YAMADA Laboratory

Department of Mechanical Engineering and Intelligent Systems
The University of Electro-Communications

This laboratory is conducting research subjects on *biomedical engineering* as new application fields of *optical and thermal engineering*. We aim to develop novel measurement methods and devices. For this aim, we actively advance collaborative researches with other research groups.

Non-Invasive Measurement Using Near Infrared Light

Fundamental and applied researches have been conducted on non-invasive measurements of physiological information using near infrared light which can penetrate into biological tissue deeper than light in the other wavelength ranges. The optical methods can be used for measurements of various factors such as oxygen concentration in a brain, blood glucose level, blood oxygen saturation ratio, water content in blood, and temperature in a tissue.

New Device Development by Thermal and Optical Engineering

We study a non-contact flowmeter for low flow rate using light, an thermal type ultra-low-flowmeter for artificial organs, and a plastic biochip fabrication using light heating. Also, methods for the measurement of cellular temperature changes are investigated. These are applied researches to biomedical and biological engineering based on advanced methods in microfabrication, measurement and control of temperature and light.

Photon Migration in the Skin for Optical Measurement of Blood Glucose Level

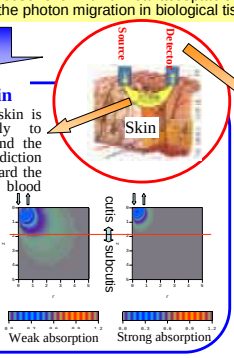
Near infrared light, the wavelength of which is a little bit longer than red light, can penetrate into biological tissue deeper than light in the other wavelength ranges and can be applied to various biomedical measurements. It is known that the change in the reflection spectra from skin tissue is related to the change in the blood glucose level, and many people have tried to measure the blood glucose level by optical methods. However, no one has succeeded in optical measurement of blood glucose level with clinical acceptable accuracy and stability mainly due to difficulty to understand the photon migration in biological tissues which strongly scatter light.

Our approach:

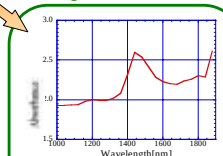
Study of photon migration in the skin

Photon migration in the skin is simulated computationally to estimate the light path and the causes of errors in the prediction of blood glucose level toward the optical measurement of blood glucose level.

The figures demonstrate the simulation results to show the difference in the light path between the cases with strong (right) and weak (left) absorption of near infrared light.



Process of optical measurement of blood glucose level



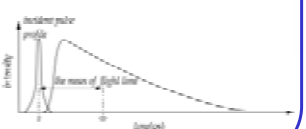
The shape of the absorption spectrum varies with the variation of blood glucose level. Statistical process such as multivariate analysis will be applied to the measured data to predict the blood glucose level.

Diffuse Optical Tomography

Physiological information such as oxygen concentration can be measured using near-infrared light, which deeply penetrates into biological tissues. However, it is very difficult to obtain its optical tomogram because intensity of light detected on a outer surface, which has passed through a tissue, is very weak and light propagation in a tissue is very complicated. In order to solve this problem, we use the time-resolved measurement method and develop an image reconstruct algorithm for oxygen concentration distribution.

Time-resolved measurement

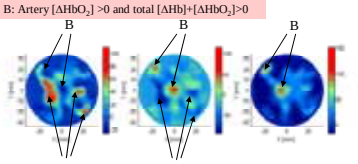
Using pico-second pulse light as incident light into a tissue, various forms of the light propagated in the tissues can be obtained at body surface points. Analyzing this detected right profile in pico-second resolution, average photon pass length and optical properties can be calculated.



Source and detection optical fibers are fixed around a forearm. The data is acquired in task states and in rest states.

A: Muscle [ΔHb]>0 and [ΔHbO$_2$]<0

B: Artery [ΔHbO$_2$] >0 and total [ΔHb]+[ΔHbO$_2$]>0



Collaborator: National Institute of Advance Industrial Science and Technology (Japan) and Tianjin University (China)

Integrating Sphere Pulse Oximeter

Pulse oximter is one of the medical instruments to measure oxygen saturation of arterial blood using two wavelengths in the red and near infrared ranges. In medical treatment, it is very important to know the oxygen saturation of arterial blood, and pulse oximeters are inevitable instruments for monitoring patients' health conditions.

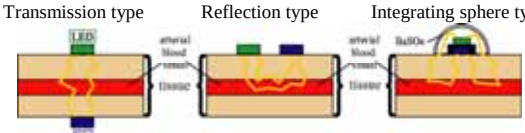
Oxygen Saturation is ...

