S cell culture. Cell viability is greater than 90 % at 24h post transfection. In addition, we have examined production of other proteins in 30 ml transfections. Our observation showed high yields of various proteins such as Human Growth Hormone (220 mg/L), EPO (7 mg/L), and Factor IX (2 mg/L).

***Keywords*:** transfection; protein expression; gene expression; cell biology; new technology

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Visualization of tumor biomarkers: *in vivo* optical imaging of transferrin receptors

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Transferrin receptors (TfR) are over-expressed in most human malignancies including breast cancer. A non-invasive imaging technique by targeting tumor biomarkers such as

TfR would be of great value in early detection of tumor; monitoring tumor progression and evaluation of chemotherapy or radiation therapy. The conventional techniques for the measurement of biomarkers, such as biopsy or resection of the tumors are sometimes problematic, which generate false negative results and involve invasive surgical procedures. In this study, we have developed a near-infrared (NIR) fluorescent conjugate of transferrin (Tf-NIR) for in vivo NIR-based optical imaging of TfR expression in a tumor mouse model. The mouse model was developed after s.c. injection of human MDA-MB-231-luc breast cancer cells in nude mice. Intravenous administration of Tf-NIR conjugate (3 moles dyes/mole Tf) showed strong fluorescent signals in the tumor. The signal was clearly detectable after 10 min and achieved maximum at about 2 h. At this maximum time point, the fluorescent intensity in tumors was 3-16 folds higher than the baseline without administration of Tf-NIR conjugate. The relative intensity ratio of tumor to muscle was in 1.5-2.3 range, depending the tumor sizes. The small size of the tumor, necrosis, and autofluorescence in the mice diet, were the main factors influencing the fluorescent intensity. To overcome the low sensitivity of imaging small tumors (< 3 mm in diameter), we developed Tf-targeted nanoparticles by linking Tf-NIR on the surface of NIR dye-encapsulated liposome (Tf–Lip-NIR) for delivering NIR dye in cancer cells. Tf–Lip-NIR nanoparticles increased the relative signal intensity of tumor to normal by 25-50 % compared to Tf-NIR conjugate. The pharmacokinetic profile of Tf–Lip-NIR nanoparticles in the tumor was found similar to that of Tf-NIR alone. The enhancement of fluorescent intensity using Tf–Lip-NIR was also confirmed by *in vitro* study using flow cytometry. The GeoMean of cell fluorescent intensity for Tf-NIR, Lip-NIR, and Tf-Lip-NIR particles was 1.8, 7.0 and 16 folds higher compared with untreated controls ($P<0.05$). Our results demonstrate that the NIR conjugate of Tf, particularly the Tf–Lip-NIR nanoparticles improved image contrast and would be useful for non-invasive real-time imaging and monitoring transferrin receptors in tumor. The same technique might be applicable for the detection of other biomarkers in cancer cell, and targeted drug delivery system.

Keywords**:** transferrin; optical imaging; biomarker; transferrin receptor; nano particle

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Discovery and development of novel tumor-targeting agents via optical imaging

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Optical imaging has been emerging as an important tool for studying molecular recognitions in biomedicines. We have been interested in applying optical imaging to the discovery and development of novel tumor targeting agents for cancer diagnosis and therapy. This presentation will provide an overview of the related molecular design, synthesis, and evaluation in our lab. The principles of our molecular design have highlighted the interactions between near infrared (NIR) fluorescent ligands and specific receptors up-regulated in tumors. Therefore, NIR fluorescent carbocyanine probes (Cypates) have been used to conjugate with diverse receptor-avid molecules such as octreotate and RGD peptide analogs for targeting the corresponding somatostatin and integerin receptors especially. We have explored different strategies for molecular design and synthesis. Especially, different Cypates have been used as optical scaffolds to construct the complex NIR fluorescent tumor-targeting molecules. The compounds have been evaluated for their receptor binding, cellular internalization, cellular localization, cytotoxicity, apoptosis, and bio-distribution in tumor-bearing nude mice. The related structure-activity relationships will be discussed. The most promising compounds we have discovered should provide some insights into their potentials in imaging the stages, progress, and therapeutic response of various cancers as well as targeted drug delivery and related cancer biology.