It is the ratio of the concentration of oxygenated hemoglobin [HbO$_2$] to that of total hemoglobin [HbO$_2$]+[Hb] in blood.

$$SpO_2(\%) = \frac{[HbO_2]}{[HbO_2]+[Hb]} \times 100$$

Pulse oximeter

Transmission type | Reflection type | Integrating sphere type



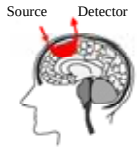
Flexibility of measurement sites and safety of reflection type

The integrating sphere type has the advantage of high flexibility and of that LED doesn't touch the tissue directly. This advantage can solve the problem such as the low temperature burns that originate in generation of heat of LED with the monitoring for a long time. Additionally, the light intensity distribution on the luminescence side becomes uniform by using the advantage of the integrating sphere, so that the more accurate measurement becomes possible.

Optical Mapping

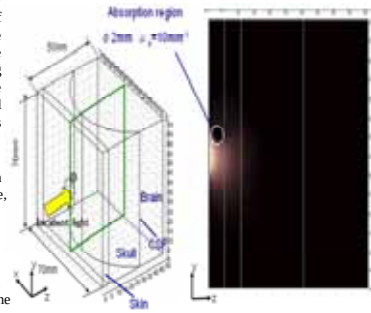
Optical topography is one of the medical apparatuses for investigating brain activity by use of near-infrared light which can penetrate bones to reach brain. Absorption of near infrared light by blood changes with the amount of oxygen in blood. By measuring the change in the absorption spectrum of near infrared, optical topography can provide the image of brain activity.

Light propagation in a head



Since near-infrared light is strongly scattered by living tissues, some of near-infrared light incident on the head surface returns back to the head surface after propagating through the shallow region of the brain. Therefore, the optical signal detected at the head surface carries the information of brain activity.

3D simulation



Higher order brain function

Optical topography images the activities of shallow region of the brain managing higher order functions like language, motion, and thinking. For example, when a human tries to speak the speech center (Broca's area) will be stimulated and more blood flows in the area. Therefore, the light propagating through the area is absorbed more strongly when the area is stimulated, and we can know the position and activation degree of the activated area by measuring the change in the detected light at the head surface.

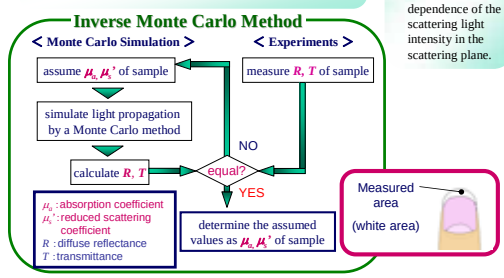
However, there exist some unresolved items. The propagation path of the light in the head is unknown, and the anatomical and functional differences from person to person affect the resultant images. The purpose of our research is to provide solutions to these items by experiment and simulation.

Measurement of the optical properties of living tissues

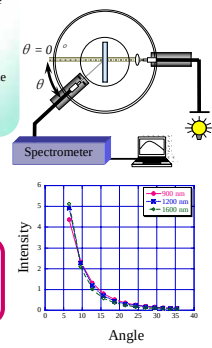
Diagnosis, treatment and bioinstrumentation using near infrared light are being developed these days. However, strong scattering and weak absorption of near infrared light by biological tissues make it difficult to know light propagation in tissues. It is necessary to measure the optical properties such as the reduced scattering and absorption coefficients in order to estimate how light propagates in various biological tissues.

①We aim to measure the optical properties of nails. By an inverse Monte Carlo method, which compares the results of experiment using a spectrometer with an integrating sphere and those of Monte Carlo simulation, we obtain the reduced scattering and absorption coefficients.

Inverse Monte Carlo Method



②We measured the single scattering phase function which describes the angular dependence of the scattering light intensity in the scattering plane.



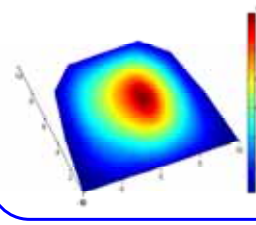
Fluorescence Tomography

Fluorescence tomography reconstructs the concentration of fluorophore injected into biological tissues with 2D or 3D tomographic images. It is possible to understand the mass transport phenomenon by observing the accumulation process of fluorophore into particular tissues some time after its injection. This can be applied to detect the positions and sizes of tumours using fluorophore which specifically accumulates in the tumours. However, it is not an easy technology to predict the distribution of fluorophore concentration inside tissues from the fluorescence light emission measured at the tissue surface. This study applies the method of DOT (diffuse optical tomography) using pico-second time-resolved measurement to the fluorescence tomography.

What is fluorescence?

Fluorescence is a phenomenon in which a material emits light with a particular wavelength when excited by light with another wavelength shorter than the emission light. Fluorescence is widely used for observing the effects of new drugs and the behaviour of molecules by introducing the drugs or proteins with fluorophore into small animals and biological cells.

Modified GPST (Generalized Pulse Spectrum Technique)

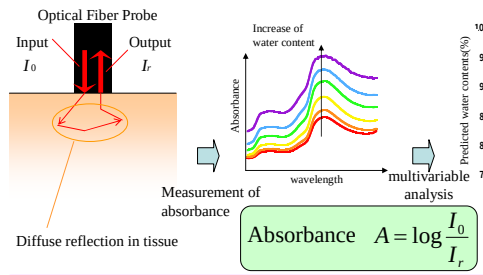


Modified GPST has been found to be effective for solving the inverse problems with the light propagation dominated by scattering. Previous studies of DOT reconstructed the tomographic images of tissue oxygenations from the measurements of near infrared light propagated through biological tissue. The purpose of this study is to reconstruct the tomographic images of concentration of fluorophore.

A numerical result of fluorescent emission distribution inside tissue simulating a rat head with the size of 20 mm using FEM for validation of the calculation of light propagation (200 pico-second after fluorophore injection).

Optical Measurement of Water Content in Tissue

Patients of kidney disease need dialysis treatment for removing waste in blood. It is necessary to know in real-time the water content in blood to prevent the serious accidents by excess water removal associated with waste removal. We are developing a method to measure water content in blood and tissue by near infrared spectroscopy.



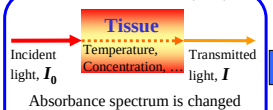
$$\text{Absorbance} \quad A = \log \frac{I_0}{I_r}$$

We measure the absorbance of blood and tissue by near infrared light in the wavelength range including water absorption band. They are preprocessed in order to reduce the errors by temperature and other factors. Then, the regression function is generated to relate the obtained spectra and water contents. It is possible to predict the water content by substituting the measured absorbance spectra of unknown samples to the obtained function. This optical method has advantages of being real-time, noninvasive and continuous.

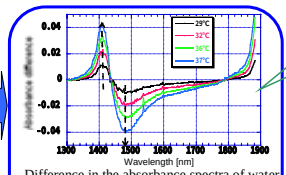
Measurement of Tissue Temperature Using Near Infrared Light

We propose a method for non-invasively measuring the temperature changes of water, which much exists in a biological tissue, using near infrared (NIR) light. The goal of this study is to measure the temperature changes of biological cells or tissues. This method is on the basis of the phenomenon that the NIR absorption spectrum of water depends on temperature.

Absorbance: $A=-\log_{10}(I/I_0)$



Absorbance spectrum is changed by various factors. Temperature is one of them.



Difference in the absorbance spectra of water from that at 26˚C. The arrows represent the direction of increasing temperature.

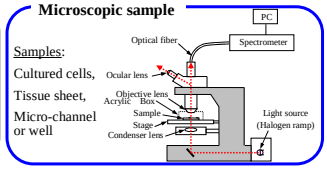
Multivariate Analysis

By statistical techniques such as multivariate analysis, a tissue temperature is predicted from the measured spectra.

Why NIR and Water?

NIR absorption spectrum of water is changed by temperature. This phenomena is caused by the changes of the connected state of water molecules. In a NIR spectroscopy, which is much studied for the noninvasive measurement of physiological information, the temperature change has been regarded as a disturbance. Contrary, making use of this phenomenon, we can measure the temperature.

Microscopic sample

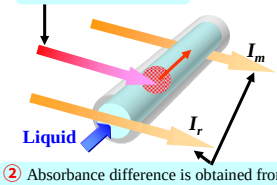


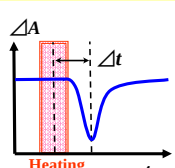
Samples:
Cultured cells,
Tissue sheet,
Micro-channel or well

Non-contact type flowmeter using near infrared ligh

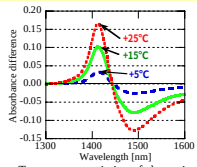
In the manufacturing process of semiconductors with the advanced miniaturized circuits, highly precise flow control is required. And in order to maintain the purity of the liquid, the flow rate must be measured without pollution. So, we are studying a new non-contact type flowmeter using near infrared light. It is expected that it can measure more precisely than the ultrasonic flowmeter.