Keywords**:** optical imaging; tumor targeting; molecular recognition; NIR fluorescent probe; peptide

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Invitro maturation and germination of microspores of *Orychophragmus violaceus*

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The male gametophyte of higher plants is an excellent system to study gene regulation, cell fate determination and cellular differentiation in plants because of its relative simplicity compared to the sporophyte and its accessibility for cytological and molecular analysis. It is reported here that some observations and investigations of pollen developmental progress and viability of *Orychophragmus violaceus in vivo* were carried on. Pollen developmental stages were confirmed and pollen of late unicellular stage was isolated *in vitro*. Liquid medium composed of MS +

White's vitamins + coconut milk (2 %) + maltose (0.5 M) was chosen which is the most appropriate for *in vitro* culture of *Orychophragmus violaceus* microspores. With this medium, the rates of in maturation and germination were 19.3 % and 4.7 %, respectively. Liquid medium composed of maltose (0.6 M) + boracic acid (100 mg/L) + $Ca(NO_3)_2$ (700 mg/L) + VB1 (10 mg/L) was chosen for germination of pollen matured *in vivo*. The rate of germination *in vitro* was 70.7 %. When pollen matured *in vitro* was cultured in the germination medium, the rate of germination was 62.7 %.

***Keywords*:** microspores; *Orychophramus violaceus*; *in vitro*; matured pollen; germination

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Get the most out of pyrosequencing: modeling and a new data processing procedure

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In cell biology, there is an increasing demand for high-throughput and inexpensive techniques for DNA sequencing. Pyrosequencing, a newly developed non-electrophoretic DNA sequencing method based on the sequencing-by-synthesis principle, could monitor DNA synthesis in real-time by a four-enzyme catalyzed cascade of reactions. DNA sequence is revealed in the form of fluorescence pulses that are contaminated by instrument noise and fake pulses caused by asynchronous synthesis of different DNA strands. The poor quality of raw data has limited this method to single nucleotide polymorphisms (SNPs) analysis and similar applications that require only sequencing of short stretches of DNA. Continuous efforts have been made to Pyrosequencing chemistry to improve the accuracy and read length of a single run, and several mathematical models of the chain reactions have been established. We designed and validated a novel procedure for Pyrosequencing post-assay data analysis based on a modified mathematical model of the reaction kinetics. The procedure could greatly improve the signal-noise ratio (SNR) by compensating the fake signals caused by asynchronous elongations in the DNA pool. It includes the following steps. (1) Measurement noise is filtered from raw fluorescence readings by smoothing, background subtraction and detrending. (2) Model parameters are estimated from the first few pulses. (3) Fluorescence readings are integrated during dNTP dispensing intervals to obtain absolute amount of photons that is emitted during each reaction cycle. (4) The integrated pulses are then compared with the model prediction to tell the fake pulses apart. (5) Parameters indicating the portion of asynchronous synthesis are recalculated from the current signal and used for future model prediction. A detailed description and analysis of this data processing procedure is provided. Raw data from assays conducted on PSQ96 (Biotage, Sweden) are used to validate the procedure, and reasonable agreement are found between procedure output and public databases when conservative segments of DNA are sequenced. The average read length of a single run has been extended so that the application of Pyrosequencing could be further expanded.

***Keywords*:** DNA sequencing; pyrosequencing; data processing; biochemical modeling

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A survey of fluorescent imaging reagents for cell biology: dyes, fluorescent proteins and quantum dot nanocrystals

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In recent years, semiconductor quantum dot nanocrystals have joined organic dye–based probes and fluorescent proteins to extend the capabilities of fluorescent labeling techniques for cell biological imaging applications. The exceptional spectroscopic properties of quantum dot nanocrystals (large cross-sections for both one- and two-photon absorption, narrow emission bandwidths and large excitation–emission separations) facilitate applications such as single-molecule tracking of receptors, emission wavelength multiplexing, and intravital imaging. Recent fluorescent protein developments include spectral palette extensions, improved transient transfection methods and photoactivatable and photoswitchable variants. In some cases, the appreciable size of fluorescent proteins (~28 kDa) causes structural perturbations resulting in nonfunctional fusion constructs. A much smaller hexapeptide motif (CCPGCC) in conjunction with an extraneously introduced biarsenical fluorophore (TC-FlAsH) provides an alternative protein tag in these cases. Increasingly there is a need to correlate fluorescence images with ultrastructural information at electron microscopic resolution. Various methods for developing fluorescent labels for electron microscopy, including diaminobenzidine photooxidation and antibodies to fluorescent haptens coupled with gold-labeled secondary antibodies, will be discussed. Together with advances in optical instrumentation these techniques are providing biologists with images of cells, tissues and organisms with continually increasing levels of spatial, temporal and

molecular identity resolution.