① Heating with laser





Temporal variation of absorbance difference



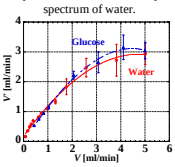
Temperature variation of absorption spectrum of water.

② Absorbance difference is obtained from the intensity ratio of the transmitted lights measured at upstream and downstream of the heating position

③ Time from the heating to the peak of absorbance change is measured and correlated with the flow rate

Absorbance difference

$$\Delta A=-\log\frac{I_m}{I_r}$$

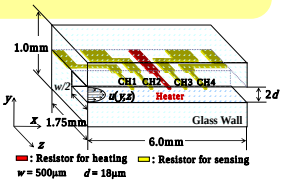


Experimental results.

Ultra-Low Flow Meter for Artificial Pancreas

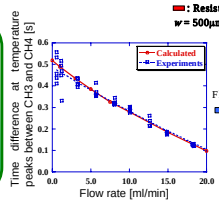
Humans obtain biological energy for living by transporting glucose into cells. Diabetic patients cannot make enough amount of insulin, which is required for transporting glucose into cells. An artificial pancreas is expected to substitute the damaged pancreas. By injecting insulin into the patients' bodies from the artificial pancreas, the diabetic patients can live like healthy people. But the amount of injected insulin must be strictly controlled in order to avoid adverse effects. Ultra-low flow meter for insulin is highly requested in the control procedure.

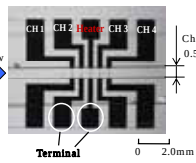
No implantable flow meter has been developed for the ultra-low flow rate in the range of several μl/min. We are developing a thermal type of flow meter, and conducting thermal analysis as well as experiments of ultra-low flow meter for the purpose of optimum designing.



Measurement principle

An electric resistance, Heater, deposited on the internal wall of the channel heats up the flow intermittently, and other electric resistances CH3 and CH4 downstream measure the temperature changes induced by the heating. Flow rate is correlated with the difference in the time at the temperature maximum between the two resistances CH3 and CH4.

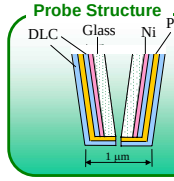




Measurement of Cellular Thermal Responses

Measurements of thermal responses in a biological micro-region, particularly a single cell, can provide new physiological information. Cellular heat productions with temperature changes are caused by reaction to certain physical or chemical stimulations. The aim of this study is to develop a micro-thermocouple probe based on a micropipette in order to measure the cellular thermal responses.

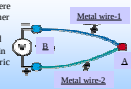
Probe Structure

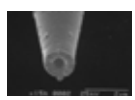


Using micro-fabrication techniques, Pt, DLC as an insulating film, Ni, and DLC are deposited sequentially on a glass micropipette. The junction of Pt and Ni is formed on the 1- μm-diameter tip, and thus the probe can be worked as a thermocouple.

What's a Thermocouple?

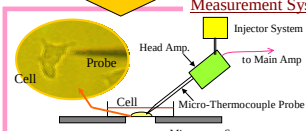
A thermocouple is constituted by pair of metals with their ends where one metal is in contact with another (point-A). Now, if the junction is heated (A) keeping the other cold (B), a voltmeter which is placed in the circuit shows the thermoelectric power generated in the circuit. Taking advantage of this phenomenon, called 'Seebeck effect,' temperature difference between A and B can be measured.





Scanning electron micrograph of the probe tip

Measurement System



The probe approaches to a cell using a micro-manipulation system. Since signals of cellular temperature changes are very small, they must be amplified. The probe based on a micropipette can also inject a substance into the cell. Therefore, the injection and the temperature measurement can be performed simultaneously.

DLC Coating on Microprobes

It is necessary to deposit and process conductive thin films and electrical insulating films in order to produce micro/nano semiconductor devices. Silicon dioxide and organic films (polyimide, etc.) have been commonly used as insulating films for some flat substrates. However, it is difficult to produce a fine insulating film for a micro-acute object such as a micropipette. Therefore, an optimum insulating material and its deposition method for the acute microprobe have been required. In this study, a diamond-like carbon (DLC) film is used as insulating film and its property is investigated.