***Keywords*:** fluorescence microscopy; nanocrystals; spectroscopy; fluorescent protein; fluorescent probes

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Isolation of sperm cells of rice (*Oryza sativa* L. Japonica)

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Isolation of sperm cells of higher plants is a basis of recognizing the mechanism of double fertilization, and isolated sperm cells can be used *in vitro* fertilization assay in which only a few sperm cells can meet requirement and in molecular studies to probe the mechanism of gametic recognition. Two methods can be used to isolate sperm cells. One is culturing pollen grains and collecting sperm cells from broken pollen. The isolated sperm cells using this method need to check their developmental maturation because they require a growth process in pollen tube *in vivo*. Another method is to isolation sperm cells from pollen tube in which both sperm cells develop nearly maturation. In this study, we conducted a protocol for the isolation of rice sperm cells from pollen grains and pollen tube, in the former en masse isolation of sperm cells can be readily obtained with the number of sperm cells correlated with the number of pollen grains, and in the later the maturation of sperm cell is more close *in vivo*. We also conducted the collection of two separate populations of the dimorphic sperm cells from pollen tubes of rice, which would be especially useful in the assay of special genes isolation of both sperm cells. In the isolation of sperm cell from pollen grain, two steps of osmotic shock were used: fresh pollen grains from just blooming flowers first were put into a solution containing 9%, 12% and 15% mannitol for 10 min, and then put in another solution with only a half osmotic pressure in low temperature. The broken pollen grains released cytoplasm including two sperm cells. In the three solutions, 9% mannitol solution was best and 70.5% pollen grains broke. Bovine Serum Albumin can protect sperm cells for longer time because without BSA sperm cells will lose FDA fluorescence after 3 h. Two sperm cells just released from pollen grain were ellipse and dimorphic in size. Then both became round soon but its dimorphism preserved. In the isolation of sperm cells from pollen tube, pollen tubes need to be induced first. There were two methods to induced pollen tube: culture pollen grains in a solution containing 10% PEG, 20% sucrose, 0.005% $CaCl_2$ and 0.01% H_3BO_3, pollen tube would germinate at 2 min after culture in room temperature, and the tubes grown very fast within first 10 min, and then the growth became slowly. When the pollen tubes were transferred into a broken solution the tube broke and released its cytoplasm content including two sperm cells. Two sperm cells isolated from pollen tube often were connected with vegetative nucleus to make a male germ unit (MGU) in which bigger sperm cell (Svn) connecting with vegetative nucleus and small one (Sua) just connecting with bigger one.

***Keywords*:** rice; *Oryza sativa* L. Japonica; sperm cell

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A dengue virus *in vitro* culture system for large-scale dengue antigen production

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With the current trend in viral serodiagnosis and the pressing need to develop effective vaccines against dengue virus, the demand for dengue antigens is increasing. This requires the development of an efficient method for large-scale antigen production. This paper reports on the optimization of a cell culture-based dengue virus production protocol for upscale production in a spinner flask system. Dengue strains representative of all four serotypes were selected from more than 300 Philippine isolates stored in the St. Luke's RBD collection. Growth adaptation of the host C6/36 *Aedes albopictus* cells to serum-depleted conditions was studied in small-scale cultures using three different commercial media. Viral infection of C6/36 cells was monitored by Immunofluorescence Assay (IFA). Viral antigen production was determined by periodic harvesting of infected culture fluid (ICF) at varying growth intervals and titration of each sample using Sandwich-ELISA. Results obtained from tube and T-25 flask cultures of dengue-2 virus by IFA showed highest degree of infection achieved in the control medium, with 2% serum, moderate in medium with reduced serum (0.125%), and no infection observed in serum-free medium. Antigen production curves showed higher yields using the two media with serum supplementation. Similar studies of dengue virus serotypes 1, 3, and 4 have also been done. Presently, the upscale culture of these dengue strains is underway using the identified optimum growth conditions. The dengue antigens from all four dengue virus serotypes will be used to make the tetravalent antigen cocktail needed to perform IgM-capture ELISA in the laboratory confirmation of dengue infection. This test

is routinely used in the community-based surveillance of dengue. There is a distinct advantage to using these antigen reagents in that these are derived from locally circulating strains of dengue virus.

***Keywords*:** dengue virus, cell culture, spinner culture, immunofluorescence assay, sandwich ELISA

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The response of maize root hair and stomata to water conditions alternating between wet and dry

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In the case of engineering and agricultural technology water-saving have made great advance, "biological water-saving" —utilization and exploitation of physiological and genetic potential of organisms themselves for the possibility of acquiring more agricultural production under identical conditions of water-supplying, can be regarded as a key pathway and last potential for advance agricultural water-saving in the future. About 40% of the land in the world is under arid and semiarid climatic conditions. Periods of drought alternating with short periods of wet conditions are common to many semiarid areas of the world. The sufficient use of precipitation and optimization of crop use efficiency are important in such conditions. Plant response to this water deficit and variable environments is complex and uncertain, because such environment present a dynamic changed water conditions, which neither similar water shortage condition along plant life, nor similar water adequate condition along plant life. Using Electricity Microscope Scanning technology, we have studied the response of maize （*Zea mays* L.) root hair and stomata to water conditions alternating between wet and dry. The results indicated that different maize variety, which planted in Chinas different region, have different response mechanism and pathway for such water conditions. For root hairs number and health under water stress, the proper order for 5 maize varieties are: Hudan4,Shandan9 (planted in shaanxi province), Nongda60 (planted in Beijing region), Shendan10 (planted in north-east region of China), Yedan13 (planted in Shandong province). For root hair re-covering capacity under re-watering, the proper orders for 5 maize varieties are: Hudan4, Nongda60, Shandan9, Yedan13, Shendan10. For stomata apertures, water stress makes the stomata both of hudan4 and shaandan9 closed significantly, but these effects are completely reversible when re-watering, which belong to the higher acclimated varieties. In both Nongda60 and Shendan10, there are stomata oscillations under water stress, and the recovering of stomata opening when re-watering is not significant. It can be concluded that maize varieties that oriented from different environment have varies mechanism response to water conditions alternating between wet and dry.

***Keywords*:** maize; root hair; stomata; water alternating between wet and dry

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Effect of water deficit on vascular bundle system of rachis in winter wheat

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Pot experiments were carried out with winter wheat under a rain shelter to study the effect of water deficit on vascular bundle system of rachis in wheat during heading stage. The changes of vascular bundle system of rachis were investigated. The results showed that the number and area of macro-vascular bundle and small-vascular bundle of rachis and the diameter of vessel and the area of phloem decreased from base to top in the rachis, but the decreased range differed with the change of the kind of vascular bundle and position of rachis and water conditions. The number of macro-vascular bundle decreased slowly from the basal part to the middle part of rachis under normal water condition, compared with the basal and the middle of rachis, it decreased 8 and 12 respectively in the top of rachis. The number of small-vascular bundle in the top of rachis was smaller 18 than that of in the basal of rachis, the diameter of vessel in the top of rachis decreased 90 μm. And the area of vascular bundle and phloem changed as the number of macro-vascular bundle did. Compared with normal water treatment, the number of macro-vascular bundle decreased 3-4 under moderate water deficit, and decreased 7-8 under server water deficit. The number of small-vascular bundle decreased 2-6 under moderate water deficit and decreased 4-17 under server water deficit. The diameter of vessel decreased 15-38 μm under moderate water deficit and decreased 20-104 μm under server deficit. The area of phloem changed little under moderate water deficit, but it decreased 3432.1-4893.9 μm^2 under server water deficit. The area of vascular bundle decreased 3081.7-5495.3 μm^2 under moderate water deficit and decreased 11452.0-17961.4 μm^2 under server deficit. This study showed that the vascular bundle system of rachis in wheat changed apparently under water deficit treatment.