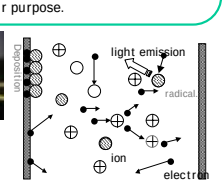
About DLC

DLC has been widely investigated due to its remarkable properties such as high electric resistivity, great hardness, low coefficient of friction, high thermal conductivity, chemical inertness, high optical (especially infrared) transparency, and biocompatibility. Therefore, DLC coatings are now used in various electronic, optical, and mechanical products. In addition, the recent evolution of micro-electromechanical systems has led to more scientific interest in DLC for applications in this field.

How to deposit DLC?

Several methods can be used to produce DLC films. However, not all methods can provide high quality DLC films over micropipette surfaces. Plasma-enhanced chemical vapor deposition (PECVD) offers several significant advantages for this purpose: low temperature deposition, few restrictions on sample geometry, and a simple apparatus. We have thus employed PECVD, and modified its electrode configuration and voltage regulation method for our purpose.

DLC-coated micropipette



Chemical reaction in plasma

DLC coating on micropipettes is also used as an interlayer insulating film of micro-thermocouples for cellular temperature measurements.

Cytometric Imaging of Cell Dynamics in Micro Flow

Measurement of platelet aggregation is effective in monitoring for anti-platelet agent or pathologic thrombosis. Conventional aggregometer, turbidmetric (or optical) method, is based on the detection of light transmitted through a cuvette containing platelet rich plasma (PRP) and is currently put on the market. It, however, has the following problems: (1) preparation of PRP is complicated and time-consuming work, and (2) PRP sample, prepared by removing erythrocytes and leukocytes, is quite different from in vivo status.

Methods

Our purpose is to develop a method for assessment of platelet aggregation in the whole blood. The whole blood is flow through micro flow path implemented between a silicon and a glass plate to reduce the light absorption by hemoglobin in erythrocytes. Platelet cells in the blood micro flow are detected with a CCD camera directly as motion-image sequence to image the generating process of platelet aggregates. The motion-image sequence processing and analysis technique is applied to investigate platelet cell dynamics in the blood micro flow in order to find a quantitative index for platelet aggregation in the whole blood.

We hope it will be useful for extending the role of the clinical testing to early detection or diagnosis of various blood diseases such as arteriosclerosis, thrombosis, myocardial infarction.

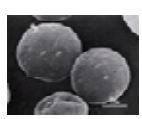


Fig. 1. Micrograph of platelets

Motion-image Sequence Analysis

We extract platelets as objects from the motion-image sequence using digital image processing technique. We measure dynamics of platelets by comparison between each image of successive frames.

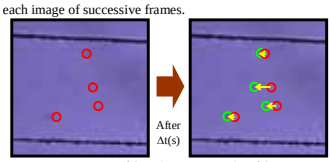
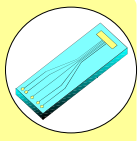


Fig. 2. Moving images of the polystyrene particles of diameter 2 μm

The red circles in the left figure represent particles at a certain time. On the other hand, right figure shows the status at the time after Δt [sec] of left figure. The particles located in red circles move to the location shown with green circles. The flow rate can be calculated by estimating the distances between each red and green circle, and it can be considered as one of the index for dynamics of the particles.

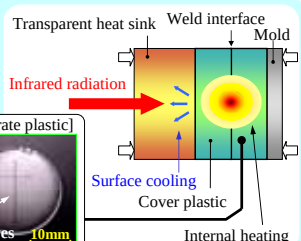
Welding of Microfluidic Chip on Plastics

Microfluidic chip is a device to extract DNA, amplify and separate genes, determine gene arrangement, etc. with high speed from ultra-small amount of blood or cells by manufacturing micro- to nanometer flow channels on one chip. It is expected to be widely used in various fields like medicine, agriculture, etc. One of the manufacturing method of micro flow channels is bonding substrate plastic printed grooves and cover plastic, and this method has advantages of low cost and mass production.



Infrared welding process

As compared to thermal conduction, radiation can weld high speed for a thermoplastic. But the surface of a plastic is overheated due to absorption of infrared radiation. This process is the solid heat sink with transparent to infrared radiation contacts with the surface of a plastic. Consequently, plastic surface is protected from thermal damage. We are studying microfluidic chip welding by this process.



YMD Lab.

2005 Members

Professor : Yukio YAMADA
yamada@net.ymdlab.mce.uec.ac.jp
Research Associate : Naoto KAKUTA
kakuta@ymdlab.mec.uec.ac.jp

13 Graduate Students (D1: 2, M2: 6, M1: 5)
4 Undergraduate Students (B4: 4)

Lab Address: 617 room, E-4 Building
URL http://www.ymdlab.mce.uec.ac.jp

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