***Keywords*:** wheat; water deficit; rachis; vascular bundle
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BD bioimaging systems: imaging tools for functional cellular analysis

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High content imaging is rapidly becoming a mainstay in pharmaceutical and life-science research laboratories. The power of imaging resides in its ability to measure not only fluorescence intensity changes within cells, but also molecular rearrangements and morphological features. In recent years, the availability of a new generation of automated imaging platforms such as the BD Pathway has created a unique opportunity to develop novel cell-based assays. To be used effectively in cell analysis, an automated imager must provide a wide range of capabilities including live-cell kinetic imaging, endpoint imaging, and high-resolution confocal imaging. These capabilities, coupled with versatile, user configurable software, allow the researcher to explore cellular events in multidimensional space and time. This presentation will explore the versatility of the BD Pathway Bioimager as a cell biology research tool and demonstrate applications on apoptosis, cell cycle, DNA damage and angiogenesis using BD Pathway system.

***Keywords*:** endpoint assay; live cell assay; automated imager; confocal imaging; data analysis
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Bacterial pathogens associated with infectious uveitis are identified by 16s rRNA gene analysis

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This paper describes the PCR-based detection and DNA sequence-based identification of bacterial pathogens from ocular samples taken from patients with infectious uveitis using 16S rRNA gene sequence analysis. Infectious uveitis is characterized by inflammation inside the eye caused by a virus, bacteria, fungus or parasite. The definitive identification of an infectious agent enables the clinician in the management of the patient, especially in the timely administration of appropriate anti-infectives. Sixty samples from infected eyes of 34 patients and 4 control samples were tested for the presence of bacterial pathogens by direct Gram staining, conventional culture techniques and Polymerase Chain Reaction (PCR) of the 16S rRNA gene. PCR of the 16S rRNA gene was positive in 16 (26.7%) of the samples from infected eyes. Of the 16 PCR-positive samples, 11 were deemed non-culturable in the three culture media used here. All four samples from control eyes were negative by direct Gram stain, conventional culture and PCR of the 16S rRNA gene. The isolates from two samples belonging to one patient was misidentified as Burkholderia cepacia by biochemical tests, but was unequivocally identified as Ralstonia mannitolylitica by 16S rRNA gene sequence analysis. Both biochemical tests and 16S rRNA gene sequence analysis identified streptococci and Enterobacteriaceae in 3 samples but were unable to assign a definite species. Using 16S rRNA gene sequence analysis, the following bacteria were identified from 4 samples that were negative by routine culture: Haemophilus influenzae, Sphingomonas sp., and Pseudomonas saccharophila. This is the first report of the application of 16S rRNA gene sequence analysis to the detection and identification of pathogenic bacteria in ocular samples from Filipino patients.

***Keywords*:** infectious uveitis; bacterial pathogens; 16S rRNA gene; PCR; DNA sequencing
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Current advances in optical Imaging for cell biology from the prospective of optical properties of biological specimens

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Recent developments in optical imaging have provided powerful research tools for cell biology. These modern imaging tools include confocal microscopy, non-linear microscopy such as multi-photon fluorescent, and second and third harmonic generation (SHG and THG) microscopy. All these tools have made three-dimensional imaging of cells and tissues possible. At the tissue level, OCT could be used for delineating tissue layers. In addition to the conventional two/three-channel detection scheme used in confocal and multiphoton fluorescent microscopy, spectral imaging has

become a powerful instrument in separating fluorphores, which have overlapping emission spectra. Background auto-fluorescence removal is also possible. Furthermore, fluorescent life time imaging is providing additional modality to separating fluorophores of similar emission spectra. Therefore imaging living cells in multi-dimensions, such as x-y-z-t, x-y-z-λ and x-y-z-τ, has become possible. Viewing the current available imaging tools and selecting the optimum instrument is an important task in the research of cell biology. In addition to the commonly used fluorescent and back scattering imaging modes, the optical characteristics of cellular organelles and extracellular matrices are allowing many additional image modalities to be used. For example, birefringent materials, such as the mitotic spindle, can be imaged by using polarization microscopy, structures exhibits bio-photonic crystal properties can be visualized by SHG, and boundaries formed by different refractive index materials can be detected by SHG.In live cell imaging, the frame rate of the imaging system, the signal strength, the wavelength and radiation dose used in the illumination system are issues that one should pay major consideration.

Keyword**:** multispectral microscopy; nonlinear microscopy

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